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Research paper

Stability of a hydrophobic drug in presence of hydrous and anhydrous lactose

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Abstract

The chemical stability of a hydrophobic Leukotriene receptor antagonist drug was investigated in the presence of lactose (both hydrous and anhydrous) under various humidity and temperature conditions. The effect of wet-granulation and direct-mixing on the stability of the drug was also studied. Mixtures of drug:lactose in the ratio 1:25, 1:50 and 1:100 were prepared and analyzed over a 6 week period after storage at 40, 83 and 97% RH (all at 25°C) and 75% RH at 40°C. The mixtures were subjected to LOD, Karl–Fischer titrimetry, HPLC and DSC analysis to evaluate the amount of moisture pickup, percent potency and presence of drug–moisture–lactose interaction. Mixtures containing lactose anhydrous picked up more moisture and exhibited greater drug degradation than those containing lactose hydrous. Also, mixtures stored under high temperature and humidity condition showed greater moisture uptake than those stored at high humidity alone. Lactose anhydrous becomes hydrated on exposure to high humidity/temperature and storage conditions. The transition state of lactose and not its stable state may be responsible for its greater interaction and subsequent degradation of the drug. Therefore, the normal belief that lactose anhydrous, which has less than 0.5% moisture, should provide greater stability as compared to lactose hydrous, needs to be properly evaluated.

Keywords: Hydrophobic; Hydrous; Anhydrous; Lactose

1. Introduction

Studies involving drug–excipient interactions are important in preformulation activities. In the presence of certain solid excipients, many drugs undergo significant physicochemical changes like: (i) Reduction in the degree of crystallinity; (ii) increased rate of chemical degradation; and (iii) formation of molecular complexes [1]. The method employed to understand drug–excipient interaction involves preparing sample mixtures of various ratios of drug and excipient, storing at elevated humidity/temperature conditions for several days/months and subsequently analyzing those mixtures using an appropriate stability indicating assay [2].

Various pharmaceutical processes like spray drying, lyophilization, wet granulation and aqueous film coating cause association of residual water with drug particles in the solid state [3]. This residual moisture could affect a variety of physico-chemical properties such as dissolution rate, chemical stability, compactability and flow pattern [3]. Also in many cases, water associated with the excipient might come in contact with the drug and affect its stability.

In this paper, we have tried to examine the effects of wet granulation and direct mixing under various humidity/temperature conditions in the presence of a commonly used tablet excipient, lactose (both hydrous and anhydrous) on the stability of a hydrophobic Leukotriene antagonist drug.

2. Experimental section

2.1. Materials

Lactose hydrous and anhydrous were purchased from Quest International (Sheffield Products, Norwich, NY). Saturated solution of the following salts (purchased from Fisher): potassium carbonate, potassium chloride and potas-

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sium dichromate, were used to generate relative humidities of 40, 83 and 97% RH, respectively in glass dessicators [4]. In addition, a controlled humidity chamber at 75% RH/40°C was also used for this study. The drug was provided by Hoffmann–La Roche (Nutley, NJ).

2.2. Methods

All the aspects of this study, including preparation of mixtures and their subsequent analysis, were carried out under subdued light due to the light sensitive nature of the drug.

2.2.1. Preparation of mixtures

Wet granulation. Lactose (either anhydrous or hydrous) was gradually added in geometric proportion to the previously weighed drug in a porcelain mortar and triturated until the addition of lactose was complete. Deionized water was then added slowly to the mixture with trituration until a wet dough consistency was obtained. Mixtures containing lactose hydrous and anhydrous consumed ≈25 and 50 ml of water, respectively. The granulation was then sieved through a 40# US standard sieve and the wet granules were dried at 45°C for ≈24 h. The dried granules were then sieved through 200# US standard sieve and stored in amber bottles in the dark. The samples are referred hereafter as LH-WG (lactose hydrous-wet granulation) and LA-WG (lactose anhydrous-wet granulation).

Direct mixing. To previously weighed drug in a porcelain mortar, lactose (either anhydrous or hydrous) was gradually added and the mixture triturated until the addition of lactose was complete. The mixtures were then blended in a V-blender for 5 min. The powder mixtures were then stored in amber bottles in the dark. The samples are referred hereafter as LH-DM (lactose hydrous-direct mixing) and LA-DM (lactose anhydrous-direct mixing).

For both the above mentioned methods of preparation, 80 g of mixture was prepared from each of the two types of lactose. The mixtures were weighed (8–9 g) separately in small amber glass bottles and stored uncapped in respective dessicators and in controlled humidity–temperature chamber. Finally, all the sample mixtures were analyzed after 15, 30 and 45 days.

Loss on drying (LOD). LOD measurements were carried out by subjecting ≈2 g of sample to constant heating at 105°C for 20 min. The mixtures were evenly spread on a thin aluminum pan before loading it onto the heating chamber of the thermal balance (Mettler Instrument, Highstown, NJ). An attached printer recorded percentage moisture loss every 2 min, before giving the net moisture loss from the sample at the end of 20 min.

Karl–Fischer titrimetry. The moisture content was determined by the Karl–Fischer method (Orion Karl–Fischer with a printer connected to an Orion balance). The reagent used for titration was Hydranal-Composite 5 from Riedel–de Haën (Germany). The Karl–Fischer apparatus was first calibrated using deionized water. The powder mixture in ≈1 g quantity was weighed accurately and introduced into the automated titrating chamber. The percentage moisture content of the sample was printed out at the end of every titration.

High performance liquid chromatography (HPLC). A HPLC system consisting of a Waters model 600 E multisolvent delivery system with a Waters WISP model 712 injector was employed to quantify the drug and determine degradation if any, in the samples. A Waters Nova-Pak C_{18} column (15 cm × 3.9 mm, i.d; 4 μm) was used. The mobile phase was pH 5.5 buffer:acetonitrile:tetrahydrofuran (52:43:5). The sample was prepared by first sonicating the mixture (equivalent to 20 mg of the drug) in the presence of 100 ml of methanol for 15 min. This was then filtered through a Millex-SR 0.5 μm filter and the filtrate injected into the column after dilution with pH 7.0 Buffer. The conditions of analysis were as follows: flow rate, 1.0 ml/min; detection wave length, 240 nm (ABI model 785A); injection volume, 40 μl; run time, 25 min. The data collection and analysis was performed using the Access*Chrom software from Perkin-Elmer Nelson Systems (Cupertino, CA).

2.2.2. Differential scanning calorimetry (DSC)

An SII differential scanning calorimeter from Seiko Instruments (Horsham, PA) was used for thermal analysis. All the samples in ≈3–7 mg quantity were weighed in standard aluminum pans which were then sealed. A pin-hole was made in the center of the caps to allow escape of moisture during scanning. Thermograms were obtained at a scanning rate of 5°C/min and a temperature range of 15–260°C. The heating chamber was continuously purged with nitrogen gas at a rate of 50 ml/min. At the end of every scan the heating chamber was rapidly brought to 15°C using liquid nitrogen before the next analysis was resumed. An attached 7550 A graphics plotter (Hewlett–Packard) recorded the DSC profile of the samples.

3. Results and discussion

Although the analyses of all the mixtures were carried out, only some of the typical results have been illustrated here. The moisture adsorbed or absorbed by the sample mixtures has been easily determined by LOD and Karl–Fischer titrimetry. Table 1 gives the moisture content of 1:50 sample mixtures at day 0 (before exposure to different humidity/temperature conditions) and after 15 days storage at 40 and 97% RH, as determined by LOD and Karl–Fischer titrimetry. For LOD, the LH-DM mixtures show greater moisture content than LA-DM mixtures. It appears that at

Table 1
Percentage moisture content by LOD and Karl–Fischer method[a]

Method of moisture determination	LOD			Karl–Fischer		
Storage period	Day 0	15 Days		Day 0	15 Days	
Percentage relative humidity		40	97		40	97
LH-WG	1.70	1.70	2.10	5.14	5.16	5.22
LH-DM	1.60	1.60	1.60	5.21	5.24	5.34
LA-WG	1.30	1.30	2.00	1.53	1.54	2.90
LA-DM	0.60	0.60	1.00	0.37	0.38	2.21

[a] For 1:50 mixtures at day 0 (initial readings) and after 15 days storage period.

high temperature of analysis (105°C), in addition to indicating the adventitious moisture, some of the crystalline moisture (bound moisture) is shown as well. This is due to heat induced breaking of the weak bonds which hold water molecules in the lactose crystal. Karl–Fischer titrimetry gives the total moisture content (adventitious moisture + crystalline moisture) of the sample. Lactose hydrous contains ~5.2% moisture in its crystal while lactose anhydrous contains less than 0.5% moisture [5,6]. Karl–Fischer data is indicative of this and hence, the moisture content of mixtures containing lactose hydrous is higher than those containing lactose anhydrous (Table 1). For mixtures containing lactose anhydrous, externally added water is adsorbed or absorbed by the mixtures and hence, LA-WG mixtures show higher moisture content than LA-DM mixtures. Also, the difference in the moisture content between LA-WG and LA-DM is relatively more than between LH-WG and LH-DM (Table 1). This is because LA-WG mixtures absorbed relatively greater moisture during wet granulation than LH-WG mixtures, which picked very little or no moisture as a result of wet granulation process.

At a lower humidity condition like 40% RH, most of the mixtures pick up very little or no moisture. This condition is similar to ambient storage conditions. Hence, at 40% RH there is very little difference in the LOD and Karl–Fischer values between 0 and 15 days storage period (Table 1). At a higher humidity condition of 97% RH however, more moisture is available for absorption or adsorption. Hence, moisture is rapidly picked up by the mixtures (Table 1), containing lactose anhydrous. LA-DM mixtures pick up relatively more moisture than LA-WG mixtures (Table 1). This explains why the difference in the total moisture content between LA-WG and LA-DM mixtures (Karl–Fischer data) decreases with increasing humidity condition (40–97% RH). Under both humidity conditions (40 and 97% RH), mixtures containing lactose hydrous pick up very little or no moisture and their total moisture content remains in the range of 5.1–5.4% (Karl–Fischer data, Table 1). Lactose hydrous already has moisture in its crystal lattice and over a short period of exposure exhibits no significant moisture pickup. The determination of moisture (adventitious and some crystalline) by LOD, coupled with the consumption of different amounts of externally added water by lactose anhydrous and hydrous containing mixtures, can be used to explain the following observations: (i) At 97% RH, LA-DM mixtures pickup most moisture followed by LA-WG mixtures; (ii) lactose hydrous containing mixtures show very little moisture pickup for both wet granulated and directly mixed mixtures.

Fig. 1a,b illustrate the Karl–Fischer data for lactose anhydrous and hydrous containing mixtures, respectively, for different humidity/temperature storage conditions for 30 days. For lactose anhydrous mixtures the moisture pickup shows the following trend: 97% RH > 75% RH/40°C > 83% RH > 40% RH (Fig. 1a). This is because a combination of high temperature and humidity increases the molecular mobility of water molecules, thereby facilitating its greater absorption by lactose anhydrous. For lactose hydrous however, there is enough moisture in the crystal lattice and no significant moisture uptake was observed (Fig. 1b).

When the samples were analyzed by HPLC, the retention time for the drug was found to be ~4.7–5.0 min. The potency of the drug was monitored by analyzing the peak area of the drug in the mixture. The potency was calculated by taking into consideration the percent moisture uptake by the mixtures. The moisture adsorbed by the lactose molecule interacts with the drug causing its subsequent degradation. The percent drug content of 1:50 sample mixtures at day 0 (initial samples) and after 15 days storage period at 40 and 97% RH are shown in Fig. 2. At 40% RH, condition drug degradation is relatively less as compared to mixtures at 97% RH (Fig. 2). Mixtures containing lactose anhydrous show higher drug degradation than those containing lactose hydrous.

Fig. 3 shows the relationship between net Karl–Fischer and HPLC data (difference between readings at 15 days and their corresponding 0 day readings) for mixtures stored at 97% RH. For all the mixtures containing lactose anhydrous, increase in moisture uptake seems to correlate well with corresponding increase in degradation of the drug. Mixtures containing lactose anhydrous, in particular LA-DM mixtures, show higher moisture uptake and subsequent drug degradation than those containing lactose hydrous. Mixtures containing lactose hydrous pick up very little or no moisture and their total moisture content remains more or less con-

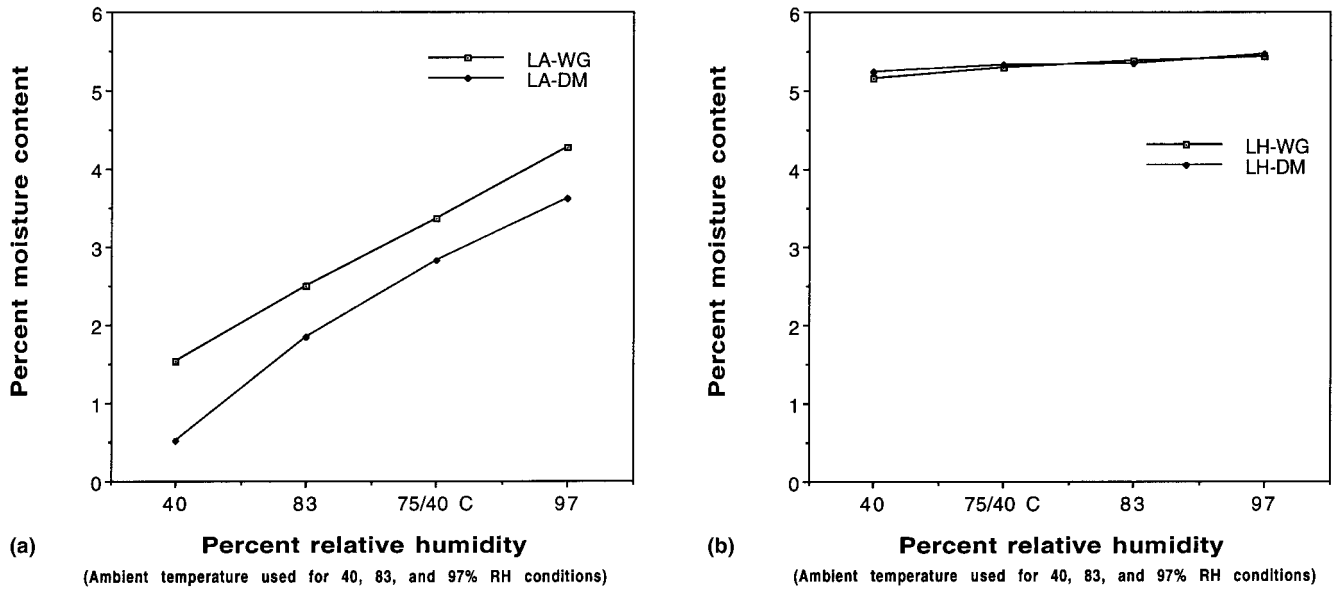

Fig. 1. (a) Karl–Fischer data for LA mixtures after 30 days storage period at different humidities; (b) Karl–Fischer data for LH mixtures after 30 days storage period at different humidities.

stant in the range of 5.1–5.4%. However, observed LH-WG mixtures show less drug degradation then LH-DM mixtures. We suspect that in the wet granulated mixtures the drug is embedded in the lactose granules and is thus less available for interaction with surface moisture, while in the dry mixtures it is more freely available for interaction. This may explain the observed discrepancy.

The DSC profile of all the samples at different humidities and periods of storage were determined. However, only some of the typical results have been illustrated here. Fig. 4a–d show the DSC analysis of lactose anhydrous, LA-WG/1:50/97% RH at 15, 30 and 45 days, respectively. In addition Fig. 5a–d illustrate the DSC analysis of lactose hydrous, LH-WG/1:50/97% RH at 15, 30 and 45 days, respectively. These figures show the endothermic transition and melting point (mp) changes between the different forms of lactose.

As a stable solid, lactose exists in the α-monohydrous, α-anhydrous and β-anhydrous forms [5,7]. When heated in a crimped sample container, α-lactose monohydrous (Fig. 5a), exhibits an endothermic dehydration peak at a temperature of ~145°C and it melts at a temperature of ~215°C. Lactose anhydrous (β-lactose), which also contains some α-anhydrous lactose, melts at around 232°C (Fig. 4a).

The α-anhydrous lactose in the β-lactose is responsible for moisture uptake. As the storage period of the mixtures increases and more moisture is picked up (Fig. 4b–d), the characteristic melting endotherm of β-anhydrous lactose

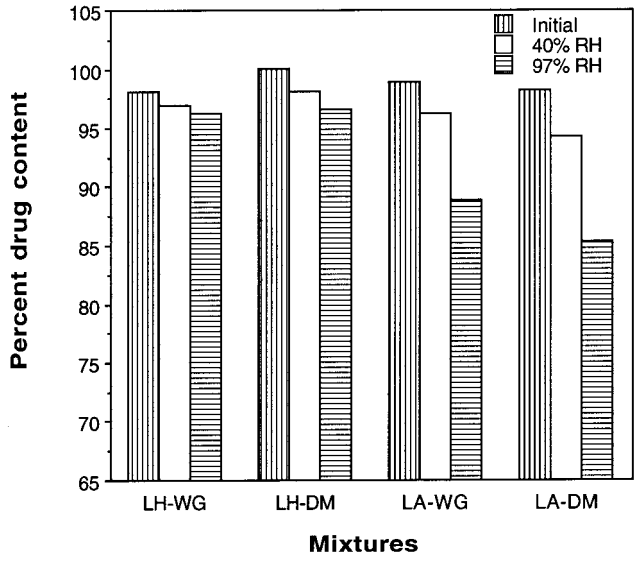

Fig. 2. Chemical stability for 1:50 mixtures at day 0 and after 15 days exposure period at 40 and 97% RH.

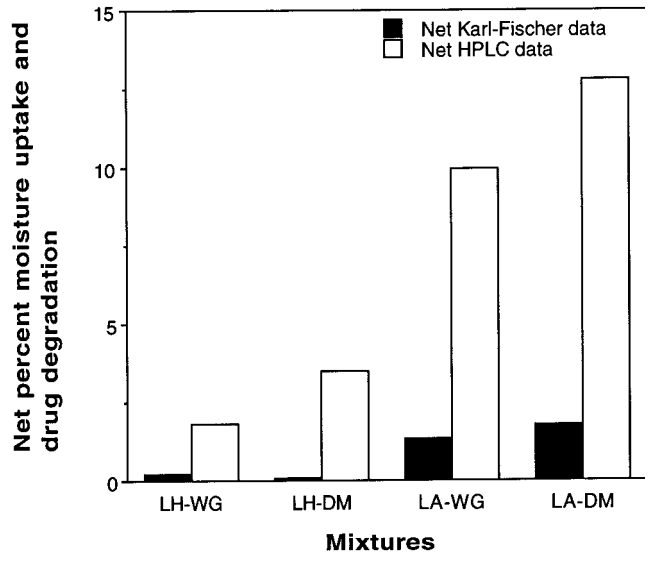

Fig. 3. Plot of net Karl–Fischer and HPLC data for 1:50 mixtures stored at 97% RH (storage period: 15 days).

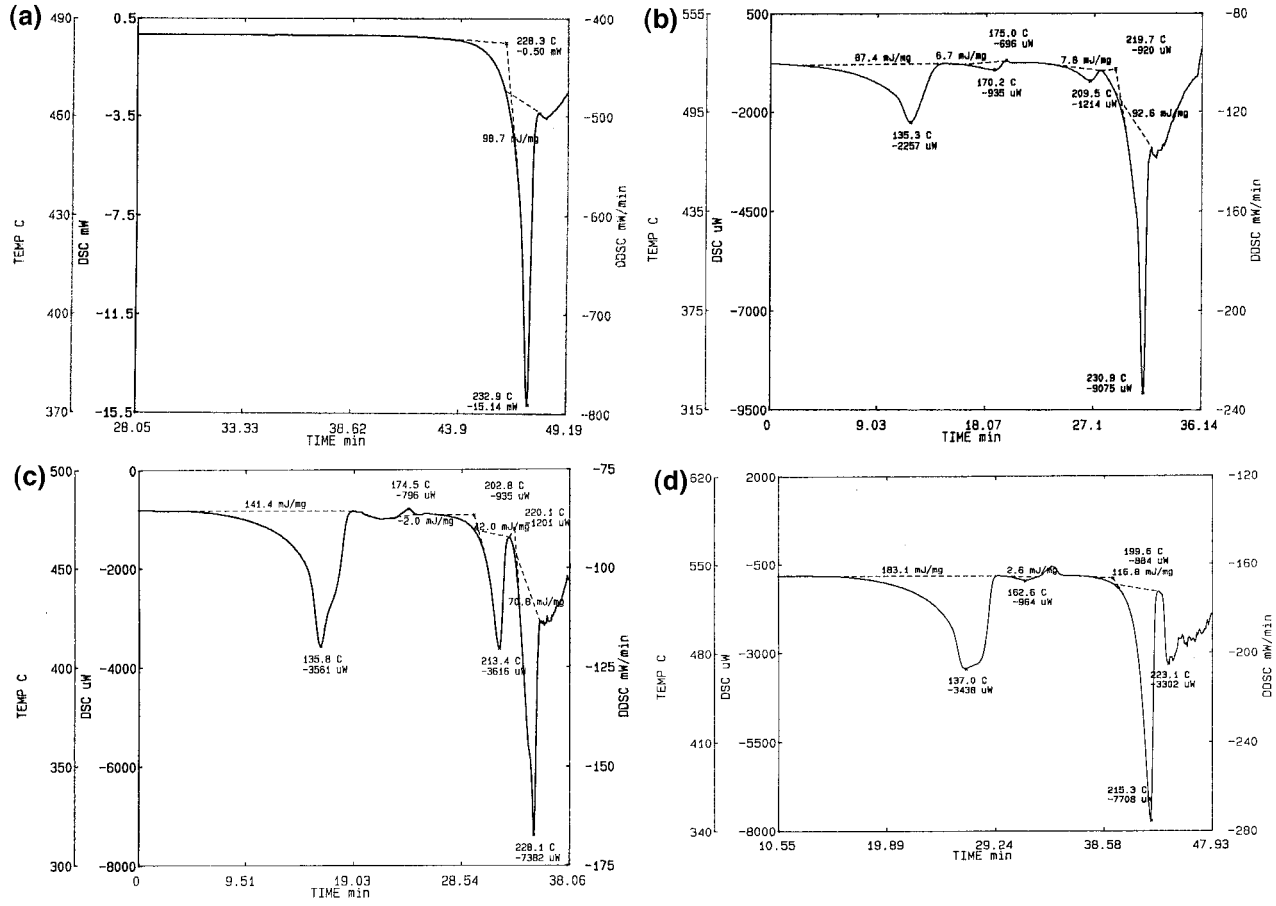

Fig. 4. (a) DSC profile of Lactose anhydrous; (b) DSC profile for LA-WG/1:50/97% RH at 15 days; (c) DSC for LA-WG/1:50/97% RH at 30 days; (d) DSC profile for LA-WG/1:50/97% RH at 45 days.

gradually diminishes and the endothermic melting transition of α-hydrous lactose becomes increasingly prominent. This is coupled by a gradual increase in enthalpies for dehydration of lactose from 87.4 to 183.1 mJ/mg (Fig. 4b–d). The lowering in mp, observed for both the forms of lactose, is due to the presence of impurities in the form of moisture and lactose of the opposite form. The β-anhydrous lactose thus undergoes gradual transition to α-hydrous lactose and this transition state may interact with the drug and cause its subsequent degradation. We observed a similar pattern in most of the formulations stored at 75% RH/40°C and 83% RH. For mixtures containing α-lactose hydrous, there is not much difference in the mp and dehydration endotherm of the lactose over increasing period of storage (Fig. 5b–d).

Water can be involved in drug–excipient interactions in the following two ways: (i) Water from the excipient can redistribute into the vapor phase and be adsorbed or absorbed by the drug; and (ii) water located at points of contact between the drug and excipient can facilitate drug–excipient interaction [1,8,9]. In both cases, water acts by virtue of its plasticizing effect [1]. In our study, the involvement of water in drug–lactose interaction is a combination of both mechanisms. The method for the preparation of different mixtures demanding extemporaneous use of water warrant the second case, while use of different humidity/temperature exposure conditions justify the first case.

4. Conclusions

Mixtures containing lactose anhydrous picked up more moisture with increase in humidity/temperature and storage period conditions than those containing lactose hydrous. HPLC analysis demonstrated that at higher humidity/temperature, mixtures containing lactose anhydrous exhibited relatively greater degradation of drug than those containing lactose hydrous. A DSC profile of individual sample mixture provided the mechanism of moisture uptake and involvement of moisture in drug–lactose interaction. Lactose anhydrous becomes hydrated on exposure to high humidity/temperature condition and the transition state of lactose and not its stable state may be responsible for its greater interaction and subsequent degradation of the drug. These results indicated that lactose anhydrous, in certain cases, may absorb significant amount of moisture, which can affect its inherent properties and can directly come in contact with the drug. For a moisture sensitive drug, it may drastically

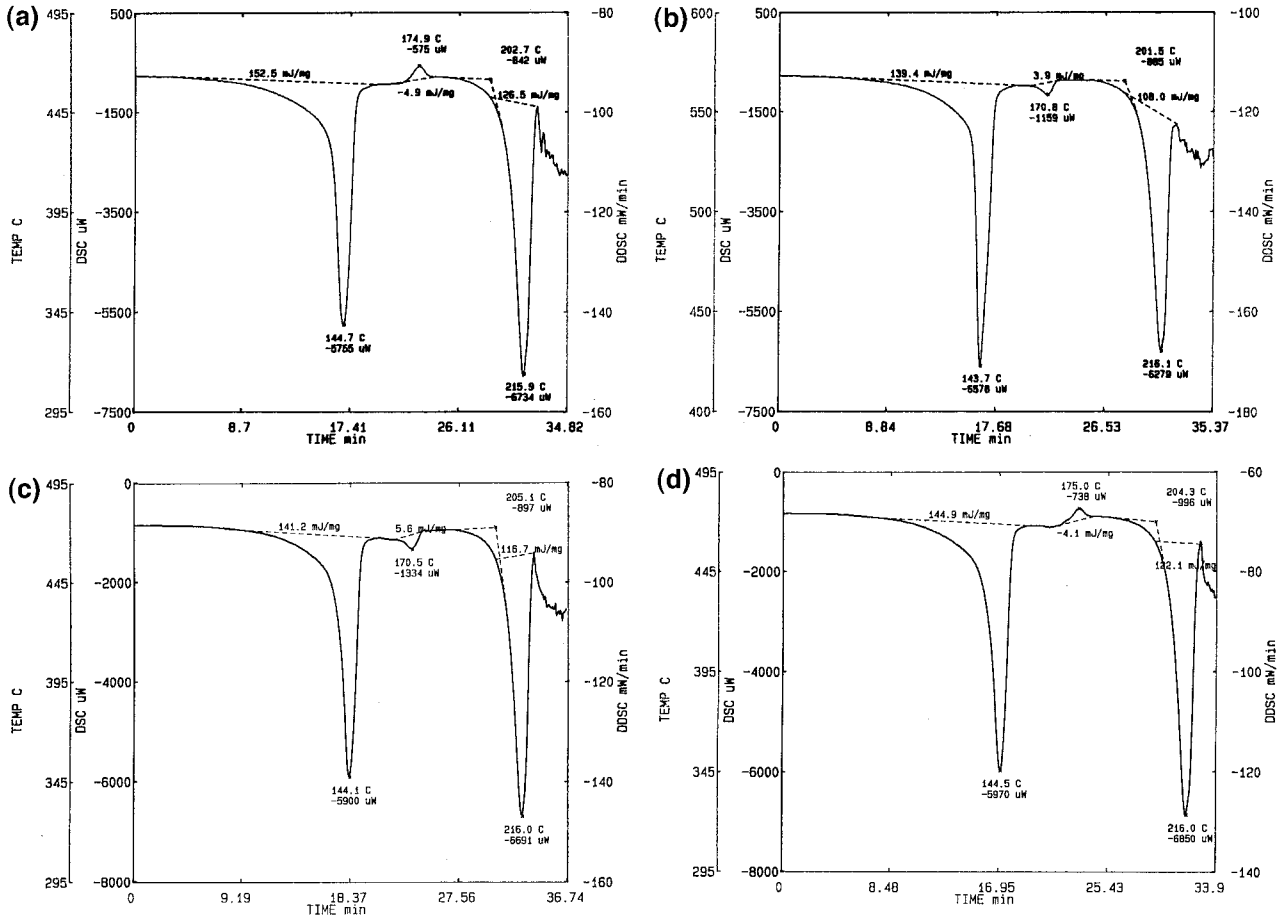

Fig. 5. (a) DSC profile of Lactose hydrous; (b) DSC profile for LH-WG/1:50/97% RH at 15 days; (c) DSC profile for LH-WG/1:50/97% RH at 30 days; (d) DSC profile for LH-WG/1:50/97% RH at 45 days.

affect the drug stability. Therefore, the general belief that lactose anhydrous, which has less than 0.5% moisture, should provide greater stability as compared to lactose hydrous needs to be properly evaluated. If not appropriately packaged lactose anhydrous may cause greater instability for the drug as compared to lactose hydrous in pharmaceutical dosage forms.

Acknowledgements

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