Pharm Res (2014) 31:2716–2723
DOI 10.1007/s11095-014-1369-4

RESEARCH PAPER

Isothermal Microcalorimetry of Pressurized Systems I: A Rapid Method to Evaluate Pressurized Metered Dose Inhaler Formulations

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Received: 18 December 2013 / Accepted: 21 March 2014 / Published online: 24 April 2014

ABSTRACT

Purpose The techniques available to study formulation stability in pressurized metered dose inhalers (pMDIs) are limited, due to the challenging conditions of working with high pressure propellants. Isothermal microcalorimetry is a valuable tool used to screen and aid in formulation development of solid and solution drug formulations; however there are currently no available methods to evaluate pMDIs. In this paper, we have developed a method that allows measurement of such pressurized systems.

Methods Samples were prepared by cold filling ampoules with propellant (HFA 134a) and drugs of interest. Ampoule caps were fitted with a specific O-ring, coated with paraffin and preconditioned prior to measurement. Samples were equilibrated at 25°C, placed in a Thermal Activity Monitor III (TAM III) system and measured isothermally at 25°C for a period of at least 24 h.

Results Using well-defined procedures and ampoule preparation techniques we were able to safely contain the volatile propellant and acquire a stable measurement baseline. We were able to rapidly determine, within 6 h, the physical stability of amorphous and crystalline drug forms of beclomethasone dipropionate and formoterol fumarate dihydrate when formulated with HFA 134a.

Conclusions Isothermal microcalorimetry in pressurized HFA propellant systems was shown to be a rapid screening tool to evaluate pMDI formulation physical stability. This method can potentially be applied to study pMDI formulation factors to expedite product development.

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KEY WORDS amorphous · formulation stability · inhalation · isothermal microcalorimetry · pressurized metered dose inhalers

ABBREVIATIONS

APIs	Active Pharmaceutical Ingredients
BDP	Beclomethasone Dipropionate
FF	Formoterol Fumarate Dihydrate
pMDIs	Pressurized Metered Dose Inhalers
TAM III	Thermal Activity Monitor III
XRD	X-ray Diffraction

INTRODUCTION

All physical and chemical processes result in the generation of a measurable heat flow, which can be studied using isothermal microcalorimetry. This technique is beneficial to formulation development because it non-destructively measures samples of any form including: solutions, suspensions, gasses, solids, and any combination thereof. Isothermal microcalorimeters, like the Thermal Activity Monitor III (TAM III), measure heat outputs on a micro/nano-watt scale, reporting the total cumulative heat (q) and heat flow (power) as a function of time. The technique is non-specific resulting in a single readout even if several reactions occur simultaneously. This allows the study of many complex reactions that are normally outside the scope of other techniques (1). However, the same non-specificity can promote erroneous analysis in the absence of appropriate controls and if sample preparation is poorly controlled.

Isothermal microcalorimetry has been employed within the pharmaceutical industry to study a variety of formulation factors including: vapour sorption, drug/excipient stability and compatibility, polymer interactions, crystallinity, and binding energies (2–6). It has been used to aid in the development of solid drug formulations, probe solute/solvent

interactions and binary mixture enthalpies, predict drug solubility of liquid formulations, and determine long term stability (7–16).

However, despite the widespread use of microcalorimetry to study solid state and solution mediated thermal events in pharmaceutical formulations, the current techniques are not transferrable to the study of pMDIs, which are becoming an increasingly popular form of drug administration as the pulmonary route is revealed to be a more efficient drug delivery pathway for many diseases (17, 18). The inability to use current analytical techniques is because of the high vapour pressure of the propellants (HFA 134a/HFA 227)—4 to 6 bars at room temperature due to their low boiling points—which makes it challenging to safely contain the gaseous propellant and produce a hermetically sealed sample. Previous isothermal microcalorimetry pMDI studies have employed model propellants (Arcton 113 or HPFP) that differ in several properties (especially compared to HFA 134a) including solubility, dielectric constant, surface tension and water miscibility (19–22).

Current analytical techniques used to directly evaluate pMDI formulation stability and product life involve lengthy experimental steps. For example, current procedures for evaluating pMDI product shelf life involve prolonged (> 6 months) high temperature/humidity cycling studies. These techniques depend on the Arrhenius equation to extrapolate degradation rates to room temperature based on the assumption that the degradation reactions will occur *via* the same mechanism at all temperatures, which however may not necessarily be true. Thus, there is a need for faster techniques to expedite direct evaluation of pMDI formulation stability and product life.

In this paper, we provide a reliable method to study actual drug-propellant interactions using isothermal microcalorimetry, demonstrating its utility to evaluate the crystallization of suspended particles in pMDI formulations. The ability to obtain a stable baseline and accurately measure the heat produced from a formulation containing HFA 134a can significantly reduce the time required to develop and optimize marketable pMDI formulations.

MATERIALS AND METHODS

Materials

HFA 134a (Fluor Ineos United, Cheshire, UK) was the main propellant used in this study as it exerts a higher pressure than HFA 227 at the same temperature. Formoterol fumarate (FF) (Vamsi Pharmaceuticals, Maharashtra, India) a long acting beta receptor agonist and beclomethasone dipropionate (BDP) (Sigma Aldrich, Castle Hill, NSW) an inhaled corticosteroid, widely used drugs to treat chronic obstructive pulmonary disease (COPD), were chosen as model drugs to test the functionality of the TAM in screening formulations. Raw FF and BDP were crystalline. 4 ml pressure ampoules were obtained from TA Instruments, fitted with specific Nitrile O-rings (Cataolg #10 N010, N & K Engineering Supplies PTY LTD, Marrickville, NSW) and coated with white soft paraffin (B.P.). Samples were measured using a Thermal Activity Monitor III (TA Instruments, Sollentuna, Sweden). HFA 134a was initially filled into Bespak 20 mL canisters (Bespak, Norfolk, UK) using Valois valves (Model#DF30 plus, Aptar Pharma, Tokyo, Japan) to allow cold transfer of the propellant to the appropriate ampoule for measurement.

Preparation of Amorphous APIs by Spray Drying

Amorphous BDP was produced *via* spray drying using a Büchi 290-Mini Spray Dryer (Büchi, Switzerland). BDP was spray dried from a 5% w/v ethanolic solution in closed loop using an outlet temperature of 40°C, inlet temperature of 60°C, aspiration setting of 35 m^3/h, pump feed rate of ~3.5 ml/min, and atomizer flow of 742 NL/h. Amorphous formoterol fumarate was spray dried from a 2% w/v methanolic solution at similar settings.

TAM Sample Preparation

pMDI canisters were cleaned with ethanol, deionized water and air dried before being crimped with Valois valves (desiccating ring removed to allow for easy removal). Crimped canisters were pressure filled with HFA 134a using a Pamasol manual pressure filler (Pamasol Willi Mäder AG, Pfäffikon Switzerland) and chilled in dry ice for 30 min. 5 mL syringes in their original packaging or pipette tips in a sealed specimen jar (protected from moisture), were simultaneously placed in a beaker surrounded by dry ice and chilled for 30 min. Ampoule caps were prepared by applying enough soft paraffin to ensure that the O-ring and the groove underneath were appropriately covered and filled (Fig. 1). The ampoule bases were placed in sealed specimen jars (to protect from moisture) and placed in dry ice for the final 10 min of the canister and syringe/pipette 30 min chilling step. Ampoule caps were stored at room temperature.

After all components had been appropriately chilled, they were transferred to a dry box (RH < 10%) and a specific amount of drug was added to the chilled ampoule. Alternatively, the drug can be weighed into the ampoule prior to chilling; however because we used amorphous drugs in this study—normally characterized by high moisture sorption rates—we added the drug after the chilling step to minimize moisture exposure. A sharp cutting plier was used to remove the valve from the chilled canister and a chilled syringe or pipette tip was used to fill the ampoule (containing the drug) with the desired volume of HFA from the canister (~4 ml). Immediately following addition the ampoule was capped,

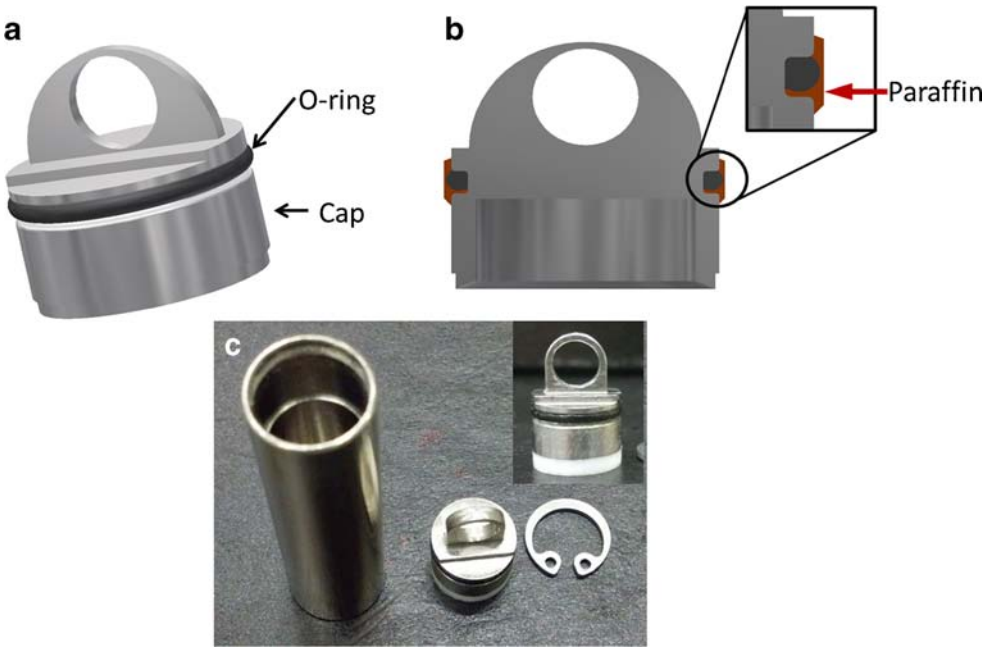

Figure 1 TAM sample ampoule cap. (**a**) Top view of the ampoule cap with the O-ring situated in the appropriate O-ring groove. (**b**) Sliced view of the ampoule cap showing the correct application of paraffin. (*Inset*) A closer view of the paraffin application: note that excess paraffin fills the gap below the O-ring in the O-ring groove. (**c**) TAM ampoule with cap and locking ring (*Inset*); side view of actual cap with Teflon sleeve and O-ring.

sealed using the locking ring, (Fig. 1c) and transferred to a water bath set at 26°C for 5 min, to roughly equilibrate the sample to the measurement temperature. This equilibration step is necessary to prevent condensation/ice forming on the outside of the ampoule, which can be detrimental to both the instrument and the accuracy of the measurement. The prepared ampoules were then transferred to the TAM III and lowered into an equilibration position for 15 min before lowering into the measurement position. Consistency in the technique and timing used to insert the ampoules into the measurement position is vital to minimize variability in friction-generated heat during insertion. The use of proper techniques eases sample comparison by limiting the equilibration period needed to ensure that an accurate signal is obtained and artefacts are minimized. Furthermore, control ampoules should always be prepared and measured concurrently to differentiate between heat signals due to equilibration and handling, and the sample of interest.

Powder X-Ray Diffraction

Powder X-Ray diffraction (XRD) patterns were obtained using a Skyscan D5000 (Siemens, VIC Australia). Samples were scanned from 3° to 40° 2θ using 15 s per step size of 0.01 2θ (~16 h scan time). Samples from measured TAM formulations were recovered by allowing the propellant phase to slowly evaporate from the ampoule (after carefully chilling and opening ampoules) in a dry box kept below 10% RH. Samples were kept desiccated using silica gel (Chem-Supply, SA Australia) until measurement.

Scanning Electron Microscopy

Samples from measured TAM formulations were recovered similarly to those obtained for XRD analysis. All SEM samples were prepared in a dry environment (RH < 10%). Samples were coated with 15 nm of gold and imaged with a beam current of 10 μA and voltage of 15 kV using a Zeiss Evo Quemscan (Zeiss, Carl Ziess Microscopy, Jena, Germany). Samples were kept desiccated until measurement.

RESULTS AND DISCUSSIONS

Key Method Factors

Sample preparation is a major component of isothermal microcalorimetric measurements. Improper handling of the sample and poorly controlled experimental procedures give rise to erroneous measurement signals that can be mistakenly interpreted as an effect of formulation design. The environmental temperature at which the samples are prepared in and the temperature at which the samples are pre-equilibrated outside of the instrument also have a measurable impact on the length of time required to achieve a stable baseline (2). There are three key controllable parameters that must be carefully considered to ensure that the signal obtained during measurement is solely attributed to formulation parameters:

1. O-ring conditioning
2. Paraffin application onto Ampoule Cap
3. HFA fill volume

O-ring Conditioning

The O-rings on the ampoule cap must be properly conditioned in order to obtain a hermetic seal and accurate measurement. To condition the O-rings, ampoules filled with HFA 134a were sealed using ampoule caps fitted with the O-rings (with paraffin applied) and stored for at least one day at room temperature. This process was performed twice consecutively to ensure that O-rings were properly conditioned. This initial conditioning allows the O-rings to swell and contract when in contact with the paraffin and propellant. Once conditioned, the O-rings can be used effectively until material failure. Failure to condition the O-rings will result in errant peaks during measurement, which can be easily misinterpreted as an effect of the formulation by an unaware user (Fig. 2).

Paraffin Application onto Ampoule Cap

The paraffin, used as a supporting sealant, must be appropriately applied as shown earlier in Fig. 1 to prevent baseline drift. Improper application of paraffin will allow the propellant to leak (at uncontrollable rates) making it impossible for the liquid and vapour phases of the propellant to reach equilibrium (Fig. 3).

Filling in the gap below the O-ring is a key component in the application of the paraffin. If the O-ring is resting against the bottom surface of the O-ring groove with paraffin above, the upward force of the propellant vapour phase will push the O-ring against the more mobile paraffin; the paraffin will be pushed out of the O-ring groove allowing the O-ring to shift during measurement. Conversely, when the O-ring is resting against the top of the O-ring groove with the paraffin filled in below, (Fig. 1) the upward force of the vapour phase pushes the more mobile paraffin against the O-ring (firmly situated against the immobile O-ring groove edge) filling the paraffin into any gaps present between the O-ring and ampoule wall resulting in an ideal seal.

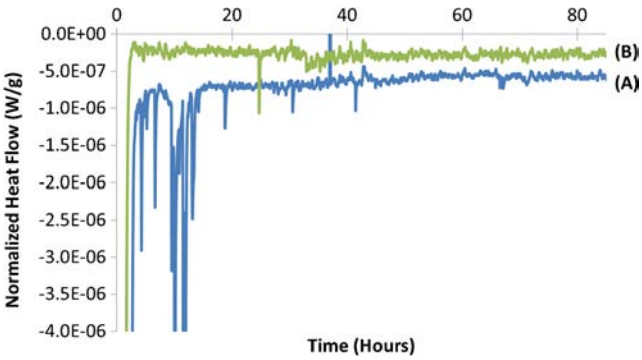

Figure 2 Failure to condition O-rings, results in relaxation and swelling of the O-ring during the measurement which produces large deviations from the baseline. (**a**) first conditioning step; (**b**) second conditioning step.

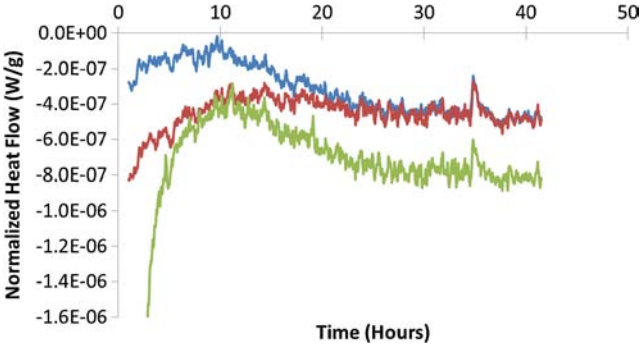

Figure 3 Unstable and irreproducible drifting baselines caused by the slow and variable leaking rate of HFA from the ampoule due to improper paraffin application.

HFA Fill Volume

The volume of HFA filled into the ampoule also affects the time required to reach a stable baseline (Fig. 4). The ampoule must be filled near its capacity volume to limit head space and promote fast equilibration times. In a closed system under pressure (as in the TAM ampoule) the propellant exists as a liquid and a vapour such that the two phases will reach a steady state.

The differing thermal conductivities of the two phases can result in large temperature gradients within the ampoule, greatly lengthening the equilibration times. Minimizing the volume of the vapour phase limits this temperature gradient to a smaller volume, resulting in quicker equilibration. Additionally, because we are interested in liquid propellant-drug interactions, maximizing the interaction volume is beneficial to signal output.

Once all the above mentioned parameters were appropriately controlled, we were able to obtain a stable and reproducible baseline with a standard deviation of $<5\times10^{-7}$ W/g after 5 h and $<3\times10^{-7}$ W/g after 10 h ($n=6$) (Fig. 5). The larger standard deviation seen within the first 5 h is due to small variations in sample preparation and environmental conditions from day to day experiments as the system

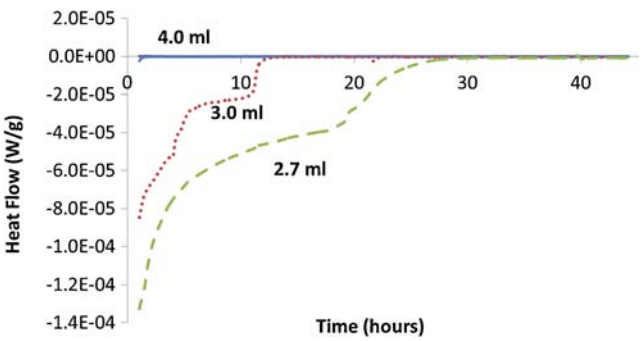

Figure 4 The amount of propellant added to the ampoule has an effect on how quickly a stable baseline is achieved. A variety of volumes ranging from 2.7 to 4.0 mL were tested.

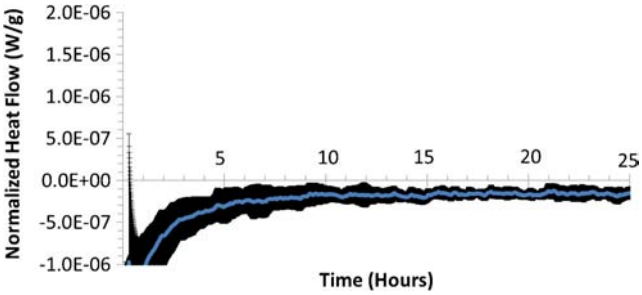

Figure 5 The baseline achieved for a sample containing only HFA 134a with the proper sample preparation techniques. All samples were conducted on different days to account for variances in environmental temperature during preparation. ($n = 6$) Mean and standard deviation shown here.

stabilizes. Thus, it is always important to conduct a control sample during each experiment.

Formulation Screening of Manufacturing Techniques and Physical Stability

Partially amorphous beclomethasone dipropionate (BDP) spherical particles (1–5 µm) particles were produced by spray drying (Fig. 6b). The partial amorphicity of these particles were verified with powder X-ray diffraction (XRD) (Fig. 7). Most amorphous drug forms are less stable than the crystalline forms because of the lack in long-range order, which result in a greater dissolution rate in a given solvent; the resulting concentrations form a supersaturated solution that promotes crystallization. Amorphous solids also exhibit a greater propensity for moisture, which leads to a decrease in the glass transition temperature and eventual crystallization (23).

We observed that partially amorphous BDP formulated with HFA 134a was physically unstable, characterized by a prominent exothermic heat flow peak from the sample within the first 5 h (Fig. 8). Analysis of large 100 µm crystalline particles recovered after the measurement confirmed that the amorphous particles underwent a dissolution-mediated crystallization in HFA 134a (Fig. 6c). The heat signal is the resultant of the wetting of the particles by propellant, dissolution of the particles in the propellant (both endothermic) and subsequent crystallization of the partially amorphous BDP particles (exothermic). The initial endothermic wetting and dissolution of the particles are not clearly visible in the heat flow measured by TAM because they most likely occur during the measurement equilibration period.

From the XRD patterns we determined that the structure of the crystals formed in the BDP-HFA134a formulation were different from the original crystalline material (Fig. 7). The difference in XRD patterns is attributed to the incorporation of HFA-134a within the crystal structure (during the solution-mediated crystallization) to form a HFA solvate of BDP, which is the most stable form under these conditions (24).

The amorphicity of the spray dried formoterol fumarate (FF) spherical particles (1–3 µm) (Fig. 9b) were verified with

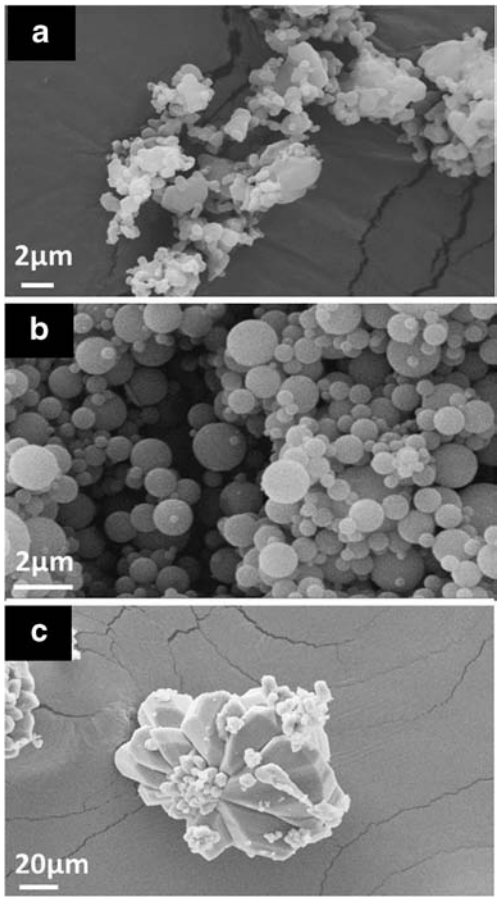

Figure 6 Beclomethasone Dipropionate SEM images (**a**) Micronized crystalline BDP (**b**) Spray dried partially amorphous BDP (**c**) Crystalline BDP recovered from the measured amorphous BDP-HFA134a formulations.

XRD (Fig. 10b). The measured heat flow for formoterol-HFA 134a formulations was identical to HFA134a only formulations (Fig. 5), indicating that the amorphous formoterol remained stable in the propellant (Fig. 11). Imaging and XRD of the recovered particles (after measurement) confirms that no visible or measurable physical changes occurred (Figs. 9c and 10c respectively).

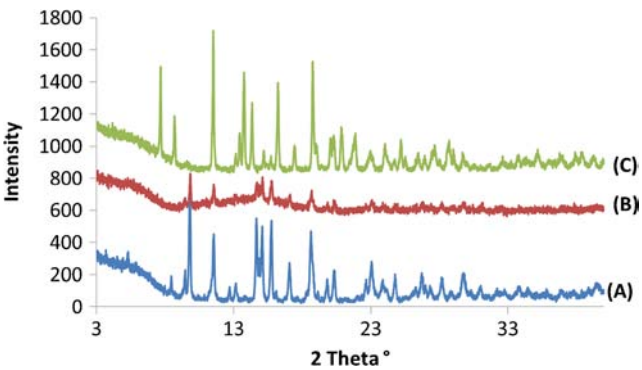

Figure 7 Beclomethasone Dipropionate XRD patterns (**a**) Micronized crystalline BDP (**b**) Spray dried partially amorphous BDP (**c**) Crystalline BDP recovered from the measured amorphous BDP-HFA134a formulations.

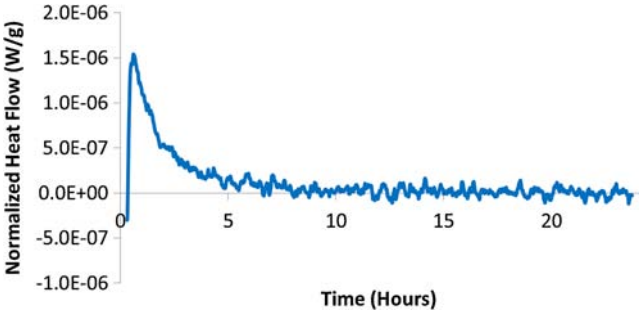

Figure 8 Normalized heat flow signal obtained for a pMDI formulation containing spray dried partially amorphous BDP in HFA 134a.

In addition to using the amorphous components of the drugs, we evaluated the raw crystalline material for formulation stability (Figs. 6a and 9a). Crystalline forms of both drugs exhibited physical stability throughout the measurement (Fig. 12), as evident from the resultant heat flows which are identical to that of a formulation containing only HFA 134a

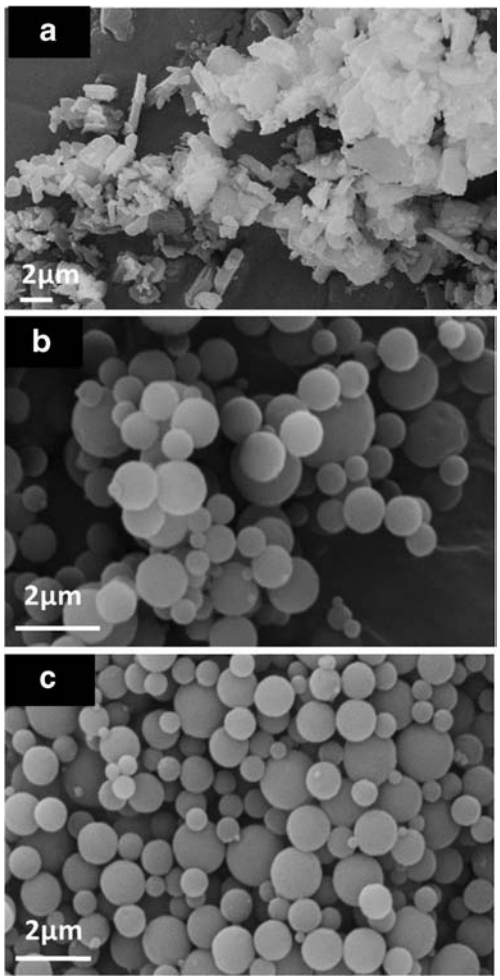

Figure 9 Formoterol Fumarate SEM Images. (**a**) Micronized crystalline FF source; (**b**) Spray dried amorphous FF; (**c**) Recovered amorphous FF from FF-HFA 134a formulations.

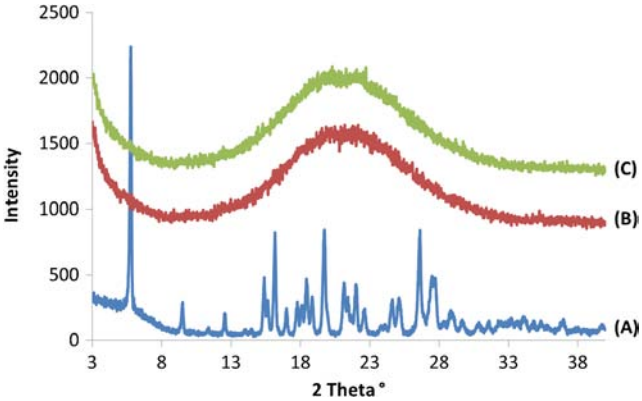

Figure 10 Formoterol Fumarate XRD patterns (**a**) Micronized crystalline FF source; (**b**) Spray dried amorphous FF; (**c**) Recovered amorphous FF from FF-HFA 134a formulations.

(Fig. 5). Despite the solvate of BDP being the most stable form of the drug, the stability of the crystalline BDP is attributed to the lack of a dissolution driving force, which is necessary to incorporate HFA 134a into the solvate crystal structure.

In an attempt to force dissolution we prepared samples containing crystalline BDP, HFA 134a and variable amounts of ethanol up to 5% w/w. Despite the use of a co-solvent, the crystalline BDP did not crystallize into the clathrate/hydrate form within the formulation during measurement (heat signals were identical to baseline signals).

POTENTIAL APPLICATIONS

It is important to reiterate that the heat signals obtained from the TAM III are all encompassing and therefore heat signals obtained may be an accumulation of various processes: simultaneous wetting, dissolution, and crystallization (includes nucleation and crystal growth). Although it is possible to obtain quantitative data from the normalized heat flow curves of these reactions (including their kinetic and thermodynamic parameters) (25–27), the rapid crystallizations measured in these studied systems make it difficult to determine if we captured the complete reaction. The measured start of the

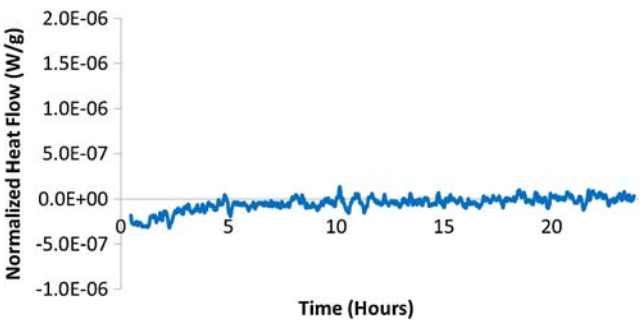

Figure 11 Normalized heat flow signal for a pMDI formulation containing amorphous FF in HFA 134a.

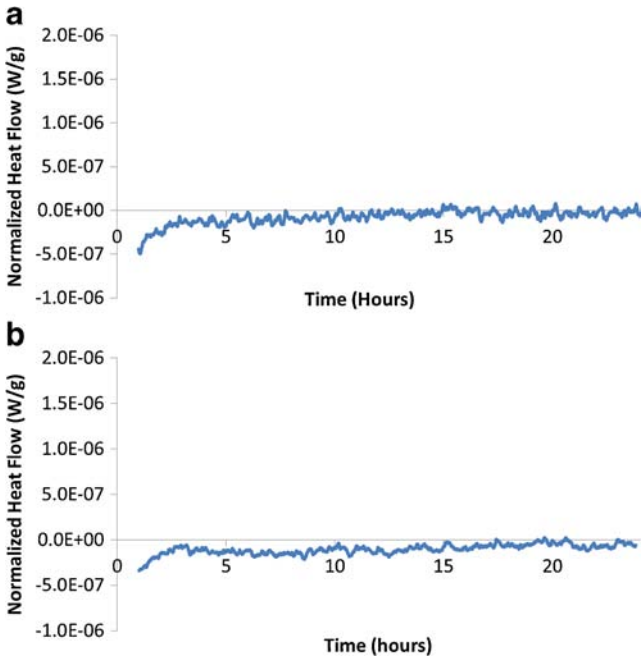

Figure 12 Normalized heat flow for the crystalline drug forms of (**a**) beclomethasone dipriopionate and (**b**) formoterol fumarate in HFA 134a. Both exhibit physical stability.

reaction may be truly unidentifiable due to the speed at which it occurs and the required equilibration period (despite the optimized equilibration parameters). Therefore, samples that exhibit delayed crystallization, reaching a stable baseline before crystallizing, would be ideal quantification models.

Even though all experiments were conducted using HFA 134a, the alternative propellant HFA 227 (which has a lower vapour pressure than HFA 134a) can also be used safely. In addition to studying crystallization, the technique presented in this paper may also be applied to evaluate excipient compatibility, moisture ingress, co-solvent concentrations, and degradation rates. The achievable stable baseline of this method combined with the high sensitivity of the TAM III provides the potential to measure drug degradation rates for drugs that degrade ≤1% per annum (28).

CONCLUSION

We have shown that it is possible to safely prepare ampoules containing HFA 134a for isothermal microcalorimetric measurements and obtain a reproducible baseline using proper preparation techniques. Using this method we were able to rapidly evaluate the physical stability of spray dried beclomethasone dipropionate and formoterol fumarate amorphous particles formulated in HFA 134a. The ability to measure actual pMDI formulations using isothermal microcalorimetry may shorten the required formulation development time for pMDIs.

ACKNOWLEDGMENTS AND DISCLOSURES

The authors gratefully acknowledge (1) the financial support of the Australian Research Council (DP120102778) and the facilities, and the scientific and technical assistance, of the Australian Centre for Microscopy and Microanalysis at the University of Sydney. D. D'Sa is also a recipient of the Australian IPRS and APA scholarships.

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