Study of *Arsenicum album* Homeopathic Medicines: Insights from UV-Visible Spectroscopy

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Abstract

Recent advances in homeopathic medicines have revolutionized the diagnosis and treatment for various health care diseases including severe acute respiratory syndrome. In UV-Vis spectroscopy, the amount of light absorbed is related to the amount of absorbing species present and hence, qualitative information about the medicinal solute species present in Arsenicum album can be obtained. The purpose of this primary research is to elucidate "Whether the medicinal solute species exist at higher potencies where the therapeutic effect is potentially high".

We have investigated different potencies of Arsenicum album procured from three manufacturers through measurements of optical absorption spectrum using a sophisticated computer controlled UV-Visible 2600 spectrometer. In order to interpret the results, a mother tincture Q sample was used to study the characteristic UV-Vis absorption behaviour of Arsenicum album at various concentrations. The results revealed that irrespective of the potencies, arsenic trioxide medicinal solute species present in the Arsenicum album medicines is in the form of quantum sized nanoclusters. The specific interaction between arsenic trioxide solute species in the Arsenicum album is expected to be reduced upon dilution. Hence, the study of the dilution series of Arsenicum album provides insights. The optical absorption results indicate that arsenic trioxide solutes dilute uniformly in the solvent medium and follow the laws of homeopathy. The study suggests that qualitative analysis of the Homeopathic medicines is possible by UV-Visible spectroscopy.

Keywords: *Arsenicum album*, Dilution Series, Homeopathy, Optical absorption, UV-Vis Spectroscopy**.**

Introduction

Homeopathic medicines have been used for more than two hundred years worldwide. Homeopathic medicines are prepared from substances derived from minerals, plants and animals.[10,21,29] Millions of people are taking homeopathic medicines for various health conditions including cough, cold, flu and related allergies, muscle pain due to body stress and strains and chronic disease conditions like Bronchial asthma, Diabetes mellitus etc.[4,15] Hahnemann, a German Physician who is considered as the Father of Homeopathic system of medicine insisted that the Homeopathic drugs retained their therapeutic power by a process named as "Potentization".[16,28] Hahnemann proposed a preparation procedure for creating high potency homeopathic drugs where by the substance is diluted and shaken vigorously over and over again, until the mixture contains no measurable trace of original matter.

There have been several reviews on the scientific evidences that homeopathic medicines are generally safe and have negligible side effects. The homeopathic drugs are not disease-specific like the drugs of other systems of medicine. Instead, homeopathic medicines can be used for different ailments based on the nature of the symptoms patients have and psycho-physical characteristics of individuals.[21]

Arsenicum album is one of the Homeopathic medicines prescribed by the homeopaths for various healthcare diseases. The *Arsenicum album* has been prepared by diluting arsenic trioxide (As_2O_3) until there is an extreme dilution, such that a pill would not even have any measurable amount of the original As_2O_3. As the potency of the *Arsenicum album* increases, the dilution factor exceeds the Avogadro's limit. For instance, *Arsenicum album* with potencies such as 30C and 200C have huge dilution factors of 10^{60} and 10^{400}. The several orders larger than Avogadro's number of 6.023 x 10^{23}.[6] In general, dilution effectively enhances the potency and the scientific evidence suggests that the effect of homeopathic drugs is mainly due to the placebo effect.[19] However, this is not true in all the cases.

In order to evaluate general homeopathic principles, *Arsenicum album* medicines have been designed for cell based *in vitro* studies. The therapeutic effect of *Aresnicum album* potencies investigated *in vitro* using a continuous cell line MT4 is an example.[12]

The effectiveness of *Arsenicum album* to biological systems was evaluated and it was reported that the high potency of *Arsenicum album* medicine reduces genotoxic effect.[2,3,11] Despite serious safety concerns, extremely diluted *Arsenicum album* is used to cure digestive disorders, food poisoning, allergies, etc. In these respects, there should not be a major concern in safety aspects on using homeopathic medicines for clinical treatments. However, one should consider the purity and source of the materials used and technological aspects of the production and manufacturer of the Homeopathic medicine.[24] The World Health

Organisation (WHO) which promotes health, has stated that with the growing popularity of use of homeopathic medicines worldwide and the rapid expansion of its global market, the safety and quality of homeopathic medicines have become a major concern for health authorities and pharmaceutical industries.[27] These conflicting study reports confirmed the necessity for further investigations for homeopathic potency preparations.

Further, an important question often discussed and debated by the critics of Homeopathy is whether the arsenic trioxide exists at higher potencies where the therapeutic effect is potentially high. Several experimental techniques such as High Performance Liquid Chromatography (HPLC), Inductively coupled Plasma - Mass Spectrometry (ICP – MS), Dielectric Dispersion, Thermoluminescence, Proton Magnetic Resonance Spectroscopy, Ultra Violet spectrometry have been often used by the researchers to test the active element present in the homeopathy medicines. Based on the results obtained from these techniques, the researchers have observed that the chemical measurement methods are less sensitive and unable to detect the active element present in the homeopath medicine.

If the techniques used are physical measurement methods, the activity of homeopathic remedies with high potency can be detected. To gain more insights and to make full use of the *Arsenicum album* medicine, the *Arsenicum album* drugs were studied using a sophisticated UV-Vis spectrometer (UV-Vis 2600, Shimadzu) available in our laboratory. With UV-Vis spectroscopy, the UV-Vis light is passed through a sample and the transmittance of light by the sample is measured. It is one of the most popular techniques because it is versatile and is able to detect nearly every solute molecule in the medium.

Material and Methods

The measurement samples used for the UV-Vis absorption studies were obtained from the three leading drug manufacturers in India and these manufacturers are denoted in this manuscript as M1, M2 and M3. Manufacturing details (Batch, date of manufacturing) of *Arsenicum album* samples M1, M2 and M3 and their potencies as tabulated in table 1 were studied using a computer controlled UV-Vis 2600 spectrometer with wavelength range from 200-800 nm. Please note that all the medicines used in the present study are within the validation period from the manufacturing date.

Prior to the UV-Vis absorption measurements, the standard cleaning procedure prescribed by the instrument supplier was followed for cleaning the Quartz cuvettes that are used for the optical absorption measurements. The UV-Vis absorption spectra were recorded at a constant temperature 298 K with laboratory humidity level between 45 and 50%. The spectrometer was switched on for about 90 minutes prior to start the measurements to minimize the electronic signal drift caused by the spectrometer. For the base line calibration, the cuvettes filled with the homeopathic liquid dilution supplied by the respective manufacturers were used. The absorption spectra were recorded multiple times (minimum two times) to check the accuracy of the data reported.

All the measurements were carried out in identical experimental conditions to avoid any systematic or random errors. A mother tincture Q sample procured from the manufacturer M2 was used to study the characteristic UV-Vis absorption behaviour of *Arsenicum album* at various concentrations. Prior to every measurement, the quartz cuvettes were rinsed with homeopathic liquid dilution several times and dried using cloth and dry air.

Results and Discussion

Figure 1 shows a typical UV-Vis absorption spectrum of *Arsenicum album* of potency 6 CH obtained from the manufacturer M1 in the wavelength region from 200 to 800 nm.

Table 1
Manufacturing details of *Arsenicum album* samples and their potencies obtained from three manufacturers M1, M2 and M3.

***Arsenicum album*/ Identification**	**Potency**	**Batch**	**Manufacturing Month/Year**
Manufacturer 1 M1	6H 200CH 10M CM	0260468 0260469 0283170 0303076	05/19 05/19 09/19 11/19
Manufacturer 2 M2	6H 200CH 10M CM	20D26090 20D26091 20D26092 20D26093	12/19 12/19 12/19 12/19
Manufacturer 3 M3	6H 200CH 10M CM	3899 5742 1206 1206	07/18 11/18 12/19 12/18

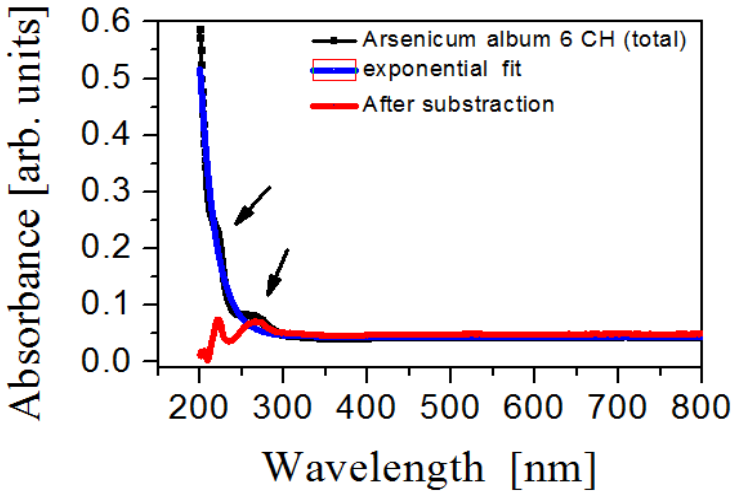

Figure 1: Optical absorption spectrum of *Arsenicum album* 6 CH potency recorded in the range 200 - 800 nm. The arrows in the figure identify the peaking regions in the absorption spectrum. The exponential fit to the original spectrum is identified by blue line. The experimental data (black line) after subtraction from the exponential fit (blue line) is depicted by red colour.

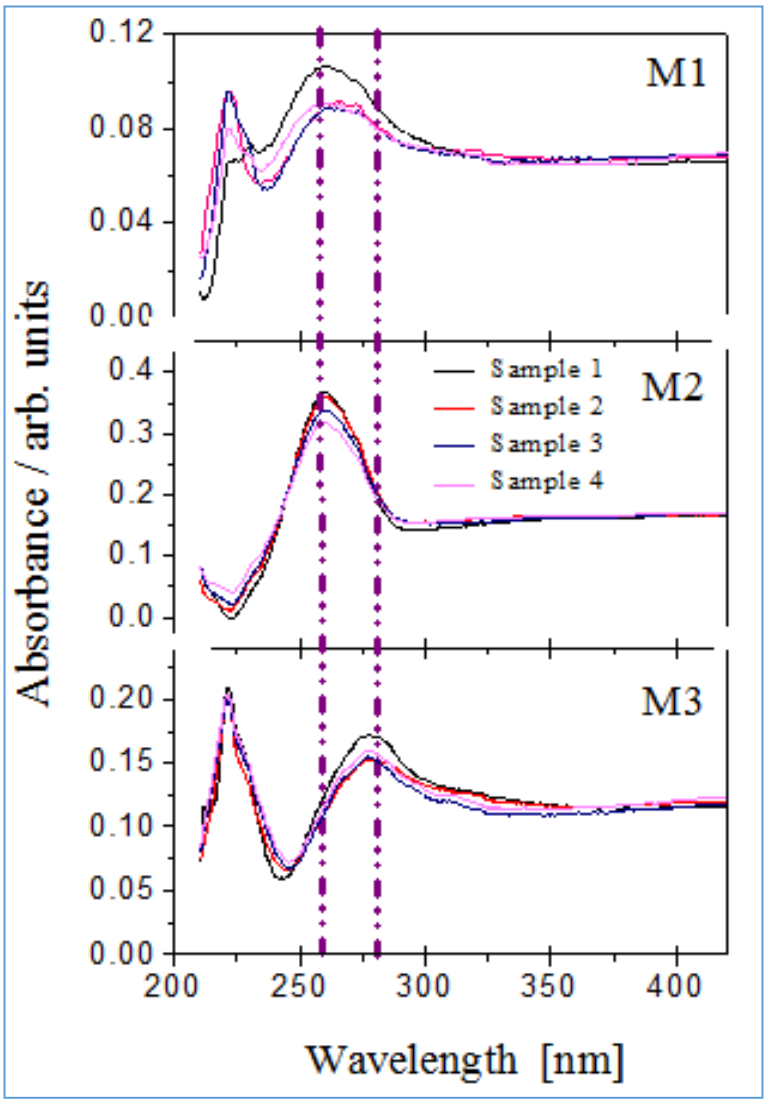

Figure 2: Variation of optical absorption intensity as a function of wavelength in the UV region 200 - 400 nm for 6 CH (Black line), 200 CH (Red line), 10 M (Navy line) and CM (Magenta) samples obtained from the three manufacturers M1 (top panel), M2 (middle panel) and M3 (bottom panel). The vertical lines in the figure identity the absorption peak positions.

The noticeable features present in the spectrum as depicted in figure 1 are low intense absorption bands in the UV region (200 - 400 nm) with well - known monotonically decreasing absorption intensity with wavelength. Since the density of optical absorption at any wavelength depends highly on the chemical structure of the solvent medium, it is considered that the deviation from the exponential behavior (absorption peaks and shoulders) is caused by the presence of absorbed species or nanostructures.[18] To resolve the absorption peaks, the original spectrum was fitted with an exponential function and subtracted from the total absorbance.

The qualitative data analysis procedure has been followed for the UV-Vis absorption analysis of the other *Arsenicum album* samples listed in table 1. It has been established that the absorption peaks that exhibit in the UV region (200-400 nm) are caused mainly by the presence of nanostructures.[1,13,14] Since the existence of any radicals in the present medicines are ruled out, it is considered that the optical absorption features evolved correspond to the existence of quantum sized As_2O_3 nanoparticles in *Arsenicum album*.

Figure 2 shows the variation in optical absorbance as a function of wavelength in the UV region 200 - 400 nm for 6 CH, 200 CH, 10 M and CM samples obtained from the manufacturers M1 (top panel), M2 (middle panel) and M3 (bottom panel). As seen in figure 2, a positive absorption peak shift of ~ 20 nm was observed in all the four potencies 6 CH, 200 CH, 10 M, CM of the sample M3 when compared to that obtained for M1 and M2. Also the absorption peak intensity of the sample M2 is found to be higher when compared to that obtained on the samples M1 and M3. To see more clarity, the absorbance versus wavelength curve of *Arsenicum Album* potency 200 CH fitted is presented separately in figure 3. After examining the results of figure 2 and figure 3, it has been revealed that the optical absorption measurements are sensitive to *Arsenicum album* nanostructures.

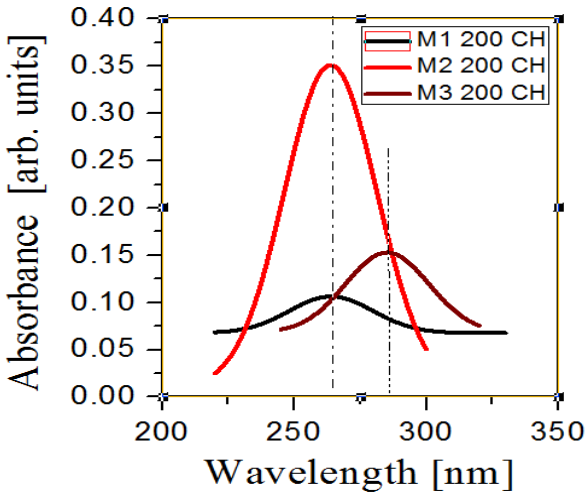

Figure 3: Fitted absorbance versus wavelength curve of *Arsenicum album* in potency 200 CH obtained from three different manufacturers M1 (black curve), M2 (red curve) and M3 (brown curve). The vertical lines in the figure identity the absorption peak positions.

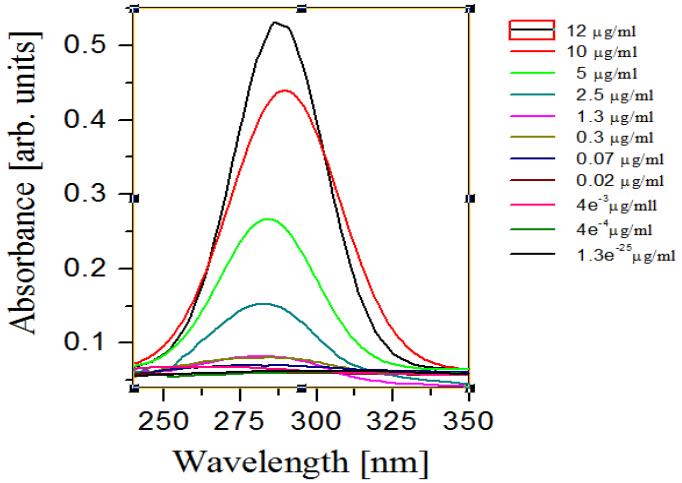

Figure 4: UV - Visible absorption spectra of *Arsenicum album* obtained in the wavelength region between 240 and 350 nm at various concentrations.

Since *Arsenicum album* is a very useful homeopathic medicine and its therapeutic effect is more at higher potency, the concentration dependent UV-Vis absorption results obtained on a mother tincture Q sample supplied by the manufacturer M2 have been analysed. Figure 4 shows the optical absorption results obtained in the wavelength region between 240 and 350 nm at various concentrations of *Arsenicum album*. It is seen that the absorption peak value decreases as the dilution of *Arsenicum album* increases.

To understand the significance of these observed results, we looked at the results of earlier literature reports. It has been mentioned that the shift of the optical absorbance band gives more knowledge about the solute species as well as the kind of interaction with solvents, whereas the intensity of the absorption band depends on the solute concentration.[9,22,23] Subastri et al through their UV-Vis spectroscopy studies reported that arsenic trioxide nanoclusters exhibit UV absorption peak in the ultraviolet region and these absorption peak shifts towards positive wavelength as the cluster grows.[26] Similar results were reported by Saad et al also.[23]

Finding the variation of absorption peak intensity with concentration of *Arsenicum album* is important from a practical point of view since the concentration dependent optical absorption properties are directly related to the density of light absorbed by *Arsenic album*. It implies that, in the absence of any absorption peak shift effect, the variation in the absorption peak intensity provides meaningful information about the nature of arsenic trioxide nanostructures along with their long range coordinated solvent molecules.

Figure 5 shows the variation of absorption peak intensity ΔI_P as a function of *Arsenicum album* concentration. The top X-axis illustrates the homeopathy potency C corresponding to the concentration of *Arsenicum album*. It is seen that initially there was a rapid decrease in the ΔI_P value upto the concentration 0.01 µg/ml value followed by a marginal decrease as the concentration of *Arsenicum album* decreases and below 0.001 µg/ml value, the ΔI_P value is independent to the concentration of *Arsenicum album*.

A way to explain the importance of the observed concentration dependent absorption behaviour is to find the attractive forces that act during the formation of *Arsenicum album*. It is to note that such interaction forces that develop across the As_2O_3 solutes in *Arsenic album* are physical in nature, not related to any chemical oxidation/reduction process and hence there is better knowledge about whether the As_2O_3 solutes modifies the ethanol solvent medium through structural co-ordination or distorts. In general, various types of interaction forces exist between As_2O_3 solutes in *Arsenicum album* solution, however, the most acceptable forces that are relevant in this context are As_2O_3 - As_2O_3 interaction (inter-molecular attraction between solute particles) and As_2O_3 - ethanol solvent medium (inter-molecular attraction between solute and solvent species).[5,7]

Since the dispersed As_2O_3 in *Arsenicum album* is in the form of nanostructures, it is considered that their surface energy contributes for the physical co-ordination of solvent molecules across the surface of As_2O_3 solutes.[8] This implies that the charges that are spread across the As_2O_3 solute structures orient the ethanol solvent molecules through the action of inter-dipolar interactions.[17,20,25] Based on the above, we presume that in the absence of solute-solute interaction, the negligible amount of As_2O_3 solute species present in the homeopathic dilution has the ability to create long range dipolar interactions between As_2O_3 and solvents as the electrostatic imprints of the As_2O_3 solute species carried by ethanol based diluent molecules.

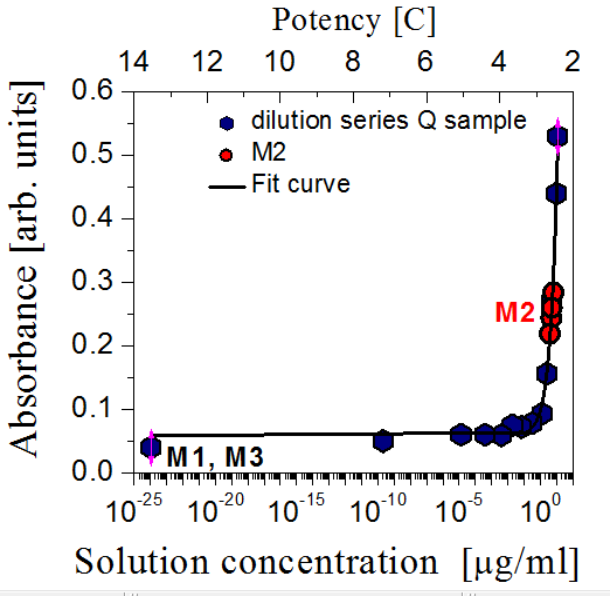

Figure 5: Variation of optical absorption peak intensity ΔI_P as a function of *Arsenicum album* concentration in µg/ml. The Homeopathy potency C values corresponding to the concentration of *Arsenicum album* are mentioned in the top X-axis.

A growing body of evidence suggests that although homeopathic medicines are in general considered to be safe when administered appropriately, the amount of starting materials present in the homeopathic medicines may depend on the method of preparation.[27]

It suggests that safety issues may arise if the differences in method of preparations are neglected. To verify the measurement consistency of the test samples, the UV-Vis absorption results obtained from the three manufacturers/suppliers M1, M2 and M3 were compared. The analysis of the optical absorption spectra revealed that absorption peak intensity of samples identified as M2 is in the region where the absorption intensity is highly dependent on the concentration of *Arsenicun Album*.

Absorption peak intensity obtained from the samples M1 and M3 is found to be lower and the values are in the constant region where the absorption intensity is independent on the *Arsenic album* concentrations. Also, it should be noted that the absorption peak position in the UV range responsible for the arsenic trioxide clusters is shifted towards positive region by 20 nm wavelength for sample M3 when compared to the samples supplied by other two suppliers M1 and M2. It reveals that there are considerable differences in the results reported.

According to the proposed theory for homeopathic medicine, the investigations of the dilution series face the fundamental problem that when the homeopathic medicine becomes dilute and exceeds the Avogadro's number limit, no original solute particles can be observed in the medicine. Remarkably, the present optical absorption data provides evidences for the presence of traces of nanostructures in the homeopathic solution. In more diluted systems, the interaction between the solute species is expected to become weak and therefore, the solute molecules can be easily solvated with the arsenic trioxide solute species. Thus the results of our study indicate that the charges delocalized at the surface of the arsenic trioxide solutes have a pronounced effect for the creation of inter-molecular interaction with the solvent medium and for the preservation of curative power in homeopathic dilution.

Conclusion

In this study, we have recorded the UV-Visible spectrum of homeopathy medicine dilution in different potencies viz. 6 CH, 200 CH, 10 M, CM of *Arsenicum album* obtained commercially from three manufacturers. The results presented here indicate that studies using UV-Visible spectrometer help in better quality control of homeopathic medicine dilution. A Gaussian decomposition approach was applied to the absorption spectra of *Arsenicum album* medicines to resolve arsenic trioxide solute component from the total absorption signals. The most important findings from the analysis of the optical absorption data of *Arsenicum album* are: i) evidences for the presence of arsenic trioxide nanostructures at higher potencies and ii) exponential variation of absorption peak intensity with concentration of *Arsenicum album*. These results suggest that the arsenic trioxide solutes dissolve uniformly in the ethanol based solvent medium and follow the laws of Homeopathy. The results of the present study provide a conclusion that there are variations in the stability aspects among the *Arsenicum album* homeopathic medicines supplied by three manufacturers. It reveals that although high potency *Arsenicum album* homeopathic medicines are considered to be safe, one has to give special attention in choosing the quality of the source materials and the excipients used in the manufacture of homeopathic medicines. Elaborate investigation of optical absorption properties on homeopathic medicines obtained from plant and animal kingdom and control of the experimental methods are necessary for future development.

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