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DSC reveals variation in enthalpy associated with free water molecules in water ethanol solution exposed to x-rays and magnetic field

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Abstract

Aqueous ethanol is the standard medium of all drugs used in homeopathy. *X-ray* and *Magnetis Poli Ambo* are two homeopathic drugs prepared by exposure of aqueous ethanol to *X-radiation* and static magnetic field, respectively to produce their mother tinctures (MTs). These MTs were successively diluted with the solvent 1:100 and succussed in several steps to prepare their centesimal potencies like 8 cH, 14 cH and 32 cH. The solvent is also processed in the same way to produce its 3 potencies. All of them, though identical in chemical composition (0.03 molar ethanol) and water content (96%), produce different therapeutic effects. One of the potencies 8cH of each was diluted with water so that it had 4, 20, 40 and 80% ethanol. The objective is to see whether these potencies show variation in free water molecules. DSC of the MTs and the potencies of each drug have revealed almost similar freezing and melting points. But they differ markedly from each other with respect to their freezing and melting enthalpies as well as free water molecules. Different dilutions of the 8^{th} potency show variation in enthalpies and free water molecules, and this variation in independent of the amount of water added. It is concluded that exposure of aqueous ethanol to x-ray and magnetic field and its subsequent dilution and succussion induce changes in the solvent involving free water molecules.

Keywords: Aqueous ethanol, x-ray, magnetic field, enthalpy, free water molecules.

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INTRODUCTION

Drugs at ultra high dilution (UHD), used in homeopathy, are chemically identical being composed of ethanol and water. But each drug is unique with respect to its symptoms as mentioned in the materia medica [1,2]. Using vibrational and Raman spectroscopy we demonstrated that these UHDs differ from each other with respect to free water molecules and hydrogen bond strength [3,4,5]. DSC has long been used as a reliable technique to determine the state of water, particularly free and bound, in different substances [6]. Free water crystallizes at 0^0C and freezable bound water does so at a temperature lower than 0^0C. But non-freezable bound water does not crystallize even at -100^0C [7]. We have applied this technique to the UHDs of two homeopathic drugs only to further confirm that free water molecules contribute to the difference in UHDs. DSC measures changes in physical properties of a sample in relation to temperature and time. It is the only method for direct determination of enthalpy associated with the phase transition in a substance [8]. Enthalpy H is the sum of the internal energy U of a body and the product of the pressure p and the volume V. A change in enthalpy at constant pressure has the form $\Delta H = \Delta V + p\Delta V$.

Two drugs, used in the present study were *X-ray* and *Magnetis Poli Ambo*. The mother tincture (MT) and three UHDs, also called potencies, 8cH, 14cH and 32cH of each drug and their control ethanol water were prepared in the laboratory following the standard procedure of successive dilution 1:100 followed by succussion using 10 vertical strokes [9,4]. Both *X-ray* and *Magnetis* MTs were prepared by exposing ethanol water to X-radiation and high intensity magnetic field, respectively [4]. For this, the MT and

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UHDs of the two drugs and their control were chemically identical being composed of ethanol and water. Each MT and its potencies contained 0.03 molar fraction of ethanol. Homeopathic medicines in aqueous ethanol are usually mixed with lactose or soaked in sucrose globules for application to patients. We have, therefore, mixed the test potencies and those of the control with lactose and obtained their DSC curves. Heys *et. al*. [10] estimated free water level in normal and cataractous human lenses by DSC and reported that bound water molecules changed to free water with age, and there was in advanced stage of cataract lower hydration of lens nucleus. The study reveals that free water plays a role in pathological condition.

Besides lactose and sucrose, homeopathic potencies are mixed with water and given to patients. The proportion of water mixed with a potency is arbitrary. How is the identity of the extremely diluted medicine is maintained in different proportions of water? In order to address this question we selected the 8^{th} potency of *X-ray*, *Magnetis* and the control, mixed them with different proportions of water and obtained their DSC curves. In a polymer mixture freezable water increases with the increase in water content [7]. The purpose here is to see whether this relationship exists in a potency mixed with different proportions of water.

MATERIALS AND METHODS

Medicines

Three centisimal UHDs 8cH, 14cH and 32cH of two drugs *X-ray* and *Magnetis Poli Ambo* and their MT, all in water ethanol, were prepared in the laboratory following

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the rules of homeopathic pharmacopoeia [9,4,5]. The control was aqueous ethanol, prepared from absolute ethanol (E-Merck, Germany). The test potencies and the control, all in 90% ethanol, were mixed with appropriate volume of double distilled water to reduce the ethanol content in each sample to 0.03M. Extrapure lactose, commonly used by homeopathic pharmacists, was obtained from SRL,Mumbai. Lactose $C_{12}H_{22}O_{11}$ is a disaccharide, formed of D-galactose and D-glucose. It occurs in α – and β – forms. Lactose used in our study contained both forms as evidenced by its melting point [11]. Each potency of a drug or the control was mixed with lactose in the proportion of 100µl/g lactose just before DSC measurement. One sample was mixed with double distilled water in the same proportion. The weight of each sample was 13.2 ± 1 mg.

DSC

DSC measurements were carried out by using 200F3 Maia model of the instrument with intra cooler 70 version (NETZSCH), Germany. The experiment was performed in dry nitrogen atmosphere with constant pressure of 0.3 bar in order to prevent any oxidation of the samples. Each sample was put into an aluminium sample pan of 5mm diametre and sealed by a sealing machine. Besides the lactose samples mixed with a potency, there was one sample of pure lactose alone without any drug or control potency. A vacant pan was measured first as a reference. DSC of the pure lactose sample was measured at a scanning rate of 10K min^{-1} in the temperature range of 70^0-200^0C to record the melting points of different forms of lactose present in the sample.

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Determination of water present in lactose samples mixed with potencies having a fixed amount of water (96%)

DSC of these samples was measured from a starting temperature of 28^0C down to -35^0C at a scanning rate of 5K min^{-1} and kept at -35^0C in an isothermal condition for 5 min. After that the temperature was raised at 3K min^{-1} upto 50^0C [10]. Thus freezing and melting points of samples were recorded, and exothermic (freezing) and endothermic enthalpies were calculated. The mass of freezable water was calculated by the formula $Wc = Q / \Delta H(g)$ where ΔH is the melting enthalpy of this type of water which is presumed to be same as bulk water ($\Delta = 333.5$ J g^{-1}), and Q is the heat absorbed during the melting process. Q is calculated according to the endothermic peak [7].

Determination of free water present in lactose samples mixed with variable amount of water in the 8^{th} potency

The 8^{th} potency (8cH) of *X-ray*, *Magnetis* and ethanol control was mixed with different proportions of double distilled water so that the ethanol content in the potency became 4%, 20%, 40% and 80%. DSC of these samples was measured in the same way as mentioned above.

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RESULTS

A DSC curve of pure lactose is presented in Figure 1 which shows two large peaks at 151.1^{0}C and 222^{0}C. The first peak belongs to water of crystallization and the second one to the melting point of α-form of lactose. The other two small peaks at 233^{0}C and 238^{0}C belong to β-form of lactose [12,13]. DSC curves of MT, potencies and water show very little difference in freezing and melting points for *X-ray* (Figs. 2,3), for *Magnetis* (Figs. 4,5) and for ethanol control (Figs. 6,7). DSC curves of different proportions of ethanol content in the 8th potency show marked difference from each other in freezing and melting enthalpies in *X-ray* (Figs. 8,9) and *Magnetis* (Figs. 10,11). Exothermic and endothermic enthalpies for the MT and potencies show marked difference from each other in case of *X-ray*, *Magnetis* and control (Table 1) in spite of the fact that their water content is same (96%). Free water molecules show marked variation in different potencies of *X-ray*, *Magnetis* and ethanol control (Table 1). Free water molecules also vary in the 8th potency mixed with different proportions of water, and this variation is not related to the amount of water added (Table 2).

DISCUSSION

Difference in freezing and melting enthalpies indicate that different quantities of energy were released and absorbed during crystallization and melting of water, respectively. The amount of water present in the samples was same. So, the difference in

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enthalpies can be attributed to the variation in free water molecules present in them (Table 1).

Present study provides further evidence in support of variation of free water molecules as obtained in our earlier study on the same three potencies of *X-ray, Magnetis* and aqueous ethanol control [4]. Three UHDs, namely 8cH, 14cH and 32cH of chamomile extract in methanol water were examined by DSC earlier. DSC curves showed small peaks in the temperature range of 40-60^{0}C. The author reported that the peaks resulted from breakage of clathrates of the diluent medium by thermal energy [13]. Elia and Niccoli [14] examined extremely diluted aqueous solutions ranging from 4cH to 12cH of acids and bases by calorimetry, electrical conductivity and pH measurement. The solutions were prepared by successive dilution and succussion. The authors reported exothermic excess heats of mixing, higher electrical conductivity and pH of extremely diluted solutions as compared to the control. They concluded that the process of dynamization could permanently alter the physico-chemical properties of the water solvent. Here also calorimetry revealed variation in water structure in UHDs of the substances. In an earlier study we observed marked change in enthalpies in UHDs of two drugs *Natrum mur* and *Sulphur* 30cH, 200cH and 1000 cH, mixed with lactose. It was suggested that UHDs modified the water structure in lactose crystals thereby changing the enthalpies associated with the loss of water of crystallization [11].

Usually, freezable water increases linearly with the increase in water content in a hydrophilic polymer [7]. In our first experiment the water content is same in all the samples (Table 1). So, the change in the freezing and melting enthalpies is due to the effect of different potencies on lactose samples. The potencies have different levels of

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free water molecules (Table 1). These free water molecules contribute to a change in melting enthalpies. As for example, *X-ray* 8 and lactose + water show some similarity in their melting enthalpies and also their level of free water molecules (Table 1). The situation is same with *Magnetis* 8 and *Magnetis* MT (Table 1).

MTs of *Magnetis* and *X-ray* have been produced by exposing aqueous ethanol to strong magnetic field and X-radiation, respectively [4]. Both X-rays and magnetic field can bring about marked changes in hydrogen bond network in water structure [15-18]. Our study simply shows that this change in water structure persists with modification during successive dilution with aqueous ethanol and succussion. In this study aqueous ethanol control also shows variation in free water molecules. This is quite possible because a potency of ethanol is itself a drug with specific symptoms [19].

Dixit *et. al*. [20] analysed alcohol-water mixture (7:3 molar ratio) by neutron diffraction and found that most of the water molecules exist as small hydrogen bonded strings and clusters bridging neighbouring alcohol OH groups through H-bonding. They reported 13% free water molecules in alcohol-water mixture having 7:1 molar ratio. The aberrant thermodynamic properties of alcohol-water mixtures may be due to the differences in energy of hydrogen bonding between water-water, water-alcohol and alcohol-alcohol molecules [20].

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CONCLUSION

1) *X-ray*, *Magnetis* and *Ethanol* and their 3 potencies 8cH, 14cH and 32cH have the same 0.03 molar fraction of ethanol. Yet they show marked variation from each other in their freezing and melting enthalpies.
2) They also differ from each other with respect to the amount of free water molecules.
3) Free water molecules in dilutions of a potency 8cH with water do not show any correlation with the water content.
4) The variation in free water molecules can be attributed to the process of successive dilution of aqueous ethanol followed by succussion or mechanical agitation.

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Table 1

Freezing points, melting points, enthalpies and free water molecules of different potencies of *X-ray, Magnetis* and *Ethanol*. MT=mother tincture. The potencies and MTs were mixed with lactose at the rate of 100 µl/g lactose. All the potencies and MTs were in aqueous ethanol having 0.03 molar fraction of ethanol.

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Sample	Cooling		Heating		Free Water molecule/%
	Freezing Point/^{0}C	Enthalpy of Freezing/^{0}C	Melting Point/^{0}C	Enthalpy of Melting/ Joule mole^{-1}	
X-Ray 8	-16.6	13.03	-0.4	7.564	12.57
X-Ray 14	-17.7	20.02	-2.7	12.75	2.22
X-Ray 32	-14.5	46.94	-0.2	37.78	11.11
X-Ray MT	-16.8	49.82	-1.4	42.75	3.75
Mag 8	-16.3	21.21	-0.7	13.59	8.7
Mag 14	-15.9	35.82	-0.2	27.99	10.73
Mag 32	-18.7	38.84	-0.5	29.85	3.99
Mag MT	-17.1	49.38	-1.3	36.49	8.23
Lactose+Water	-17.9	50.48	0.2	45.58	13.4
Ethanol 8	-16.8	12.54	-0.3	12.54	3.67
Ethanol 14	-17.7	43.85	-0.4	44.72	12.89
Ethanol 32	-17.7	40.42	-0.4	41.07	11.88

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Table 2

Freezing points, melting points, enthalpies and free water molecules of the 8th potency of *X-ray*, *Magnetis* and *Ethanol*. MT = Mother Tincture. The potency and MT were in aqueous ethanol with 0.03 molar ethanol. The potency had different proportions of distilled water added to it. All were mixed with lactose in the proportion of 100 µl/g lactose.

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Sample	Cooling		Heating		Free Water molecule/%
	Freezing Point//^{0}C	Enthalpy of Freezing//^{0}C	Melting Point/^{0}C	Enthalpy of Melting/Joule mole^{-1}	
XRAY 8 (4%)	-16.6	46.94	-0.4	42.75	12.57
X-Ray 8 (20%)	-18.0	36.58	-1.1	27.21	8
X-Ray 8 (40%)	-18.3	35.78	-0.2	25.59	7.52
X-Ray 8 (80%)	-19.8	27.55	-0.1	19.95	5.86
Mag 8 (4%)	-16.3	35.82	-0.7	29.85	8.77
Mag 8 (20%)	-20.1	19.03	-1.3	12.42	3.65
Mag 8 (40%)	-22.4	6.851	-5.0	2.278	0.67
Mag 8 (80%)	-18.5	21.64	-1.0	15.08	4.43
Ethanol 8 (4%)	-16.8	12.54	-0.3	12.54	3.67
Ethanol 8 (20%)	-18.3	45.99	-0.4	45.62	13.52
Ethanol 8 (40%)	-16.7	50.64	0.1	47.5	14.89
Ethanol 8	-16.0	8.75	-0.7	8.65	2.57

(80%)					

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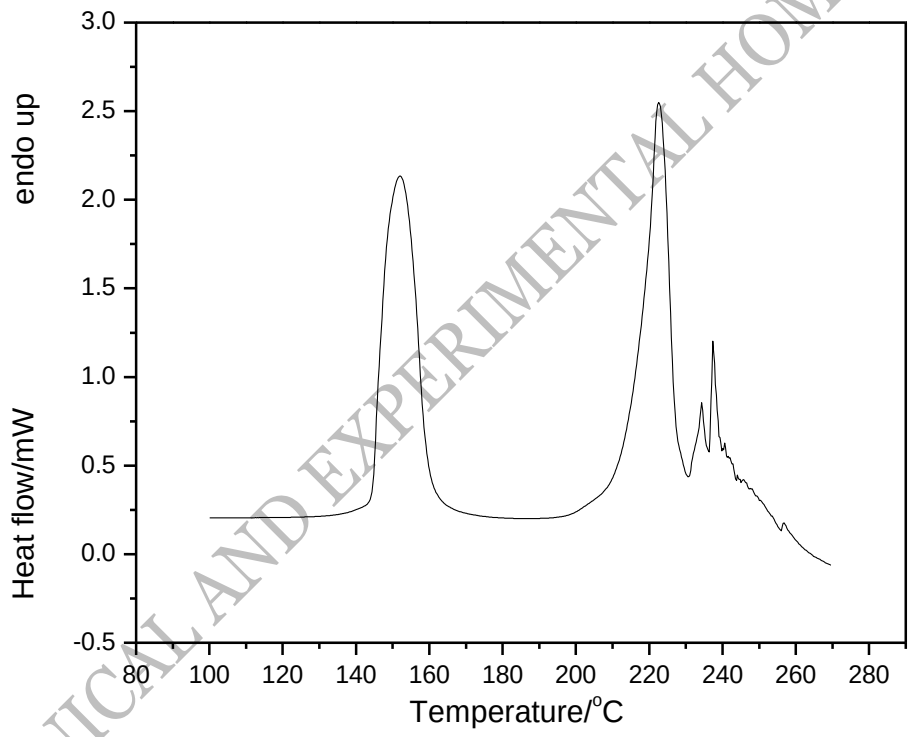

Fig.1. DSC curve of lactose showing the 1st exothermic peak of water of crystallization at 151^{0}C and the 2nd peak of melting point at 222 ^{0}C and 2 small peaks of melting point 233^{0}C and 238^{0}C.

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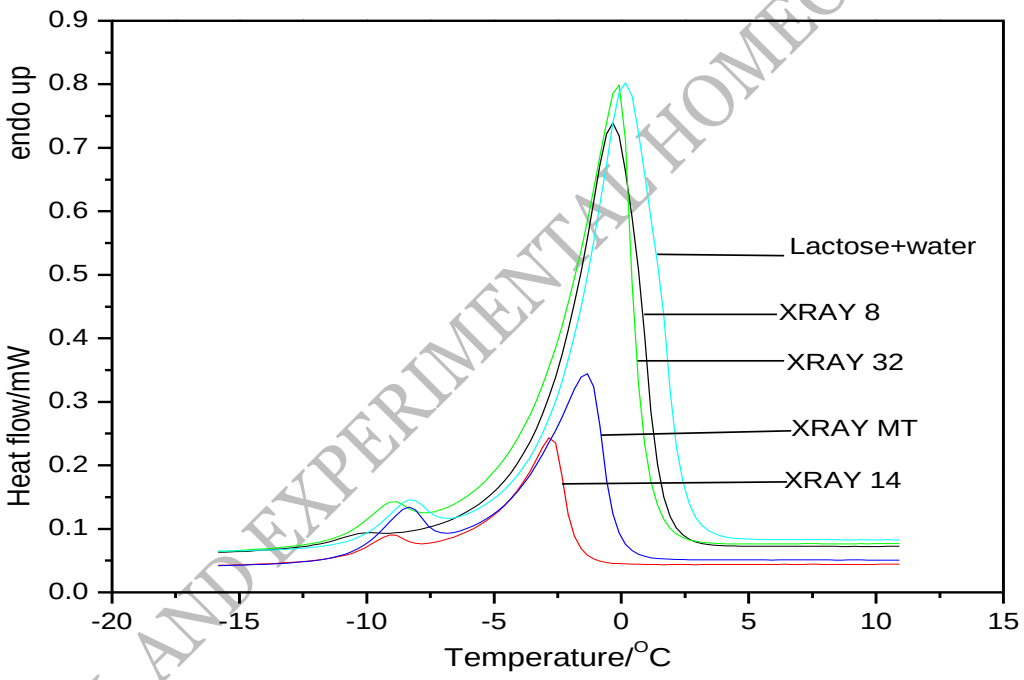

Fig.2. DSC curve showing melting points of mother tincture/MT and 3 potencies 8cH, 14cH and 32cH of a drug *X-ray* and water mixed with lactose at 100µlg^{-1} lactose. The MT and potencies were in aqueous ethanol having 0.03 molar ethanol. The samples were cooled from 28^{0}C down to –35^{0}C at 5K min^{-1}.

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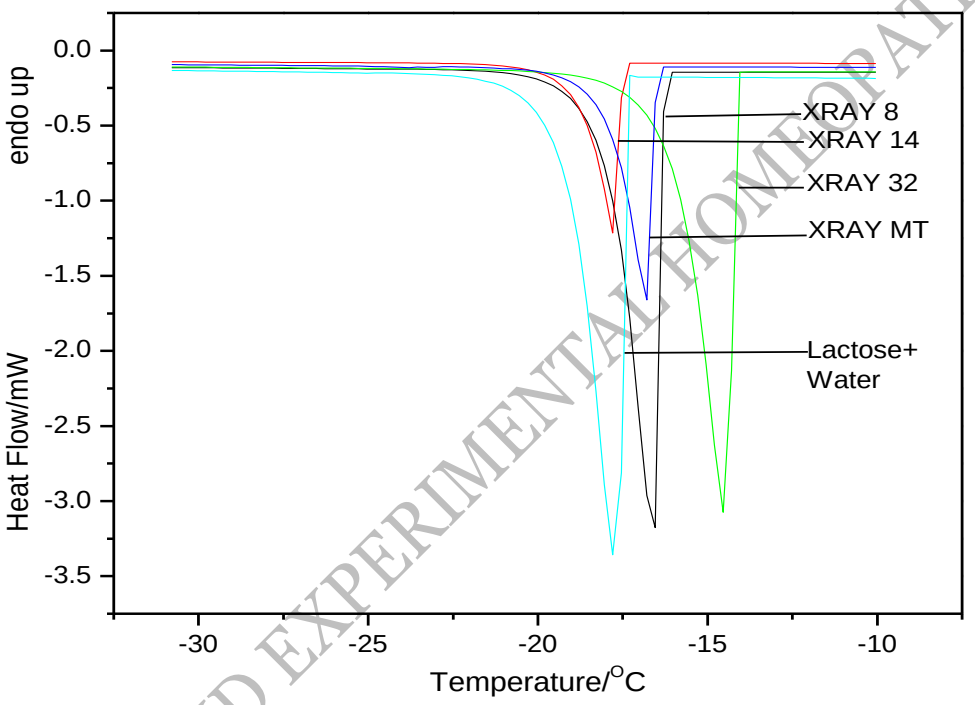

Fig.3. DSC curve showing freezing points of mother tincture/MT and 3 potencies 8cH, 14cH and 32cH of *X-ray* and water mixed with lactose at 100µlg^{-1} lactose. The MT and potencies were in aqueous ethanol having 0.03 molar ethanol. The samples were cooled from 28^{0}C down to –35^{0}C at the rate 5K min^{-1}, and heated upto 50^{0}C at 3K min^{-1}.

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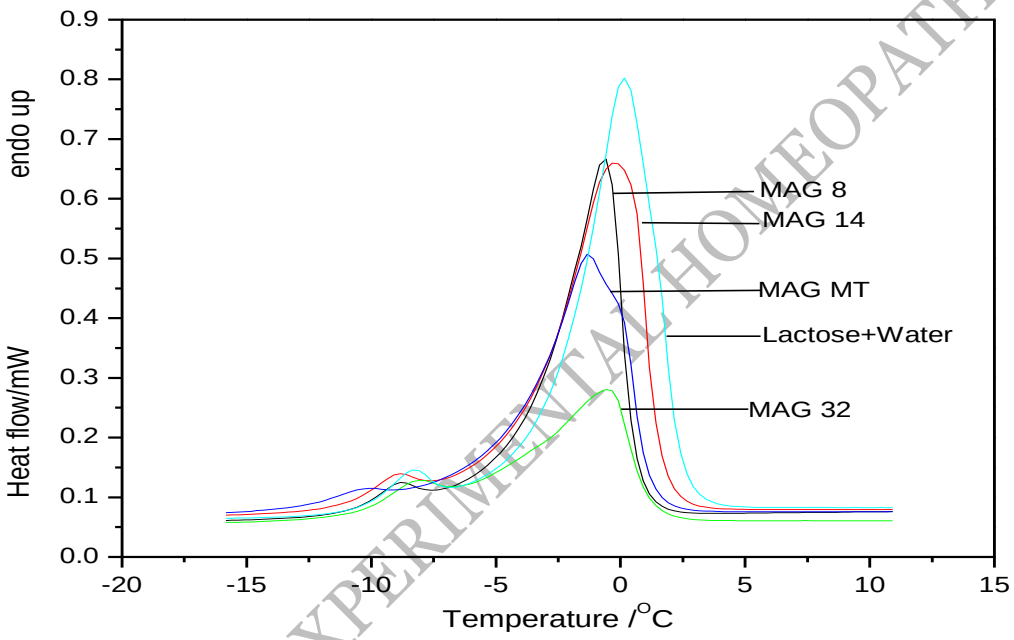

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Fig.4. DSC curve showing melting points of mother tincture/MT and 3 potencies 8cH, 14cH and 32cH of the drug *Magnetis* and water mixed with lactose at 100µlg^{-1} lactose. The MT and potencies were in aqueous ethanol having 0.03 molar ethanol. The samples were cooled from 28^{0}C down to –35^{0}C at the rate 5K min^{-1}, and heated upto 50^{0}C at 3K min^{-1}.

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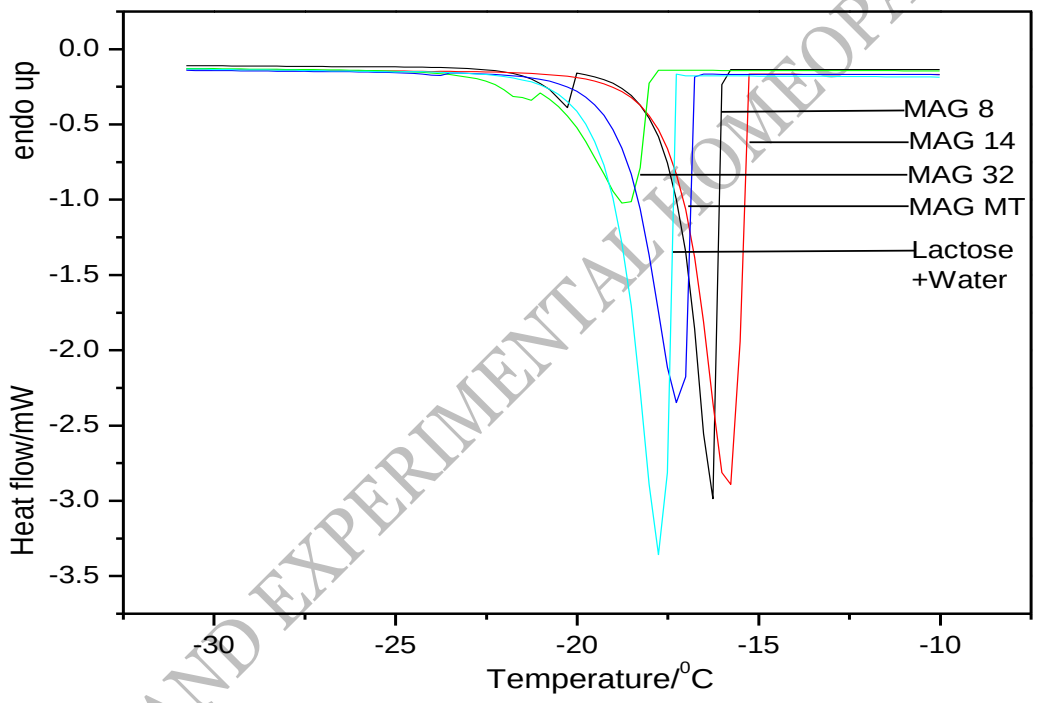

Fig.5. DSC curve showing freezing points of mother tincture/MT and 3 potencies 8cH, 14cH and 32cH of *Magnetis* and water mixed with lactose at 100µl/g lactose. The MT and potencies were in aqueous ethanol having 0.03 molar ethanol. The samples were cooled from 28^{0}C down to –35^{0}C at the rate 5K min^{-1}, and heated upto 50^{0}C at 3K min^{-1}.

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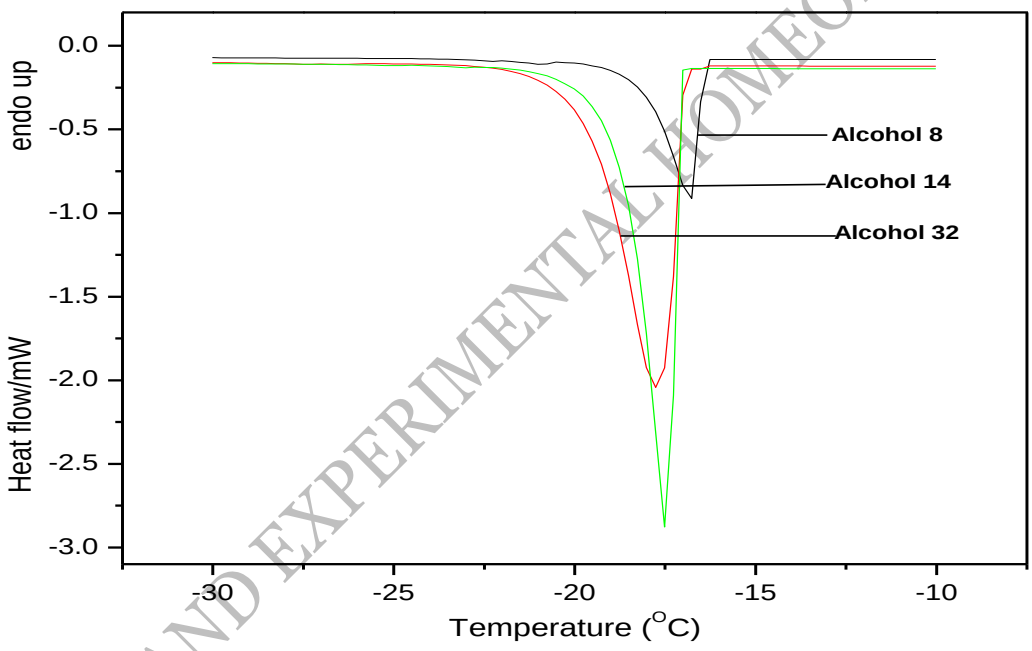

Fig.6. DSC curve showing freezing points of three potencies 8cH, 14cH and 32cH of alcohol (ethanol) and water mixed with lactose at 100µlg^{-1} lactose. The potencies were in aqueous ethanol having 0.03 molar ethanol. The samples were cooled from 28^{0}C down to -35^{0}C at the rate of 5K min^{-1}.

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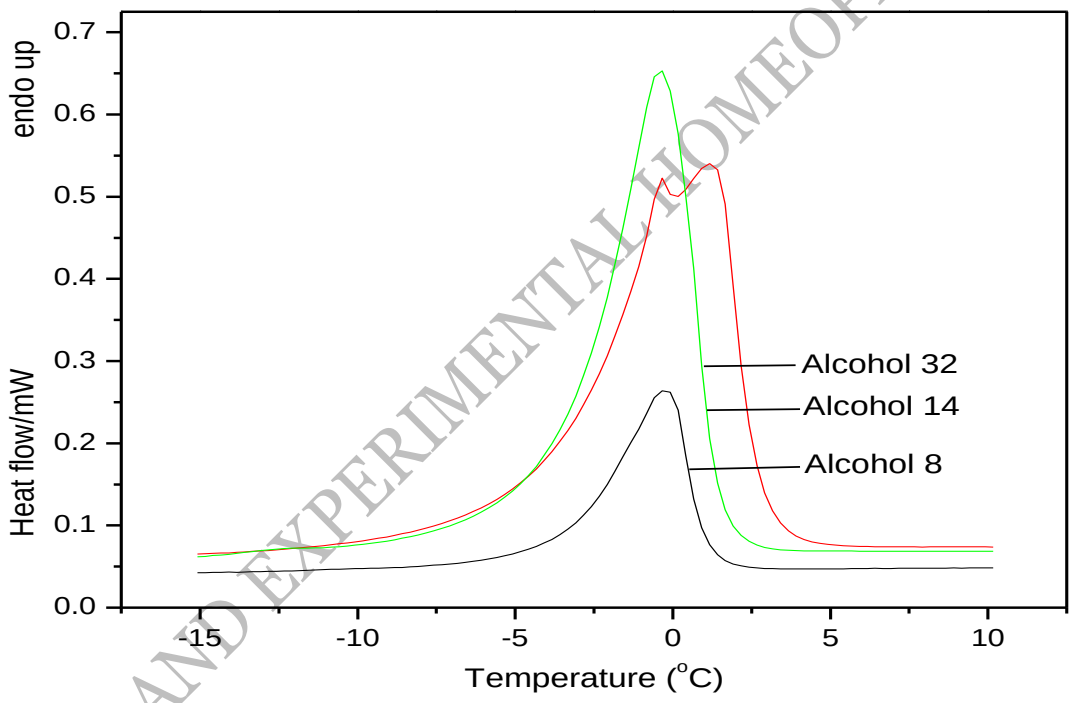

Fig.7. DSC curve showing melting points of three potencies 8cH, 14cH and 32cH of alcohol/ethanol and water mixed with lactose at 100µlg^{-1} lactose. The potencies were in aqueous ethanol having 0.03 molar ethanol. The samples were cooled from 28^{0}C down to -35^{0}C at the rate of 5K min^{-1}.

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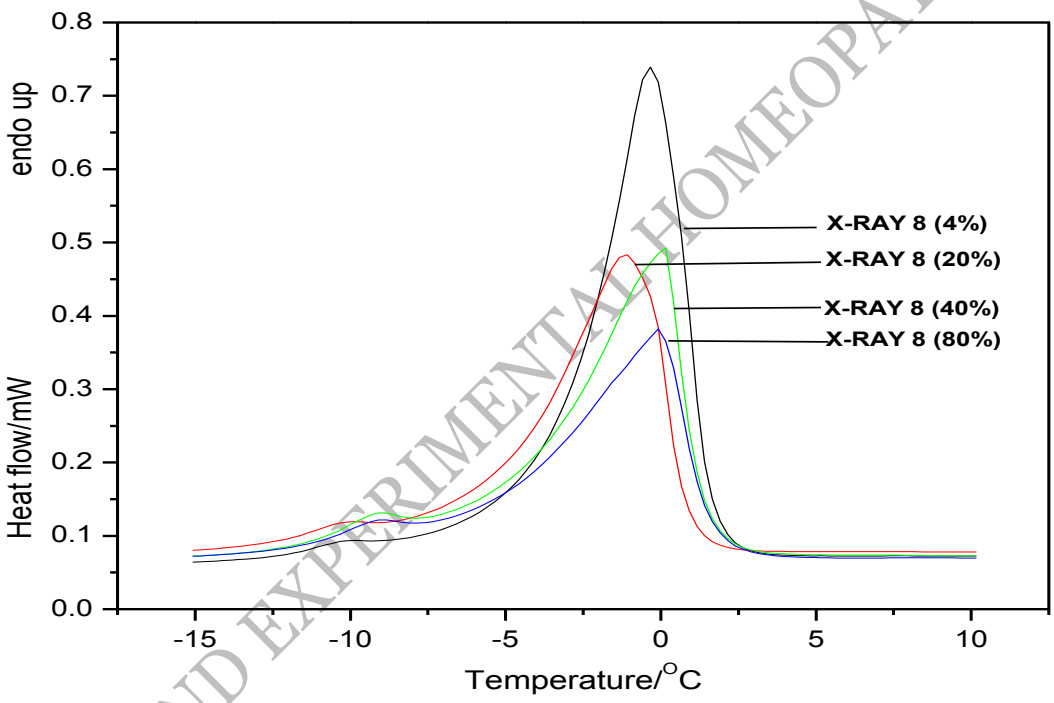

Fig.8. DSC curve showing melting points of the 8th potency of *X-ray*, diluted with different proportions of water so as to make ethanol content 4%, 20%, 40% and 80% . Each dilution was mixed with lactose at 100µlg^{-1} lactose. The potency was in aqueous ethanol with 0.03 molar ethanol. The samples were cooled from 28^{0}C down to –35^{0}C at 5K min^{-1}.

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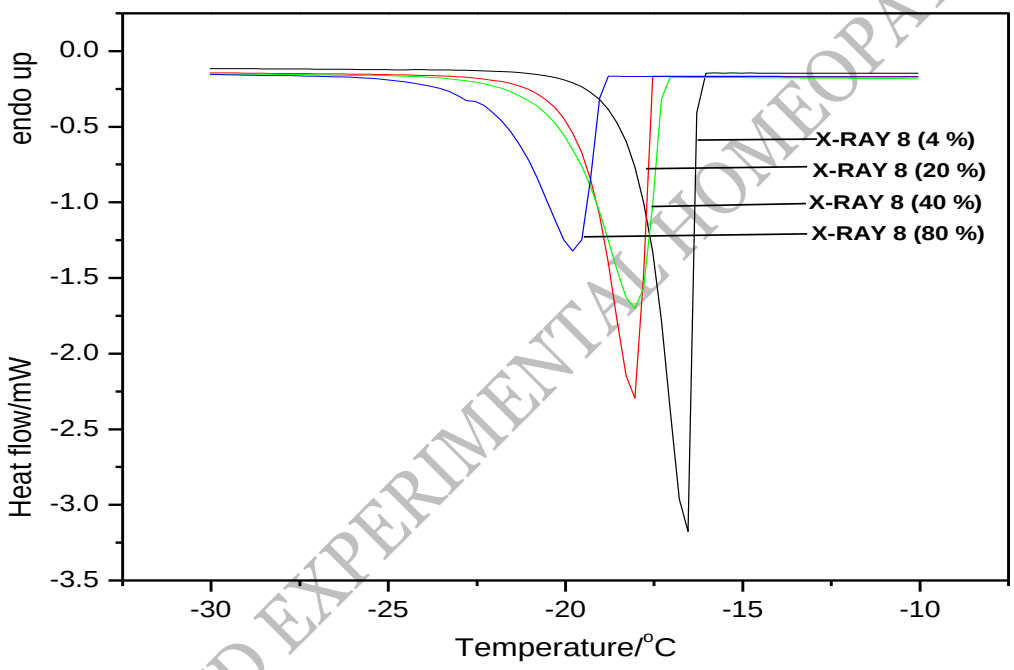

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Fig.9. DSC curve showing freezing points of the 8th potency of *X-ray* diluted with different proportions of water so as to make ethanol content 4%, 20%, 40% and 80% . Each dilution was mixed with lactose at 100µlg^{-1} lactose. The potencies was in aqueous ethanol having 0.03 molar ethanol. The samples were cooled from 28^{0}C down to –35^{0}C at 5K min^{-1}, and then heated to 50^{0}C at 3K min^{-1}.

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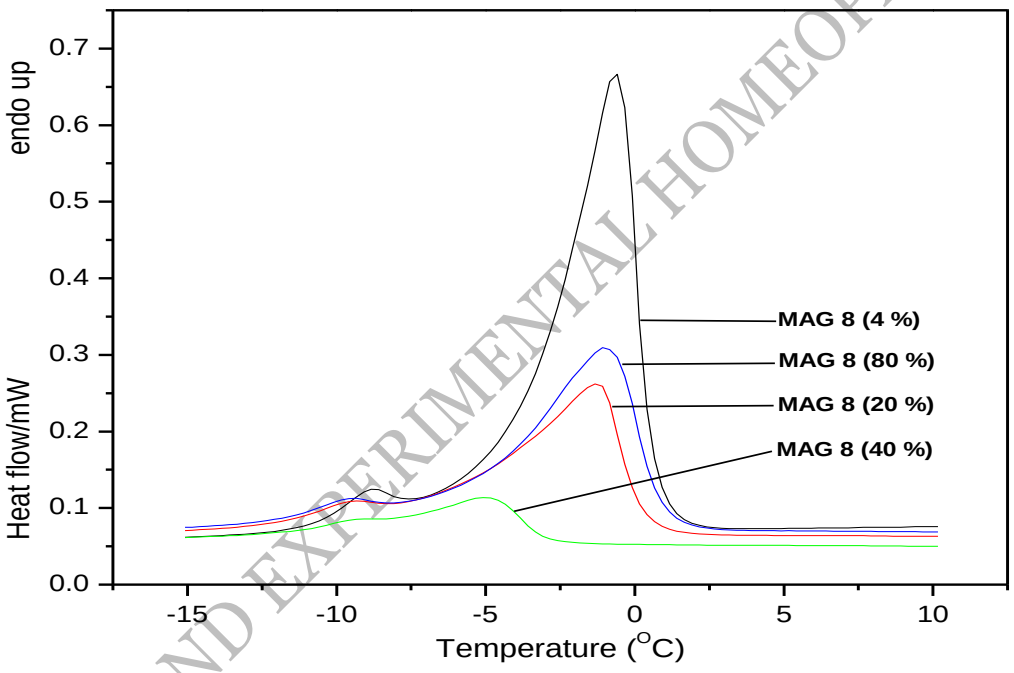

Fig.10. DSC curve showing melting points of the 8th potency of *Magnetis Poli Ambo*, diluted with different proportions of water so as to make ethanol content 4%, 20%, 40% and 80% . Each dilution was mixed with lactose at 100µlg^{-1} lactose. The potency was in aqueous ethanol with 0.03 molar ethanol. The samples were cooled from 28^{0}C down to –35^{0}C at 5K min^{-1}.

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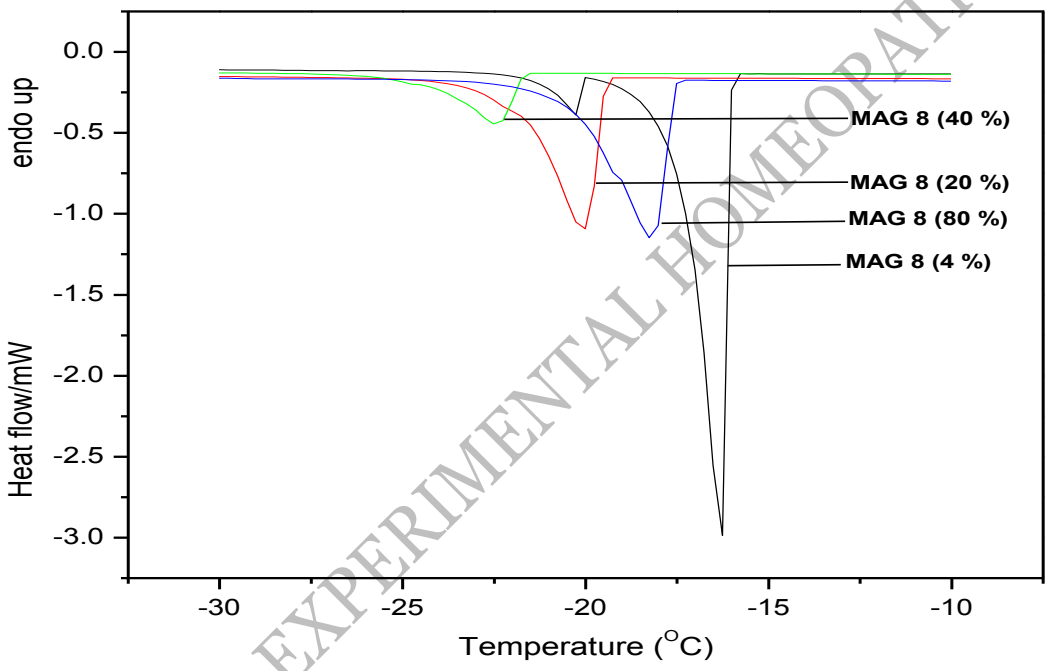

Fig.11. DSC curve showing freezing points of the 8th potency of *Magnetis Poli Ambo* diluted with different proportions of water so as to make ethanol content 4%, 20%, 40% and 80% . Each dilution was mixed with lactose at 100µlg^{-1} lactose. The potencies was in aqueous ethanol having 0.03 molar ethanol. The samples were cooled from 28^{0}C down to –35^{0}C at 5K min^{-1}, and then heated to 50^{0}C at 3K min^{-1}.

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