Examination of Homeopathic Preparations by Fourier Transformation-Infrared Spectroscopy
Preliminary Results

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Abstract: We have used Fourier transformation-infrared spectroscopy (FT-IR) to test for differences between homeopathic preparations and control samples. We assessed the differences in FT-IR spectra at various peaks between: (1) succussed water samples and control samples (i.e., plain water, not succussed), (2) sonicated 90% ethanol samples and control samples (i.e., 90% ethanol, not sonicated), and (3) sonicated 10% ethanol samples and control samples (i.e., 10% ethanol, not sonicated). Succussed water and corresponding control samples were different with significance ($P<0.05$) at peaks 3293 and 2123 cm^{-1}. No statistically significant differences were seen between succussed water and controls at peak 1636 cm^{-1}. No statistically significant differences were seen between sonicated 90% ethanol samples and corresponding control samples, or between sonicated 10% ethanol samples and corresponding control samples. FT-IR appears to be able to detect differences between some homeopathic preparations and control samples. Additional work in this field is warranted.

Keywords: homeopathy and infrared spectroscopy, FT-IR spectroscopy experiments, succussed water, sonicated ethanol

1. Introduction

Homeopathy is a commonly used, but controversial complementary or alternative medicine therapy that originated in the 1800s with the work of a German physician, Samuel Hahnemann.(1) The 'Law of Similars' in homeopathy holds that a substance capable of producing symptoms in a healthy individual may act as a curative agent in a diseased individual exhibiting the same symptoms.(2) Similar theories have been used in Chinese medicine as the basis for treatment for thousands of years, and were the subsequent basis for the development of vaccination by Edward Jenner in 1798. Today in vaccination, inactive or weakened pathogens are used to stimulate immunity and/or enhance protection against disease.

The controversy surrounding homeopathy stems mainly from the use of very high dilutions in homeopathic remedies. The remedies are prepared by potentization, which involves serial dilution and succussion.(3) Any remedy diluted and succussed beyond Avogadro's number, that is, a 10^{-24} (or 12C, i.e., 12-fold 1:100) or greater dilution, will theoretically contain zero molecules of the original substance, and may appear, therefore, chemically indistinguishable from unprepared pure water.

Most research in homeopathy has concentrated on showing possible effects from remedies.(4) Though some studies have shown no apparent differences between homeopathic remedies and placebo therapy in humans,(5) and in veterinary medicine,(6) other studies have reported biological responses or protective effects with remedies as dilute as 10^{-27}M (7), 10^{-30}M (8,9), 10^{-36}M (10), 30C (or 10^{-60}M)(11-13), 200C (or 10^{-400}M)(14), and 1000C (or 10^{-2000}M).(15) In addition, Meta-analyses of clinical trials(16-18) have demonstrated that the therapeutic effects of homeopathy cannot be attributed solely to placebo effects.

Considerable controversy in homeopathy also stems from the seeming lack of a plausible mechanism for the reported therapeutic effects of the high dilution remedies. While the mechanism of action of homeopathy is not well understood, it may involve alterations in the water itself. Extensive experimental and theoretical work has been conducted in chemical physics in order to better understand the structures and properties of water clusters.(19-26) Water cluster models provide a framework for understanding the processes involved in possible

preparations.

Several theories and models in homeopathy (27-29) indicate that the action of dilution and succussion imprints information or a pattern on the liquid water. Water cluster models in homeopathy (30,31) suggest that the homeopathic remedies contain specialized groupings of H_2O molecules that either do not occur in unprepared water or occur in different proportions.

Spectroscopic methods, e.g., nuclear magnetic resonance (NMR), Raman laser, ultraviolet (UV), and infrared (IR) spectroscopy, may be used to determine whether homeopathic preparations differ from unprepared water. While some NMR (32,15) and Raman laser spectroscopy studies (33,34) have shown differences between homeopathic preparations and control samples, other NMR (35,36) and UV spectroscopy studies in homeopathy (37,38) have not.

The few experimental studies in chemical physics that have been conducted on water clusters using IR spectroscopy (39-41) measured the IR spectra of water clusters in the gas (as opposed to the liquid) phase. To the best of our knowledge, only one study (42) has used IR to analyze homeopathic remedies, and it has been concluded (43,44) that the results were artifactual. Moreover, no studies to date have analyzed homeopathic preparations by Fourier transformation-infrared (FT-IR) spectroscopy.

FT-IR has several major advantages over conventional IR spectroscopy: a much greater signal-to-noise ratio, an extremely high resolving power, and much less time is required to obtain a spectrum. Therefore, we have conducted experiments using FT-IR to test for differences between homeopathic preparations and control samples. Specifically, we assessed the differences in FT-IR spectra at various peaks between: (1) succussed water samples and control samples (i.e., distilled, deionized water, not succussed), (2) sonicated 90% ethanol samples and control samples (i.e., 90% ethanol, not sonicated), and (3) sonicated 10% ethanol samples and control samples (i.e., 10% ethanol, not sonicated).

2. MATERIALS AND METHODS

2.1 Reagents

Distilled, deionized water was obtained from a Millipore Milli-Q gradient water purification system. Acetone (min 99.5%, GC resolv grade), and methylene chloride (analytical grade) were purchased from Fisher Scientific (Pittsburg, PA, USA). Ethanol (assay min 99.5%) was purchased from Spectrum Chemical Mfg. Corporation (Gardena, CA, USA).

2.2 Sample Preparation

Water and ethanol were chosen for experiments as these are the most commonly used solvents in homeopathic preparations. Succussed water has been used to treat patients, and sonicated ethanol preparations have been shown to have a protective effect.(12) Acetone was chosen to help determine the sensitivity of the FT-IR, as it is an IR active compound that does not exchange hydrogens with the ambient water. Acetone samples ranged from 0.2 to 0% acetone in distilled, deionized water.

All water, acetone, and ethanol samples were prepared in 8 ml glass vials with Teflon caps. All vials were precleaned three times in methylene chloride, rinsed six times with distilled, deionized water, and dried in a constant temperature oven. Sample volumes were 6 ml. Small sample volumes and containers were used because possible processes occurring as a result of succussion may be more active near vessel walls,(45) and may be enhanced, therefore, with the use of a high sample volume to container volume ratio, as in this study. All succussed samples were mixed at a rate of 120 successions/min for one min from a height of approximately one foot. All sonicated ethanol samples were placed in a Branson 2510 ultrasonic cleaner (Branson Ultrasonics Corp., Danbury, CT, USA) in water for thirty sec at 42 KHz (+/- 6%) output frequency.

2.3 Quality Assurance

Samples were introduced onto the ZnSe crystal or the CaF_2 sample cells (see Section 2.4 Instrumentation) using a new pipette tip each time. New pipette tips were used in order to eliminate possible memory effects, as water adhering to inner surfaces of some laboratory equipment has been shown to cause memory effects,(46) limiting accurate measurements. The ZnSe crystal and the CaF_2 sample cells were cleaned with distilled, deionized water and thoroughly dried after each run.

Succussed water and controls, sonicated 90% ethanol and controls, and sonicated 10% ethanol and control samples were paired. Each sample was analyzed an average of five times, and each group of experiments were repeated four to six times for quality assurance. For example, after five measurements were obtained from a control water (or ethanol) sample, the vial containing the original sample was succussed (or sonicated), and five new measurements were immediately taken on the freshly prepared sample. This process was repeated four to six times; each experimental subgroup, therefore, contained an average of five samples.

To correct for possible long-term instrument variability over time, paired samples from all three groups of experiments were analyzed over an average of three days during a three-month period. To

eliminate possible short-term instrument variability over time, samples were analyzed at least thirty minutes after the instrument had stabilized.

2.4 Instrumentation

All samples were analyzed with a Perkin Elmer Spectrum One FT-IR spectrometer, equipped with a $LiTaO_3$ detector. A ZnSe-HATR (Horizontal Attenuated Total Reflectance) system and CaF_2 sample cells were used for analyses. The ZnSe crystal was used for water and acetone samples, and the CaF_2 sample cells were used for ethanol samples. Operation conditions included: 4000-650 cm^{-1} scan range, 4 cm^{-1} resolution, 64 scans, and an autocorrection for water vapor and CO_2.

2.5 Data Analysis

The Perkin Elmer Spectrum v3.02 software program was used to quantify peak areas in all experiments. The most major peaks or peaks not at saturation were chosen for analysis. Peak areas were defined by the start and end abscissa values and two baseline points for baseline correction. Start and end abscissa values and peak heights were selected from scans without knowledge of the type of sample, and identical abscissa, baseline, and peak height values were used for treated samples and corresponding controls. Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and STATA 9.0 (StataCorp LP, College Station, TX, USA) were used for statistics. The data were analyzed using paired two-tailed t-tests with Bonferroni correction (which adjusts for multiple comparisons) for an alpha level of 0.05 over all tests.

3. RESULTS

Results of experiments to gauge the sensitivity of the instrument using the HATR system are shown in Figure 1. Samples containing as little as 0.005% acetone (0.68 mM/L) produced quantifiable peaks vs. control samples containing distilled, deionized water (i.e. 0% acetone). More dilute samples (e.g. 0.0025% acetone; data not shown) show negative peaks that cannot be discerned from controls. The detection limit of the instrument is, therefore, approximately 0.68 mM/L concentration.

We searched for possible new peaks in the spectra of homeopathic preparations vs. controls, but none were detected. We quantified various peak areas for the three groups of experiments, and statistical results are listed in Table 1. Succussed water and corresponding control samples were different with significance ($P<0.05$) at peaks 3293 and 2123 cm^{-1}. No statistically significant differences were seen between succussed water and controls at peak 1636 cm^{-1}. No statistically significant differences were

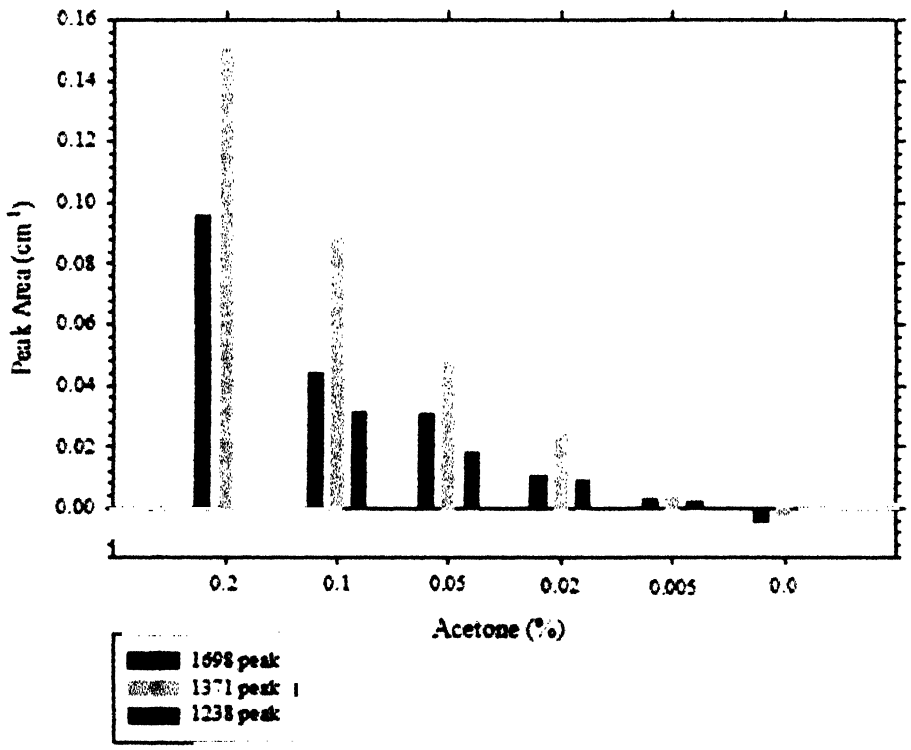

Fig. 1: Excellent method and instrument sensitivity shown by acetone % as a function of peak area (cm^{-1}). Samples containing as little as 0.005% acetone (corresponding to a concentration of 0.68 μmole) produced quantifiable peaks vs. control samples containing only distilled, deionized water (i.e., 0% acetone).

seen between sonicated 90% ethanol samples and corresponding control samples, or between sonicated 10% ethanol samples and corresponding control samples.

Table 1. Statistical results[a]

Peaks (cm^{-1})	Samples[b] Succ. Water& Controls	Sonic. 90% Ethan.& Controls	Sonic. 10% Ethan.& Controls
Water			
3293	P=0.03	n/a	n/a
2123	P=0.03	n/a	n/a
1636	P=1	n/a	n/a
Ethanol			
2136	n/a	n/a	P=1
1925	n/a	P=1	n/a
1656	n/a	P=1	n/a
1455	n/a	P=0.6	n/a
1383	n/a	P=1	n/a
1086	n/a	n/a	P=1
1045	n/a	n/a	P=1

[a]n/a=not applicable

[b]Succ.=succussed; Sonic.=sonicated; Ethan=ethanol

4. DISCUSSION

FT-IR, in general, is an excellent technique with a low detection limit as well as high sensitivity and reproducibility. When equipped with HATR, the detection limit and sensitivity can be increased more than ten-fold. In this study, we searched for changes in the vibrational spectra between water and ethanol preparations and controls, as a possible indicator of water restructuring in homeopathic preparations based on IR characteristics. Three IR active peaks in the range of 650 – 3400 cm^{-1} were examined. They are 3293, 1636, and 2123 cm^{-1}, in order of prominence. From the gas phase IR data of the water cluster (39-41) one could expect water cluster peaks to shift to a lower absorption energy when changing from gas phase to a more polarizable media such as water. These shifts also depend on the dipole moment of each vibrational mode. The larger the dipole moment, the greater the shift. One should not expect the shift to be the same for all IR peaks. For this reason, it is reasonable to associate the 3293, 1636, and 2123 cm^{-1} IR peaks with O-H stretching vibrations, H-O-H bending modes, and overtones of intermolecular modes or a combination of the 1636 cm^{-1} peak with an intermolecular mode, respectively.(47)

The statistically significant differences seen between succussed water and control samples in two of the three peaks analyzed suggest that FT-IR may be a useful tool for detecting differences between some homeopathic preparations and controls. These differences may be an indicator of water clusters present in succussed samples in high enough concentrations and strong enough oscillations to influence the observed peaks, and be measurable.

The lack of statistical significance between sonicated ethanol samples and controls may be due, in part, to the sonication frequency used. A *Nux vomica* 30C remedy prepared with 90% ethanol and sonicated at 20 KHz was effective in reducing the sleep time in mice.(12) If the protective effects seen by Sukul, et al. (1999) were due to differences in water clustering, our output frequency of 42 KHz (+/- 6%) may have been too high for the stability of some water clusters.

The lack of statistical significance between sonicated ethanol samples and controls may also be due to the experimental set-up. The CaF_2 crystals used in these experiments provided a single-path window, whereas the HATR set-up (used for succussed water samples and controls) was a multi-bounce, multi-path system. The detection limit using the HATR system, therefore, should be 100-1000 times better than that of the CaF_2 crystals. This may explain why we did not see a significant difference between ethanol samples and controls. The CaF_2 crystals, however, are the only choice of accessory that is compatible with the analysis of OH peaks at the high concentrations of water and ethanol used in this study. Alternatively, the lack of a difference between ethanol samples and controls may be because these samples were too concentrated; ethanol peaks may have obscured IR active peaks associated with water clustering, which should be very weak.

Additional research is needed to corroborate the results of this study. Limitations of the present work include the resolution of the instrument, the selected number of scans, and the number of samples and peaks analyzed. Future research should be performed using a greater than 4 cm^{-1} resolution, more than 64 scans, larger sample sizes, additional peaks and homeopathic preparations, and test for possible signal changes as a function of time and temperature, among other conditions.

5. CONCLUSIONS

Our FT-IR instrument set-up and developed method using the multi-bounce HATR system produced excellent sensitivity. FT-IR with HATR appears to be able to detect some differences between recently succussed water samples and controls. Much more work in this field is warranted.

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