

Original Article

## Raman spectroscopy reveals variation in free OH groups and hydrogen bond strength in ultrahigh dilutions

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### Abstract

**Objective:** To decipher the nature of water structure in two ultrahigh diluted (UHD) homeopathic drugs by Laser Raman Spectroscopy.

**Method:** 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 were used in the present study. Laser Raman spectra of all the samples were obtained in the wave number region of 2400 – 4200 cm<sup>-1</sup>. The intensity ratio at vibration frequencies between 3200 and 3420 (R1) and that between 3620 and 3420 (R2) were calculated for each UHD of the samples.

**Results:** The spectra show a marked difference in intensities in the stretching vibrations of CH and OH groups of all the samples. R1 values for three UHDs of *Calc.* and *Sep.* show negative and positive relationships, respectively. In the case of R2 values, the relationship in three UHDs is 8<202>1002 for *Calc.*, and 8> 202 < 1002 for *Sep.* In the case of control (ethanol UHDs) both R1 and R2 show a negative relationship.

**Conclusion:** R1 denotes a relative number of OH groups with strong and weak hydrogen bonds. R2 indicates the relative number of OH groups with broken and weak H-bonds. Therefore, 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.

**Keywords:** *Calcarea carbonica*, *Sepia*, high dilution, hydrogen bond, free OH, Raman spectroscopy, homeopathy.



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## Introduction

Drugs prepared by successive dilution with aqueous ethanol followed by succussion or mechanical agitation (potentization) are used in homeopathy for a couple of centuries. During potentization the drug molecules are progressively depleted. Beyond 12cH the dilutions cross Avogadro constant and thus the original drug molecules disappear<sup>1</sup>. In our earlier study, we have demonstrated that these ultrahigh dilutions (UHD) have water structures specific to their UHD rank and original drug. The water structures in UHDs mainly relate to free OH and hydrogen bond strength as evidenced by Raman and vibrational spectroscopy<sup>2-4</sup>. UHDs of different drugs are known to produce specific therapeutic effects on the patients depending on their individual symptoms<sup>5-6</sup>. Since UHDs do not contain original drug molecules we have to confirm that water structures relating to free OH and H-bond strength actually characterize UHDs. Thus in this work we have analyzed Raman spectra of UHDs of two more drugs *Calcarea carbonica* (*Calc.*) and *Sepia officinalis* (*Sep.*) and their diluents medium aqueous ethanol to confirm that the two factors, free OH groups, and H-bond strength, contribute to the variation in water structures in these two drugs.

*Calc.* is produced from the middle layer of an oyster shell<sup>7</sup> whereas *Sep.* is prepared from ink in the ink sac of a marine cuttle-fish<sup>7</sup>. The crude substance of each drug is triturated according to the rules of homeopathic pharmacopeia with lactose up to the 3<sup>rd</sup> potencies using a mortar and pestle. One part of the 3<sup>rd</sup> solid potency is mixed with 50 parts by weight of distilled water and the mixture is stirred. Now 50 parts by weight of ethanol are added to the aqueous mixture in a glass vial.

The vial is shaken by 10 downward strokes and the 4<sup>th</sup> potency is prepared. This is the way of conversion of solid potencies of drugs, insoluble in aqueous ethanol, into a liquid form of potencies. Subsequent potencies are prepared by successive dilution of the 4<sup>th</sup> potency with aqueous ethanol 1:100 followed by potentization.<sup>1</sup>

A brief note on laser Raman spectroscopy has been given in our earlier paper<sup>4</sup>. Raman spectroscopy provides a fingerprint by which molecules can be identified. The monochromatic laser beam interacts with molecular vibrations resulting in the energy of laser photons being shifted up and down. The shift in energy provides information about the vibrational mode in the system. In the present study, we have focused our attention on the OH stretching vibrations of ethanol-water which constitutes the diluent medium of our drugs as well as the control. In UHDs only the two components of the medium, ethanol and water, exist because the dilutions used have crossed the Avogadro's constant.

## Materials and Methods

### Medicines

Three potencies, 6cH, 200cH and 1000cH of *Calc.* and *Sep.* were products of Dr. Reckeweg, Germany, purchased from Kolkata in sealed vials. The potencies were in 90% ethanol as mentioned in the labels of the vials. Each of the potencies was further diluted with 90% ethanol of our laboratory and succussed 10 times in two more steps to raise the potency to two upper ranks. Thus the final potencies for experimentation were 8cH, 202cH and 1002cH having a uniform ethanol content (90%). Two potencies of ethanol 8cH and 202cH were prepared in the laboratory following the standard procedure and used a



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control<sup>1</sup>. All potencies of medicines and ethanol were mixed with an appropriate volume of HPLC water to reduce their ethanol content to 25%. The optical density of all the test samples was measured by a UV-VIS spectrophotometer and found to be same. This was to confirm the uniformity of ethanol content in all the samples.

### Raman spectra

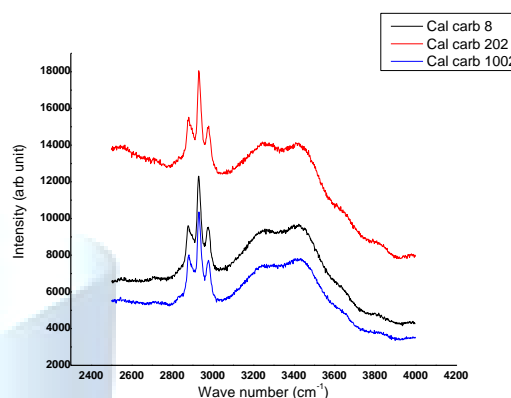
Raman measurements of the samples were taken at 23°C using a micro-Raman setup (Lab Ram HR, Jobin Yuon) equipped with an Argon ion laser of wavelength 488nm and CCD detector. The spectra of drug samples, control, and HPLC water were obtained in the wave number region of 2400-4000  $\text{cm}^{-1}$  and analyzed after suitable baseline correction. The experiments were repeated thrice and the results were similar. The data from the second experiment were taken here.

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) gives information about the relative number of OH groups with strong and weak hydrogen bonds. The ratio of intensities at 3620 and 3420  $\text{cm}^{-1}$  (R2) suggests the relative number of OH groups with broken and weak hydrogen bonds<sup>8-9</sup>. In the present study, we have calculated the two ratios R1 and R2 from the Raman spectra of all the test samples as well as the control.

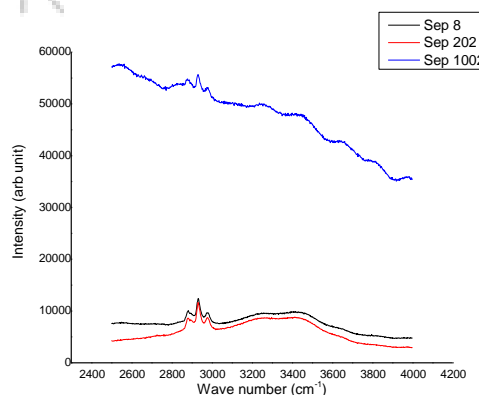
### Results

Spectra of the potencies of *Calc.*, *Sep.* and ethanol together with water is presented in Figures 1, 2, 3 respectively. All the spectra

show a marked difference in intensities in relation to potency ranks. The sharp peaks of high intensities belong to CH stretching band of the ethanol content of potencies of drugs as well as the control. Water has no such peak (Fig. 3). The broad dome-shaped peaks of lower intensities belong to OH stretching band of both water and ethanol (Figs 1, 2, 3). Water lies in a position between Ethanol 8cH and 202cH (Fig.3).



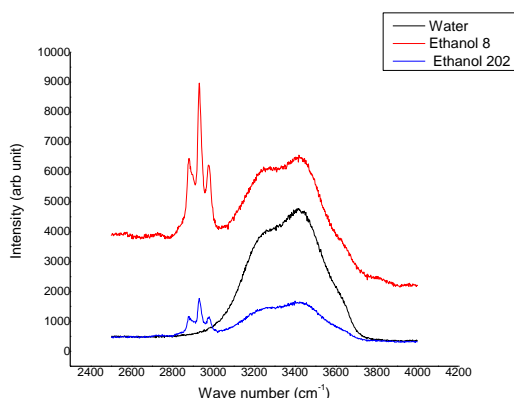
**Figure 1:** Raman spectra of three potencies 8cH, 202cH, and 1002cH of *Calc.*, all in 25% ethanol v/v, showing CH and OH bands.



**Figure 2:** Raman spectra of three potencies 8cH, 202cH and 1002cH of *Sep.*, all in 25% ethanol v/v, showing CH and OH bands.



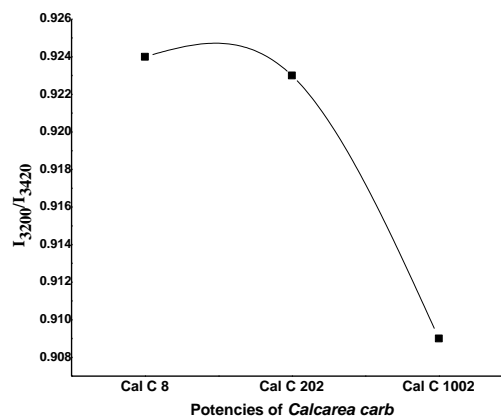
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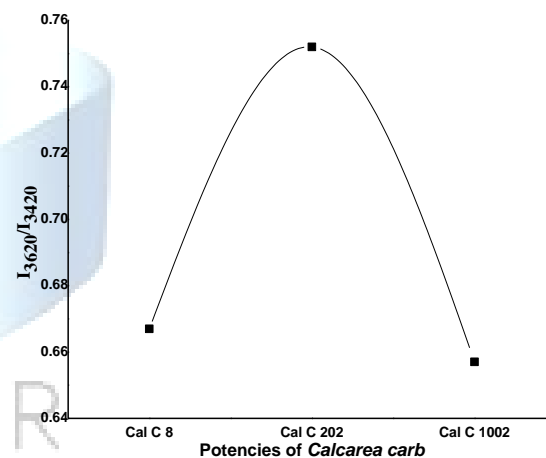
**Figure 3:** Raman spectra of two potencies of ethanol (25% v/v) and water.

### Intensity ratios

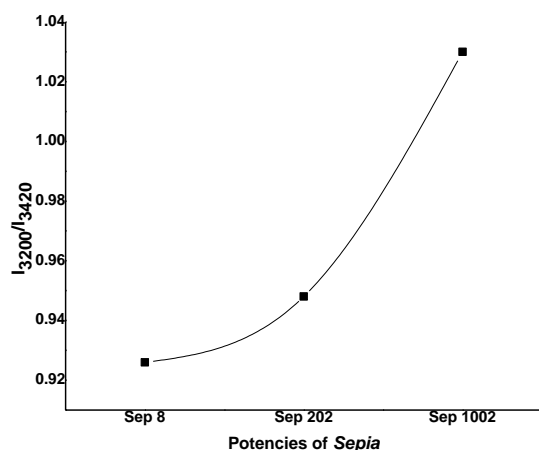
The ratios R1 and R2 for the three potencies of each drug and the two potencies of the control show marked difference from each other (Figs. 4-9). *Calc.* 8cH, 202cH and 1002cH show a negative relationship with respect to R1 values such as 8>202>1002 (Fig.4). R2 values for *Calc.* potencies show different relationship like 8<202>1002 (Fig.5). For *Sep.* potencies, R1 values show positive relationship like 8<202<1002 (Fig.6). R2 values for the same drug show different relationship like 8>202<1002 (Fig.7). For ethanol potencies, both R1 and R2 show similar negative relationship like 8>202 (Fig.8, 9). This means the higher is the potency of ethanol, the weaker is the H-bond strength of OH groups and, the fewer is the free OH groups.



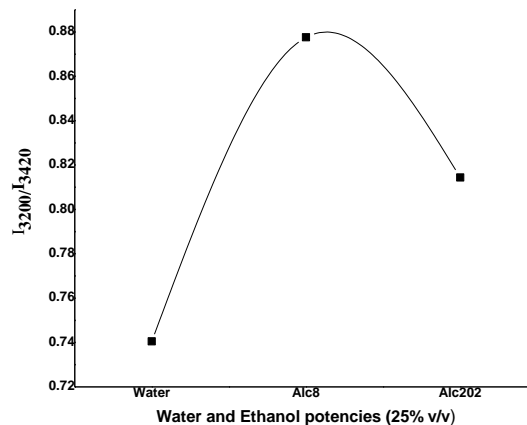
**Figure 4:** Intensity Ratio  $I_{3200}/I_{3420}$  (R1) showing the negative relationship in potencies of *Calc.* All in 25% of ethanol (v/v).



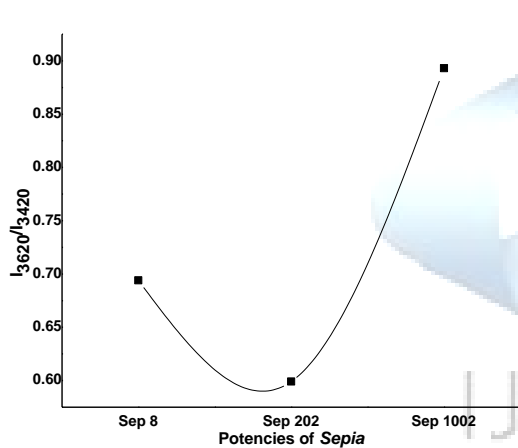
**Figure 5:** Intensity Ratio  $I_{3620}/I_{3420}$  (R2) showing the difference in three potencies of *Calc.* All in 25% of ethanol (v/v).



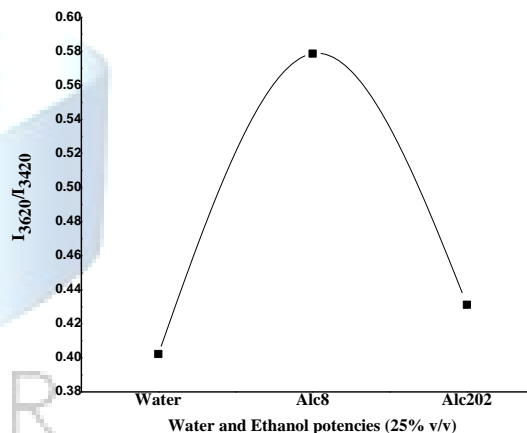
**Figure 6:** Intensity Ratio  $I_{3200}/I_{3420}$  (R1) showing the positive relationship in potencies of *Sep*. All in 25% of ethanol (v/v).



**Figure8:** Intensity ratio  $I_{3200}/I_{3420}$  (R1) showing the negative relationship in two potencies of ethanol. All in 25% ethanol v/v.



**Figure 7:** Intensity Ratio  $I_{3620}/I_{3420}$  (R2) showing the difference in three potencies of *Sep*. All in 25% of ethanol (v/v).



**Figure 9:** Intensity ratio  $I_{3620}/I_{3420}$  (R2) showing the negative relationship in two potencies of Ethanol. All in 25% ethanol v/v.

## Discussion

Different potencies of the drugs and also of control showed marked variation in intensities. The intensity of Raman scattering is proportional to the electric dipole-electric dipole polarizability of change. Raman spectra are dependent on the vibrational energy levels



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of the ground electronic states of the samples (Wikipedia, 2016). So we can say that different potencies show distinct variation with respect to the electric dipole-electric dipole polarizability of water-ethanol molecules. When a molecule is placed in a static electric field its positively charged nucleus is attracted to the negative pole, and negatively charged electrons attracted to the positive pole. This causes an induced dipole moment in the molecule, which is thus polarized.

### Intensity ratio

In the case of *Calc*, potencies show a negative relationship with R1 values like  $8 > 202 > 1003$ . This finding agrees well with the potencies of *Sulphur* and *Natrum mur*<sup>4</sup>. This means the lower is the potency the stronger is the H-bond of OH groups. However, in the case of *Sep.*, the higher is the potency the stronger is the H-bond of the OH (Fig.6). R2 values suggest that the higher is the UHD rank, the more abundant is the free OH groups or free water molecules as with *Sulphur* and *Natrum mur*<sup>4</sup>. However, no such positive relationship exists with respect to the R2 values of *Calc.* and *Sep.* (Figs 5, 7). This shows that each drug is unique with respect to hydrogen bond strength and free OH groups or non-hydrogen bonded water molecules. Our study reveals variation in only two factors, free OH and H-bond strength in different UHDs. We do not rule out other factors contributing to this variation in UHDs. This distinctive feature of homeopathic medicines constitutes the central theme in homeopathic Materia Medica<sup>5,6</sup>.

It is interesting to note that in the case of ethanol potencies both R1 and R2 show the negative relationship (Fig. 8, 9). In other words, the higher is the potency the weaker is the H-bond of OH groups and lesser is the

number of free water molecules. It may be mentioned here that potentized ethanol is a homeopathic medicine having specific symptoms<sup>10</sup>.

Since UHDs are basically a mixture of ethanol and water we should understand the physicochemical properties of this mixture. Using neutron diffraction technique Dixit *et al*<sup>11</sup> analyzed alcohol-water mixture (7:3 molar ratio) at the molecular level. According to them most of the water molecules exist as small hydrogen-bonded strings and clusters bridging neighboring methanol OH groups through H-bonding. This shows incomplete mixing of alcohol water at the molecular level. They further report that single water molecules exist in the alcohol-water mixture (7:1 molar ratio) to the extent of 13%. The remaining 87% water molecules occur in the form of clusters or strings containing 2 to 20 or more molecules<sup>11</sup>. The aberrant thermodynamic properties of water-alcohol mixtures are thought to be due to the differences in energy of hydrogen bonding between water-water, water-alcohol, and alcohol-alcohol molecules. The energy of H-bonding in 20% ethanol (v/v) has been calculated to be highest as revealed by Raman spectroscopy. This supports the presence of clathrate-like structures in water-ethanol solution around the concentration of 20% ethanol<sup>12</sup>. All of our samples are in 25% ethanol, but the hydrogen bond strength is not same. The H-bond strength shows variation with respect to drugs and their potency ranks. Obviously, this may be due to the nature of the starting substance of the drug, and also the specific process of preparation of UHDs by potentization. The process of mechanical agitation makes the non-homogeneous mixture of alcohol water more homogeneous



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and compact<sup>13</sup>. Therefore, compactness, homogeneity, hydrogen bond strength and free OH groups together contribute to the uniqueness of each UHD and its specificity of therapeutic effect. Potencies appear to contain clathrate-like structures.

## Conclusion

1. The three potencies 8cH, 202cH and 1002cH of *Calc.* and *Sep.* show marked variation in the intensity of Raman scattering spectra. The intensity is related to electric dipole polarizability change.
2. Each UHD rank like 8cH, 202cH and 1002cH of *Calc.* and *Sep.* maintains its unique identity with respect to the hydrogen bond strength and free OH groups or water molecules, although their ethanol content is same i.e. 25% v/v.

**Conflict of Interest:** None declared.

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