# Variation in Fourier Transform Infrared Spectra of Some Homeopathic Potencies and Their Diluent Media

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## ABSTRACT

**Objective:** The aim of this study was to determine whether potentized homeopathic drugs and their diluent media differ from each other with respect to their Fourier transform infrared (FTIR) spectra.

**Design:** FTIR spectra of *Nux vomica* 30C, *Lycopodium* 30C, *Santonin* 30C, *Cina* 30C, *Cina* 206C, *Cina* 1006C, and their diluent media 90% ethanol and *Ethanol* 30C were obtained in the wave number range of 2000–1000 cm<sup>-1</sup> at 20°C. Potassium bromide powder soaked with the potencies, pressed into pellets, and air dried were used to measure the spectra. Because water structures in homeopathic potencies are thought to carry specific information on drug molecules and because O-H bending vibrational band (v<sub>2</sub>) exclusively belongs to water, the study was restricted to the bands in that wave number region. Alcohol has no absorption in the O-H bending region.

**Results:** The potencies were found to differ from each other and their diluent media in the number of  $v_2$  bands, their wave number (cm<sup>-1</sup>), shape, and half-width (cm<sup>-1</sup>) of the bands.

**Conclusions:** The number and other characteristics of the  $v_2$  band represent the number of hydrogen-bonded water species and their hydrogen-bonding strength, respectively. The potencies and their diluent media therefore differ from each other in the number of hydrogen-bonded water species and their hydrogen-bonding strength. The observation that KBr pellets soaked with a potentized drug retains its specific spectral absorption properties simply confirms that medicated sucrose globules, used in homeopathic dispensing, are capable of retaining the therapeutic properties of the drug.

## **INTRODUCTION**

**P**otentized homeopathic drugs are prepared and stored in aqueous ethanol. Sucrose globules soaked with liquid potencies retain therapeutic properties of the drugs for a long time. Water also serves as a good medium but it does not keep the properties of a potency for long.<sup>1</sup> It has been suggested that water structures in a potentized drug are responsible for carrying the information of drug molecules or particles present in the mother tincture.<sup>1–5</sup> Ethanol molecules are thought to promote or to preserve water structures characteristic of a potentized drug.<sup>1</sup> A basic quality of a hydrogen-bonded solvent such as water is the hydrogen bond strength. Physicochemical properties of the water in aqueous alcohol mixtures have been studied widely by such techniques as X-ray or light scattering, dielectric relaxation, nuclear magnetic resonance imaging et cetera. Among these methods, infrared (IR) spectroscopy is one of the most promising for the study of the distribution of hydrogen-bonding strengths of the water molecules in the mixtures because of the short time scale of measurements.<sup>6</sup> There are two kinds of fundamental vibrations for molecules: (1) stretching, in which the distance between two atoms increase or decrease but the atom remains in the same bond axis; and (2) bending, in which the position of the atom changes relative to the original bond axis. Infrared radiation causes vibrational excitation of the molec-

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ular framework of a compound. In aqueous alcohol O-H stretching vibrational bands of water ( $v_1$  and  $v_3$ ) overlap the alcoholic O-H band. For this the IR spectra in the stretching region are of no use for studying hydrogen bonds of the water molecules in water/alcohol mixtures. In the region of bending vibrational band of water ( $v_2$ ), alcohols have no absorption bands.<sup>6</sup>

The purpose of the present work is to study  $v_2$  bands through Fourier transform infrared (FTIR) spectroscopy in 90% ethanol, *Ethanol* 30C, and some potentized drugs such as *Nux vomica* 30C, *Lycopodium* 30C, *Santonin* 30C, *Cina* 30C, *Cina* 206C, and *Cina* 1006C prepared in 90% ethanol. Conventionally vibrations are labeled in decreasing frequency within their symmetry type. The symmetric vibrations of H<sub>2</sub>O are labeled  $v_1$  for the highest fully symmetric frequency (3651.7 cm<sup>-1</sup>) and  $v_2$  for the next highest (1595.0 cm<sup>-1</sup>).<sup>7</sup> FTIR spectroscopy provides simultaneous and almost instantaneous recording of the whole spectrum in the infrared region while minimizing background noise.

#### **MATERIALS AND METHODS**

## Drug

Nux vomica 30C, Lycopodium 30C, Santonin 30C, and *Cina* 30C were prepared by successive dilution (1:100 v/v)with 90% ethanol followed by succussion in 30 steps from the respective mother tinctures in this laboratory.<sup>8</sup> Cina 200C and Cina 1000C, purchased from M. Bhattacharyya and Co. (Calcutta, India), were further diluted (1:100) and succussed with 90% ethanol in 6 more steps to prepare Cina 206C and Cina 1006C. All of these potencies have the same absorbance (3.135) at 255 nm, showing similar concentrations of ethanol (90%). The purpose was to replace the manufacturer's aqueous ethanol in Cina 200C and Cina 1000C with the ethanol in this laboratory so that the diluent medium (90% ethanol) of all the test potencies would be of the same quality. Ethanol was obtained from Bengal Chemical and Pharmaceuticals Ltd. (Calcutta, India). Sterile deionized and double-distilled water was added to absolute ethanol to prepare 90% ethanol, which



**FIG. 1.** Fourier transform infrared (FTIR) spectra of (A) 90% ethanol, (B) *Ethanol* 30C, (C) *Nux vomica* 30C, (D) *Lycopodium* 30C, (E) *Santonin* 30C, (F) *Cina* 30C, (G) *Cina* 206C, and (H) *Cina* 1006C obtained by a Jasco FTIR spectrometer (Model 420, Japan) at 20°C in the wave-number region of 2000–1000 cm<sup>-1</sup>.



FIG. 1. (Continued).

served as the diluent medium of all potenties as well as the control.

## RESULTS

## Spectroscopy

FTIR spectra were measured at 20°C by a Jasco FTIR spectrometer (Jasco, model 420, Japan). The wave number resolution was 4 cm<sup>-1</sup>. Spectra were obtained in the wave number range of 2000–1000 cm<sup>-1</sup>. Potassium bromide powder (~150 mg) was soaked with 90% ethanol (~0.15 mL) or any of the six potencies tested. The drug-soaked powder was mixed thoroughly with a mortar and pestle, spread in thin film (1 mm deep) in a petri dish, and allowed to dry at 30°C (50% humidity). The powder was then pressed into small equal-sized pellets. The KBr pellets, which simulate sucrose globules soaked with a potency, were exposed to IR radiation in the spectrometer. Five pellets were prepared for each drug or the diluent medium, and the IR spectra were observed for each pellet to see the range of variation of IR spectra of each potency.

The spectra of 90% ethanol, *Ethanol* 30C, *Nux vom* 30C, Lycopodium 30C, Santonin 30C, Cina 30C, Cina 206C, and Cina 1006C are shown in Figures 1(A-H, respectively). In the wave number region observed, 90% ethanol and Ethanol 30C have three absorption bands, Nux vom 30C 4, Lycopodium 30C 6, Santonin 30C 6, Cina 30C 5, Cina 206C 6, and Cina 1006C 6. The position of bands at wave numbers, their half-width  $(cm^{-1})$  (in the middle) and percent absorption are shown in Table 1. Data were analyzed by oneway analysis of variance. Table 1 shows that different potencies and their diluent media (90% ethanol, Ethanol 30C) differ significantly (p < 0.01) from each other with respect to the positions of bands in the wave number regions, their half-widths, and their absorption intensities except the wave numbers in column 5 (Table 1). Figure 1 (A-H) shows difference in the shape of the bands in different potencies. The first  $v_2$  band of Nux vom 30C (1649.4 cm<sup>-1</sup>) corresponds to the second v<sub>2</sub> band of 90% ethanol, Ethanol 30C, Santonon 30C, Cina 206C, and Cina 1006C. The absorp-

Drug	Wave number $(cm^{-1}, (top number), half-width (middle number), and absorption intensities (bottom number) of bands$							
	1	2	3	4	5	6	7	8
90% Ethanol	1923.2 <sup>f</sup>	1652.2 <sup>d</sup>		1455.7 <sup>a</sup>				
	65.9 <sup>f</sup>	166.1 <sup>e</sup>		399.2 <sup>f</sup>				
	75.3 <sup>a</sup>	97.9°		99.2 <sup>e</sup>				
Ethanol 30C	1922.0 <sup>e</sup>	1647.0 <sup>a</sup>			1387.7°			
	66.8 <sup>f</sup>	166.2 <sup>e</sup>			399.8 <sup>d</sup>			
	75.3 <sup>a</sup>	98.0 <sup>c</sup>			98.8 <sup>d</sup>			
Nux vomica 30C		1649.4 <sup>b</sup>	1542.4		1385.5 <sup>b</sup>			1121.8 <sup>b</sup>
		61.0 <sup>b</sup>	12.3		13.9 <sup>a</sup>			23.4 <sup>a</sup>
		58.1 <sup>a</sup>	53.9		49.6 <sup>a</sup>			46.2 <sup>a</sup>
Lycopodium 30C	1729.4 <sup>b</sup>			1458.4 <sup>b</sup>	1387.2 <sup>c</sup>	1286.8 <sup>b</sup>	1271.4 <sup>c</sup>	1122.6 <sup>c</sup>
	51.2 <sup>e</sup>			29.8 <sup>d</sup>	23.8 <sup>b</sup>	61.1 <sup>c</sup>	61.1 <sup>d</sup>	42.4 <sup>f</sup>
	99.5°			97.2 <sup>d</sup>	95.5°	98.6 <sup>d</sup>	98.9 <sup>d</sup>	95.8 <sup>f</sup>
Santonin 30C	1732.2°	1648.6 <sup>b</sup>		1455.8 <sup>a</sup>		1287.0 <sup>b</sup>	1270.3 <sup>b</sup>	1124.1 <sup>d</sup>
	39.1°	63.8 <sup>c</sup>		17.9 <sup>a</sup>		45.5 <sup>a</sup>	46.1 <sup>a</sup>	26.2 <sup>b</sup>
	98.5°	97.7 <sup>b</sup>		95.6°		93.9 <sup>b</sup>	94.0 <sup>b</sup>	90.5 <sup>d</sup>
Cina 30C	1727.9 <sup>a</sup>			1458.2 <sup>b</sup>	1382.8 <sup>a</sup>		1274.3 <sup>e</sup>	1120.5 <sup>a</sup>
	42.3 <sup>d</sup>			37.9 <sup>e</sup>	31.1°		59.6 <sup>c</sup>	36.3 <sup>e</sup>
	96.2 <sup>b</sup>			91.3 <sup>a</sup>	88.5 <sup>b</sup>		92.0 <sup>a</sup>	89.6°
Cina 206C	1733.8 <sup>d</sup>	1651.1°		1458.1 <sup>b</sup>		1285.2 <sup>a</sup>	1269.1 <sup>a</sup>	1125.7 <sup>e</sup>
	32.3 <sup>a</sup>	73.6 <sup>d</sup>		19.8 <sup>b</sup>		55.3 <sup>b</sup>	53.9 <sup>b</sup>	29.0°
	98.8°	99.6 <sup>d</sup>		96.5°		95.6°	96.2°	93.2 <sup>e</sup>
Cina 1006C	1731.9°	1649.3 <sup>b</sup>		1458.6 <sup>b</sup>		1286.9 <sup>b</sup>	1272.1 <sup>d</sup>	1125.7 <sup>e</sup>
	36.3 <sup>b</sup>	59.8 <sup>a</sup>		23.4 <sup>c</sup>		61.2 <sup>c</sup>	60.5 <sup>d</sup>	33.4 <sup>d</sup>
	97.8°	97.0 <sup>b</sup>		94.2 <sup>b</sup>		92.3ª	91.7 <sup>a</sup>	86.6 <sup>b</sup>

TABLE 1. WAVE NUMBER (cm<sup>-1</sup>) OF FOURIER TRANSFORM INFRARED (FTIB) ABSORPTION BANDS, THEIR HALF-WIDTH (cm<sup>-1</sup>), AND ABSORPTION INTENSITIES IN PERCENT OF 90% ETHANOL, *ETHANOL* 30C, AND POTENTIZED DRUGS IN 90% ETHANOL

*NOTE:* Columns contain matched wave numbers cm<sup>-1</sup>. Spectra were obtained by an FTIR Spectrometer (JASCO, Model 420, Japan) AT 20°C.

<sup>a-f</sup>Indicates significant differences (p < 0.01, analysis of variance) in the same column for corresponding figures in each box.

tion intensity of this band is the lowest in *Nux vom* 30C (58.1%) compared to the same in the corresponding band in other potencies. *Nux vom* 30C is unique in having the third  $v_2$  band at 1542.4 cm<sup>-1</sup>. The half-width (cm<sup>-1</sup>) and shape of the  $v_2$  bands of aqueous ethanol and other drugs show marked variation from each other (Table 1, Fig. 1). However, *Santonin* 30C, *Cina* 206C, and *Cina* 1006C show some similarity in this respect. The second  $v_2$  band is missing in *Lycopodium* 30C and *Cina* 30C (Table 1, Fig. 1). The first  $v_2$  band of 90% ethanol and *Ethanol* 30C show a marked blue shift (1923, 1922 cm<sup>-1</sup>) compared to the same in the potencies tested. The bands in 90% ethanol and *Ethanol* 30C. show close similarity. However, the fifth band is absent in 90% ethanol and so is the 4th one in *Ethanol* 30C.

#### DISCUSSION

Because all KBr pellets were prepared under similar conditions, it is quite unlikely that they have different amounts of water in them. In earlier work the present authors observed a marked variation in O-H bending vibration among 90% ethanol, Nux vom 30C (unsuccussed), and Nux vom 30C succussed.<sup>5</sup> The results of the present study show that potentized drugs differ from each other and also from their diluent medium, 90% ethanol, in the number of  $v_2$  bands. The number of observed v<sub>2</sub> bands should provide the number of water species with different hydrogen-bonding strengths.<sup>6</sup> There may be a few more water species than those actually observed by v<sub>2</sub> bands in the spectra. According to Mizuno (personal communication, June 2003), IR spectroscopy has superior power in that different water species are distinctive from each other, but it is very difficult to resolve the curve into components. Mizuno further observed that there was no linearity in the absorption intensities of different bands. Thus different potentized drugs have different water species with different hydrogen-bonding strengths. The v<sub>2</sub> bands have different half-widths in different potencies. The broadening of v2 bands has been attributed to the distribution of hydrogen-bonding strengths and vibrational coupling.<sup>6</sup> The v<sub>2</sub> band of pure water has an unusually broad width of 82 cm<sup>-1</sup> at half-maximum. The v<sub>2</sub> band is found to be narrower with an increase in the alcohol concentration. The narrowing of the v2 band is con-

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sidered to be caused by the weakening of the vibrational coupling as a result of dilution by the alcohol.<sup>6</sup> The concentration of ethanol was the same (90%) in all the potencies tested. The variation in the half-width of the  $v_2$  band may thus be caused by influence of original molecules at the start of the dilution process and also by succussion. Previously the present authors observed that succussion caused blue shift of the  $v_2$  in *Nux vomica* 30C.<sup>5</sup>

In each column of Table 1 the band of different drugs showed either a blue or red shift. Blue shifts represent the formation of stronger hydrogen bonds among water molecules. This has also been confirmed by <sup>1</sup>H-NMR studies.<sup>6</sup> It has long been known in clinical practice that sucrose globules soaked with a liquid potentized drug retain all the therapeutic properties of the drugs. FTIR spectra of KBr pellets soaked with potentized drugs simply confirm the long-standing clinical observation.

Cowan et al.<sup>9</sup> demonstrated that the three-dimensional structure of liquid water loses its memory of molecular arrangement through the H-bond network in about 50 fs. The work was based on O-H stretching vibrations of pure H<sub>2</sub>O. Pure water is not comparable to a homeopathic potency that is prepared by successive dilution and succussion from a mother tincture and preserved in 90% ethanol. Ethanol molecules with large nonpolar parts can preserve or promote water structures specific to a homeopathic potency. The efficacy of a homeopathic potency prepared in pure water is very short-lived.<sup>1</sup> An electrostatic component is usually the dominant force contributing to H-bonding.<sup>10</sup> Succussion or any mechanical agitation would therefore make the H-bonding stronger in a homeopathic potency. In ethanol solution the sequential H-bond dissociation and reassociation occur between the same OH groups.<sup>11</sup>

In water the broken bonds probably reform to give the same H-bond.<sup>12</sup> Dissociation is a rare event occurring only twice a day, that is, once for every 10<sup>16</sup> times the H-bond breaks. Thus clusters can persist for much longer times.<sup>13</sup> The relative proportions of different polymers of water preserved by ethanol are at dynamic equilibria of specific geometric configurations. It is assumed that this dynamic geometric configuration of water clusters in a collective way confers specificity on a potentized homeopathic drug.<sup>8</sup> The homeopathic potencies used in the present study were prepared in 90% ethanol and soaked in KBr pellets. Here water structures were preserved by ethanol and their random motion restricted in fine capillaries of KBr pellets.

## CONCLUSIONS

Based on the study findings several conclusions can be drawn. First, in the FTIR spectra of aqueous alcohol mixtures O-H bending vibrational bands (v<sub>2</sub>) exclusively belong to water. *Nux vomica* 30C, *Lycopodium* 30C, *Santonin* 30C, *Cina* 30C, *Cina* 206C, and *Cina* 1006C differ from each other and also from their diluent medium, 90% ethanol, in the number of  $v_2$  bands, their wave-number (cm<sup>-1</sup>), their shape, and half-width (cm<sup>-1</sup>) in the FTIR spectra. Second, the number of  $v_2$  bands and other parameters of the same represent, respectively, the number of hydrogen-bonded species of water and their hydrogen bonding strengths. Thus the potencies and their diluent medium differ from each other with respect to the number of H-bonded water species and their H-bonding strengths. Third, KBr pellets soaked with potentized drug, such as medicated sucrose globules used in homeopathic dispensing, retain specific spectral properties of the drugs concerned. Finally, homeopathic potencies can be differentiated from each other by FTIR spectra with respect to the O-H bending vibrational band.

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