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Free and Bound Water in Lactose Mixed with Ultra High Dilution of Sulphur, Sodium Chloride and Ethanol as Revealed by Thermogravimetry

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Abstract *Natrum mur* 200 cH was prepared by successive dilution of NaCl solution with 90% ethanol 1:100 followed by succussion in 200 steps. Sulphur powder was first triturated and then processed similarly to prepare the 200th potency. Each of the two potencies and 90% ethanol was diluted with water 1 : 1000 and mixed with lactose 100 µl/2 g lactose. Each sample was then subjected to themogravimetric analysis (TGA) using a Perkin Elmer (STA 6000) instrument. The first phase (s) of mass loss below 100°C in TGA curve indicates evaporation of free water from the samples. The next phase of mass loss upto 180°C suggests removal of bound water. Thus *Sulphur* 200 cH, *Natrum mur* 200 cH and Ethanol contain 35.82, 9.5 and 3.71% free water molecules, respectively. Bound water constitutes 64.17% for *Sulphur* 200 cH, 90.5% for *Natrun mur* 200 cH and 96.29% for Ethanol. DTA curve shows average heat flow of 7.06 mW for *Sulphur* 200 cH, 11.38 mW for *Natrum mur* 200 cH and 13.55 mW for Ethanol. The phases of water loss are associated with the endothermic peaks in DTA curves indicating higher rates of heat flow there. It is concluded that the three drugs tested show marked variation from each other with respect to the quantity of free and bound water molecules.

Keywords *Sulphur, Natrum mur*, High dilution, Free water, Bound water, Thermogravimetric analysis.

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Introduction

Ultra high dilutions (UHD) of drugs have been used in homeopathy for more than a couple of centuries. In homeopathy a drug solution in aqueous ethanol, called mother tincture (MT), is diluted with the same solvent or distilled water and mechanically agitated by succussion to produce a potency. In case of centesi-

mal potency the MT is diluted with a solvent 1:100 and succussed 10 times to produce the first potency called 1 cH. Rank of potency denotes the number of steps applied to a MT to produce that potency. Thus 200th potency means the MT has been subjected to 200 steps of successive dilution followed by succussion (Anonymous 1920, Sukul and Sukul 2004). Obviously, the 200th potency of a drug crosses the Avogadro number an is devoid of original drug molecules. Thus all homeopathic potencies, 12 cH and above, are identical in chemical composition being made up of ethanol and water. Yet they produce different therapeutic effects on patients.

In a series of experiments involving Fourier Transform Infra Red (FTIR) spectroscopy, Raman spectroscopy and Differential Scanning Calorimetry (DSC) we have demonstrated that physico-chemical basis of potency consists of two factors, free water molecules and hydrogen bond strength of the OH groups of water (Chakraborty et al. 2014, Sarkar et al. 2016a, b, Sarkar et al. 2017). The purpose of the present study is to further confirm the variation in quantity of free water molecules in the 200th potency of two drugs *Sulphur* and *Natrum mur*, and their solvent medium ethanol water using another technique thermogravimetry. In this technique the progressive loss of weight in a sample is monitored in relation to the gradual rise in temperature. Usually, non-hydrogen bonded free water molecules evolve first at relatively lower temperatures within 100^{o}C, and bound water evolves later. Higher energy is needed to break free the hydrogen bonded bound water or water in crystal structure.

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Materials and Methods

Both sulphur powder and sodium chloride were purchased from the market. Sulphur is not soluble in water. So sulphur powder was triturated with lactose up to the 3rd centesimal potency and then mixed with aqueous ethanol and succussed 10 times to produce the 4th potency following the standard procedure of preparation of homeopathic potencies. The 200th potency of sulphur was prepared by successive dilution with aqueous ethanol 1:100 followed by succussion in the laboratory (Anonymous 1920, Sukul and Sukul 2004). Sodium chloride mother tincture (500 mM NaCl solution in distilled water) was serially diluted and succussed to prepare *Natrum nur* 200 cH following the same Korsakovian method as in the case of sulphur. The succussion machine was designed in the laboratory. The mechanical arm 40 cm long, has a 400 g iron cage at the tip holding a 30 ml vial. The vial is filled 2/3rd with the drug solution (90% EtOH). Ten successions / 30 sec are given for each rank of potency. Ethanol (E Merck), obtained from the market, was diluted with distilled water to prepare 90% solution. Each potency was diluted with distilled water 1:1000 just before the experiment to minimize the ethanol content (0.09%), Ethanol was similarly diluted.

A sample of lactose, obtained from SRL, Mumbai, was mixed with the drug solution (1:1000) at 2 g lactose/100 µl drug solution. Lactose has been traditionally used as a medium to dispense with homeopathic potencies for oral application on patients. Thermal gravimetric analyzer, Perkin Elmer (STA 6000) was used to measure the amount of free and bound water in the lactose samples in terms of mass loss as a function of temperature. While bound water evolves at higher temperature, free water does so at relatively lower temperature. This is because higher energy is needed to remove bound water compared to free water. Aluminium sample pan containing each sample was placed in platinum crucible. The temperature was ramped at 10^{o}C min^{-1} from room temperature 30^{o}C to 180^{o}C. The differential thermal analysis (DTA) curve shows the rate of heat flow in milliwatt (mW) versus temperature.

Results

Results are presented in three figures showing both TGA and DTA curves in each figure. TGA and DTA curves for *Sulphur* 200 cH are shown in Fig. 1. TGA curve of *Sulphur* 200 cH shows 2.88% weight loss at 33–47^{o}C, 0.209% weight loss at 47.5–114^{o}C, 4.86% weight loss at 114–166.6^{o}C and 0.092% weight loss at

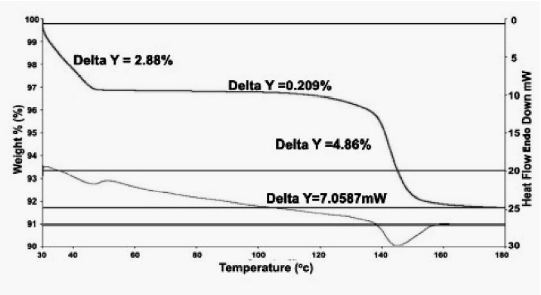

Fig. 1. Thermogravimetric analysis (TGA) curve above shows mass loss in % from lactose mixed with *Sulphur* 200 cH in aqueous ethanol (0.09%) at 2 g lactose/100 µl drug soln with increasing temperature (10°C min^{-1}). Differential thermal analysis (DTA) curve of the same sample shows rate of heat flow in milliwatt (mW) as a function of temperature. Heat required for endothermic decomposition is 7.059 mW.

166.6–180°C. Thus the total water content in this lactose sample is 8.046%. Water loss in the first phase or free water is 35.82% of total water, and the rest is bound water. The ratio between the free (F) and bound (B) water is 35.824/64.175=0.558. Fig. 1 further shows that DTA has an endothermic peak close to 50°C coinciding with the first phase of water loss as shown by the TGA trace. There is another DTA peak at 146.6°C which coincides with the largest amount of water loss as shown by the TGA trace (Fig. 1). The DTA curve further shows that the heat required for the endothermic process is 7.051 mW.

TGA and DTA curves for *Natrum mur* 200 cH are shown in Fig. 2. Here TGA curve shows 0.614% weight loss at 30–100°C, 5.543% weight loss at 100–160°C and 0.3098% weight loss at 100–180°C. Here the first phase of weight loss up to 100°C indicates loss of free water. The second and third phase of weight loss indicate loss of bound water. So the total water loss is 6.468%. Out of this first phases of water loss constitutes 9.498%, and the last two phases of water loss 90.50%. Here the free water is 9.498% and bound water 90.50%, and the ratio between the two is 0.105%. The DTA curve for *Natrum mur* 200 cH shows first peak at 60°C, second one at 90°C and the third one at 147.8°C. The last peak coincides with the largest phase

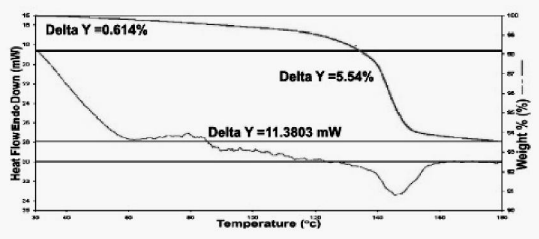

Fig. 2. TGA curve above shows mass loss in % in a sample of lactose mixed with *Natrum mur* 200 cH (100 µl drug/2 g lactose) as a function of temperature. DTA curve shows rate of heat flow in mW through the same sample as a function of temperature. Heat required for endothermic decomposition is 11.38 mW.

of weight loss as shown by the TGA curve (Fig. 2). Here the heat needed for endothermic transition is 11.38 mW.

TGA and DTA curves of ethanol are shown in Fig. 3. Here TGA trace shows 0.195% weight loss at 30–100°C and 4.83% loss at 100–155°C and 0.234% loss at 155–180°C. Here free and bound water are 3.708% and 96.29%, respectively. Here the ratio between free water and bound water is 0.038. The DTA curve shows a single peak at 146.6°C which coincides with largest phase of water loss as shown by the

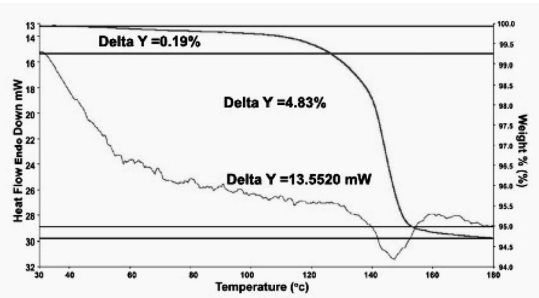

Fig. 3. TGA curve above shows mass loss in % in a sample of lactose mixed with ethsnol (0.09%) at 100 µl EtOH/2 g lactose) as a function of temperature. DTA curve shows rate of heat flow in mW through the same sample as a function of temperature. Heat required for endothermic decomposition is 13.55 mW.

TGA trace (Fig. 3). In this case heat involved in endothermic dehydration is 13.552 mW. Largest endothermic peak of DTA curve occurs at 30 mW in the temperature range of 137–157°C for *Sulphur* 200 cH. For *Natrum mur* this peak occurs at 31.7 mW in the temperature range of 135–160°C. In case of ethanol this peak occurs at 31.5 mW in the temperature range of 135–160°C.

Discussion

It is evident from the results that the two homeopathic potencies, *Sulphur* 200 cH and *Natrum mur* 200 cH have different amounts of free and bound water molecules, and their ratios F/B are totally different from each other. All the three drugs are composed mainly of water and a very small amount of ethanol (0.09%). Although the medium of all homeopathic potencies are aqueous ethanol, the latter is also used as a homeopathic drug (Farrington 1928). Based on the amount of free water and the value of the ratio (F/B), the three test drugs can be ranked as *Sulphur* >*Natrum mur*>Ethanol. However, from the perspective of the average rate of heat flow the test drugs show the reverse relationship: Ethanol>*Natrum mur*>*Sulphur*. Heat flow is measured in joules per g and milliwatts. This means that *Natrum mur* 200 cH has a lower heat capacity than ethanol, and *Sulphur* 200 cH has a lower heat capacity than *Natrum mur* 200 cH. Heat capacity is the heat energy in joules required to raise the temperature of a body by 1°kelvin. In other words heat capacity is heat transferred / temperature rise. Heat flow is the transfer of energy away from a body, here drug soaked lactose sample, into the surroundings by conduction, convection and radiation or evaporation. Endothermic peaks in DTA curves indicate higher rate of heat flow due to phase changes by way of breakdown of bound water or release of water of crystallization. Water of crystallization is released from lactose crystals at a temperature 140–165°C (Konar et al. 2017). It is interesting to note that the largest endothermic peak in the DTA curves of all the three drugs occur in this temperature zone (Fig. 1, 2, 3). So this peak indicates loss of water of crystallization. Heat flow in the largest DTA peak is more or less same (30–31.7 mW) for all the three drugs. It appears from the results that higher is the ratio F/B, the lowest is the flow of heat.

Conclusion

The same ultra high dilution of three drugs *Sulphur, Natrum mur* and *ethanol*, used in homeopathy, vary from each other with respect to the relative proportion of free and bound water molecules.

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