

Original article

Investigation of the Influence of clathrate hydrate crystals on the Structuring of Homeopathic High Dilutions

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Abstract

High dilutions (HD) of drugs used in homeopathy are mostly too dilute to contain original drug molecules. But evidence supports their specific biological and therapeutic effects. The reason behind this is thought to be the water structure characteristic of the original drug. Spectroscopic studies indicate that the specific water structure in HDs can be resolved into free water molecules, the hydrogen bonding strength of water hydroxyl, the number of hydrogen bonds, and clathrate hydrate crystals (CHC). HDs are prepared in EtOH water solution by serial dilution and mechanical agitation and are called potencies. The objective of the present study is to further confirm the presence of CHCs in the two potencies of three drugs. Electronic spectra of the HDs of the potencies indicate two broad peaks and marked differences in intensities of absorption. Fourier Transform Infrared (FT-IR) spectra of the test potencies and their control show difference in intensity shift and contour shape of OH stretching and bending bands. All the experimental data indicate the presence of CHCs in varying amounts in the test potencies. Potentization of drugs involves charge transfer (CT) interaction.

Keywords: High dilutions, FT-IR spectra, UV spectra, Clathrate hydrate crystal, Water structure, Charge transfer interaction.

Introduction

High dilutions of drugs (HDs) have been used in homeopathy for more than 200 years. This therapeutic system was introduced by Dr. Samuel Hahnemann, a German physician, in 1756 [1,2]. The HDs, prepared by serial dilution followed by mechanical agitation or succussion in several progressive steps, are called potencies. The drugs are diluted in 90% EtOH in the proportion of 1:100. The twelfth potency has a dilution of 10^{-24} which crosses the Avogadro number. So the twelfth and higher potencies are too dilute to contain original drug molecules. This makes homeopathy scientifically untenable. But there is a large number of evidence that shows that these potencies have biological and therapeutic effects [3,4].

Scientists believe that the biological effects of potencies are due to characteristic water structures [5-8]. In a series of experiments, we have demonstrated that water structures in the potencies involve free water molecules, hydrogen bond strength of water hydroxyl, and several hydrogen bonds. We have recently demonstrated that the potencies of two homeopathic drugs contain clathrate hydrate crystals (CHC) in addition to other factors already reported [9]. The objective of the present study is to further confirm the presence of CHCs in the potencies of other drugs. These crystals occur in methane gas trapped in ice crystals and break down at higher temperatures [10,11]. Homeopathic



potencies are prepared in ethanol water solution where water maintains hydrogen bonds at room temperature as in the ice state. The hydrogen bonding is further strengthened by chemical components present in alcohol-based drinks [12]. We have selected three drugs *Nux vomica*, *Natrum muriaticum*, and Sulphur because these drugs have been used earlier for spectroscopic studies and biological effects. Moreover, they are used very often by homeopathic physicians.

Materials and Methods

Drugs

Two potencies (30 cH and 200 cH) of three drugs *Nux vomica*, *Natrum muriaticum*, and Sulphur were purchased in sealed vials from the market at Kolkata. The drugs were products of Dr. Reckeweg, Germany, and were in 90% EtOH (Nux 30 and 200 Lot number-4992IN379160, 4999IN370240. Nat mur 30 and 200 Lot number-4871IN376080, 4877IN375050. Sulph 30 and 200 Lot number - 6197IN377150, 6204IN378120). To standardize the samples with our 90% EtOH we increased two ranks of each potency by the standard procedure of serial dilution 1:100 followed by manual succussion. The purpose is to make the test potencies, here 32 cH and 202 cH, uniform concerning our 90% ethanol control, which was prepared from absolute ethanol (Merck, Germany, Lot number-K51355683917) by adding deionized and distilled water (DD) in the proportion 9:1. The percentage of ethanol was further checked by UV-absorption spectra in our laboratory. All the test samples were further diluted with DD water to reduce the EtOH content to 20%.

UV-spectra

Using a UV-VIS spectrophotometer (Shimadzu UV-VIS 1900i software Lab Solution UV-VIS) UV-spectra of all the test samples were obtained in the wavelength range 200-300 nm, with scan speed-medium and data interval of 0.5 nm. The control 20% EtOH was used as a baseline for all the test samples. Five spectra of each test sample were obtained and their average was calculated. The margin of error was determined from the following equation: $MOE = Z^* \times \sigma / \sqrt{n}$, where Z^* = Z score, σ = SD, n = number of samples [13,14].

FT-IR Spectra

Fourier Transform Infrared (FT-IR) spectra of all the test samples were obtained with the help of a Shimadzu IR Affinity -1S Fourier Transform Infrared spectrophotometer (Spectrum two) on the attenuated total reflection (ATR) method. The energy resolution was 4 cm^{-1} . The baseline was corrected for atmospheric humidity and CO_2 . One drop of each test sample was placed in the sample groove, and the tip of a single reflection pure diamond crystal was brought in contact with the sample drop to record the whole spectrum in the wave number range of 4000 to 500 cm^{-1} . Forty-five scans were averaged for each spectrum to improve the signal-to-noise ratio. To quantify the contour shape of the OH-stretching band the ratio of absorbance (A) intensities at 3240 cm^{-1} (strong hydrogen bonding) and 3360 cm^{-1} (weak hydrogen bonding) was determined [15]. In the case of the OH bending (ν_2) band, the ratio was calculated between A 1580 cm^{-1} and 1690 cm^{-1} . The ratio values were determined after the normalization of the FT-IR spectra.

Both UV and FT-IR Spectra were recorded at room temperature and humidity maintained in the laboratory at 20°C and less than 50%, respectively.

Results

UV-Spectra



Spectra of the two potencies of three drugs show two distinct broad peaks one between 207 and 212 nm and another 264 and 265 nm. They both show positive and negative absorption. In the case of Sulphur and *Nux vomica*, the 202 potency shows higher absorption intensity than the 32 potencies. But in the case of *Natrum muriaticum*, the 32 potency shows higher absorption intensity than 202 (Figs.-1, 2, 3). The MOE values, given in the legends to figures, are very low, and for this, the difference between the spectra is significant.

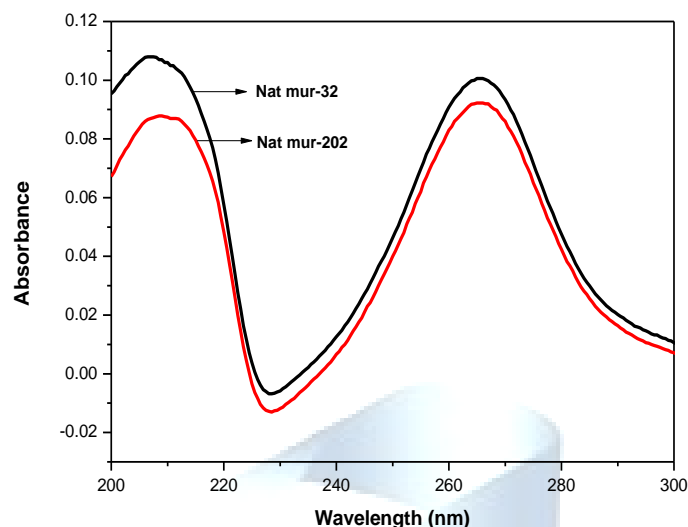


Figure-1: Electronic spectra of the two potencies of *Natrum muriaticum* in 20% EtOH. Each spectrum represents an average of 5 spectra, with a Baseline of 20% EtOH as in all potencies. The margin of error (MOE) between Nat mur-32 and Nat mur-202 0.0029%. Since the MOE is very low the difference between the paired spectra is significant.

FT-IR Spectra

The peak frequency of the OH stretching band is plotted against the test potencies of each drug in Fig.- 4. The potencies of the three drugs show a difference from each other concerning the stretching band. Sulph 202 shows the lowest peak frequency 3251.06 and Sulph 32 shows the highest peak at 3571.77 (Fig.-4). The frequency of the ν_2 band (OH bending) is plotted against the test potencies of three drugs. The potencies differ from each other concerning the ν_2 band (Fig.-5). Sulph 32 shows the lowest frequency at 1646.44 and EtOH, Nat-m 32, and Nux 202 show the highest frequency at 1654,95 (Fig.-5). The control ethanol water was not included in the figures, because it merges with the baseline.

The ratio values of both OH stretching (Fig.-6) and bending bands (Fig.-7) show a difference from each other in the potencies of the drugs. The ratio value of OH stretching is lowest with Sulph 32 (0.64) and highest with Sulph 202 at 1.07 (Fig.-6). In the case of the OH bending band, the ratio value is lowest with Nat mur 32 (0.32) and highest with Sulph 32 at 1.01 (Fig.-7).

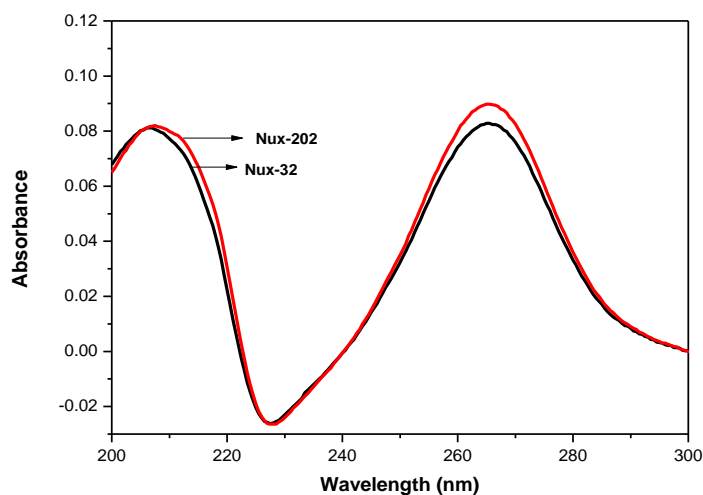


Figure-2: Electronic spectra of the two potencies of *Nux vomica* in 20% EtOH. Each spectrum represents an average of 5 spectra, with a Baseline of 20% EtOH as in all potencies. The margin of error (MOE) between Nux-32 and Nux-202 is 0.00034%. Since the MOE is very low the difference between the paired spectra is significant.

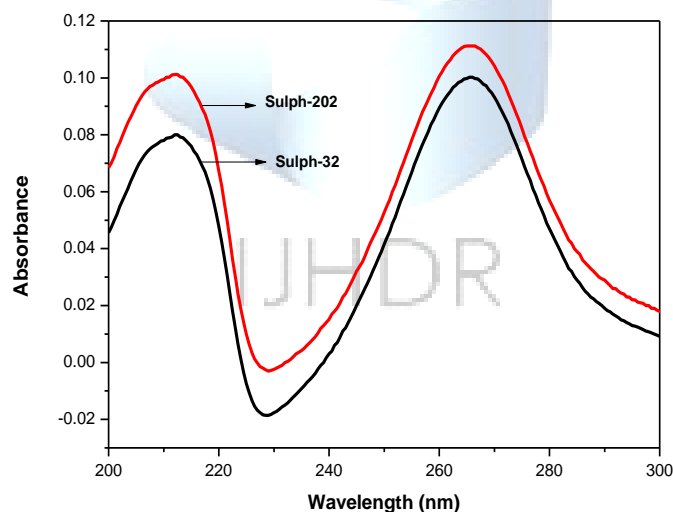


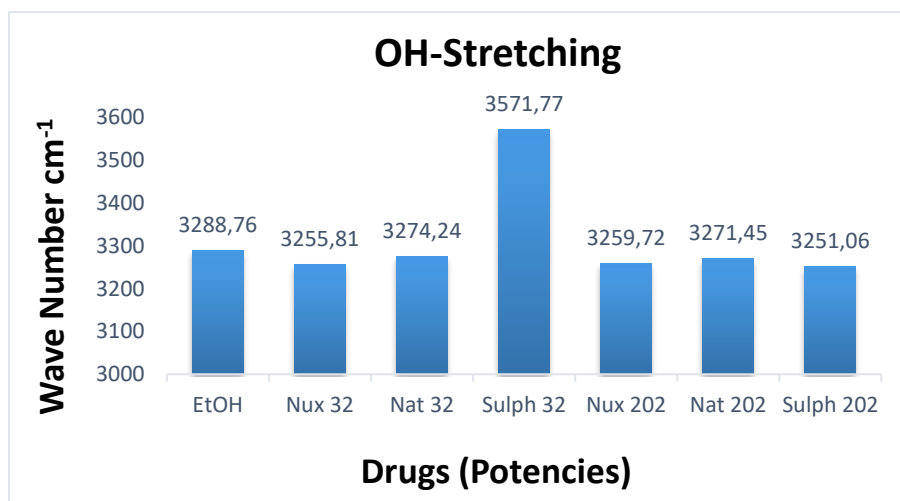
Figure-3: Electronic spectra of the two potencies of *Sulphur* in 20% EtOH. Each spectrum represents an average of 5 spectra, with a Baseline of 20% EtOH as in all potencies. The margin of error (MOE) between Sulph-32 and Sulph-202 is 0.0051%. Since the MOE is very low the difference between the paired spectra is significant.

FT-IR Spectra

The peak frequency of the OH stretching band is plotted against the test potencies of each drug in Fig.- 4. The potencies of the three drugs show differences from each other concerning the stretching band. Sulph 202 shows the lowest peak frequency 3251.06 and Sulph 32

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7).

Figure 4: Histogram showing frequency shift of OH stretching band in EtOH control and different potencies 32 and 202 cH of Nux, Nat mur and Sulph, all in 20% EtOH.

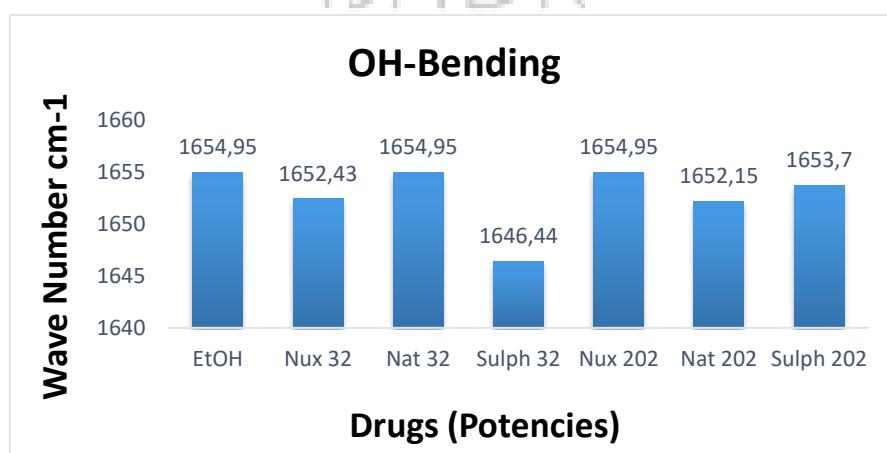


Figure 5: Histogram showing frequency shift of OH bending band in EtOH control and different potencies 32 and 202 cH of Nux, Nat mur and, Sulph, all in 20% EtOH.

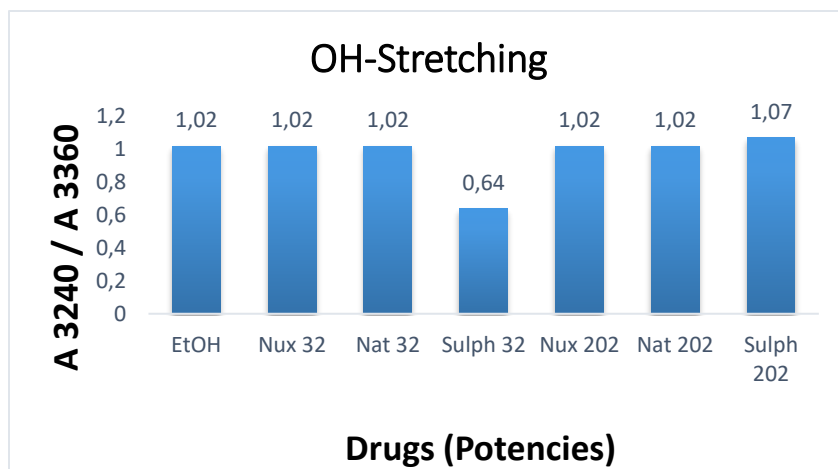


Figure 6: Ratio of absorbance intensities of OH stretching band of two potencies 32 and 202 cH of Nux, Nat mur, and Sulph in 20% EtOH at wave numbers 3240 cm^{-1} and 3360 cm^{-1} . 20% blank EtOH control was also included. The calculation was done after normalization.

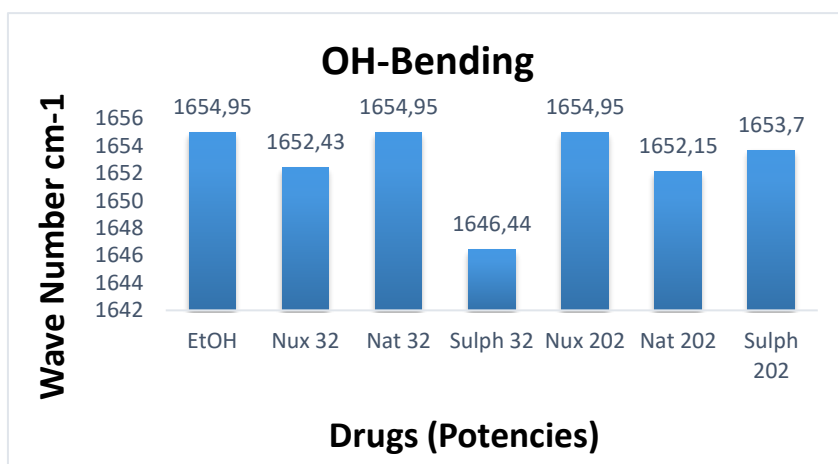


Figure 7: Ratio of absorbance intensities of OH bending (ν_2) band of two potencies 32 and 202cH of Nux, Nat mur, and Sulph in 20% EtOH at wave numbers 1590 cm^{-1} and 1680 cm^{-1} . 20% blank EtOH control was also included. The calculation was done after normalization.

Discussion

Sulphur is an element and Natrum mur is a compound NaCl. But Nux vomica is a plant extract containing many compounds. Each of them influences the water structure in its way. All the compounds in Nux vomica together take part in shaping the water structure in a specific way.

UV-Spectra

In UV spectra water usually absorbs around 191 nm and EtOH around 204 nm. Water and ethanol do not form a homogeneous mixture but have aggregates of ethanol and water. There are also free water molecules [16,17]. We have used 20% ethanol in our study. In EtOH, water mixture hydrogen bonding strength is a maximum of around 15-20% of ethanol [15]. This is due to the difference in

hydrogen bonding energy between alcohol-water, alcohol-alcohol, and water-water molecules [18]. This finding serves as evidence for the presence of clathrate hydrate structure in water-ethanol mixtures around 20% EtOH [18].

Peak I at lower wavelength region may represent water-rich clathrate hydrates. But peak II at the higher wavelength region stands for charge transfer (CT) interaction as evidenced by supporting literature [19-21]. The difference between potencies of the same drug is significant because the MOE is extremely low (Figs.-1-3). The difference in intensities may be due to the difference in the number of clathrate hydrate crystals. The negative absorption in electronic spectra has been reported in water, EtOH, and chloroform [22]. The phenomena may be due to clathrate hydrate crystals in the test potencies.

FT-IR Spectra

The difference in a frequency shift in the OH stretching band in the test potencies and EtOH control can be attributed to the quantities of clathrate hydrate crystals (Fig.- 4) and free water molecules. Lower ratio values point to a higher number of free water molecules, and higher ratio values indicate a higher amount of bound molecules [15]. The ratio values of the test potencies show some difference in the Sulph potencies but not in others (Fig.-6).

In the case of the ν_2 band the higher the concentration of EtOH the greater the blue shift [23]. In the present study, the concentration of EtOH is fixed. So the variation in the frequency shift of the ν_2 band is due to the difference in hydrogen bond strength in water hydrogen in the test potencies and EtOH control (Fig.-5). The variation in the ratio value in the ν_2 band indicates the difference in clathrate hydrate crystals in the test potencies and EtOH control (Fig.-7). The lower ratio values relate to a higher number of free water molecules, and higher ratio values represent a higher number of hydrogen bonded molecules [15]. So the variation in the ratio value as observed in our study indicates variation in free water molecules in different test potencies and EtOH control (Figs. - 6,7).

The present study shows that high dilutions of drugs contain clathrate hydrate crystals in addition to such factors as free water molecules, hydrogen bond strength, and a number of hydrogen bonding. All these factors together are assumed to contribute to the biological effect vis-à-vis the therapeutic effect of HDs.

Conclusions

1. Potencies of drugs prepared by serial dilution and mechanical agitation generate clathrate hydrate crystals.
2. Besides the crystals HDs contain free water molecules, a number of hydrogen bonding, and hydrogen bond strength.
3. Preparation of potencies involves charge transfer interaction.

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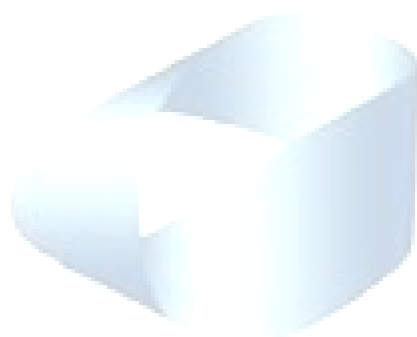
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