

## Thermoluminescence in Ultra-High Dilution Research

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### ABSTRACT

**Objective:** Thermoluminescence of homeopathically prepared ultra-high dilutions of lithium chloride in deuterium oxide ( $D_2O$ ) ( $10^{-30}$  g  $cm^{-3}$ ; C15 LiCl in  $D_2O$ ) and similarly prepared  $D_2O$  (C15  $D_2O$ ) was compared in three experiments varying (1) time between preparation of substance and time of experimentation, and (2) time between irradiation and thermoluminescence recording. Data were also compared with a normal solution 0.1 M LiCl in  $D_2O$  and with pure  $D_2O$ .

**Methods:** To record thermoluminescence, the solutions were frozen in liquid nitrogen (77 K,  $-196^\circ C$ ), irradiated with 1 kGy, and after a succession of definite storage periods, were progressively rewarmed to 238 K ( $-35^\circ C$ ).

**Results:** Thermoluminescence *patterns* of C15 LiCl in  $D_2O$ , C15  $D_2O$ , and  $D_2O$  were similar. The average thermoluminescence *intensity* of C15 LiCl in  $D_2O$  tended to be less than C15  $D_2O$  when tests were performed shortly after preparation of the substances and with short storage time between irradiation and recording of thermoluminescence. Thermoluminescence of  $D_2O$  was significantly lower than from C15  $D_2O$ , particularly when the time between irradiation and recording was increased.

**Conclusion:** The nature of the phenomena here described still remains unexplained. Nevertheless, data suggest that thermoluminescence might be developed into a promising tool to study homeopathically prepared ultra-high dilutions.

### INTRODUCTION

Thermoluminescence is the emission of light from a substance when it is heated. It is not to be confused with the light spontaneously emitted from a material when it is heated to incandescence. It is the thermally stimulated emission of light following the previous absorption of energy from radiation. Thermoluminescence is a common tool to study the structure of solids, and has been utilized following irradiation of samples at room temperature and below room temperature for the purpose of dosimetry, age determination, geology, or solid-state defect structure analysis.<sup>1</sup> More recently, thermoluminescence has been investigated as a future dating technique for icy bodies in the solar system.<sup>2</sup>

Low-temperature thermoluminescence was also utilized to study properties of liquids by turning them into stable solids with the use of very low temperatures.<sup>3</sup> Utilizing ther-

moluminescence, an anomalous effect was observed with ultra-high dilutions of lithium chloride ( $10^{-30}$  g  $cm^{-3}$ ), prepared in successive steps alternating vigorous mechanical stirring and 1:100 dilution with deuterium oxide. Although theoretically, such dilutions consist of pure deuterium oxide ( $D_2O$ ), thermoluminescence was reported to differ from that of pure  $D_2O$  without previously dissolved and diluted lithium chloride.<sup>4</sup> Such data resulted in discussions about the memory capacity of ultra-high dilution regarding original salts dissolved initially.<sup>5</sup>

$D_2O$  frozen in liquid nitrogen and then irradiated demonstrated thermoluminescence consisting of peak 1 near 125 K ( $-150^\circ C$ ) and a peak 2 near 166 K ( $-107^\circ C$ ). The two peaks differ in their induction by irradiation.<sup>4</sup> Peak 1 luminescence demonstrated an increase greater than 2 orders of magnitude via a dose range between 1 and 20 kGy. Peak 2 showed similar thermoluminescence intensity and an increase in intensity via a dose range of 0.02 to 0.4 kGy. The

two peaks also differ in emission spectrum. The spectrum of the thermoluminescence of peak 1 was  $\sim 350$  nm (peak 1A) and 660 nm (peak 1B), whereas the spectrum of peak 2 demonstrated peaks at 450 nm, 485 nm, 525 nm, and 575 nm.

For the purpose of replicating these observations, however, the study was lacking information regarding (1) variability in thermoluminescence of peak 1 and 2 in identically frozen, irradiated and measured samples, (2) time between preparation of a sample and its utilization in the experiment; and (3) time between irradiation of a sample and measurement of thermoluminescence. The influence of time on thermoluminescence can be twofold. A change, by succession, in the physical properties of a substance during the preparation of an ultra-high dilution can decrease over time while in storage. A change in the activated state following irradiation can also spontaneously decrease.

The present study was an attempt to provide information on these phenomena. New data were collected for: (1) D<sub>2</sub>O; (2) 0.1 M lithium chloride in D<sub>2</sub>O; (3) ultra-high dilutions of D<sub>2</sub>O prepared by 15 times (C15 D<sub>2</sub>O), a dilution of 100-fold alternated with vigorous mechanical stirring; and (4) ultra-high dilution of lithium chloride in D<sub>2</sub>O by 15 times (C15 LiCl), a dilution of 100-fold with D<sub>2</sub>O alternated with vigorous mechanical stirring. Thermoluminescence of these samples was determined (1) 3 weeks after their preparation and with only 1 week at very low temperature storage between irradiation and recording; (2) 7 weeks after preparation and with 2 weeks of storage; and (3) 12 weeks after preparation and 3 weeks of storage.

## MATERIALS AND METHODS

### Materials

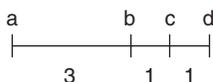
D<sub>2</sub>O and LiCl were obtained from Sigma-Aldrich, Zwijndrecht, The Netherlands (Bottles, 20 mL, brown glass, hydrolytic Class 2) were utilized untreated from original packaging; all bottles were from the same package. Dilutions were prepared by VSM Geneesmiddelen bv (Alkmaar, The Netherlands) according to a standard protocol. The first dilution was prepared by solving 0.1 g of lithium chloride (Sigma-Aldrich, Zwijndrecht, The Netherlands) in 9.9 mL of deuterium oxide (D<sub>2</sub>O; Sigma-Aldrich, Zwijndrecht, The Netherlands). Following solutions were made by dissolving 1 (mass) part of the preceding solution in 99 (mass) parts of D<sub>2</sub>O. Each solution was prepared in a new, clean glass bottle. Between each diluting step, the solutions were mechanically succussed (using a Klinkhamer apparatus), in a vertical direction (100 strokes in 15 seconds). This procedure was repeated until a "concentration" of  $10^{-30}$  was reached (C15 LiCl). The control preparation (C15 D<sub>2</sub>O) was prepared according to the same procedure with the exception

that the first solution existed of 1 part of D<sub>2</sub>O in 99 parts of D<sub>2</sub>O. Cleaning between potentizing different substances was performed according to Dutch pharmaceutical regulations. The dilutions and the solutions of 0.1 M LiCl in D<sub>2</sub>O and pure D<sub>2</sub>O were stored in tightly closed brown bottles under nitrogen at room temperature. All preparations were made from the same batch of D<sub>2</sub>O and at the same time. The four solutions were utilized throughout the experiment.

### Experimental design

At 3, 7, and 12 weeks after preparation, the four substances were utilized in a thermoluminescence experiment. These experiments are indicated as A, B, and C, respectively. In each of the three experiments, three samples from each of the four solutions were frozen, irradiated, and analyzed for thermoluminescence. The three samples for all three experiments were taken from the same bottle. Within the same experiment, freezing and irradiation of the 12 samples were performed at the same time. The freezing and irradiation were identical for all 36 samples in the three experiments. Time between irradiation and analysis of thermoluminescence was different in the three experiments (Fig. 1). Time of freezing after sample preparation and time between irradiation and measurement were not independently varied in this study, partly because of limitations in capacity. Thermoluminescence recordings in each experiment were carried out within a period of 36 hours.

#### Experiment A



#### Experiment B



#### Experiment C



a = Preparation of substances  
 b = Freezing  
 c = Irradiation  
 d = Thermoluminescence measurement

**FIG. 1.** Experimental design of experiments A, B and C, indicating time between preparation of substance (a), freezing (b), irradiation (c) and thermoluminescence measurement (d).

### *Freezing and blinding procedure*

One mL of each solution was placed in aluminum test cups of 20-mm diameter and 2-mm depth and frozen to 253 K ( $-20^{\circ}\text{C}$ ). Each test cup had a number and the numbers corresponding with each of the four solutions were noted. The frozen samples were kept 1 day at 253 K to achieve stability of the crystallization pattern. Then, the cups were randomly placed by another person on top of each other in 2 special holders and immersed into liquid nitrogen and maintained at 77 K ( $-196^{\circ}\text{C}$ ) for 1 week in a large liquid nitrogen—containing Dewar vessel before irradiation. Samples were maintained in the same ordered arrangement in the liquid nitrogen during irradiation. The sequence of the cups in the holder was essentially unknown until after their thermoluminescence was recorded. The thermoluminescence was recorded by a third person who was not present in any of the previous stages (coding, freezing, or irradiation). This person took the samples one after the other from the holder, starting with the upper cup. By recording the number of the cup after the thermoluminescence, it was evident what the sequence in the holder was and which thermoluminescence data corresponded with which of the four solutions.

### *Irradiation*

Irradiation of the two holders with cups was performed with the Co-60 source Gammacell 220 Excell (GC220E) from MDS Nordion (Fleurus, Belgium). A special Dewar vessel in a size to fit in the radiation chamber was used. The dose selected was 1 kGy. The average dose rate was approximately 30 cGy/second; irradiation time was 58 minutes. This dose was selected because it is in the range that thermoluminescence of both peak 1 and peak 2 are directly related with the dose.<sup>4</sup> The exact absorbed dose distribution in a vertical plane through the central vertical axis of the irradiation chamber is known. After irradiation, all samples were transferred into the large liquid nitrogen container for 1, 2, or 3 weeks before thermoluminescence measurements.

### *Thermoluminescence equipment and procedure*

Thermoluminescence equipment (JMD électronique, Montelier, France) was equipped with a Statop 4849 temperature controller (Chauvin Arnoux, Vaulx en Velin, France). For each measurement, the Dewar of the thermoluminescence equipment was filled with liquid nitrogen. When the aluminum temperature block had reached 78 K, a cup was transferred rapidly to the block and heating was started. The average time to increase the temperature of the block was approximately 20 minutes before the sensors registered a linear increase of temperature in time. Temperature increase was  $6^{\circ}\text{C}$  per minute from 85 K to 235 K. Thermoluminescence produced by the sample reached the

photomultiplier at a distance of 216 mm, utilizing a steel tube with reflecting walls.

### *Photomultiplier*

The photomultiplier connected to the thermoluminescence equipment was a 9235QB, selected type, photomultiplier in a single-counting mode from Electron Tubes Limited (Ruislip, England). The photomultiplier has a window of 52-mm diameter and a spectral sensitivity range of 200 to 650 nm, allowing optimal detection of peak 1A and peak 2. The front ring of the photomultiplier was vented inside, avoiding the condensation of moisture at the quartz window. The photomultiplier was maintained at a temperature of 238 K ( $-25^{\circ}\text{C}$ ) to reduce the dark current. The dark current during the experimental period was  $5.1 \pm 0.3$  cps.

## RESULTS

### *D<sub>2</sub>O and solution of 0.1 M LiCl in D<sub>2</sub>O*

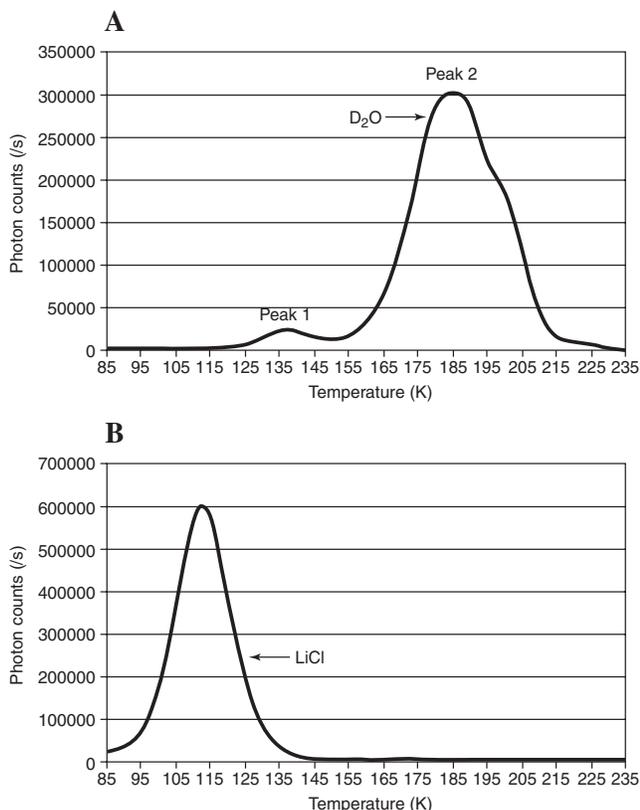
Thermoluminescence between 85 K and 235 K of 1 kGy-irradiated D<sub>2</sub>O resulted first in a small peak at 120 K–140 K. This was followed by the major emission between 165 K and 210 K (Fig. 2A). The peak at 120 K–140 K is indicated as peak 1 and the major emission peak is indicated as peak 2. Thermoluminescence of 0.1 M LiCl in D<sub>2</sub>O was substantially different from that of pure D<sub>2</sub>O. Data showed that 0.1M LiCl erased the D<sub>2</sub>O peak. Instead, a strong peak occurred at approximately 110 K (Fig. 2B).

### *Effect of succussion*

The stepwise repetition of succussion and dilution of D<sub>2</sub>O with nonsuccussed D<sub>2</sub>O resulted in C15 D<sub>2</sub>O. Similarly, such a stepwise procedure, starting with LiCl, resulted in potentized LiCl in D<sub>2</sub>O (C15 LiCl in D<sub>2</sub>O). Figure 3 shows the average thermoluminescence patterns of LiCl, D<sub>2</sub>O, C15 D<sub>2</sub>O, and C15 LiCl in D<sub>2</sub>O in the experiments A (Fig. 3A), B (Fig. 3B) and C (Fig. 3C).

These experiments differed in time after preparation of substances and time between irradiation and analysis of thermoluminescence. In experiment A, samples were tested 3 weeks after their preparation and with only 1 week of storage at 78 K, after their radiation. In experiments B and C the samples were tested at 7 and 12 weeks after preparation, and with 2 and 3 weeks of storage at 78 K, respectively. Quantitative information including mean and standard error of the counts of thermoluminescence in the different peaks is given in Table 1.

Our experiments clearly show that the variability of the peak 2 thermoluminescence increases with its mean. Consequently, for further statistical analysis, we took all data to its logarithm, thus achieving homogenous variances across

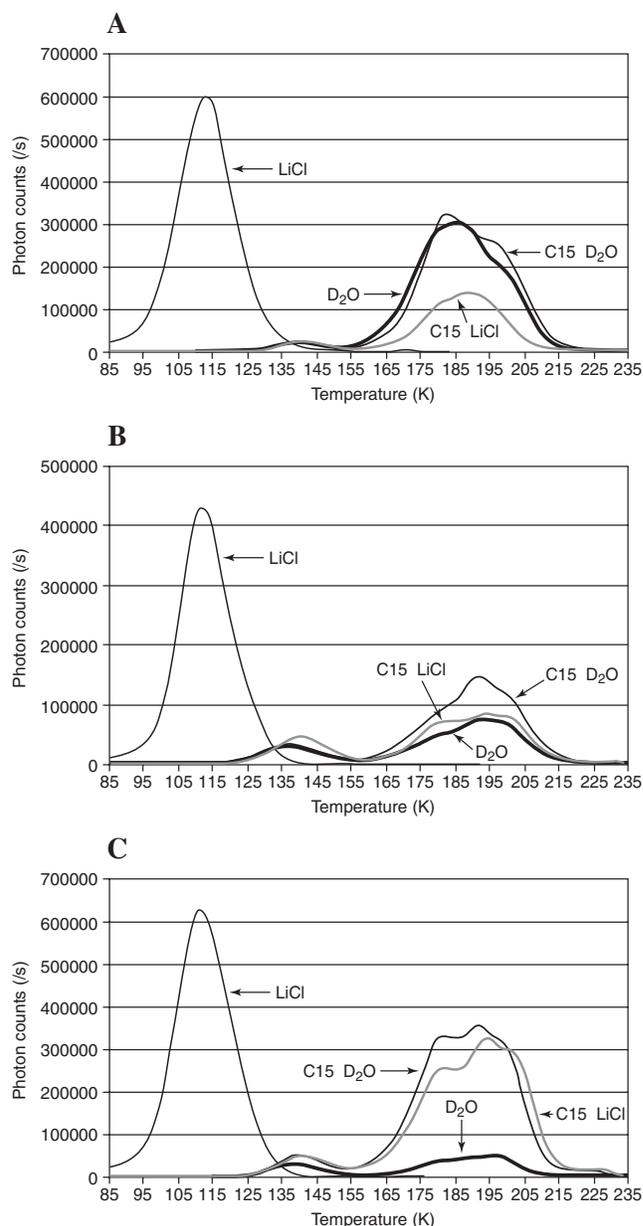


**FIG. 2.** Thermoluminescence of deuterium oxide ( $D_2O$ ) (A) and of a 0.1 M LiCl solution in  $D_2O$  (B) irradiated at 78 K by  $\gamma$  rays (1 kGy).

all conditions and substances. Next, we fitted a two-way analysis of variances (ANOVAs) to the data where the conditions (A, B, or C), the substances ( $D_2O$ ,  $C15 D_2O$ , and  $C15 LiCl$ ) and the condition-substance interaction were taken as fixed factors.

From this model we can conclude that  $C15 LiCl$  did not significantly differ from  $C15 D_2O$  in experiments B and C ( $p = 0.72$  and  $p = 0.63$ ; ANOVA  $t$ -tests). In experiment A, there tended to be a difference between both groups ( $p = 0.059$ ; ANOVA  $t$ -test). Moreover, in experiment C,  $D_2O$  statistically differed from  $C15 D_2O$  ( $p < 0.0001$ ; ANOVA  $t$ -test).

It has been suggested that peak 2 could be connected to the hydrogen-bond network within the ice, while peak 1 is closely related to the molecule.<sup>4</sup> To compare peak 2 counts for the different conditions and substances, the emitted light in peak 2 could also be normalized taking peak 1 as the reference. The ratio of peak 2/peak 1 is shown in Table 1. In doing so, we were able to conclude that in the two-way ANOVA  $t$ -test there was a significant difference between LiCl  $C15$  and  $C15 D_2O$  in experiment A ( $p = 0.0128$ ) but not in experiments B and C ( $p = 0.60$  and  $p = 0.73$ , respectively). In experiment C,  $D_2O$  statistically differed from  $C15 D_2O$  ( $p < 0.0004$ ).



**FIG. 3.** Thermoluminescence of 0.1 M LiCl in deuterium oxide ( $D_2O$ ),  $D_2O$ ,  $C15 LiCl$  in  $D_2O$ , and  $C15 D_2O$ , irradiated at 78 K by  $\gamma$  rays (1 kGy). A. Freezing was 3 weeks after preparation of substances; very low temperature storage for 1 week occurred between irradiation and recording. B. Freezing was 7 weeks after preparation; storage between irradiation and recording was 2 weeks. C. Freezing was 12 weeks after preparation; storage between irradiation and recording was 3 weeks.

## DISCUSSION

We report here differences in thermoluminescence between  $C15 D_2O$  and  $C15 LiCl$ , which correspond with the observations reported by Rey.<sup>4</sup> However, the difference from all of these recordings of these substances was not statistically significant. The present data suggest that such a

TABLE 1. PHOTON COUNTS IN THE THERMOLUMINESCENCE PEAK 1 (125K–150K) AND PEAK 2 (160K–215K) FROM DEUTERIUM OXIDE (D<sub>2</sub>O), C15 D<sub>2</sub>O, AND C15 LiCl IN D<sub>2</sub>O, IRRADIATED AT 78 K BY  $\gamma$  RAYS (1 kGy)

Exp.	Counts ( $\times 10^6$ ) $\pm$ SE ( $\times 10^6$ ) peak 1 (125 K–150 K)			Counts ( $\times 10^6$ ) $\pm$ SE ( $\times 10^6$ ) peak 2 (160 K–215 K)			Ratio peak 2/peak 1		
	D <sub>2</sub> O	C15 D <sub>2</sub> O	C15 LiCl	D <sub>2</sub> O	C15 D <sub>2</sub> O	C15 LiCl	D <sub>2</sub> O	C15 D <sub>2</sub> O	C15 LiCl
A	6.01	3.32	4.54	174.72	164.76	38.26	29.07	49.63	8.43
A	4.50	3.61	4.22	49.19	52.75	23.63	10.93	14.61	5.59
A	2.15	4.09	4.14	41.53	63.16	44.98	19.32	15.44	10.86
<b>A</b>	<b>4.22 <math>\pm</math> 1.12</b>	<b>3.67 <math>\pm</math> 0.22</b>	<b>4.30 <math>\pm</math> 0.12</b>	<b>88.48 <math>\pm</math> 43.17</b>	<b>93.56 <math>\pm</math> 35.73</b>	<b>35.62 <math>\pm</math> 6.30</b>	<b>19.77 <math>\pm</math> 5.24</b>	<b>26.56 <math>\pm</math> 11.54</b>	<b>8.29 <math>\pm</math> 1.52</b>
B	10.39	5.34	3.60	35.33	49.63	18.68	3.40	9.29	5.18
B	3.88	9.12	11.77	14.48	46.40	37.20	3.73	3.16	3.16
B	3.48	3.06	6.41	18.37	28.08	58.23	5.28	9.18	9.08
<b>B</b>	<b>5.91 <math>\pm</math> 2.24</b>	<b>5.84 <math>\pm</math> 1.77</b>	<b>7.26 <math>\pm</math> 2.40</b>	<b>22.73 <math>\pm</math> 6.40</b>	<b>41.37 <math>\pm</math> 6.71</b>	<b>38.04 <math>\pm</math> 11.42</b>	<b>4.14 <math>\pm</math> 0.58</b>	<b>7.21 <math>\pm</math> 2.03</b>	<b>5.81 <math>\pm</math> 1.74</b>
C	6.10	11.17	12.50	24.10	110.98	183.69	3.95	9.94	14.70
C	3.12	5.09	6.33	8.55	92.11	54.28	2.74	18.10	8.58
C	4.25	7.50	4.27	9.56	131.83	71.93	2.25	17.58	16.85
<b>C</b>	<b>4.48 <math>\pm</math> 0.87</b>	<b>7.92 <math>\pm</math> 1.77</b>	<b>7.70 <math>\pm</math> 2.47</b>	<b>14.07 <math>\pm</math> 5.02</b>	<b>111.64 <math>\pm</math> 11.47</b>	<b>103.30 <math>\pm</math> 40.52</b>	<b>2.98 <math>\pm</math> 0.51</b>	<b>15.21 <math>\pm</math> 2.64</b>	<b>13.38 <math>\pm</math> 2.42</b>

Mean and standard error were determined in three experiments, utilizing 3 thermoluminescence data sets of each substance per experiment. Exp., experiment; SE, standard error.

difference tended to be significant only when the experiment was carried out 3 weeks after preparation of the substances and with a short time between irradiation and thermoluminescence recording. Another observation in the present study was the difference in thermoluminescence between C15 D<sub>2</sub>O and D<sub>2</sub>O. Data suggest an increased difference during long storage conditions.

We can only speculate about the significance of the observed opposite trends. Given that the original pure D<sub>2</sub>O had not been modified and was also the basis for the preparation of succussed ultra-high dilutions, it is proposed that it would not be additionally activated after irradiation in the experiments carried out at 3, 7, and 12 weeks after its use for the preparation of the succussed ultra-high dilutions. It is more likely that the obvious thermoluminescence decreasing trend of D<sub>2</sub>O compared to C15 D<sub>2</sub>O under long storage conditions suggests the possibility that stored irradiation energy in D<sub>2</sub>O might be released more easily at very low temperatures. Be that as it may, it appears that the structural state of a solution made in D<sub>2</sub>O can be modified by strong succussion. The explanation of the trend observed in the difference between C15 D<sub>2</sub>O and C15 LiCl might be just the opposite. It has been proposed that LiCl suppresses hydrogen bonds. With such strong hydrogen-bond suppression as a "ghost effect" after succussion in the ultra-high dilution, one can imagine that this effect is more likely to be present shortly after preparation. Such a ghost effect would be lost after a long storage of the substance. With this in mind, the hypothesis to explain the data include (1) succussion stabilizes the D<sub>2</sub>O physical structure in such a way that stored radiation energy can be kept for a longer period; and (2) the ghost effect of LiCl after preparation interferes with this stabilization process, but only for a limited time.

The nature of the phenomena here described still remains unexplained. Generally, liquid water is described as a disordered ensemble of highly polar molecules linked via a fluctuating network of intermolecular hydrogen bonds. Recent studies demonstrated the efficiency of energy redistribution within the hydrogen-bonded network and that water loses the memory of persistent correlations in its structure within 50 fs. Energy-transfer dynamics studies of a water molecule that is enclosed by acetone molecules have demonstrated hydrogen-bond dynamics with a time constant of 1.3 ps.<sup>7</sup> This means that the exact nature of the phenomena presented in this paper is not, at present, in agreement with current theories on the fluctuations in the network of intermolecular hydrogen bonds.

It is evident that the results need further study. The point must be stressed that we obtained a very good qualitative reproducibility of the thermoluminescence pattern, but the quantitative reproducibility was rather poor, and *p*-values should be interpreted in the sense of descriptive statistics. Subsequent sample size calculations showed that 18 peak 2 measurements (9 with each group) were needed to detect a difference between C15 LiCl and C15 D<sub>2</sub>O with a

power of 90%. The variation between samples can only partially be explained by the time between substance preparation and testing, and/or time between irradiation and recording. The variation is slightly reduced if we normalized the emitted light readings in peak 2 utilizing peak 1 as the reference.<sup>4</sup> To study the origin of the fluctuations within one set of data, we have looked at the following variables: (1) Filling the cups was kept constant. The thermoluminescence was not correlated filling the cups in sequence or placing them in the freezer. (2) The samples were frozen under similar conditions. (3) The procedure for irradiation was constant. The location of the cups inside the irradiation chamber was compared to the absorbed dose-distribution in the irradiation chamber, and no correlation was observed between the location in the chamber and the luminescence recorded. (4) The procedure for recording thermoluminescence was kept constant. Although some variation was observed in time before temperature increased linearly, this linear increase always began when the temperature was at approximately 87 K, demonstrating no correlation with the differences in luminescence intensity. Comparing all these steps in the experimental protocol, it can be suggested that the freezing process causes the variation in thermoluminescence. It is well known that in this process, variations arise as they do in ice crystalization patterns. Yada et al.<sup>2</sup> observed that peak 2 is extremely dependent upon oxygen content. Further studies are required to study the role of oxygen in the variation.

## CONCLUSIONS

In summary, the present data suggest that the thermoluminescence technique can contribute to the challenge of succussion and ultra-high dilution preparation procedures commonly applied to homeopathic medication preparation. In order to improve the protocol, our data suggest further research utilizing a large number of observations to include (1) different time periods between the preparation of substances and their use in an experiment; and (2) different time periods between irradiation and thermoluminescence registration. If such protocol were to be developed, it could be utilized to compare different procedures currently used in homeopathic pharmaceutical practice.

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