

Original Article

Free water molecules and hydrogen bonding form the basis of variation in homeopathic potencies as revealed by vibrational spectroscopy

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Abstract

Objective: Using Fourier Transform Infrared spectroscopy (FTIR) we have demonstrated that homeopathic potencies of *Natrum mur*, *Cantharis*, *Nux vomica* and *Sulphur* show differences with respect to the number of free water molecules and strength of hydrogen bonding. The purpose of the present study is to confirm this phenomenon in three potencies of two more drugs *Calcarea carb* and *Silicea*.

Design: The potencies used for each of the two drugs were 30cH, 200cH and 1000cH. The control was 90% ethanol as also the potentized drugs. The control, as well as the potencies, were diluted with distilled water to reduce the level of ethanol to 0.03 molar fraction in each of them. FTIR spectra of all the potentized drugs, control and sterile distilled water (reference water) were taken in the wave number region of 4000-2800 cm⁻¹. The full width at half maximum (fwhm) of OH band was measured for each spectrum. The width was divided into two in the middle. The difference spectrum (absorbance of drug solution - absorbance of reference water) for each potency and the control was obtained after normalization of the spectrum at 3410 cm⁻¹. One difference spectrum so obtained for a potency was subtracted from another to find out if there is a difference between two different potencies.

Results: The half width half maximum (hwhm) in both the high and low-frequency sides of the OH band is far less narrow in potencies than in the control as compared to that in water. The difference spectra for different potencies show different levels of fall in intensity at the wave number region of dip at 3630 cm⁻¹. The level of dip at 3630 cm⁻¹ and subsequent rise in intensity in the lower frequency region represent the quantity of free water molecules and strong alcoholic OH bond around 3250 cm⁻¹, respectively. The results of subtraction between two different potencies are not zero but have marked positive or negative values.



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Conclusion

- (i) Potencies have stronger intermolecular interactions and a higher number of chemical environments than the control, as revealed by the data on hwhm.
- (ii) The three potencies of each of the two drugs show distinct variation in the number of free water molecules and strength of hydrogen bonding.
- (iii) There exists both inter-drug and inter-potency variation as revealed by the difference spectra and results of subtraction between two difference spectra.

Keywords: *Calcarea carb*, *Silicea*, potencies, free water molecules, hydrogen bonding.

Introduction

Homeopathic potencies 12cH and above surpass the Avogadro constant and are, therefore, expected to be devoid of original drug molecules. The medium of all potencies is a mixture of water and ethanol. In a series of experiments we have demonstrated that water carries the information of original drug.¹⁻⁵ Using Fourier Transform Infrared spectroscopy (FTIR) we showed that variation in homeopathic potencies relates to the number of free water molecules and strength of hydrogen bonding.⁶⁻⁷ We studied 8cH and 32cH of *Natrum muriaticum*, *Cantharis* and *Nux vomica*.⁶ We also studied 30cH, 200cH and 1000cH of *Sulphur* and *Natrum mur*.⁷ In every case the basis of variation relates to free water molecules and hydrogen bonding. The present study is aimed at further confirming the results in two more drugs *Calcarea carb* and *Silicea* in 30cH, 200cH and 1000cH potencies. The action of homeopathic medicine is a warranted area for exploration. We cannot understand the mechanism of action of a potency unless we know its physicochemical basis. The IR spectroscopy helps in deciphering the exact nature of the water structure characteristic of a potency.

Materials and Methods

The drugs (*Calc* 30cH, 200cH, 1000cH and *Sil* 30cH, 200cH, 1000cH.), obtained in sealed

vials from the local market at Kolkata, were products of Dr. Reckeweg & Co., Germany. All drugs were in 90% ethanol as mentioned in their labels. The control, 90% ethanol, was prepared from absolute alcohol (Merck). All the drugs, as well as the control, were reduced to 0.03 molar fraction of ethanol by adding the appropriate volume of sterile distilled water to them.

The IR absorption spectra were recorded by a FTIR spectrophotometer (Perkin Elmer, Spectrum 100) at 22°C and relative humidity 65% in nitrogen atmosphere using ATR accessory at 2cm⁻¹ resolution. One drop of a drug, control solution or reference water was put in between calcium fluoride plates and covered with a lid. All measurements were taken in the wave number region of 4000-2800 cm⁻¹. We observed herein mainly the stretching vibration of O-H at 3800-3000 cm⁻¹. A small hump, observed at 2977 cm⁻¹, belonged to C-H stretching vibration. The full width at half maximum (fwhm) of the OH band was measured from each spectrum. Each spectrum was then divided in the middle and the width in each half was measured (hwhm). The width of water spectrum was taken as the standard. The hwhm of each drug or control spectrum was deducted from the corresponding hwhm of water and the values are given in Table 1. The intensity of each



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absorption spectrum was normalized at 3410 cm^{-1} close to the peak position.

The difference spectra for each drug and the control (ethanol solution at 0.03 molar fraction) was obtained by subtracting the intensity of normalized reference water spectrum from each drug or the control. This difference reflects the role of water in the water-alcohol solution of each drug and the control.⁸ In order to see whether the difference spectra show any variation from each other we have further subtracted one difference spectrum from another.

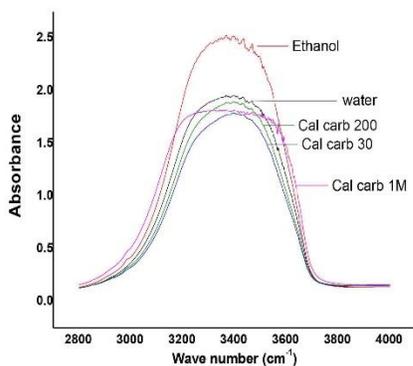


Figure 1. FTIR spectra of *Cal carb* 30CH, 200CH and 1000CH, Pure water, Ethanol (0.03M). The medium of all *Cal carb* potencies is aqueous ethanol (0.03M).

The C-H stretching vibration occurs as a small hump in different potencies of the drugs as well as ethanol at 2977 cm^{-1} and its absorbance intensity follows the same sequence as that of the O-H stretching vibration (Figures 1, 2).

Results

The FTIR spectra of all the three potencies mentioned of each drug, aqueous ethanol control and reference water are presented in Figures 1, 2. For *Calc.* the sequence of absorbance from the highest to the lowest is ethanol, water, *Calc.* 200cH, *Calc.* 1000cH, *Calc.* 30cH (Figure 1). For *Sil.* the sequence of absorbance from the highest to the lowest is ethanol, *Sil.* 1000cH, water, *Sil.* 200 cH, *Sil.* 30cH, (Figure 2).

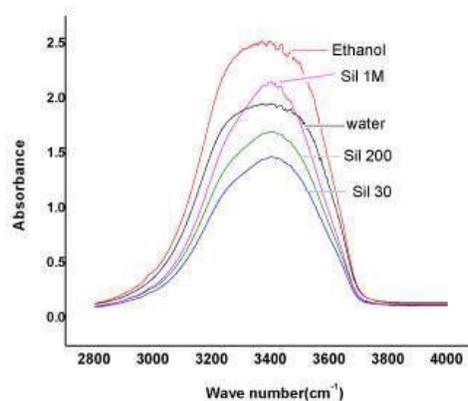


Figure 2. FTIR spectra of *Silicea* 30CH, 200CH and 1000CH, Pure water, Ethanol (0.03M). The medium of all *Silicea* potencies is aqueous ethanol (0.03M).

The half width at half maximum (hwhm) data on the high and low-frequency side of OH stretching vibration at 3400 cm^{-1} in different drugs and the diluent medium 90% ethanol relative to water are shown in **Table 1**.



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Potencies of drugs and medium 90% ethanol	hwhm in the high frequency side relative to water	hwhm in the low frequency side relative to water
Medium 0.03 molar	Narrower by 70 cm ⁻¹ .	Narrower by 142 cm ⁻¹ .
<i>Calcarea carb</i> 30 cH	Narrower by 28 cm ⁻¹ .	Narrower by 28 cm ⁻¹ .
<i>Calcarea carb</i> 200 cH	Narrower by 22 cm ⁻¹ .	Narrower by 33 cm ⁻¹ .
<i>Calcarea carb</i> 1000 cH	Wider by 22 cm ⁻¹	Wider by 34 cm ⁻¹
<i>Silicea</i> 30 cH	Narrower by 27 cm ⁻¹ .	Narrower by 33 cm ⁻¹ .
<i>Silicea</i> 200 cH	Narrower by 27 cm ⁻¹ .	Narrower by 33 cm ⁻¹ .
<i>Silicea</i> 1000cH	Narrower by 43 cm ⁻¹ .	Narrower by 49 cm ⁻¹ .

Table 1: Data on the half width at half maximum (hwhm) in the high and low frequency side of the OH stretching vibration at 3400 cm⁻¹ in different potencies of homeopathic drugs and their diluent medium 90% ethanol relative to reference water. All the potencies and their diluent medium have been equilibrated at 0.03 molar fraction of ethanol by adding appropriate volume of water to each potency tested.

Difference spectra (Absorbance of drug solution - Absorbance of reference water) in the wave number region of 4000-2800 cm⁻¹ are shown in Figures 3, 4. Difference between

two different potencies of *Calc.* and of *Sil.* is shown in Figures 5 and 6, respectively. Difference between the same potencies of *Calc.* and *Sil.* is shown in Figure 7

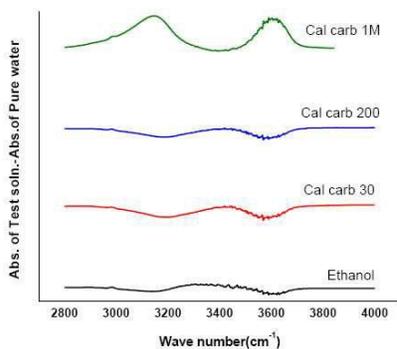


Figure 3: Difference spectra (absorbance of test soln. at 0.03 molar fraction - absorbance of pure water). All spectra have been intensity normalized at 3410 cm⁻¹.

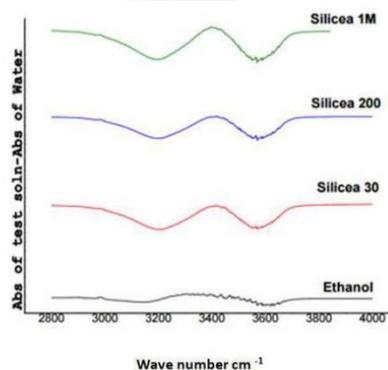


Figure 4: Difference spectra (absorbance of test soln. at 0.03 molar fractions - absorbance of water). All spectra have been intensity normalized at 3410 cm⁻¹.



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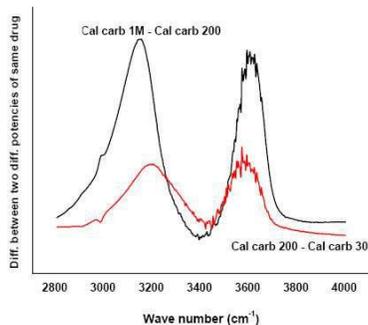


Figure 5: Difference spectra between Cal carb 1000CH- Cal carb 200CH and Cal carb 200 CH- Cal carb 30CH. Each potency of Cal carb represents absorbance of drug soln. – absorbance of pure water after intensity normalization at 3410 cm⁻¹.

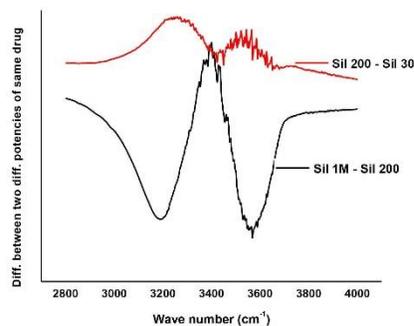


Figure 6: Difference spectra between Silicea 1000CH- Silicea 200CH and Silicea 200 CH- Silicea 30CH. Each potency of Silicea represents absorbance of drug soln. – absorbance of pure water after intensity normalization at 3410 cm⁻¹.

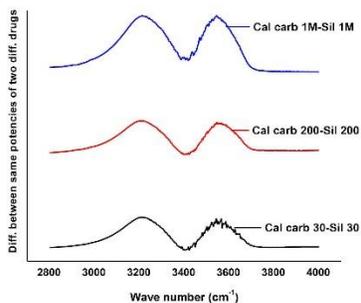


Figure 7: Difference spectra between same potencies of different drugs. Each potency of each drug represents absorbance of drug soln. – absorbance of pure water after intensity normalization at 3410 cm⁻¹.



The absorbance intensity at the dip at 3600 cm⁻¹ varies in the different drug solutions and the diluent medium (Figures 3,4). Different potencies of the same drug vary from each other (Figures 5,6). The same potencies of the two drugs tested also show variation from each other (Figure 7).

Discussion

FTIR spectra of the potencies of a drug show variation in width of OH stretching band (Figures 1, 2, Table 1). Width of IR bands depends on the number of chemical

environments which is related to the strength of intermolecular interactions such as hydrogen bonding. So the number of H-bonds and their strength in a sample constitute the chemical environment. Since the number and strength of hydrogen bonds differ with different chemical environments, the force constant varies. For this, the wave number at which these molecules absorb infrared light also varies.^{9,10} In other words the peak position would vary. The higher is the number and strength of intermolecular interactions the broader is the IR band.



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In our samples, OH stretching bands are mostly narrower in both the high and the low frequency side as compared to reference water (Table 1). This indicates weak intermolecular interactions leading to lower number of chemical environments in the potencies studied.^{9,10} In one potency such *Calc.* 1000cH the band is a little wider relative to water. This means this potency has strong hydrogen bonding and a higher number of chemical environments. However, the OH band in all the potencies are markedly wider, in most cases by more than 50%, in both the high and low-frequency sides as compared to the aqueous ethanol control. Thus all the potencies have stronger hydrogen bonding and a higher number of chemical environments than the control. It appears that the process of successive dilution followed by succussion might have contributed to the variation in the strength of intermolecular interaction vis-à-vis hydrogen bonding in the samples studied. Starting material in the mother tincture of a drug also plays a role in this regard as is evident in the variation in width in different drugs (Table 1). Nishi *et al*⁸ observed in the difference spectra (absorbance of water-ethanol mixtures – absorbance of pure water) that the deepest dip in intensity at 3600 cm⁻¹ and subsequent rise in the lower frequency region varied exactly according to the concentrations of ethanol (0.01, 0.02, 0.03 molar). The authors attributed this fall and rise in intensity to the level of free water molecules and stronger O-H bond around 3250 cm⁻¹, respectively. In the present study, we observed a marked variation in intensity decrease at 3600 cm⁻¹ and rise in intensity around 3440 cm⁻¹ in different potencies of a drug (Figures 3-4) although their ethanol concentration was

same (0.03 molar). The deeper was the dip, the lesser was the level of free water molecules and vice-versa. It is interesting to note that higher potencies show lesser dip compared to lower potencies. This is evident in *Calc.* potencies (Figure 3). The situation, however, varies from drug to drug. As for example *Sil.* potencies do not follow this condition where the 30th potency shows least depth (Figure 4). In other words, potencies show distinct variation in free and bound water molecules as well as hydrogen bonding. Since the three potencies of a drug vary from each other with respect to the depth at 3600 cm⁻¹ and also the level of rise in the subsequent lower frequency regions we can assume that they differ from each other with respect to the cause of this fall and rise. This may be due to the process of successive dilution followed by mechanical agitation involved in the preparation of homeopathic potencies. Starting substances may also contribute to this factor. In this study, two different starting substances were used. *Calc.* is prepared from the oyster shell which is rich in carbonate of lime, but also contains other compounds. *Sil.* is prepared from pure flint which is rich in silica, but contains other compounds.^{11,12} None of them is soluble in aqueous ethanol, and is prepared initially by trituration with lactose, and later by dilution and succussion with aqueous ethanol following the standard procedure of preparation of homeopathic potencies.¹³ During this process of successive dilution followed by succussion water structure in a potency undergoes changes, and these changes are specific to the starting substance. We can, therefore, assume that a starting material somehow causes directional changes in the vehicle, i.e., aqueous ethanol, during the process of dynamization.



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Conflicts of Interest

None declared.

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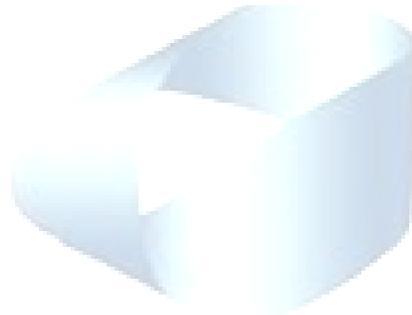
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