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Use of Raman spectroscopy in standardization of homoeopathic medicine: A review to the importance of emerging technologies in homoeopathy

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Abstract
Homoeopathy, a system of alternative medicine, relies heavily on the precise preparation and standardization of its medicines. With emerging technologies, such as Raman spectroscopy, there arises an opportunity to enhance the quality control and standardization processes within the field of Homoeopathy. This review aims to explore the application of Raman spectroscopy in the standardization of Homoeopathic medicines, highlighting its importance in ensuring efficacy and safety. By examining recent advancements and studies, this review elucidates the potential of Raman spectroscopy to revolutionize quality assurance practices in Homoeopathic pharmacy.

Keywords: Homoeopathy, quality control, raman spectroscopy, standardization, ultrahigh dilutions

Introduction

Homoeopathy is a therapeutic system of medicine which treats the diseases by administering the medicine that can produce exact similar symptoms in a healthy individual which has stated in its “Similia Similibus Curentur” idiom. Homeopathy is a gentle and natural healing system that works with the body to relieve symptoms, restore vitality, and improve overall health which is modern requirement of day to day life [1].

This system of medicine is promulgated by Dr. Christian Friedrich Samuel Hahnemann in late 18th century. Central of which is principle of ancient healing that is like cures like, where substances that produce symptoms in healthy individuals are used to treat similar symptoms in the sick. This holistic approach to medicine emphasizes individualized treatment and the stimulation of the body's innate healing mechanisms [2].

Homoeopathy is also based on certain fundamental principles which are quite distinct and different from other medical treatment. They are as the Law of similia, Law of simplex, Law of minimum, Doctrine of drug proving, Theory of chronic disease, Theory of vital force, Doctrine of drug dynamization [3].

Homeopathic medicines are prepared by vigorous agitating/shaking in a scientific manner known as potentization. The process of potentization is supposed to make the drug capable of treating any condition. Homeopathic practice includes the use of potentized drugs routinely in high dilutions [4]. Apart from controversy regarding high dilutions and mechanisms of action, a major concern with homoeopathy is the lack of strong quality control measures and verified markers that may be linked to biological efficacy. The issue of standardisation is escalated by the wide variety of sources employed in the preparation of high dilutions. Monographs in various countries' pharmacopoeias set different specifications and methods of preparation for the same medications. This contributes to the unwanted debate on quality and efficacy of homoeopathic medications [5].

Modern analytical procedures, such as chromatographic techniques, are employed to standardise and control the quality of homoeopathic medication mother tinctures.

However, even advanced chemical and analytical assays fail to standardise high dilutions in the absence of well-defined active principles. Consequently, the standardisation of high dilutions becomes an intractable issue. Bioassays are used to standardise medications that cannot be tested using sensitive chemical or analytical procedures [5].

Raman Spectroscopy

Raman spectroscopy relies upon inelastic scattering of photons, known as Raman scattering. When light interacts with a molecule, the incident photon can either be scattered elastically (Rayleigh scattering) or inelastically (Raman scattering). Inelastic scattering, the scattered photon has the same energy (frequency and wavelength) as the incident photon. In inelastic scattering, the scattered photon has a different energy than the incident photon. The difference in energy is due to the transfer of energy between the photon and the molecule [6].

The energy transferred between the photon and the molecule can be either positive or negative. If the molecule gains energy from the collision, the scattered photon will have a lower energy (longer wavelength) than the incident photon. This type of Raman scattering is called Stokes Raman scattering. If the molecule loses energy from the collision, the scattered photon will have a higher energy (shorter wavelength) than the incident photon. This type of Raman scattering is called anti-Stokes Raman scattering [6].

The energy difference between the incident and scattered photons is characteristic of the vibrational modes of the molecule. By measuring the intensity of the Raman scattered light as a function of the energy difference (Raman shift), a Raman spectrum can be obtained. The Raman spectrum can be used to identify the molecule and to study its structure [7].

In 1928, Dr. C. V. Raman noted that the Raman signal tends to be faint, with only one in every hundred million incident photons, necessitating intense illumination for detection. It is the modern advancements in laser technology and highly sensitive detectors, this scattering phenomenon can now be utilized for analysing samples that are more intricate than the original clean liquids or gases. Raman peaks, usually narrow in spectral width (a few wave numbers), and often correspond to the vibration of specific chemical bonds within molecules or the dominant vibration mode of a single functional group [8].

Spectroscopy is the study of interaction of electromagnetic radiation with matter. Spectroscopic methods can be based on phenomena of emission, absorption, fluorescence or scattering. Different spectroscopic methods are frequently used for the characterization of a wide range of samples of different interest. The qualitative analysis is performed to establish the identity of sample while quantitative analysis is performed to estimate the concentration of analyte in sample. Some of the spectroscopic methods (e.g. UV–Vis Spectrophotometry) are used as a screening method since it gives the tentative identification of sample and are not specific in nature while other spectroscopic methods (e.g. Infrared Spectroscopy and Mass Spectrometry) are used as a confirmatory method since they give the reliable identity of sample and are specific in nature [10-12].

Methods

Application of Raman spectroscopy in ex vivo tissue detection [10]

What happens if doctors have a tool that could analyse a tiny piece of tissue and tell them exactly what's going on, all without harming the patient. Seems nice, if so, to the patients and common man as well as, to the medical world Raman spectroscopy is emerging as a powerful technique for just this purpose. It works by shining a special light on tissue samples, both from biopsies and other sources, and analysing the way the light bounces back. This tells scientists about the chemical makeup of the tissue, which can help identify diseases. The ideal situation would be to use fresh tissue samples, but these can be difficult to maintain in their natural state.

Application of Raman spectroscopy for *in vivo* tissue detection [10]

It can directly analyze living tissues without any need for processing or injecting markers. This live analysis is achieved using portable Raman systems equipped with specialized probes. The collected data can then be used to create diagnostic models and validate the effectiveness of Raman-based *in vivo* detection.

There are two main approaches for using Raman spectroscopy in clinical *in vivo* detection minimally Invasive Approach and direct tissue detection. Minimal invasive approach method combines a Raman system with medical endoscopy, allowing doctors to examine tissues within the body (in situ). It's particularly useful for reaching organs like the lungs and digestive system through natural openings.

Direct Tissue Detection is used when endoscopy isn't feasible due to location, Raman systems can be used for direct detection of living tissues. This approach has been explored for studying cervical cancer and preterm birth in both humans and animals. Additionally, a variation called spatially offset Raman spectroscopy shows promise for non-invasive skin cancer screening.

By this Raman Spectroscopy can become a real-time way to examine tissues and potentially diagnose diseases at an early stage.

Clinical applications of Raman spectroscopy [10]

Over the past decades, multiple researchers have explored the potential of Raman spectroscopy for clinical applications, continuously identifying compelling medical challenges where the remarkable sensitivity of Raman scattering shows significant potential. Cancer is the top target for Raman spectroscopy in clinical research, and for good reason. Some of the areas of scope in such conditions can be discussed.

First cervical cancer remains one of the leading causes of cancer-related death among women in these regions, accounting for a staggering 90% of the 265,000 global deaths annually. Raman spectroscopy has been evaluated as an early diagnostic tool for cervical cancers and pre-cancers over the past two decades. Our group demonstrated that Raman scattering was sensitive to normal, benign, low grade, and high-grade dysplastic tissues *in vivo*.

Skin cancers are among the easiest to study with optical techniques; however, the complex, turbid nature of the skin makes it one of the most challenging clinical targets for optical diagnostics and monitoring. Early studies used Raman spectroscopy to extract water concentration profiles in human skin while demonstrating the feasibility of *in vivo* Raman spectroscopy for clinical monitoring. Since then, much of skin Raman research has focused on the investigation of Raman based diagnostics.

For gastrointestinal tract developing Raman spectroscopy can be used as a diagnostic tool. The challenge is in the development of a fibre probe that can be inserted through the endoscope and placed in contact with the tissue of interest in a stable manner for the duration of data

acquisition.

Discussion

Raman Spectroscopy in Homoeopathy

- Sarkar et al shows difference in drugs at ultrahigh dilution (UHD) prepared with stepwise mechanical agitation, decoding the nature of the water structure of two ultrahigh diluted homeopathic drugs by Laser Raman Spectroscopy. Sulphur and *Natrum muriticum* in three UHD 30cH, 200cH and 1000cH were studied in Raman spectra of the drugs and their medium (90% ethanol) were obtained in the wave number region of 2600-3800 cm-1. The intensity ratio at vibration frequencies between 3200 and 3420 (R1) and that between 3620 and 3420 (R2) was calculated for each UHD as well as the control. This study has shows stretching vibrations of CH and OH groups of UHD. The three UHDs of each drug shows an inverse relationship with respect to the R1 values providing information about the relative number of OH groups with strong and weak hydrogen bonds. However, for R2 the relationship of UHD for each drug is positive, suggestive the relative number of OH groups with broken and weak hydrogen bonds. Interpreting, the lower the UHD, stronger the H-bond of the OH group. Whereas, higher the UHD, ample the free OH group [13].
- Bhattacharya et al advocated vibrational spectroscopy as a tool for providing information on different states of hydrogen bonding as an effect of potentization. Objective of the study was , the changes in hydrogen bonding due to dilution followed by potentization of 91% ethanol and two homeopathic medicines *Chininum purum* and *Acidum benzoicum* have been studied with the help of vibrational spectra and the result of correlated with the changes in the electrical property of the system. The voltage generated across two symmetrically placed platinum rods were measured by using a U shaped glass tube electrochemical cell, where one arm contained bi-distilled water and the other arm alcohol/ homoeopathic medicine (the arm being separated by platinum foil). It was observed that potentization affected the intensity of OH stretching bands, corresponding to strong hydrogen bond, weak hydrogen bond and broken hydrogen bond, respectively. With the increase in potency, in the presence and absence of the two medicines in ethanol, the number of OH groups linked by strong hydrogen bonds decreased, while the number of OH groups with weak hydrogen bonds increased. With the increase in potentization, the number of OH groups with broken hydrogen bonds showed a difference in the presence and absence of the medicine. The voltage measurements for ethanol show that, with succussion, the magnitude of voltage increased with the two medicines at lower potencies, but not at higher potency where the voltage is lower [14].
- Konar et al suggested that the UHDs of the two drugs and the control are different from each other with respect to hydrogen bond strength of OH groups and the number of free OH groups or non-hydrogen bonded water molecules. Study wanted to decipher the nature of water structure in two ultrahigh diluted (UHD) homeopathic drugs by Laser Raman Spectroscopy. Two homeopathic drugs *Calcarea carbonica* (Calc.) and *Sepia officinalis* (Sep.) in 8cH, 202cH, and 1002cH and their diluent medium 90% ethanol in 8cH and 202cH. Laser Raman spectra of all the samples were obtained in different wave number region. The intensity ratio at vibration frequencies has show a marked difference in intensities in the stretching vibrations of CH and OH groups of all the samples [15].
- Grosan et al has evaluated the morphological characteristics of ethanol- and water-based highly diluted solutions of gold; three levels of potentization (6C, 30C, and 200C) were examined for each type of solution. Through transmission electron microscopy (TEM) investigations. Moreover, Raman spectroscopy and deep learning (DL) algorithms were employed for the analysis of the three potentization levels of purified water, unpurified water, and purified water-based gold solutions. Three batches were assessed for each considered category, and the ability to discriminate between all investigated classes, between the potencies within each group or between the classes within the same level of potentization was presented and discussed in correlation with the TEM findings [16].
- Joshi et al. suggested that vibrational Raman Spectroscopy can establish the primary standards of homoeopathic formulation of *Baryta Muriaticum* at lower potencies. 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. 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 wave lengths along with intensities. The spectra were collected within the wave number region of (50 4000 cm) and analysed after suitable baseline correction. 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 (saccharum lactis). This confirms the presence of *Baryta Muriaticum* in the given sample [17].

Conclusion

Raw materials for homoeopathic medicines are procured from many different sources, like plants animals, minerals etc. Further currently, the technologies are also advanced in respect of the conventional methods of preparation of homoeopathic medicines. This arises the requirement of advancements in the methods of standardization techniques, both for qualitative and quantitative analysis. Different researches in this field have shown the utility of Raman Spectroscopy and viberational spectroscopy for the detection of distinctive features of homoeopathic medicines at ultra- high dilution. It enables rapid, non- destructive measurements, the technique appears a most promising tool for monitoring and analysis of homoeopathic dilution even in higher potencies. The utilization of Raman spectroscopy in the standardization of homoeopathic medicine represents a significant stride towards ensuring the quality and efficacy of these remedies. Traditional methods of standardization often fall short in providing precise and objective

assessments, making it challenging to maintain consistency across different batches of homoeopathic preparations. However, by harnessing the capabilities of Raman spectroscopy, homoeopathic practitioners and manufacturers can overcome many of these limitations.

Conflict of Interest
Not available

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Reference

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