

Drugs at Ultra High Dilution Induce Changes in Enthalpy Associated with the Loss of Water of Crystallization in Lactose

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Abstract Drugs at ultra high dilution (UHD) are used in homeopathy. Lactose is used as a medium for dispensing UHD drugs. FTIR and Raman spectroscopy have shown that different UHD drugs, though devoid of original drug molecules, have different quantities of free water molecules, and also have variation in hydrogen bond strength. The purpose of the present study is to see whether specific water structures in UHDs could specifically modify the water structure in lactose, particularly the water of crystallization in the crystal structure of lactose. Three UHDs, also called potencies like 30cH, 200cH and 1000cH, of two drugs *Natrum mur* and *Sulfur* were mixed with lactose samples separately in the propor-

tion of 100 ul/g lactose. Differential scanning calorimetry (DSC) of the samples was measured. The thermograms of potencies, mixed with lactose, differ from each other with respect to the temperature and enthalpy associated with the removal of water of crystallization from lactose. It is thought that the UHDs tested have modified the water structure in lactose thereby changing the enthalpy for the removal of water of crystallization.

Keywords Homeopathic drugs, DSC, Lactose, Crystallization, Hydrogen bond.

Introduction

Drugs at ultra high dilution (UHD) are used in homeopathy, a therapeutic system introduced by the German physician Dr Samuel Hahnemann about a couple of centuries ago. Lactose has long been used as a medium for dispensing UHD drugs. UHDs are prepared in aqueous ethanol by successive dilution 1:100 of a crude drug with water ethanol followed by mechanical agitation or succussion in several steps (The American Homeopathic Pharmacopoeia, 1920). As the UHD reaches 12th rank or potency, it crosses the Avogadro number and becomes devoid of the starting material or original drug molecules. It is thought that water structures in the water ethanol medium

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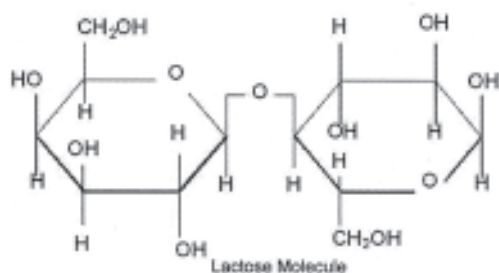


Fig. 1. Molecular structure of lactose.

carries the information of original drug [1]. Analysis of UHDs by FTIR and Raman spectroscopy shows that the specific water structures in UHDs mainly relate to hydrogen bond strength and free water molecules [2-4]. The question is whether the specific water structures could bring about any specific change in the molecular organization of lactose.

Lactose $C_{12}H_{22}O_{11}$ or sugar of milk is a disaccharide formed of D-galactose and D-glucose. It exists in α and β -forms. Crystalline lactose has three modifications α -form, β -form and α -form monohydrate having melting points $223^{\circ}C$, $252^{\circ}C$ and $202^{\circ}C$ respectively. Lactose is soluble in diluted ethanol. The molecular structure is given in Figure 1. We have analysed lactose mixed with UHDs and control medium by differential scanning calorimetry (DSC) with a view to understanding the change in enthalpy during phase transition of lactose involving loss of water of crystallization. Enthalpy H is the sum of the internal energy U of a body, here lactose sample, and the product of the pressure P and the volume V . $H = U + pV$. A change in enthalpy at constant pressure has the form

$$\Delta H = \Delta U + p \Delta V$$

Earlier Aragnostatos [5] studied aqueous solutions of chamomile extract and also 3 UHDs (6cH, 12cH and 14cH) of the same in methanol water solution by DSC. The potencies showed small peaks in the temperature range of $40-60^{\circ}C$. The author interpreted the peaks in terms of breakage of clathrates by thermal energy.

Materials and Methods

Lactose, commonly used by homeopathic pharmacists, was obtained from the market in Kolkata. Homeopathic medicines used for the experiment were *Sulfur* and *Natrum mur*. Each medicine has three potencies 30cH, 200cH and 10000cH, all of which were products of Dr Reckeweg, Germany. Potencies 30cH, 200cH and 1000cH indicate ranks of dilution. Thus the 30th potency (30cH) means that the original drug or the starting material has been diluted with aqueous ethanol and succeeded in 30 steps following the rules of Homeopathic Pharmacopoeia. All of them were purchased in sealed vials from Kolkata. The medicines were in 90% ethanol as mentioned in their labels. *Sulphur* and *Natrum mur* were prepared from *Sulphur* and Sodium chloride, respectively. All the UHDs, which are in 90% ethanol, were reduced to 25% EtOH by adding deionized double distilled water in appropriate volume to them. The control was prepared from absolute ethanol (E-Mark, Germany). The optical density of all samples were measured by a UV-VIS spectrophotometer at 200nm and found to be same, thereby confirming their ethanol content.

Each diluted (25% EtOH) potency (30,200 or 1000cH) as mixed thoroughly with an aliquote of lactose in the proportion of $100\mu g$ lactose. Thus there were 7 test samples. The control (25% EtOH) was similarly mixed with lactose. Each sample, in the form of a semi-solid paste, was put into an aluminium sample pan of 5mm diam. The pan was sealed by a sealing machine. There was one more sample of lactose without any drug or control. The eight of the samples in each pan varied from 12-14 mg. Differential scanning calorimetry (DSC) of the samples was measured, one at a time, by an instrument NETZSCH DSC 200 F3 MAIA, Germany at a scanning rate of $10^{\circ}C/min$ in the temperature range $70-200^{\circ}C$. A vacant pan was measured first as a reference. A sample of lactose was also analyzed by DSC in the temperature range from 70 to $260^{\circ}C$ with a view to confirming the melting points. All measurements were replicated thrice.

Results and Discussion

DSC thermograms of potencies of *Natrum mur* together with ethanol control and lactose alone are

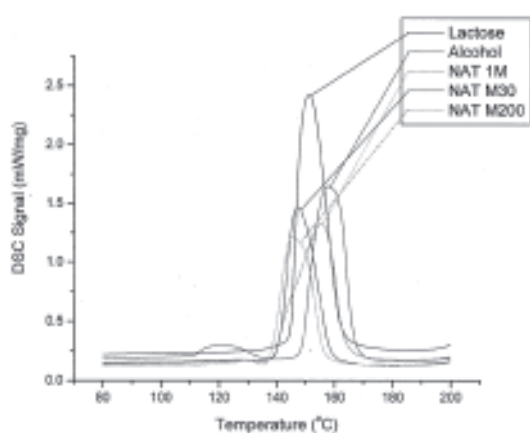


Fig. 2. DSC thermogram of lactose mixed with ethanol, *Natrum mur* 30, 200, 1000cH (all in 25% ethanol) at 100 μ l/g lactose and lactose alone.

shown in Fig. 2. The thermograms of *Sulfur* potencies are shown in Fig. 3. The thermograms show two peaks, one small around 94.2-129.9°C and another large around 136.5-157.6°C (Figs. 2,3). Enthalpies for the two peaks were calculated and presented in Table 1. The table shows different enthalpies for different drugs like *Natrum mur* and *Sulfur* with their potencies. Lactose tested in the temperature range 70-260°C showed three more at 222°C, 233°C and 238°C (Fig. 4). DSC of lactose in the temperature range 70-260°C confirms that the lactose used in our study contains three forms but abundant in α - form (Fig. 4)

The small peak indicates loss of surface water. The surface water forms a few layers of free water molecules over lactose molecules [6]. The second larger peak represents loss of water of crystallization of lactose crystals. Water of crystallization of lactose occurs within the crystal lattice. This water of crystallization strongly links together oxygen atoms from four different lactose molecules [6]. Further, hydrogen bonding regulates the crystallization, packing and morphology of polyurethanes [7]. In order to remove lactose molecule from the crystal structure a number of bonds must be broken. Different levels of thermal energy are needed to remove both free water molecules and water of crystallization from lactose. It has been demonstrated by FTIR and Raman spectroscopy that *Sulfur* and *Natrum mur* potencies have H-bond

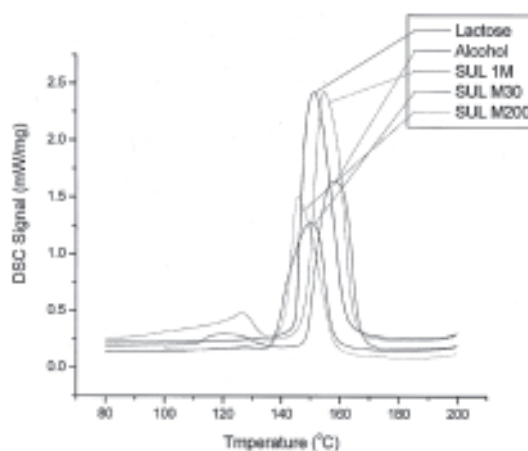


Fig. 3. DSC thermogram of lactose mixed with ethanol, *Sulfur* 30, 200, 1000cH (all in 25% ethanol) at 100 μ l/g lactose and lactose alone.

strength related to their potency ranks. The lower is the potency rank the stronger is the H-bond. Further, the higher is the potency the more abundant is the number of free water molecules [2, 4]. It appears that hydrogen bond strength in different UHDS Contrib-

Table 1. Temperature and enthalpy associated with the loss of surface water and water of crystallization in lactose soaked with different potencies of drugs in the proportion of 100 μ l/g lactose as revealed by differential scanning calorimetry. Drugs were in 25% ethanol. Control=only 25% ethanol. *Mean of 3 replicates \pm SE.

Lactose + drugs	Temperature °C*		Enthalpy ΔH_m (J/G)*	
	Peak 1	Peak 2	Peak 1	Peak 2
<i>Natrum mur</i> 30cH	-	147.1 ± 0.00	-	85.34 ± 0.002
<i>Natrum mur</i> 200cH	129.9 ± 0.002	145.6 ± 0.003	0.8238 ± 0.062	76.31 ± 0.004
<i>Natrum mur</i> 1000cH	127.3 ± 0.002	155.3 ± 0.005	0.3458 ± 0.056	101.9 ± 0.012
<i>Sulfur</i> 30cH	127.8 ± 0.001	150.0 ± 0.001	0.461 ± 0.090	86.97 ± 0.012
<i>Sulfur</i> 200cH	-	145.6 ± 0.001	-	82.38 ± 0.030
<i>Sulfur</i> 1000cH	126.2 ± 0.003	154.4 ± 0.013	6.399 ± 0.003	140.2 ± 0.012
Ethanol 25%	120.6 ± 0.004	157.6 ± 0.002	9.27 ± 0.02	109.2 ± 0.002
No drug or control	-	151.1 ± 0.001	-	137.1 ± 0.001

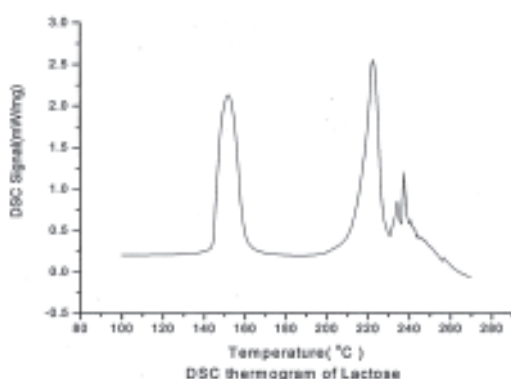


Fig. 4. DSC thermogram of lactose alone in the temperature range upto 260°C showing water of crystallization (1st peak), and melting points at higher temperatures.

uted to the difference in energy for removal of free water molecules over lactose molecules and also of water of crystallization (WC) from lactose crystals.

Again since lactose dissolves in polar solvents like aqueous ethanol, a number of solvent molecules, here water and ethanol, form weak bonds with each lactose ion. The net energy gained would also contribute to the removal of lactose molecule from the crystal. Hydrogen bond strength and free water molecules for different UHDs can also modify the net gain in energy differently. It is evident from Table 1 that the temperature needed to remove WC from lactose in order from high to low is : aqueous ethanol > *Nat mur* 1000cH > lactose alone > *Nutrum mur* 30cH > *Natrum mur* 200cH for *Nat mur*, and aqueous ethanol > Sul 1000cH > lactose alone > Sul 30cH > Sul 200cH. The sequence is same for two different drugs. As regards the change in enthalpy from high to low the sequence for *Nat mur* is lactose alone > aqueous ethanol > *Nat mur* 1000cH > *Nut mur* 30cH > *Nat mur* 200cH, and for *Sulfur* sul 1000cH > lactose alone > aqueous ethanol > Sul 30cH > Sul 200cH. Difference in H-bond strength and the number of free water molecules in different UHDs have contributed to the sequences in UHDs of two drugs with respect to temperature and change in enthalpy. This means that UHDs tested have modified the number of hydrogen

bonds and their strength in the crystal structure of lactose.

Conclusion

1. DSC of lactose mixed with different UHDs of two homeopathic drugs like *Natrum mur* and *Sulfur* has shown that different thermal energy is needed to remove water of crystallization from lactose.
2. The UHDs also have contributed to the change in enthalpy associated with the removal of water of crystallization from lactose.
3. UHDs tested might have modified the number of hydrogen bonds and their strength in the crystal structure of lactose.
4. Specific water structures in liquid aqueous ethanol medium are transferable to solid medium of lactose.

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