

**Original Article**

## **Raman spectroscopy shows difference in drugs at ultrahigh dilution prepared with stepwise mechanical agitation**

**Tandra Sarkar, M.Sc<sup>1,3</sup>; Atheni Konar, M.Sc<sup>1,3</sup>; Nirmal Chandra Sukul\*, Ph.D<sup>1,2</sup>; Dipanwita Majumdar, M.Sc<sup>4</sup>; Achintya Singha, Ph.D<sup>4</sup>; Anirban Sukul, Ph.D<sup>1</sup>**

<sup>1</sup>Sukul Institute of Homeopathic Research, Santiniketan, West Bengal, India.

<sup>2</sup>Department of Zoology, Visva-Bharati University, Santiniketan, WB, India.

<sup>3</sup>Centre for Health Care Science and Technology, IEST, Shibpur, WB, India.

<sup>4</sup>Department of Physics, Bose Institute, Kolkata, WB, India.

\*Corresponding author. Email: [ncsukul@gmail.com](mailto:ncsukul@gmail.com)

### **Abstract**

**Objective:** The present study aims at deciphering the nature of the water structure of two ultrahigh diluted (UHD) homeopathic drugs by Laser Raman Spectroscopy.

**Method:** Two homeopathic drugs *Sulphur* and *Natrum mur* in three UHD 30cH, 200cH and 1000cH were selected for the study. Raman spectra of the drugs and their medium (90% ethanol) were obtained in the wave number region of 2600-3800 cm<sup>-1</sup>. 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.

**Results:** Raman spectra shows differences in intensities in different UHDs and their control in the stretching vibrations of CH and OH groups. The three UHDs of each drug show an inverse relationship with respect to the R1 values. However, for R2 the relationship of UHD for each drug is positive.

**Conclusion:** R1 provides information about the relative number of OH groups with strong and weak hydrogen bonds. R2 suggests the relative number of OH groups with broken and weak hydrogen bonds. Judged from R1 values the lower is the rank of UHD, the stronger is the H-bond of the OH groups. In the light of R2 values, the higher is the UHD rank the more abundant is the free OH groups. So, hydrogen bond strength and free OH groups together make an effective UHD rank relating to *Sulphur* and *Natrum mur*.

**Keywords:** Sulphur, Natrum mur, high dilution, hydrogen bond, free OH.



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## Introduction

Drugs at ultrahigh dilution (UHD) are frequently used in homeopathy. The concentration of original drug molecules in the 12<sup>th</sup> centesimal dilution reaches to a dilution beyond  $10^{24}$ , therefore, UHDs (beyond Avogadro) are likely to be devoid of original drug molecules. However, the UHDs show specificity in their biological effects as reported in various studies. Viewed from the perspective of their biological function the UHDs appear different from each other and also from their diluent medium, in spite of the fact that their chemical composition is same as ethanol and water. This paper attempts to address the pertinent question, "What makes them different from each other?" using Laser Raman spectroscopy. Earlier studies using NMR, electronic, vibrational and Raman spectroscopy have shown differences in UHDs<sup>1-7</sup>.

The difference has been attributed to the difference in the hydrogen-bonded water structures. But the exact nature of the water structures in UHDs has not yet been elucidated. Recently, an FTIR study by our group revealed variation in free water molecules and hydrogen bonding in different UHD's<sup>8</sup>.

The Raman spectroscopy is complementary to Infrared spectroscopy<sup>9</sup>. For this, we have studied Raman spectra of *Natrum muriaticum* (*Nat-m*) and *Sulphur* (*Sulph*) in 30cH, 200cH, and 1000cH potencies with a view to exploring the differences among them. When a beam of light passes through a transparent material a small amount of radiation energy is scattered. The scattered energy consists of radiation mostly of incident frequency called Raleigh scattering. However, certain discrete frequencies above and below the frequency of

the incident beam are scattered, and this is referred to as Raman Scattering.

When a sample of molecules, water-ethanol in UHDs, is subjected to radiation beam of a particular frequency the electric field experienced by each molecule varies, and the induced dipole also undergoes oscillations of the same frequency. In addition, the molecule undergoes internal motion such as vibration or rotation. The vibrational or rotational oscillation of the molecule would be superimposed on the oscillating dipole of the same molecule. This results in changes in the polarization of the molecule. When a molecule is put in a static electric field its positively charged nucleus and negative electrons are attracted to the negative and positive pole of the field, respectively. This causes an induced dipole moment in the molecule, which is thus polarized.

In this study, we examined the vibrational Raman Spectra of our samples using laser as a source of radiation. The laser provides a narrow monochromatic coherent beam. The rare gas laser, here Argon, gives intensities which are one million times greater than that of sunlight<sup>10</sup>.

## Materials and Methods

**Medicines:** UHDs of *Natrum muriaticum* (*Nat-m*) and *Sulphur* (*Sulph*) in 30cH, 200cH, and 1000cH potencies were used for the experiments. All drugs, purchased in sealed vials from the local market, Kolkata, were products of Dr. Reckeweg, Germany. The drugs were in 90% ethanol as mentioned on their label. The control consisted of 90% ethanol prepared from absolute ethanol by adding the appropriate volume of High Performance Liquid Chromatography (HPLC) water. In this study, we did not use UHDs of



alcohol as a second control because UHD alcohol may have some biological action. Obviously, a drug cannot serve as a control for another drug<sup>11</sup>. Aliquots of the control, as well as the drugs, were mixed with an appropriate volume of HPLC water to reduce the ethanol content to 25%. The optical density of all the samples was measured by a UV-VIS spectrophotometer at 200nm and found to be same.

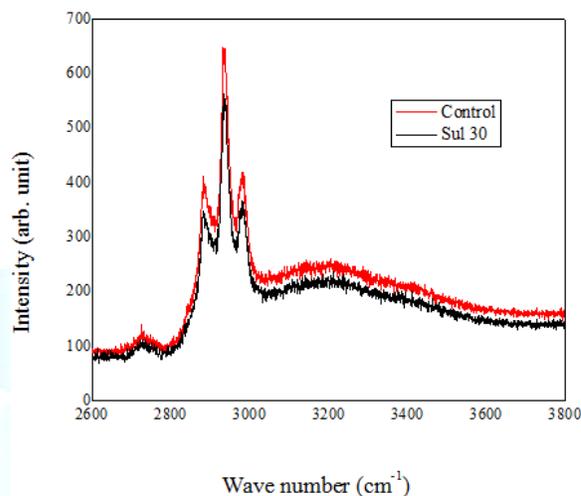
**Raman spectra:** Raman measurements were carried out at 23°C using a micro-Raman setup (Lab Ram HR, Jobin Yvon) equipped with an Argon ion laser of wavelength 488nm and a CCD detector. The spectra were collected within the wave number region of 2600-3800  $\text{cm}^{-1}$  and analyzed after suitable baseline correction. The experiments were repeated thrice and the results were similar. Here, we have considered the results of the second experiment.

In water-ethanol solution OH bond undergoes changes in integral intensity and contour shape. The intensity ratio of vibrational frequencies at 3200 and 3420  $\text{cm}^{-1}$ (R1) provides information about the relative number of OH groups with strong and weak hydrogen bonds. The intensity ratio of the modes at 3620 and 3420  $\text{cm}^{-1}$ (R2) suggests the relative number of OH groups with broken and weak hydrogen bonds<sup>12,13</sup>. In this study, we have calculated these two ratios from the Raman spectra of all the drug samples and the control used.

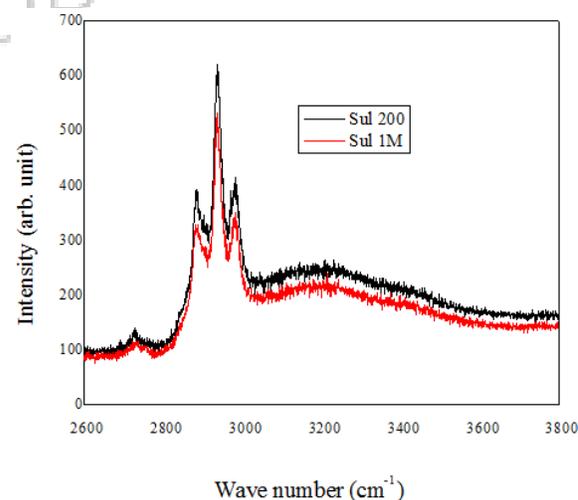
## Results

Raman spectra in the region of stretching vibrations of CH (2800-3000 $\text{cm}^{-1}$ ) and OH (3100-3700 $\text{cm}^{-1}$ ) are presented for different UHDs of drugs and their aqueous ethanol control. Fig. 1 shows the spectra of *Sulph*

30cH and ethanol control, and Fig. 2 *Sulph* 200cH and 1000cH. In all cases, CH bands of ethanol have distinct sharp peaks and OH bands of ethanol and water broad peaks (Figs.1, 2). The peaks of CH and OH bands of the drug (*Sulph* 30cH) and the control and also of *Sulph* 200cH and 1000cH with the same ethanol content 25% show variation in intensities.



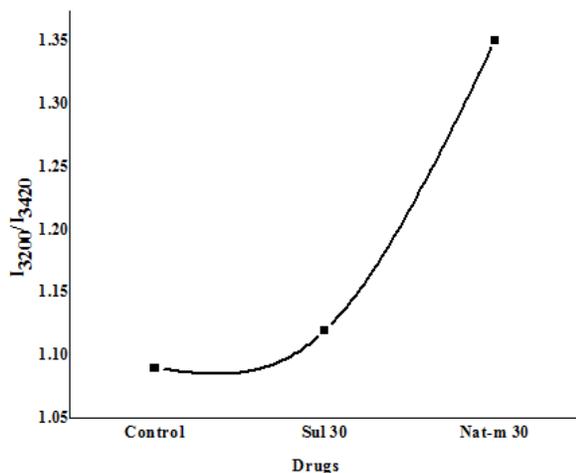
**Fig.-1:** Raman spectra of *Sulph* 30cH and aqueous ethanol control, both in 25% ethanol in the region of CH and OH stretching vibration.



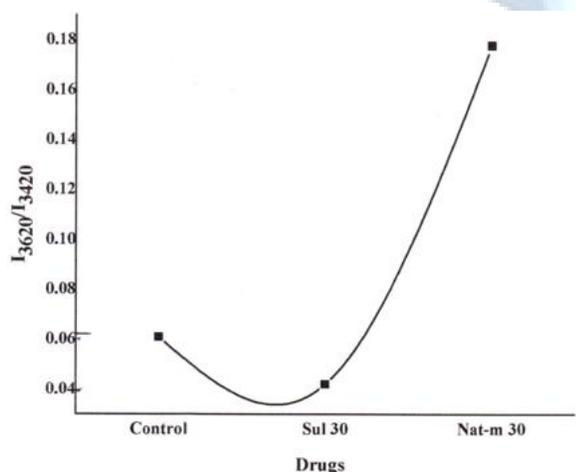
**Fig.-2:** Raman spectra of *Sulph* 200cH and 1000cH in 25% ethanol in the region of CH and OH stretching vibration.



**Intensity Ratio:** The intensity ratios R1 and R2 for control, *Sulph* 30cH and *Nat-m* 30cH in 25% ethanol show marked difference from each other (Figs 3, 4). The ratio values rise gradually with *Sulph* 30cH at the bottom and *Nat-m* 30cH at the top with the control lying close to *Sulph* (Figs.3, 4).



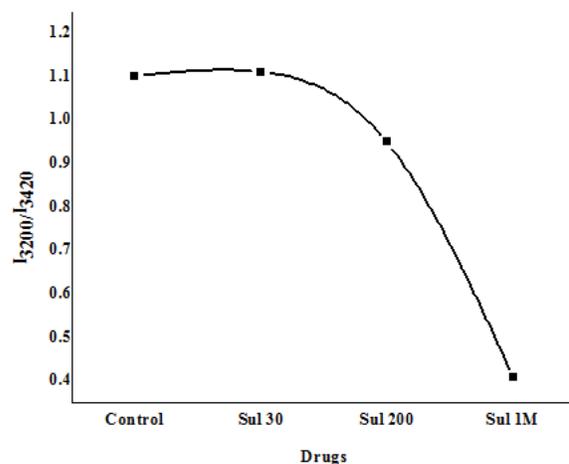
**Fig.-3:** Intensity ratio  $I_{3200}/I_{3420}$  showing the difference in Control, *Sulph* 30cH and *Nat-m* 30cH. All in 25% ethanol.



**Fig.-4:** Intensity ratio  $I_{3620}/I_{3420}$  showing the difference in control, *Sulph* 30cH and *Nat-m* 30cH. All in 25% ethanol.

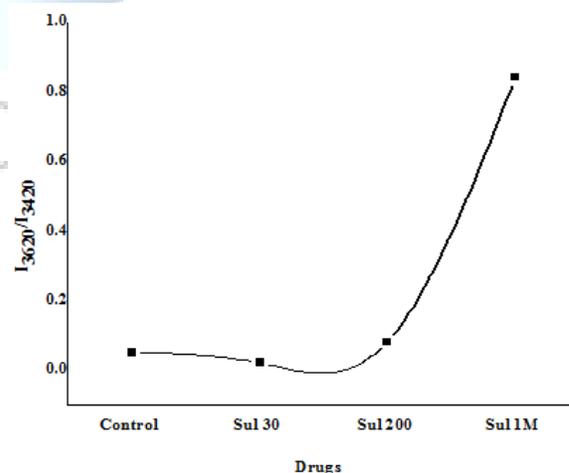
The intensity ratio R1 for control, *Sulph* 30cH, *Sulph* 200cH and *Sulph* 1000cH is inversely related to the UHD ranks (Fig.5). The position

of the control is slightly lower than that of *Sulph* 30cH (Fig.5).



**Fig.-5:** Intensity ratio  $I_{3200}/I_{3420}$  showing negative relation in three potencies of *Sulph* All in 25% ethanol.

The intensity ratio R2 for control and three different UHDs of *Sulph* rises gradually with the increase in UHD ranks from the 30<sup>th</sup> to the 1000<sup>th</sup> (Fig.6).

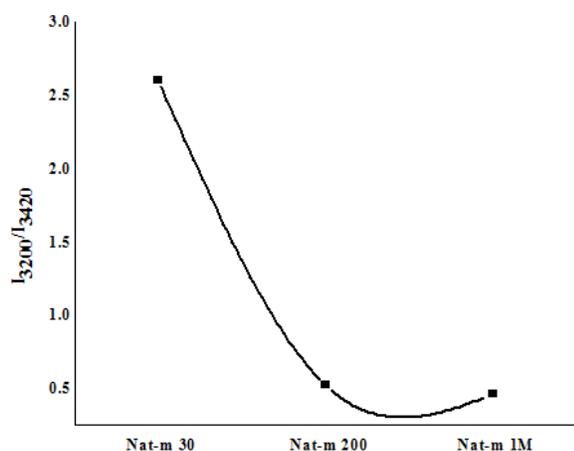


**Fig.-6:** Intensity ratio  $I_{3620}/I_{3420}$  showing positive relation in three potencies of *Sulph*. All in 25% ethanol.

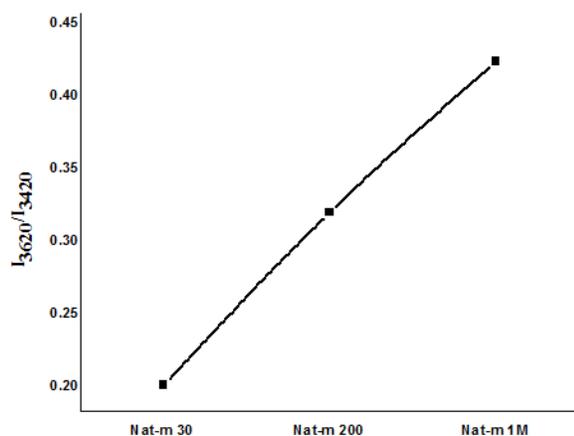
The control lies slightly above *Sulph* 30cH. Like *Sulph*, intensity ratio R1 shows a negative relation between the three UHD



ranks of *Nat-m* (Fig. 7). The ratio R2 shows a positive relation with three UHD ranks of *Nat-m* (Fig.8) as in *Sulph*.



**Fig.-7:** Intensity ratio  $I_{3200}/I_{3420}$  showing negative relation in three potencies of *Nat-m*. All in 25% ethanol. The position of the intensity ratio of the control is at 1.09.



**Fig.-8:** Intensity ratio  $I_{3620}/I_{3420}$  showing positive relation in three potencies of *Nat-m*. All in 25% ethanol. The position of the intensity ratio of the control is at 0.06.

## Discussion

The amplitudes of CH and OH stretching bands increase with the increase in ethanol content in aqueous ethanol solutions<sup>13</sup>. In our study, the amplitudes of CH and OH

stretching bands vary between the control and the drugs (Fig.1) and in different UHD ranks of the same drug (Fig. 2) in spite of the fact that ethanol content is same (25%) in all the samples. The difference in hydrogen bond and in bond strength may be responsible for this.

**Intensity Ratio:** The intensity ratio R1 is maximum at 20-25% ethanol concentration indicating strongest hydrogen bonding of OH groups of water and ethanol<sup>12,13</sup>. At this concentration of ethanol, there occurs structural rearrangement leading to stabilization and strengthening of water structure by ethanol molecules, and strengthening of hydrogen bonds between OH groups<sup>13</sup>.

The ratio R2 decreases as ethanol concentration increases in water-ethanol solutions<sup>13</sup>. The authors hypothesize that with higher ethanol concentration free OH groups, not linked with a hydrogen bond, decreases both in water and ethanol as the ethanol content increases<sup>13</sup>. But in our study *Nat-m* 30cH has strong hydrogen bond and maximum free OH groups followed by *Sulph* 30cH (Figs 3,4). Control is close to *Sulph*. Thus, we see that in 25% ethanol the drug, which has the strongest hydrogen bonding of the OH groups, also has a maximum number of free OH groups. Judged from the clinical perspective *Nat-m* is a deep and long-acting drug<sup>14,15</sup>. Although control and *Sulph* are close to each other with respect to the free OH groups, the latter has stronger hydrogen bonding of OH groups than the control (Fig.3). This makes *Sulph* an effective drug distinct from the control. It may be mentioned here that UHDs of aqueous ethanol control also has some biological effect



which could be reduced or eliminated by dilution with water 1:1000<sup>16</sup>.

In the case of the three UHD ranks of *Sulph* and *Nat-m*, the highest rank 1000cH occupies the lowest position while the lowest rank 30<sup>th</sup> the highest position as evidenced by the ratio R1 (Figs. 5,7). It means the lower is the rank the stronger is the H-bond of the OH groups. But as per the ratio R2, the higher is the rank the more abundant is the free OH groups (Figs 6, 8). In homeopathy, the higher is the UHD rank of a drug the deeper is its therapeutic effect. For *Sulph* as well as *Nat-m* the deeper efficacy depends more on the free OH groups rather than on the hydrogen bond strength of the OH groups. Or, it may depend on both the factors taken together.

Why do hydrogen bond strength and free OH groups vary in different UHDs although their ethanol content is same? The answer lies in the method of preparation of HD drugs. The method involves successive dilution of a mother tincture or a starting material and mechanical agitation<sup>1</sup>. Therefore, these two factors may contribute to the variation in H-bond strength and free OH groups in UHDs. It is known that mechanical agitation or sonication could change the solution structure in aqueous ethanol, and this change could produce a biological effect. Haseba et al<sup>17</sup> observed that weak ultra-sonication of aqueous ethanol solution accelerates the thermal motion of water molecules in the solution. This acceleration disperses ethanol molecules amongst water molecules making the solution more compact and homogeneous as compared to the non-sonicated solution. The authors further observed that sonicated aqueous ethanol behaves like a mature spirit being tastier and less toxic than ordinary alcohol. This means that different UHDs of

drugs might have acquired different levels of compactness and homogeneity of water molecules due to different degrees of mechanical agitation applied. Sonication, instead of succussion, also makes an effective UHD<sup>1</sup>. Therefore, we can say that three factors like hydrogen bond strength, free OH groups and compactness cum homogeneity of water molecules together make an effective UHD with specificity.

## Conclusion

The intensity ratio R1 provides information about the relative number of OH groups with strong and weak hydrogen bonds. The ratio R2 suggests the relative number of OH groups with broken and weak hydrogen bonds. Judged from R1 values the lower is the UHD rank, the stronger is the H-bond of the OH groups. In the light of R2 values, the higher is the UHD rank the more abundant is the free OH groups. So, hydrogen bond strength and free OH groups together make an effective UHD relating to *Sulphur* and *Natrum muriaticum*.

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## Conflict of Interest

None declared.

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