Environment and Ecology 37 (2) : 628—633, April—June 2019
Website: environmentandecology.com ISSN 0970-0420

Free and Bound Water in Three Different Concentrations of a Homeopathic Drug *Mercurius corrosivus* 200 cH and its Vehicle Ethanol

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Received 4 January 2019 ; Accepted 7 February 2019 ; Published on 28 February 2019

Abstract *Mercurius corrosivus* 200 cH, prepared by successive dilution followed by succussion and ethanol without succussion are both in 90% ethanol. Each of them was diluted first with water 1 : 1000 and then each dilution as a unit was further diluted with appropriate volume of water to make 0.5, 2 and 4% solution. Each of these diluted samples of *Merc cor*/ethanol was mixed separately with lactose in the proportion of 50 µl per 1g lactose. One lactose sample was mixed with water alone at 50 µl per 1g lactose. All the lactose samples were separately tested by thermogravimetry (TG) to assess the amount of free and bound water. Each test sample was heated from 30°C to 200°C at the rate of 10°C per min. While TG curve indicates mass loss (%) as a function of temperature, differential thermogravimetry (DTG) shows rate of change of mass loss in relation to temperature. Lactose samples containing 0.5, 2 and 4% ethanol unit have 7.7, 10.7 and 7.9% free water, respectively. Lactose samples with 0.5, 2 and 4% *Merc cor* 200 cH unit have 12.3, 5.2 and 8.3% free water, respectively. Lactose sample with water (1 : 50µl) has 3.5% free water at 36.4°C temperature and 5% bound water at 147.7°C. Bound water varies between 3.5 and 4.6% in both ethanol and *Merc cor* samples at a temperature of 146–147.7°C. Loss of free water in both *Merc cor* and ethanol occurred between 45.3–63.3°C. There is no linearity in relationship between free water and total water content and between thermal energy needed to remove free water and the total water content in all the test samples.

Keywords Free water, Bound water, Thermogravimetry, Homeopathy, *Mercurius corrosivus* ethanol.

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Introduction

In homeopathy drugs are very often used in extreme dilutions beyond Avogadro number. These extremely diluted drugs, called potencies, do not contain original drug molecules from which they have been prepared by successive dilution followed by mechanical agitation or succussion (Anonymous 1920). Yet these potencies produce therapeutic effects on patients. Experimental evidences show that homeopathic

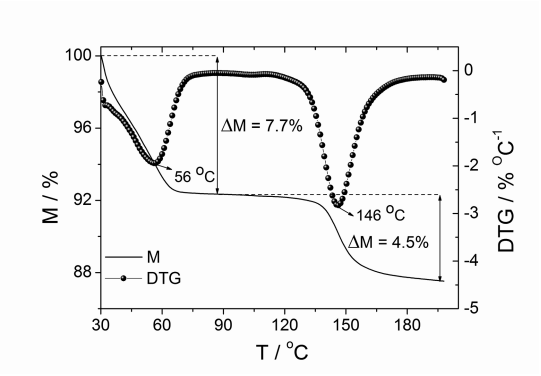

Fig. 1. Lactose + 0.09% EtOH with 99.5% added water (1 g : 50 µl) : TG curve (solid line) showing evolution of water (%) as a function of temperature and DTG curve (dotted line) showing rate of change of mass with temperature.

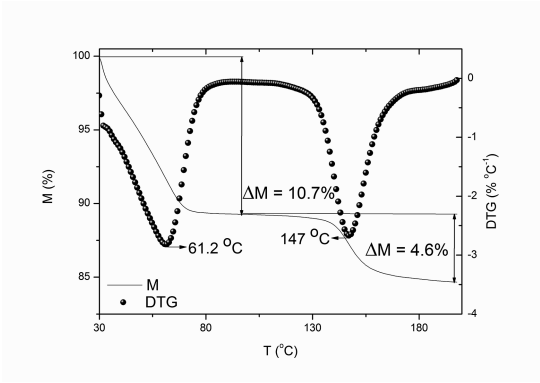

Fig. 2. Lactose+0.09% EtOH with 98% added water (1g: 50µl): TG curve (solid line) showing evolution of water (%) as a function of temperature and DTG curve (dotted line) showing rate of change of mass with temperature.

potencies produce significant effect on plants and animals (Sukul and Sukul 2004). Potencies also produce *in vitro* effects including those on proteins in a cell-free medium (Witt et al. 2007, Konar et al. 2015). Physical basis of homeopathic potencies, as revealed by FTIR and Raman spectroscopy, consists of 2 factors, free water molecules (FW) and hydrogen bond strength (HBS) of OH groups (Chakraborty et al. 2014, Sarkar et al. 2018). Differential scanning calorimetry (DSC) and thermogravimetry analysis (TGA) further confirm that potencies differ from each other with respect to FW and HBS of OH groups. Homeopathic potencies are prepared and preserved in aquous ethanol, usually 90% ethanol. The potencies are normally diluted with water and then administered orally on patients and this dilution is arbitrary. Again the standard method of preparation of a centesimal potency involves dilution with water in the proportion 1 part: 100 parts water (v/v). In a recent study we observed that potencies diluted with water retain their characteristic uniqueness with respect to the 2 factors, FW and HBS (Konar et al. 2018). The objective of the present study is to see whether the dilution above 1:1000 of potentized *Merc cor* and unpotentized ethanol could influence the 2 factors FW and HBS. For this we used a homeopathic potency *Mercurius corrosivus* 200 cH (*Merc cor* 200 cH). This potentized drug was prepared from the mother tincture of mercuric chloride ($HgCl_2$ solution in water) called *Merc cor* θ following the standard method of dilution with water 1:100 followed by succussion in 200 steps (Anonymous 1920, Sukul and Sukul 2004). *Merc cor* 200 cH, thus prepared, was finally preserved in 90% ethanol. *Merc cor* 200 cH and the vehicle 90% ethanol diluted with deionized and distilled (DD) water 1:1000 without succussion to minimize EtOH content to 0.09%. It may be mentioned here that ethanol is also used as a homeopathic drug. We prepared 3 concentrations 0.5, 2 and 4% of each diluted test sample by adding appropriate volume of DD water to these. Each of the 3 different concentrations was mixed with lactose, a traditional medium for dispensing homeopathic potencies, for estimation of FW. We used thermal gravimetry (TG) and derivative of thermogravimetry (DTG) for determination of free water (FW) and bound water (BW) in the lactose samples mixed with drugs / water control.

Materials and Methods

Merc cor θ (147 mM $HgCl_2$ solution in DD water) was serially diluted with DD water 1:100 and succussed 10 times using a vertical stirrer in 200 steps in the laboratory to produce *Merc cor* 200 cH. This 200^{th} potency was kept in 90% ethanol (Konar et al. 2018). The vehicle 90% EtOH was prepared from absolute ethanol (E Merck, Germany). We used Korsakovian method to prepare *Merc cor* 200 cH (Sukul and Sukul 2004). *Merc cor* 200 cH in 90% EtOH or the blank 90% ethanol was diluted with DD water 1:1000 and again each dilution as a unit was further diluted with DD water in 3 proportions, 1 part: 99.5 parts water,

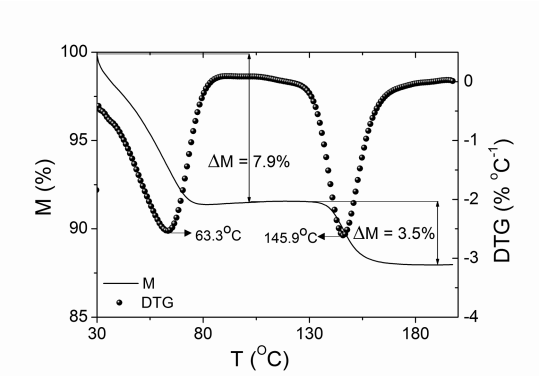

Fig. 3. Lactose + 0.09% EtOH with 96% added water (1 g : 50 µl) :TG curve (solid line) showing evolution of water (%) as a function of temperature and DTG curve (dotted line) showing rate of change of mass with temperature.

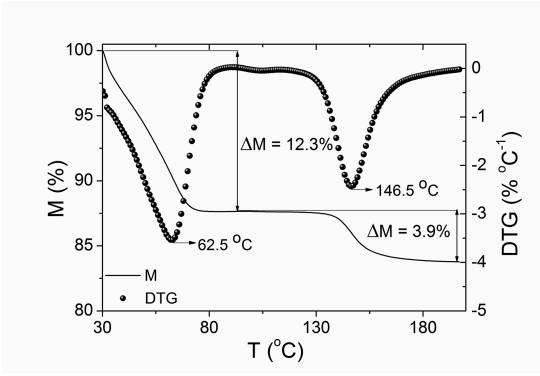

Fig. 4. Lactose+*Merc cor* 200 cH in 0.09% EtOH with 99.5% added water (1 g : 50 µl) :TG curve (solid line) showing evolution of water (%) as a function of temperature and DTG curve (dotted line) showing rate of change of mass with temperature.

1:98 parts water and 1 : 96 parts water v/v, so that the original diluted unit (in 0.09% EtOH) of either *Merc cor* 200 cH or ethanol became 0.5, 2 and 4% solution, respectively. Each test sample was then thoroughly mixed with lactose in the proportion of 50 µl of a test sample per 1g lactose. After mixing, each sample was wrapped by pressure in a piece of aluminium foil to prevent slow evaporation. Thermal gravimetry analyser (STA 449 F3 Jupiter of Netzsch, Germany) was used to measure the free and bound water in the test samples in terms of mass loss as a function of temperature. Free water evolves first but bound water requires higher temperature for its removal (Fessas and Schiraldi 2001). The thermogravimetry (TG) curves were obtained by scanning the test sample at constant linear heating rate of 10°C min^{-1} from room temperature (30ºC) to 200°C. UHP N_2 (99.999%) was used as the protective gas in the instrument. The sample and reference crucibles used for the study were made of alumina. Thermogravimetry curve shows mass loss in percent (%) in relation to temperature while the differential thermogravimetry (DTG) curve shows the rate of change of mass with temperature. The mass loss here is due to dehydration and not decomposition of the material tested within 200ºC.

Results

The results obtained from the thermogravimetry study of the samples are presented in Figs. 1—7 while the

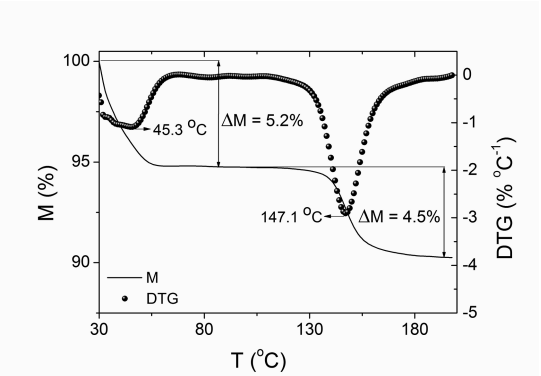

Fig. 5. Lactose + *Merc cor* 200 cH in 0.09% EtOH with 98% added water (1 g : 50 µl) :TG curve (solid line) showing evolution of water (%) as a function of temperature and DTG curve (dotted line) showing rate of change of mass with temperature.

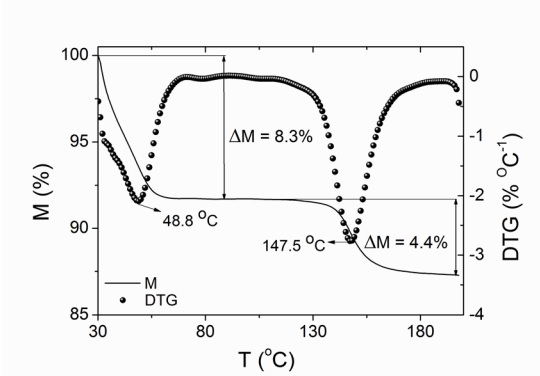

Fig. 6. Lactose+*Merc cor* 200 cH in 0.09% EtOH with 96% added water (1 g : 50 µl) :TG curve (solid line) showing evolution of water (%) as a function of temperature and DTG curve (dotted line) showing rate of change of mass with temperature.

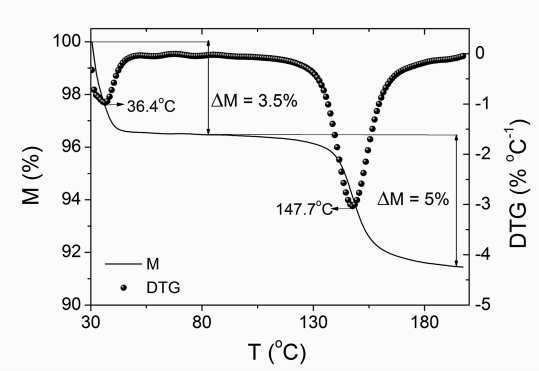

Fig. 7. Lactose + water (1 g : 50 µl) :TG curve (solid line) showing evolution of water (%) as a function of temperature and DTG curve (dotted line) showing rate of change of mass with temperature.

step-wise mass loss and DTG peak values estimated from these figures are presented in Table 1. Each figure shows a TG curve and a DTG curve. The TG curve shows 2 events of mass loss, 1 between 36.4–63.3°C in the time range of 3.6–6.6 min and another between 146–148.7°C in the time range of 14.3–14.9 min. The mass loss is associated with the DTG curve which shows 2 exothermic peaks, 1 in the range of 36.4–63.3°C and another in the range of 146–148.7°C. The first event of mass loss in the TG curve represents free water and the second one of mass loss indicates bound water. The first exothermic peak in the DTG curve indicates fall in the rate of change in temperature due to evaporation of free water and the second one that for bound water.

Lactose samples containing 0.5, 2 and 4% ethanol solution had 7.7, 10.7 and 7.9% free water, respectively (Figs. 1, 2, 3). DTG curve shows 2 exothermic peaks at 56°C and 146°C for 0.5% EtOH, 61.2°C and 147°C for 2% EtOH ((Fig. 2) and 63.3°C and 145.9°C for 4% EtOH (Fig. 3). Bound water occurred in 4.5, 4.6 and 3.5% in 0.5, 2 and 4% EtOH, respectively (Figs. 1, 2, 3 and Table 1). For *Merc cor* 200 cH free and bound water were 12.3 and 3.9%, respectively in lactose samples with 0.5% drug solution (Fig. 4). In case of 2% *Merc cor* 200 cH solution free and bound water were 5.2 and 4.5%, respectively (Fig. 5, Table 1). In case of 4% *Merc cor* 200 cH free and bound water were 8.3 and 4.4%, respectively (Fig. 6, Table 1). DTG curve shows 2 dips at 62.5°C and 148.7°C for 0.5% *Merc cor* 200 cH (Fig. 4), at 45.3°C and 147.1°C for 2% *Merc cor* 200 cH (Fig. 5) and at 48.8°C and

Table 1. Free and bound water in 3 different concentrations (0.5, 2 and 4%) of *Merc cor* 200 cH and ethanol, mixed with lactose in the proportion of 50 µl of drug solution/g lactose. Both *Merc cor* 200 cH and ethanol were in 90% ethanol. They were diluted with deionized and distilled (DD) water 1:1000 to minimize ethanol content to 0.09%. One lactose sample was mixed with DD water in the same proportion. The ratio between free and bound water for each concentration is shown in parentheses.

Water/ drug solution mixed with lactose	0.5% (99.5% water) Free water % (F)	0.5% (99.5% water) Bound water % (B)	2% (98% water) Free water % (F)	2% (98% water) Bound water % (B)	4% (96% water) Free water % (F)	4% (96% water) Bound water % (B)
Ethanol	7.7% at 56°C	4.5% at 146°C (1.71)	10.7% at 61.2°C	4.6% at 147.7°C (2.33)	7.9% at 63.3°C	3.5% at 145.9°C (2.56)
Merc cor 200 cH	12.3% at 62.5°C	3.9% at 148.7°C (3.15)	5.2% at 45.3°C	4.5% at 147.1°C (1.15)	8.3% at 48.8°C	4.4% at 147.5°C (1.89)
Water	Free water % (F)	Bound water % (B)				
	3.5% at 36.4°C	5% at 147.7°C (0.70)				

147.5°C for 4% *Merc cor* 200 cH (Fig. 6). Lactose sample containing 0.5% water only (control) had 3.5% free water and 5% bound water(Fig. 7, Table 1). DTG curve in this control sample shows 2 peaks at 36.4°C and 147.7°C (Fig. 7, Table 1).

Discussion

It is evident from the results obtained that there is a wide variation in the percentage of free water in all the lactose samples tested (3.5–12.3%). However, the percentage of bound water shows relatively lesser variation (3.5 and 5). The bound water is actually the water of crystallization in lactose crystals (Konar et al. 2017, Listohadi et al. 2009). The second exothermic peak in the DTG curve shows much higher temperature (145.9–147.7°C) as compared to that of the first peak. The thermal energy needed to break free bound water in different samples is much higher than that for free water. However, there is a wide variation in the first exothermic peak of DTG curve (36.4–63.3°C) corresponding to the free water in the test samples containing the same percentage of drug solution or control (Table 1). DTG curve in the water control shows the lowest temperature (36.4°C) for free water (Table 1). Free water needs higher temperature for removal from ethanol-soaked lactose sample with an increase in ethanol content. But in case of lactose samples with *Merc cor*, free water needs lesser thermal energy for their removal with increasing EtOH content. The higher is the EtOH content, the higher is the ratio value between free and bound water (F/B) for ethanol samples. But in case of *Merc cor* 200 cH the situation is reverse. Here the temperature is highest for the lowest ethanol content (Table 1). In this case the higher the EtOH content the lesser is the ratio value (F/B). In both ethanol and *Merc cor* free water is higher in amount than bound water in each of the 3 concentrations (Table 1). But in case of water control free water is much lower than the bound water.

In case of *Merc cor* free water molecules are more strongly bound to each other by hydrogen bonds when EtOH content is low. This may be due to the fact that hydrogen bond strength is higher between water molecules when EtOH content is lower. In case of ethanol the situation is reverse. Free water molecules are loosely bound to each other when ethanol is totally absent, as in the case of water control (Table 1). Both ethanol and *Merc cor* are used as drugs in homeopathy. In the present experiment we used a potency of *Merc cor* and ordinary ethanol without dynamization. So dynamization might have added to the strength of hydrogen bonds in *Merc cor* 200 cH due to mechanical agitation during the preparation of the potency (Sarkar et al. 2016). Hydrogen bond is actually an electrostatic attraction between 2 close water molecules. Oxygen with partial negative change in 1 H_2O is attracted by hydrogen with partial positive charge of another H_2O by electrostatic force. Oxygen shares its lone pair of electrons with electron deficient hydrogen forming hydrogen bond. It is a weak bond because electron sharing is not as strong as in a covalent bond of a water molecule H-O-H. The energy of hydrogen bond varies from 1 to 40 Kcal mol^{-1} (Compendium of chemical terminology 1997, Steiner 2002). In case of water-water and alcohol-alcohol the H-bond strength is 5.0 Kcal mol^{-1} (Markovitch and Agmon 2007). During the process of dynamization the hydrogen bond might have gained strength as in potentized *Merc cor* but not in unpotentized ethanol. Again the starting substance like $HgCl_2$ plays an important role in both free water content and hydrogen bond strength in a homeopathic potency.

Conclusion

Free water molecules vary widely in 3 different concentration of *Merc cor* and ethanol tested. There is no linearity in relationship between water content and free water. Water control has the lowest amount of free water. Bound water content varies little in all the test samples in spite of variation in their water content. This means that water of crystallization is not much affected by the amount of water added. Free water molecules are more strongly bound to each other when water content is highest as in 0.5% *Merc cor*. Here the high bond strength between water molecules may be due to mechanical agitation. The nature of the starting material ($HgCl_2$) Might have contributed to the H-bond strength.

Acknowledgement

Financial support for the thermogravimetry analyzer

(STA 449 F3 Jupiter) from the Department of Science and Technology (DST), Govt. of India through FIST–2010 to the Department of Physics is gratefully acknowledged. PM thanks the Sukul Institute of Homeopathic Research for a research fellowship.

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