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DEVELOPING AN APPLICATION OF RAMAN SPECTROSCOPY IN HOMOEOPATHIC PHARMACEUTICAL ANALYSIS OF *BARYTA MURIATICUM*

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ABSTRACT

Background: Raman Spectroscopy is very beneficial and effective tool for qualitative and quantitative analysis of various pharmaceutical products or formulations. This helps in scientific validation and accurate standardization of numerous drug products. Homoeopathic medicines are conventionally prepared from various complex organic and simple inorganic substances. Trituration and succussion are two methods of preparation of homeopathic medicines. Following certain steps of mathematical dilution, it crosses even subatomic level and practically becomes undetectable on even recent cutting-edge instruments as well. In this study homoeopathic formulation of *Baryta Muriaticum* is specifically chosen at lower potency to establish the primary standards for homoeopathic medicines quality control through the vibrational Raman Spectroscopy.

Objective: The primary objective of this study was to perform Raman spectroscopic evaluation of homoeopathic preparations of '*Baryta Muriaticum*' (barium chloride) and its various potencies namely (3X, 6X and 12X) for pharmaceutical quality control.

Methodology: The homoeopathic formulations of *Baryta Muriaticum* viz., 3X, 6X and 12X were prepared according to guidelines set by Indian Homeopathic Pharmacopeia, which were further analysed by Micro Raman spectroscope to assess the Scattering of molecule at different wavelengths along with intensities at [(UGC-DAE, CSR) Raman Laboratory (DAVV), Indore (Madhya Pradesh), India]. The spectra were collected within the wavenumber region of (50 - 4000 cm⁻¹) and analysed after suitable baseline correction.

Results: Significant structural alterations were seen in Raman spectra for homoeopathic preparations of *Baryta Muriaticum* 3X, 6X and 12X when compared to the vehicle control of lactose (sachrum lactis). This confirms the presence of *Baryta Muriaticum* in the given sample. Using linear regression model for goodness of fit, the (r²) values [3X (r² = 0.6), 6X (r²= 0.6), and 12X (r²=0.8)], indicate that Raman spectroscopy was effective tool in detecting the variability in Raman shift (cm⁻¹) when plotted against intensity (a.u.) which in turn possibly differentiated those three triturations of *Baryta Muriaticum*.

Conclusion: This study concluded that Raman vibrational spectroscopy is a sensitive and effective application in detecting variability among various homoeopathic triturations of *Baryta Muriaticum* and thus helpful technique in standardization and validation of homoeopathic medicines.

Key words

I. INTRODUCTION

Raman spectroscopy is an important non-destructive technique for the qualitative as well as quantitative analysis of the material (Vankeirsbilck et al., 2002). It is principally based on inelastic scattering of the light at different wavelengths (Lines et al., 2020). This is classically used to find out vibrational modes of molecules (Wei et al., 2015). It provides fingerprint to recognize precise molecule (Pasteris and Beyssac, 2020). Homoeopathic medicines often lack standardization due to their ultramolecular nature which further makes it more implausible for even cutting-edge analytical methods (Dole et al., 2012b; Gosavi et al., 2011; Witt et al., 2007). Trituration is a mathematico-mechanical process for step-by-step enhancing remedial power of the solid drug substances and succession is a liquid counterpart of this entire potentization process (Dole et al., 2012a; Gosavi et al., 2012; Kalliantas et al., 2021). The present study was aimed at evaluating the role of Raman spectroscopy in the validation of homoeopathic preparations of *Baryta Muriaticum* [barium chloride ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) (3X, 6X and 12X)]. *Baryta Muriaticum* is widely used for the treatment of hypertension in a homoeopathic clinical practice especially in lower potencies (3X, 6X and 12X) (Boericke, 2002). The purpose of standardization of these 3 triturations viz. (*Baryta Muriaticum* 3X, 6X and 12X) was to develop the 'Raman spectroscopy' as a rational application for uniform quality control of homoeopathic preparations. This was also to be helped in differentiating all the three (3X, 6X and 12X) chosen potencies of homoeopathic preparations of *Baryta Muriaticum* through Raman spectroscopic analysis.

II. MATERIAL AND METHODS

The present study was conducted at [(UGC-DAE, CSR) Raman Laboratory (D.A.V.V.), Indore (Madhya Pradesh), India]. Raman measurements were carried out at 23°C using a micro-Raman setup (Lab Ram HR, Jobin Yvon) equipped with Diode laser of wavelength 473 nm and a CCD detector. The homoeopathic preparations of *Baryta Muriaticum* viz., 3X, 6X and 12 X were all set according to rules and regulations of Indian Homeopathic Pharmacopeia in the ratio of [1:9 (Drug: Vehicle)] (Robusta, 2000). Analytical grade (AR/ACS) crude barium chloride dihydrate ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) was procured from Finar Chemicals, Andheri, Mumbai, Maharashtra and Sugar of Milk (Lactose) ($\text{C}_{12}\text{H}_{22}\text{O}_{11} \cdot \text{H}_2\text{O}$) was procured from SBL, India. The process of trituration was carried in Metrex electric Triturater machine at department of Homoeopathic Pharmacy, Bharati Vidyapeeth (Deemed to be University) Homoeopathic Medical College, Pune, Maharashtra, India.

The homoeopathic triturations of [*Baryta Muriaticum*(3X, 6X and 12X)] and control (lactose) were analyzed by Micro Raman spectroscope to assess the Scattering of molecule at different wavelength (Raman Shift) along with intensity. The selection of wavelength was justified on the basis of wavelength analysis of Silicon (SiO_2) which was (520 cm^{-1}). These homoeopathic triturations (*Baryta Muriaticum* 3X, 6X and 12X) and control (lactose) were subjected to monochromatic light in sample detector channel with the help of CCD detector. Thereafter the digital recordings of the Raman spectrometer were analyzed, the Intensity (a.u.) was plotted on Y axis and wavelength (Raman Shift) (cm^{-1}) was plotted on X axis. With the increase of potency, a change in the Intensity was demonstrated and plotted graphically and the peak of the Raman Shift (cm^{-1}) was also calculated. The spectra were collected within the wavenumber region of ($50 - 4000 \text{ cm}^{-1}$) and analyzed after suitable baseline correction.

All data were analysed using Origin 8.5 and Graph Pad Prism 7.0 data analysis software. Linear regression model for goodness of fit [(r^2) value] was used for detecting the variability in Raman shift (cm^{-1}) when plotted against intensity (a.u.).

III. RESULTS

A Raman spectrum of *Baryta Muriaticum* ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) is shown in (Figure 1).

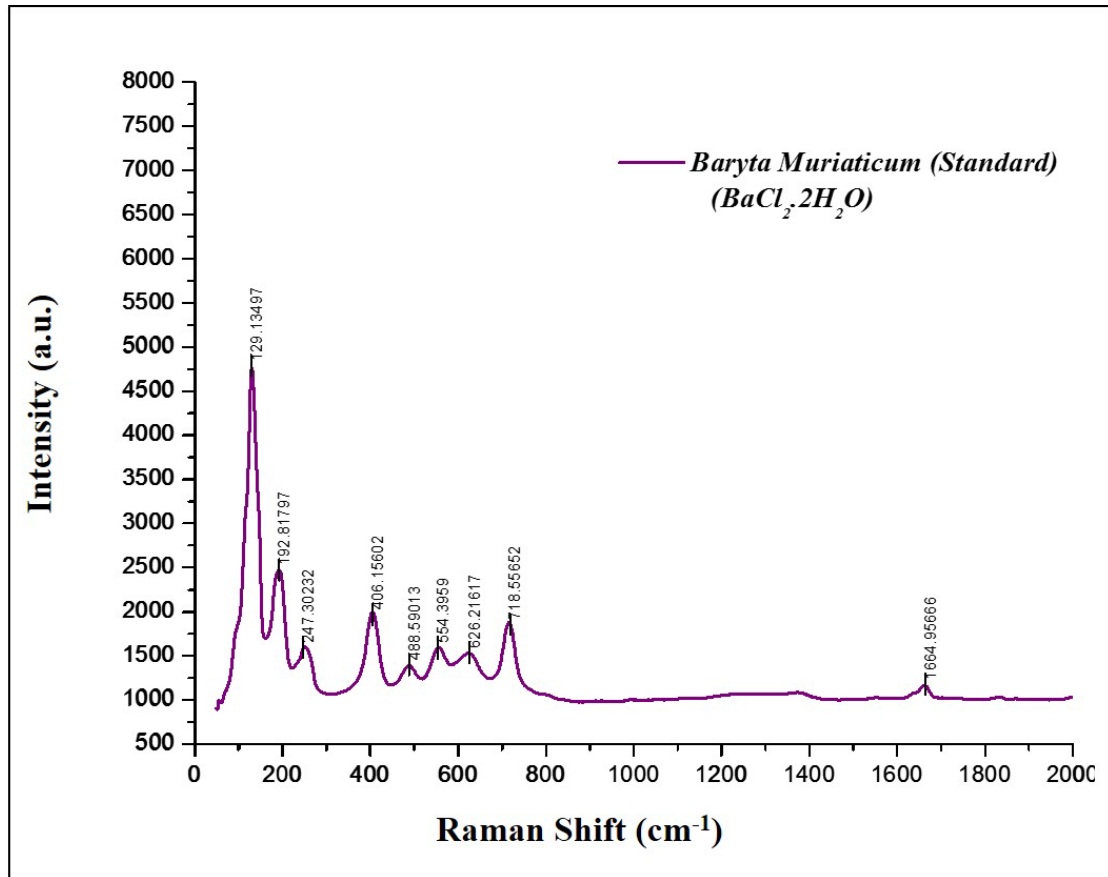


Figure 1: Raman data analysis of *Baryta Muriaticum* (Standard) BaCl₂·2H₂O

The classic Raman shift was evident at (554 cm⁻¹), similarly a Raman spectrum for Silica (SiO₂) at (520 cm⁻¹) is also shown in (Figure 2).

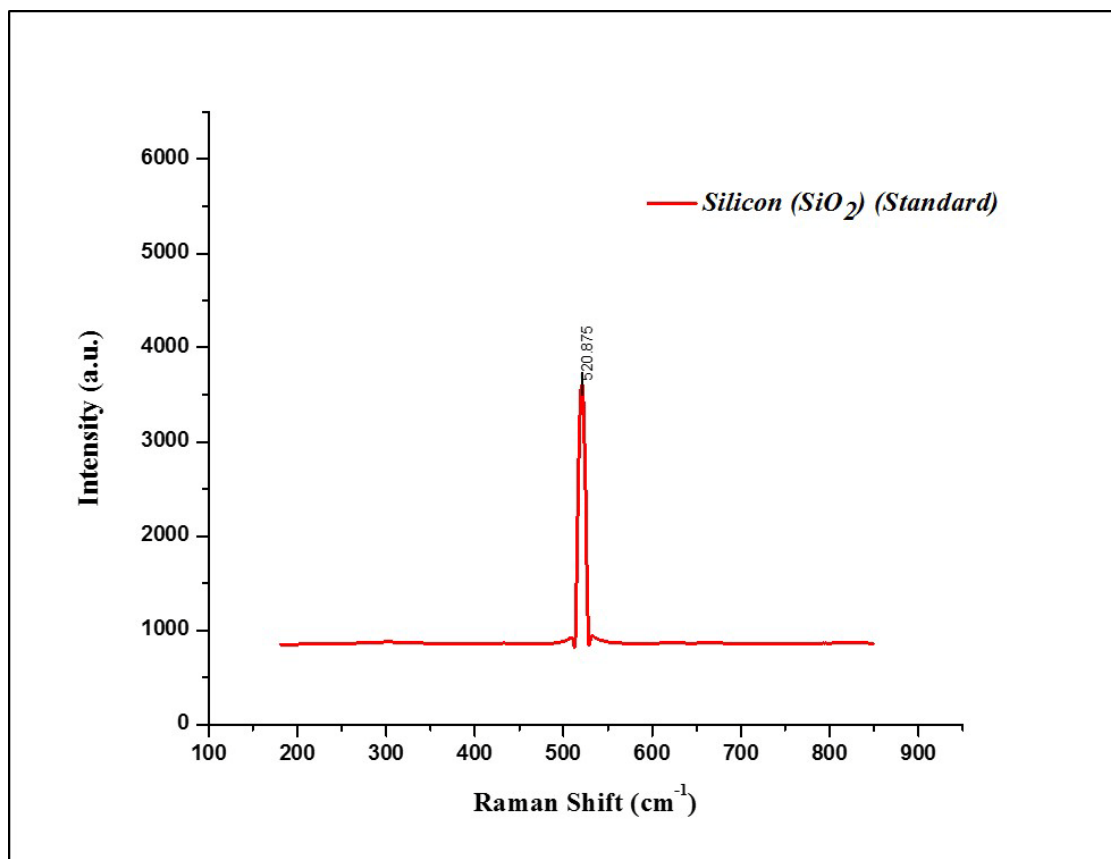


Figure 2: Raman Data analysis of Silicon (Standard) SiO₂

Significant structural changes were seen in the peaks of Raman spectra for homoeopathic preparations of *Baryta Muriaticum* 3X, 6X and 12X when compared with vehicle control namely (lactose). This confirms the presence of *Baryta Muriaticum*(BaCl₂.2H₂O) in the given sample. Using linear regression model for goodness of fit, the (r²) values were [3X (0.6698), 6X (0.6743), and 12X (0.8187)] which may possibly indicate that Raman spectroscopy was useful in detecting the variability in Raman shift (cm⁻¹) when plotted against intensity (a.u.). Comparative illustrations of Raman spectra of various triturations of homoeopathic preparation of *Baryta Muriaticum* 3x, 6x and 12x versus barium chloride, silicon and lactose standard are given Figure 3-6.

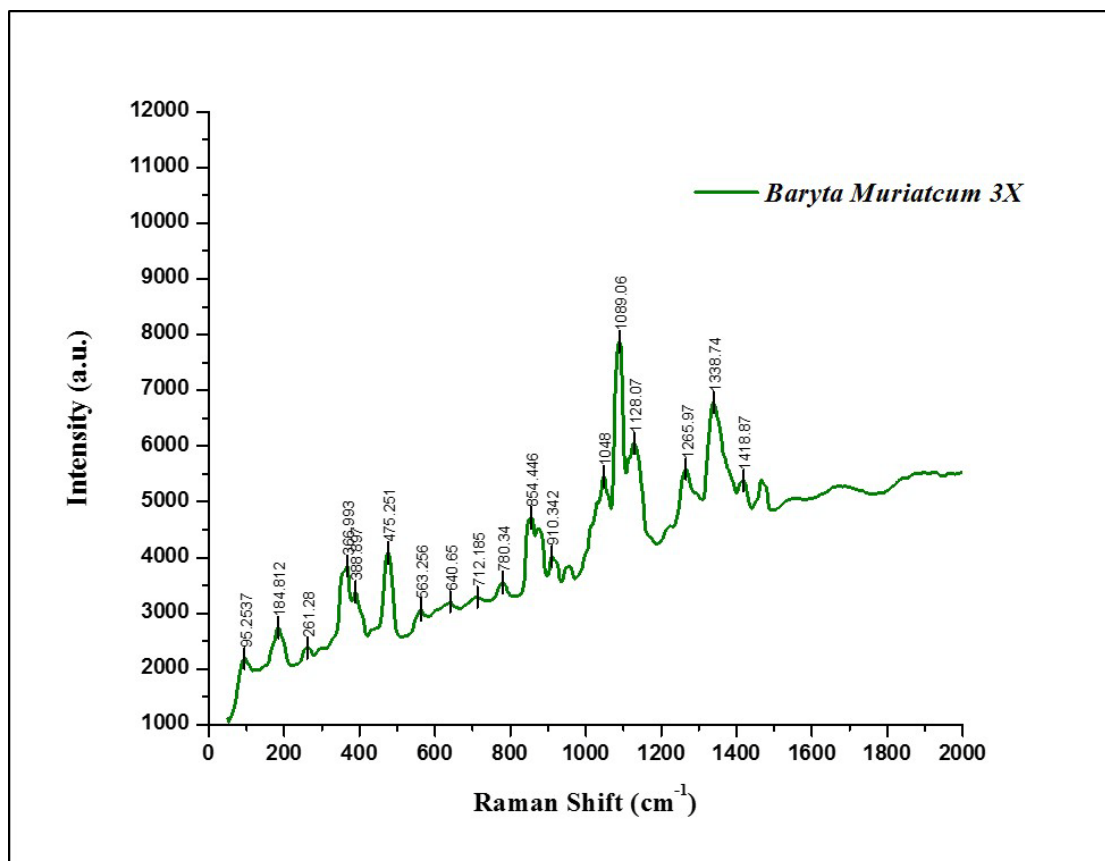


Figure 3: Raman data analysis of *Baryta Muriaticum* 3X

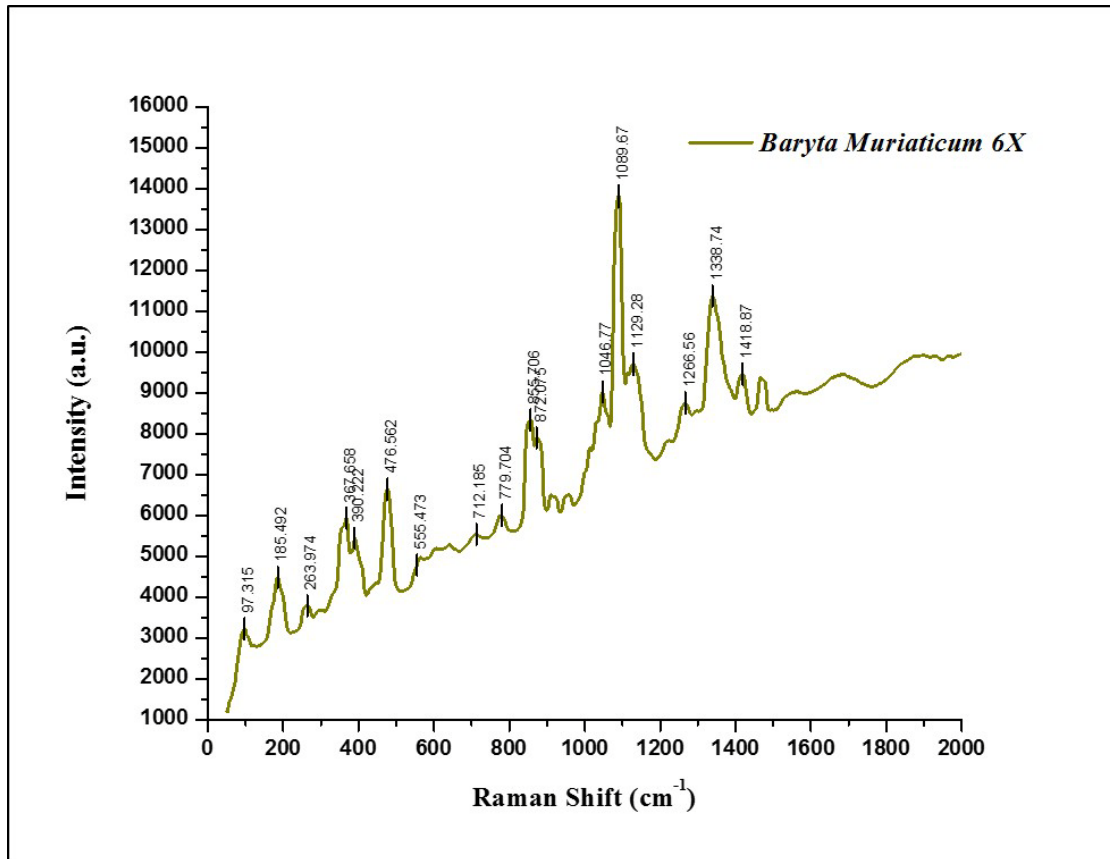


Figure 4: Raman data analysis of *Baryta Muriaticum 6X*

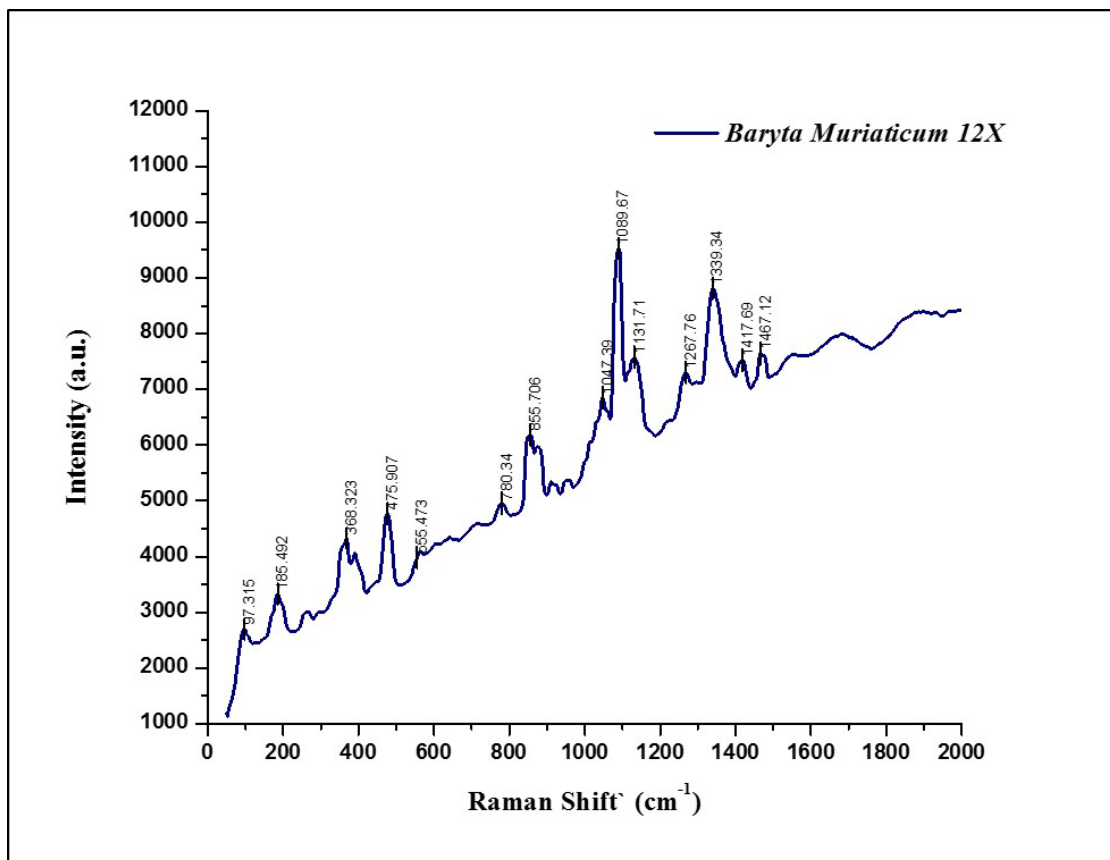


Figure 5: Raman data analysis of *Baryta Muriaticum 12X*

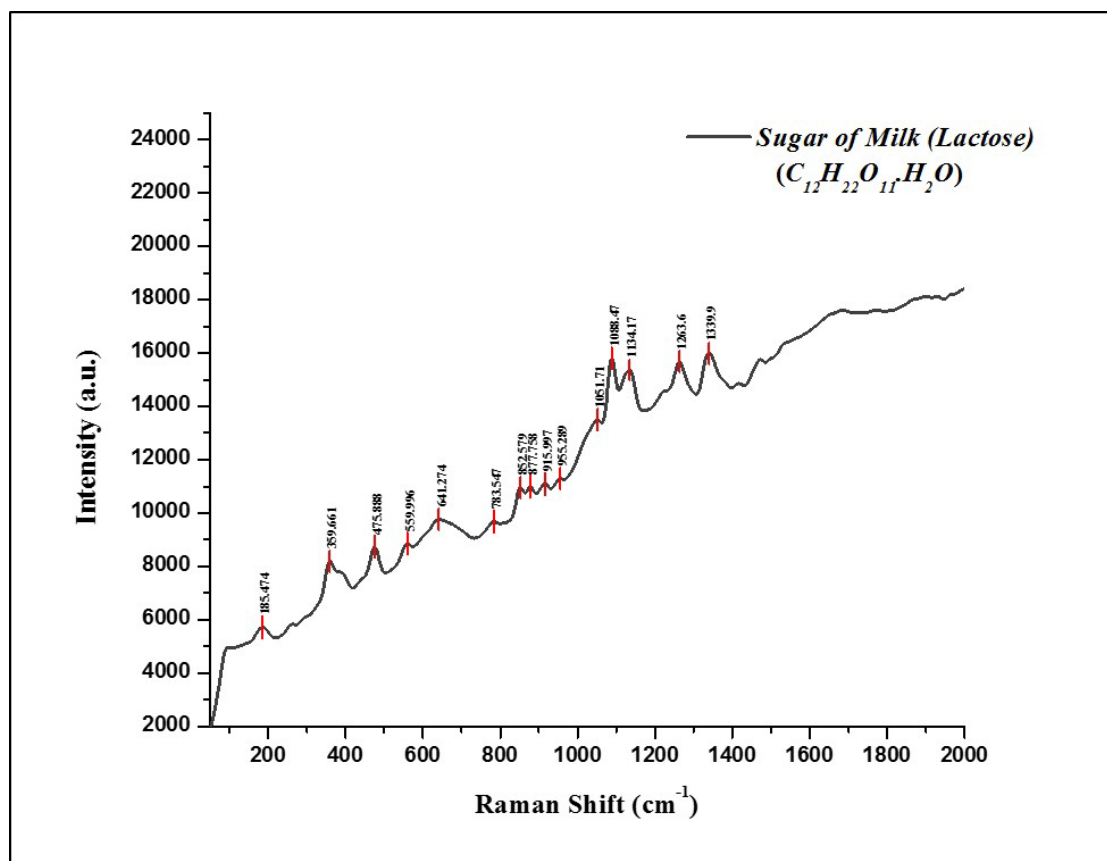


Figure 6: Raman data analysis of Sugar of milk (control group)

IV. DISCUSSION

The Present study was conducted for standardization and validation of homoeopathic pharmaceutical formulations. In this study we had used homoeopathic preparations of Barium chloride dihydrate ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) which is also termed as *Baryta Muriaticum* in homoeopathic literature. The aim of this study was to develop an application of Raman Spectroscopy in Homoeopathic pharmaceutical analysis of *Baryta Muriaticum*. The choice of potencies in our study was mainly based on medicinal properties of Barium chloride dehydrate (*Baryta Muriaticum*) in its lower potencies, hence *Baryta Muriaticum* 3X, 6X and 12X triturations were used to analyze the presence of molecules. The Raman Spectroscopy method was selected for investigation as it provides key information about chemical composition and material structure.

Researcher have demonstrated an infrared Raman spectrum of $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ and $\text{BaCl}_2 \cdot 2\text{D}_2\text{O}$ (Venkatesh and Neelakantan, 1966). Another experiment conducted by Bhattacharya *et al.*, investigated the origin of voltage generation from the vibrational spectra and changes in hydrogen bonding through Raman spectroscopy (Bhattacharya *et al.*, 2019). Rao *et al.* (2007) have illustrated that Raman spectroscopy can distinguish between the different potencies of a homoeopathic medicine (Rao *et al.*, 2007). Two homeopathic drugs Sulphur and Natrum mur in three ultra-high dilutions (UHD) namely, 30cH, 200cH and 1000cH were investigated by laser Raman spectra in as per study published by Sarkar *et al.*, juxtaposing free OH groups and hydrogen bond strength together made an effective rank of Sulphur and Natrum mur UHDs (Sarkar *et al.*, 2021).

Luu *et al.* (1982) investigated Bryonia (C1-C30) with Raman laser spectroscopy using control of ethyl alcohol (Luu *et al.*, 1982). Researcher observed different peak heights of Raman lines among Bryonia and control until C7 (Luu *et al.*, 1982). Raman intensities of ethyl alcohol were greater than Bryonia. Byron and Luu-D-Vinh found that most spectra differ after heating and recooling in comparison to unheated control when tested by Raman spectroscope. Similarly, for heating and cooling phase different spectra were obtained. In this study several homoeopathic medicines including Bryonia were used in C1-C30 potencies. Also, another study by same authors demonstrated individual curves of homoeopathic remedies such as Aurum met, Ars-I, Nat-m, Sulphur, Sulf-I in potencies C1 to C30 when compared against ethyl alcohol using Raman spectroscope (Boiron and Luu-D-Vinh, 1980).

The Raman spectroscopy was found useful method in our study for lower triturations of *Baryta Muriaticum* after applying simple linear regression model for goodness of fit for all the triturations [3X ($r^2 = 0.6$), 6X ($r^2 = 0.6$), and 12X ($r^2 = 0.8$)]. This indicates that Raman spectroscopy could be useful in detecting variations among all subsequent triturations of homeopathic preparations of *Baryta Muriaticum*.

V. CONCLUSION

This study concluded that Raman vibrational spectroscopy is a sensitive and useful application in detecting variability among all 3X, 6X, and 12X triturations of homeopathic preparations of *Baryta Muriaticum*.

Conflict of Interest

The author has no conflict of interest among them in the whole research experimental study.

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