

Comparative Study of Ultra Molecular Homeopathic Dilutions and Potentised Preparations by UV Spectroscopy in Relation with Beer's Law.

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Summary

Apposite assay to create an evidence for homeopathic system of medicine assembles basic, preclinical as well as clinical research. In foundation of all these studies the only clue which remained is to search for mechanism of action of medicines. Ultra molecular dilutions of homeopathically potentised drugs proved clinical efficacy in number of disease conditions. But same potencies fail to reproduce standard tests in conventional medicine. UV spectrophotometric analysis carried in different experiments proved logical. Detection of drug substance cannot be appeased in homeopathic potencies but altered physicochemical dimensions and properties could be relevant to the issue. In conventional medicine concentration of drug substance is according to Beer's law, on the contrary homeopathic potencies shows nonlinear phenomena at 210nm when compared to serial dilutions of same drugs and dispensing alcohol as a standard control.

Key words: UV spectroscopy, homeopathy, Beer's law, dilutions, potencies.

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Introduction

Explanation of the much greater effect of the solution is prepared than the dry undissolved extract through the variety of experiments all over world². There is a good deal of talk about the purely dynamic action of the drugs, the incredibly small quantity of them that will suffice for the cure, and the absolute superiority in point of power of the weakest on chiefly in relation to the exalted susceptibility present in disease, for it is stated that same doses have no effect on the healthy or on those patients for whose disease the drug is not suitable; but there is in this essay no allusion to an increase of power by process of trituration and succussion, indeed no particular mention is made of any peculiarity in the homeopathic pharmaceutical processes.

Diminution of the dose was advised nominally for the sake of preventing the too violent action of the remedy given according to the new therapeutic principle, the sensibility being so much exalted for such medicines in the diseased state and this doctrine is again precisely and explicitly expressed in a short essay published in 1809⁵.

The Beer-Lambert law (or Beer's law) is the linear relationship between absorbance and concentration of an absorbing species. There array of limitations to the Beer's law explaining nonlinearity which stray the results but none of them can give corroboration to the nonlinear phenomena of homeopathically potentised drugs.

Keeping one's countenance doesn't show any remedial answer for this corollary. Explicit theories can explain some causes by considering the limitations to them and homoeopathy. Today the homeopathic dynamics is completely not revealed but through variety of clinical and statistical analyses; the critical solution can be drawn in favor of system. Basic research can prove a major step of help for future study and analogies.

Material and Methods

All homeopathic medications, dispensing alcohol and their respective machine made dilutions and potencies were procured from K.R. Homeo Pharmacy, Pune. Distilled water was prepared in the laboratory using distillation assembly. Cuvettes were procured from Optiglass Ltd. - 20/Q/485. UV spectrophotometer used was JASCO version 530, made in Japan. Graph Pad Prism data analysis software was procured from Graph Pad Inc. USA.

According to rules and regulations of Indian homeopathic pharmacopeia; dispensing alcohol was subjected to UV spectroscopic analysis at 210 nm. Various homeopathic centesimal (c) dilutions, 30c, 200c, 1000c (1M) of *Conium maculatum*, *Argentum metallicum*, *Tarentula hispanica* respectively were taken and controls (succussed potentization medium) were prepared in sterile conditions, along with dispensing alcohol and dilutions were also prepared and after preparation randomized and blinded. The code was only disclosed after data analysis was completed. Three separate sets of samples of each potentization level were filled in test tubes. The dilutions were analysed by UV spectroscopy to assess the absorbance at a fixed wavelength of 210 nm. The selection of wavelength was justified as the wavelength of analysis of alcohol was also 210 nm. These dilutions were subjected to UV light in quartz cuvettes along with a blank which was distilled water. The clear side of the quartz cuvette was exposed to the UV light and the hazy surface was used to hold the cuvette with fingers while placing it in the holder. Thereafter the digital recordings of the UV spectrophotometer were analysed. Absorbance was plotted on Y

axis and wavelength was plotted on X axis. The increasing dilutions demonstrated a change in the absorbance which was plotted graphically. The slope of the line and its equation was calculated. This provided us with the knowledge about the linearity and non linearity of the behavior of the dilutions. The various curves were analysed using Microsoft excel and Graph Pad Prism V software. r^2 value was calculated and the equation of the line was framed depicting the spectroscopic molecular nature of each dilution. $Y= mx+C$ formula was used to determine the equation of the line for each dilution and potency. The experiment was framed and executed as to discernibly differentiate between the various dilutions and potencies using UV spectroscopy.

Statistical Analysis: All data was analysed using Microsoft excel and Graph Pad Prism V.

Results

Dispensing Alcohol:

Dispensing alcohol was subjected to UV spectroscopic analysis at 210 nm. From the figure.1 it is evident that **dispensing alcohol** exhibited a series of absorbance values at when measured at 230, 250 and 270 nm using monochromatic UV Vis spectroscopy. It is known that the solution follows Beer Lambert's law when the absorbance is less than 1. Hence the investigational solution was found to obey Beer Lambert's law. This reading was plotted and is depicted in the graph. At 230 nm the absorbance was 0.358, at 250 nm the absorbance was 0.350 and at 270 nm the absorbance was found to be equal to 0.110.

Dilution- 30D

From the figure 2 it is evident that **Conium** dilution 30D showed an absorbance value of 0.998 at 210 nm when measured using monochromatic UV Vis spectroscopy. It could be seen that the absorbance was nearing 1. It is known that the solution follows Beer Lambert law when the absorbance is less than 1. Hence the investigational solution was found to obey Beer Lambert's law. This reading was plotted and is depicted in the graph.

Dilution- 200D

From the figure 2 it could be elucidated that **Conium** dilution 200D exhibited an absorbance value of 0.500 at 210 nm when measured using monochromatic UV Vis spectroscopy. Solutions which have an absorbance value of less than 1 obey Beer Lambert's law. Hence the sample solution obeyed Beer Lambert's law. This reading was plotted and is depicted in the graph.

Dilution- 1000D

The graph 2 shows that at a dilution of 1000D **Conium maculatum** demonstrated an absorbance value of 0.100 when subjected to a monochromatic UV light at 210 nm. The solution was found to be in concert with Beer Lambert's law. The reading obtained was plotted and depiction is discernable in the graph.

Potency-30C

From the figure 3 it could be seen that **Conium** potency 30C showed an absorbance value of 0.895 at 210 nm when measured using monochromatic UV Vis spectroscopy. It is known that Solutions which have an absorbance value of less than 1 obey Beer Lambert's law. Hence the sample solution obeyed Beer Lambert's law. This reading was recorded and is depicted in the graph.

Potency-200C

From the figure 3 it is evident that *Conium* potency200C showed an absorbance value of 0.300 at 210 nm when measured using monochromatic UV Vis spectroscopy. It could be seen that the absorbance was nearing 1. It is known that the solution follows beer lambert law when the absorbance is less than 1. Hence the investigational solution was found to obey beer lambert's law. This reading was plotted and is depicted in the graph.

Potency-1000C

The graph 3 shows that at a dilution of 1000C *Conium maculatum* demonstrated an absorbance value of 0.200 when subjected to a monochromatic UV light at 210 nm. The solution was found to be in concert with Beer Lambert's law. The reading obtained was plotted and depiction is discernable in the graph.

Dilution- 30D

From the figure 4 it is evident that *Argentum met* dilution 30D showed an absorbance value of 0.887 at 210 nm when measured using monochromatic UV Vis spectroscopy. It could be seen that the absorbance was nearing 1. It is known that the solution follows beer lambert law when the absorbance is less than 1. Hence the investigational solution was found to obey beer lambert's law. This reading was plotted and is depicted in the graph.

Dilution- 200D

From the figure 4 it could be elucidated that *Argentum met* dilution 200D exhibited an absorbance value of 0.555 at 210 nm when measured using monochromatic UV Vis spectroscopy. Solutions which have an absorbance value of less than 1 obey Beer Lambert's law. Hence the sample solution obeyed beer lamberts' law. This reading was plotted and is depicted in the graph.

Dilution- 1000D

The graph 4 shows that at a dilution of 1000D *Argentum met* demonstrated an absorbance value of 0.256 when subjected to a monochromatic UV light at 210 nm. The solution was found to be in concert with Beer Lambert's. The reading obtained was plotted and depiction is discernable in the graph.

Potency- 30C

From the figure 5 it could be seen that *Argentum met* potency 30C showed an absorbance value of 0.660 at 210 nm when measured using monochromatic UV Vis spectroscopy. It is known that Solutions which have an absorbance value of less than 1 obey beer Lambert's law. Hence the sample solution obeyed beer Lambert's law. This reading was recorded and is depicted in the graph.

Potency- 200C

From the figure 5 it is evident that *Argentum met* potency200C showed an absorbance value of 0.280 at 210 nm when measured using monochromatic UV Vis spectroscopy. It could be seen that the absorbance was nearing 1. It is known that the solution follows beer Lambert law when the absorbance is less than 1. Hence the investigational solution was found to obey beer Lamberts' law. This reading was plotted and is depicted in the graph.

Potency- 1000C

The graph 5vshows that at a dilution of 1000C *Argentum met* demonstrated an absorbance value of 0.150 when subjected to a monochromatic UV light at 210 nm. The solution was found to be in concert with Beer Lambert's law. The reading obtained was plotted and depiction is discernable in the graph.

Dilution- 30D

From the figure 6 it is evident that *Tarentula* dilution 30D showed an absorbance value of 0.5440 at 210 nm when measured using monochromatic UV Vis spectroscopy. It could be seen that the absorbance was nearing 1. It is known that the solution follows beer Lambert law when the absorbance is less than 1. Hence the investigational solution was found to obey beer lambert's law. This reading was plotted and is depicted in the graph.

Dilution- 200D

From the figure 6 it could be elucidated that *Tarentula* dilution 200D exhibited an absorbance value of 0.3340 at 210 nm when measured using monochromatic UV Vis spectroscopy. Solutions which have an absorbance value of less than 1 obey beer Lambert's law. Hence the sample solution obeyed beer Lambert's law. This reading was plotted and is depicted in the graph.

Dilution- 1000D

The graph 6 shows that at a dilution of 1000D *Tarentula hispanica* demonstrated an absorbance value of 0.1220 when subjected to a monochromatic UV light at 210 nm. The solution was found to be in concert with Beer Lambert's law. The reading obtained was plotted and depiction is discernable in the graph.

Potency-30C

From the figure 7 it could be seen that *Tarentula* potency 30C showed an absorbance value of 0.7750 at 210 nm when measured using monochromatic UV Vis spectroscopy. It is known that Solutions which have an absorbance value of less than 1 obey Beer Lambert's law. Hence the sample solution obeyed Beer Lambert's law. This reading was recorded and is depicted in the graph.

Potency-200C

From the figure 7 it is evident that *Tarentula* potency200C showed an absorbance value of 0.3000 at 210 nm when measured using monochromatic UV Vis spectroscopy. It could be seen that the absorbance was nearing 1. It is known that the solution follows Beer Lambert's law when the absorbance is less than 1. Hence the investigational solution was found to obey Beer Lambert's law. This reading was plotted and is depicted in the graph.

Potency-1000C

The graph 7shows that at a dilution of 1000C, *Tarentula hispanica* demonstrated an absorbance value of 0.1500 when subjected to a monochromatic UV light at 210 nm. The solution was found to be in concert with Beer Lambert's law. The reading obtained was plotted and depiction is discernable in the graph.

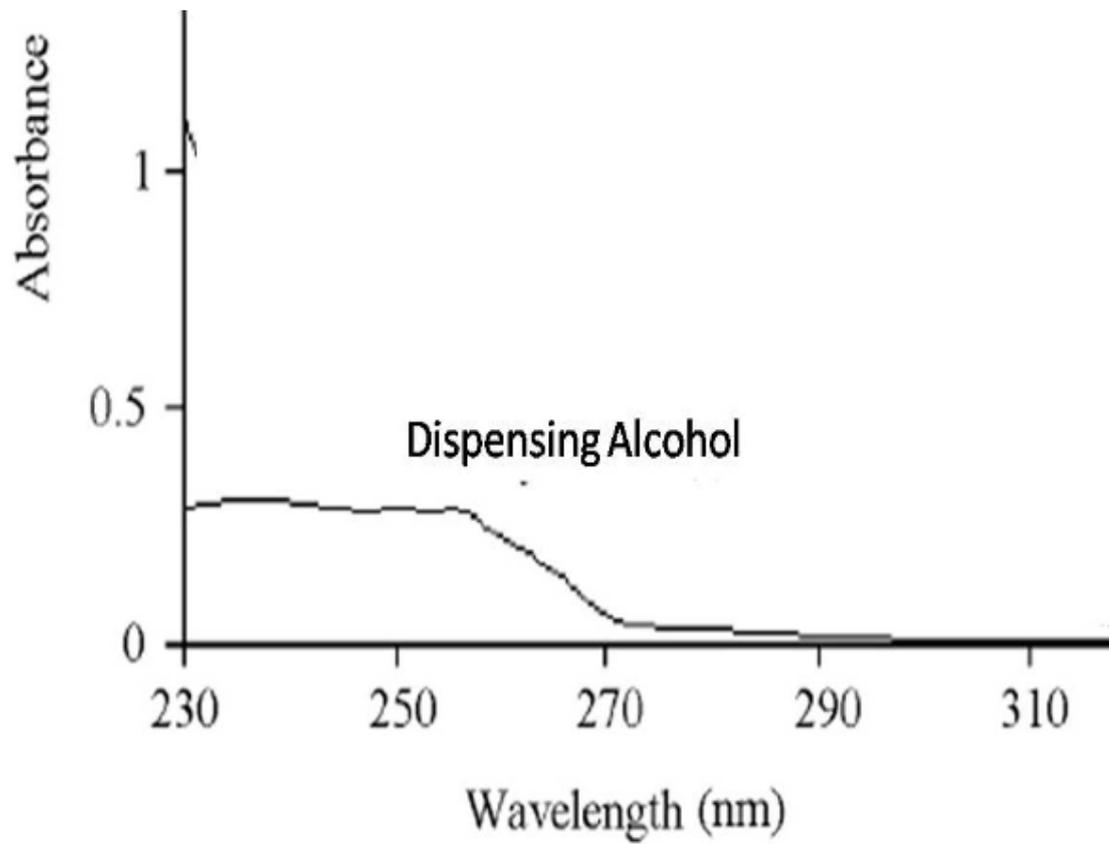


Fig:1 Figure showing the UV analysis of dispensing alcohol at 210 nm

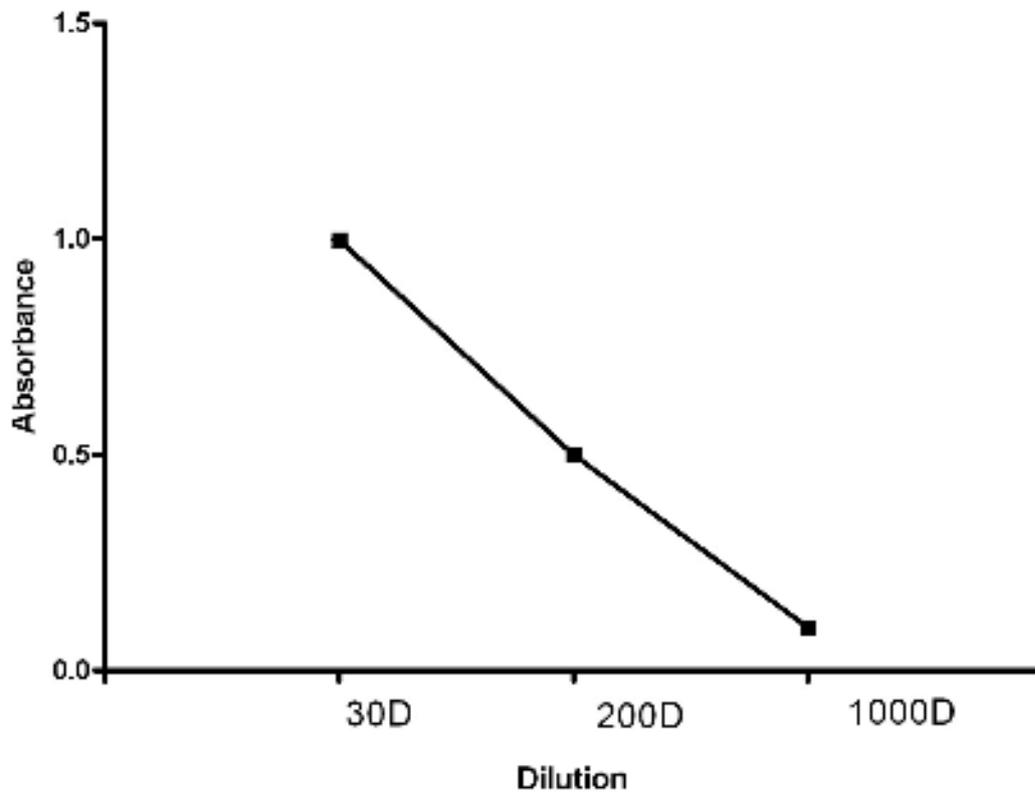


Fig.2 Figure showing the UV analysis of *Conium* dilution at 210 nm.

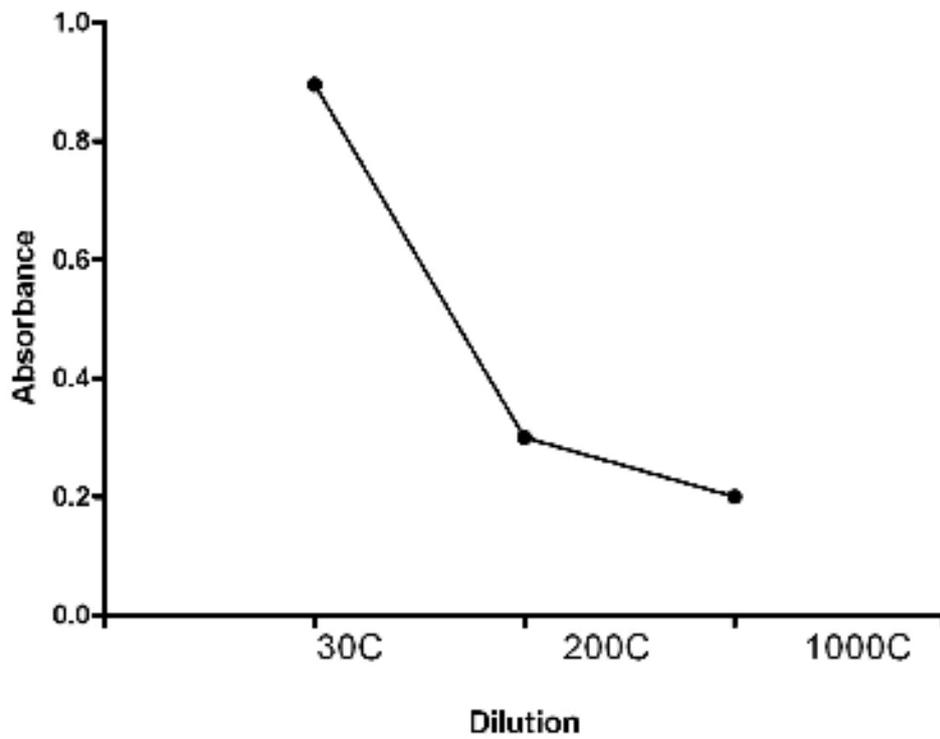


Fig.3 Figure showing the UV analysis of *Conium* potency at 210 nm.

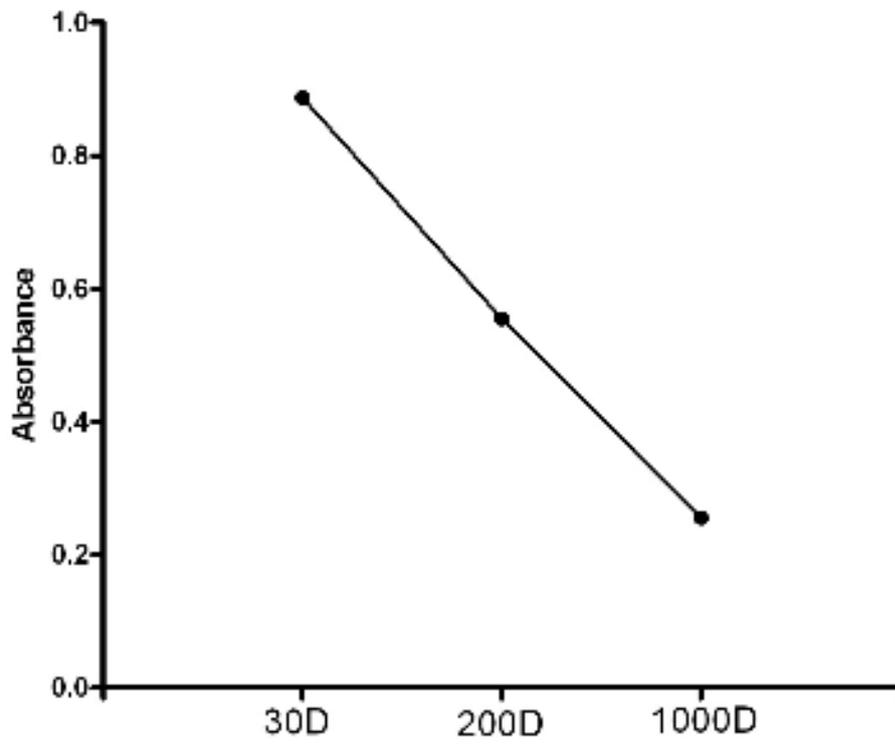


Fig.4 Figure showing the UV analysis of *Argentum met* dilution at 210 nm.

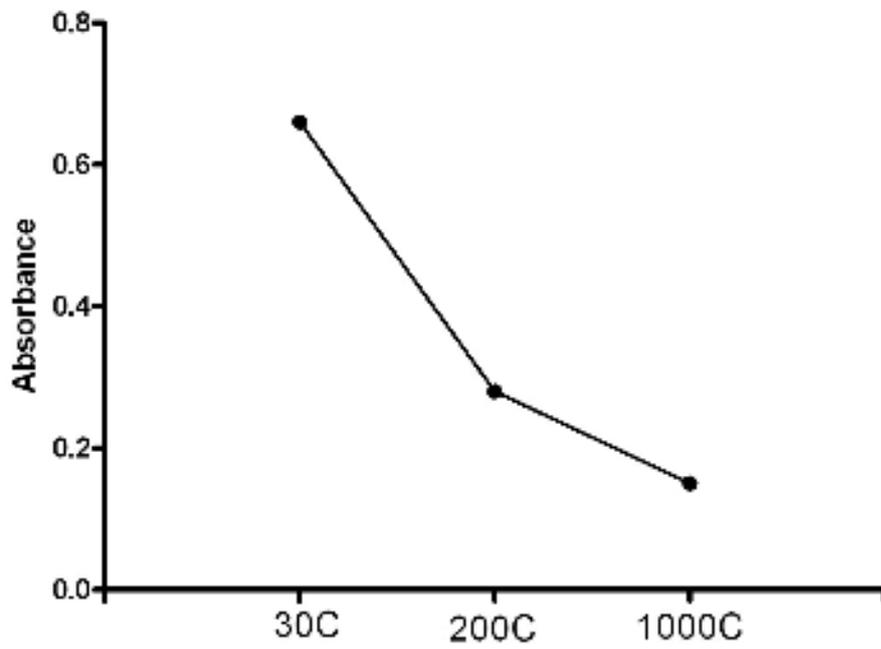


Fig.5 Figure showing the UV analysis of *Argentum met* potency at 210 nm.

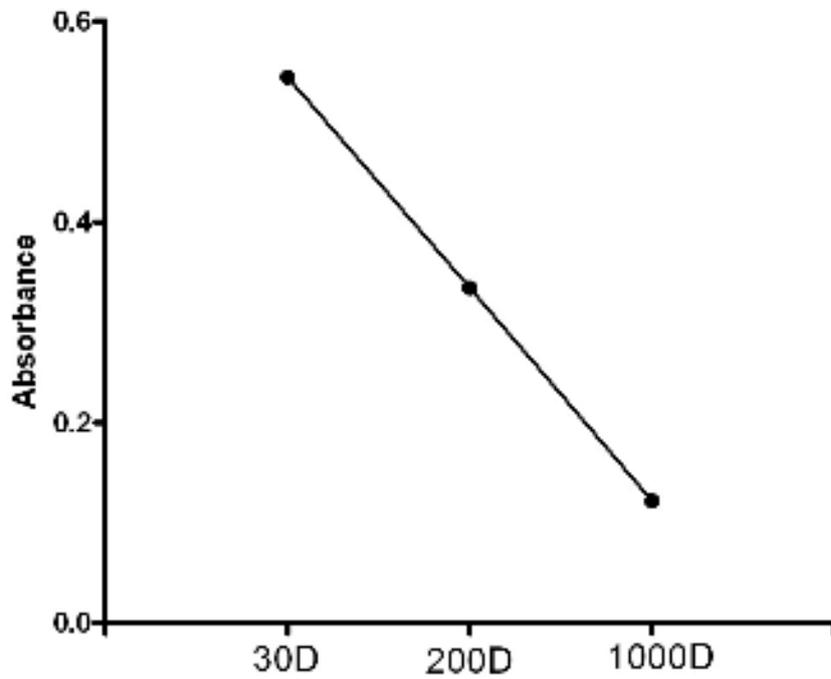


Fig.6 Figure showing the UV analysis of *Tarentula hispanica* dilution at 210 nm.

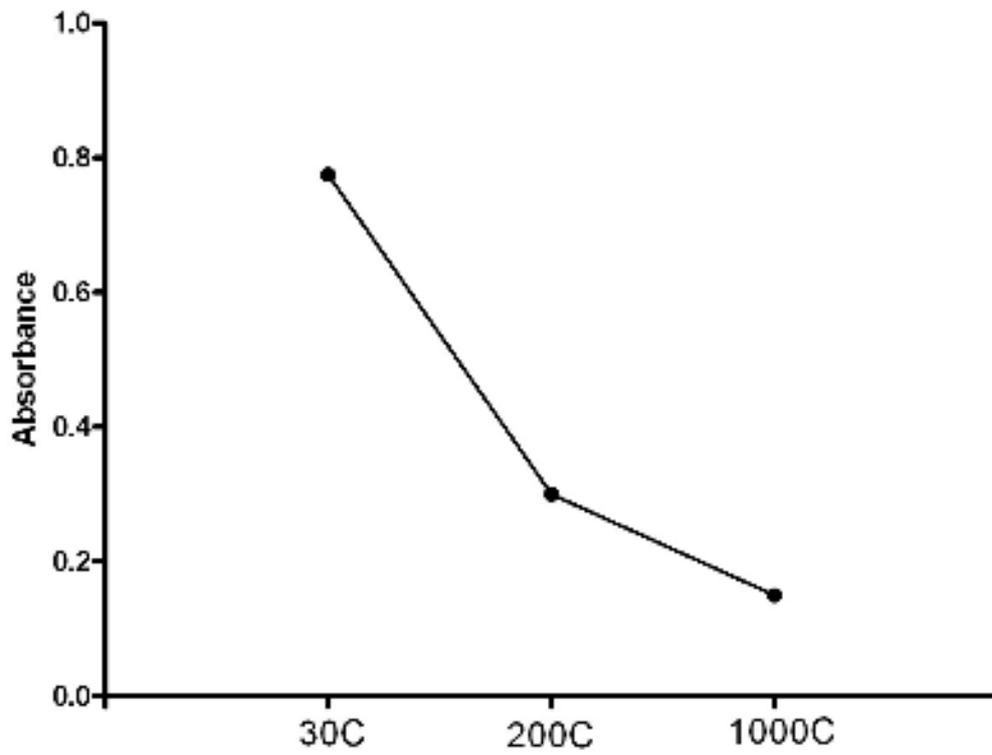


Fig.7 Figure showing the UV analysis of *Tarentula hispanica* potency at 210 nm.

Discussion

Beer-Lambert's law has been used considerable in research to determine the behaviour of the solutions under the effect of dilution. The transmittance is inversely proportional to the concentration of the solution^{1,2}. However, it could be seen that UV absorbance followed a linear pattern with dilution of each homoeopathic drug where as the potencies followed a nonlinear pattern. This demonstrates that there is a discernable difference between UV absorbance of dilutions and potencies. It is reiterated the homoeopathic concept of potentiation. It is evident from the UV readings that dilution is primarily linear process where as in potentiation on the transmission of UV light through the potentiated solution. A similar pattern of a graphical representation was visible at *Conium maculatum*, *Argentum metallicum*, and *Tarentula hispanica*. Our results Provide evidence to the work carried out by Wolf et al., 2009 who carried out extensive studies to determine the effect of UV on the machine made homoeopathic dilutions and potencies. Our observations are in concert with Endler et al., 2009. Hence, in the light of previous research work carried out before our studies and our own observations, the homoeopathic principles laid down by Hahnemann seem to hold true¹². However, careful and systematic studies need to be carried out to unearth the molecular mechanism behind such results. Our study paves the way for the way of evidence based experimental procedures and systematic documentation of the details which may open novel vistas in the field of research in homoeopathy. This study provides an innuendo in to possible gross alterations in the various physicochemical properties of the solutions brought about by the process of dilution. However it is needed of the hour to carry out experiments to unearth miniscule or molecular changes that are responsible for the exhibition of linear and non linear properties of dilution.

Conclusion

It could be concluded from present investigation that when the mother tinctures of *Conium maculatum*, *Argentum metallicum*, *Tarentula hispanica* are diluted, the resulting solutions adhere to the Beer-Lambert's law and their absorbencies is linearly altered.

However when these drugs are potentised along with simultaneous dilutions the resulting solutions behaves independent of Beer-Lambert's law.

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