Pharm Res (2015) 32:714–722
DOI 10.1007/s11095-014-1499-8

RESEARCH PAPER

Isothermal Microcalorimetry of Pressurized Systems II: Effect of Excipient and Water Ingress on Formulation Stability of Amorphous Glycopyrrolate

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Received: 17 April 2014 / Accepted: 20 August 2014 / Published online: 6 September 2014

ABSTRACT

Purpose Use isothermal microcalorimetry to directly evaluate the effects of excipients and water content to produce a stable amorphous glycopyrrolate pressurized metered dose inhaler (pMDI) formulation.

Methods Amorphous glycopyrrolate particles with and without excipients (Distearoyl-sn-glycero-3-phosphatidylcholine (DSPC) or β-cyclodextrin (βCD)) were spray dried and cold filled along with HFA 134a into customized thermal activity monitor (TAM) measurement ampoules. When applicable, a known amount of water was also pipetted into the ampoule. Sample ampoules were hermetically sealed, equilibrated to 25°C and measured isothermally for at least 24 h using the TAM III (TA Instruments, Sollentuna, Sweden).

Results Amorphous glycopyrrolate particles were highly unstable and crystallized rapidly when suspended in HFA 134a. Co-spray drying the glycopyrrolate with DSPC failed to mitigate this instability, but co-spray drying with βCD protected the amorphous glycopyrrolate from crystallization, resulting in a stable formulation at low water contents (≤100 ppm).

Conclusions This study shows that isothermal microcalorimetry can easily differentiate between physically stable and unstable pMDI formulations of glycopyrrolate within a few hours. Furthermore, it allows rapid screening of various formulation factors (drug form, excipients, water ingress), which can greatly reduce the time required to develop marketable products with acceptable shelf life.

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KEY WORDS amorphous formulations · crystallization · excipient compatibility · long-term stability · water ingress

ABBREVIATIONS

($CaCl_2$)	Calcium chloride
(COPD)	Chronic obstructive pulmonary disease
(DSPC)	Distearoyl-sn-glycero-3-phosphatidylcholine
(FTIR)	Fourier transform infrared
(GP)	Glycopyrrolate
(pMDIs)	Pressurized metered dose inhalers
(TAM III)	Thermal activity monitor III
(T_g)	Glass transition temperature
(βCD)	β-Cyclodextrin

INTRODUCTION

Glycopyrrolate (GP) was recently found to demonstrate sustained bronchodilation for>24 h with a rapid onset of action to treat chronic obstructive pulmonary disease (COPD) [1–3]. Despite this new discovery, a limited number of products are available and inhalable glycopyrrolate is yet to be approved for treatment of COPD in the United States. Currently, Novartis has the only two marketed dry powder inhalable formulations of glycopyrrolate (Seebri and Ultibro Breezhalers), while Pearl Therapeutics is in phase III clinical trials of two glycopyrrolate pressurized metered dose inhalers (pMDI) (PT001, PT003). The limited number of products pursued is most likely attributed to the highly unstable nature of amorphous glycopyrrolate produced during typical manufacturing processes and the lengthy developmental timelines to evaluate product stability.

Manufacturing techniques such as spray drying, micronization, and spray-freeze drying are required to control drug particle size and morphology to enable efficient delivery to the lungs. However, the large mechanical stresses experienced during micronization and the fast solvent removal associated

with spray drying normally produce partially or fully amorphous particles. Although amorphous drugs dissolve and are absorbed *in-vivo* faster than the crystalline form [4–7], they are usually more unstable and can transform into larger crystalline particles when formulated with HFA 134a/HFA 227, leading to blockages in the pMDI valve and a reduction in the respirable particle fraction. Furthermore, the amorphous glycopyrrolate glass transition temperature (T_g) drops below room temperature when exposed to even a slightly humid environment (20–40% RH) [8], and thus can be difficult to prepare formulations that remain stable throughout the manufacturing process.

To mitigate such instabilities in pMDI formulations, various excipients like phosphatidylcholines and cyclodextrins can be employed. DSPC is a common phosphatidylcholine used in DPIs and pMDIs such as Nektar's Pulmospheres™ and Pearl's Porous Particles™ [9–13]. DSPC is often spray dried with $CaCl_2$ to increase the particle T_g and thus particle stability [14]; while cyclodextrins form non-covalent drug-inclusion complexes, which result in greater stability due to the protection of the complexed drug molecule [15]. Although excipients are employed to improve stability, the use of an incompatible excipient can exacerbate physical instabilities and accelerate drug degradation [16]. Such incompatibilities may not be noticeable instantaneously, but can develop over an extended storage time.

Thus, long-term stability must be evaluated to ensure excipient-drug particles remain stable during storage. In storage, pMDI formulations can be largely affected by water, which can enter the pMDI formulation by moisture diffusion through the valve components [17]. Accumulation of water in a formulation can lead to changes in particle size distribution, particle adhesion, crystal deliquescence, crystallization, formation of hydrates, and may induce hydrolysis or act as an intermediary between solid components [18–20].

Current techniques for evaluating pMDI long term stability and excipient compatibility require prolonged exposure (>6 months) to elevated temperature and humidity environments, and are limited by the low boiling temperature of propellants used in formulation. In part one of this series, we developed a technique using isothermal microcalorimetry to directly evaluate pMDI formulations [21]. This paper aims to apply the technique as a rapid tool to evaluate whether DSPC/$CaCl_2$ or β-Cyclodextrin can stabilize amorphous glycopyrrolate in a pMDI formulation. The technique was also used to assess the effect of water content on formulation stability, mimicking water ingress expected during normal shelf-life.

MATERIALS AND METHODS

Spray Drying of APIs

Spray dried particles were produced using a Büchi 290-Mini Spray Dryer (Büchi, Switzerland) using the parameters shown in Table I. Raw crystalline glycopyrrolate was supplied by Boerhinger Ingelheim (New Castle, Pennsylvania USA). Distearoyl-sn-glycero-3-phosphatidylcholine/calcium chloride (Sigma Aldrich, Castle Hill, NSW) particles were spray dried from a dispersion in a 2:1 ratio (*w*/*w*) dispersed in 60°C deionized water using a high shear mixer (Silverson, J L Lennard Pty. LTD, Sydney NSW). β-Cyclodextrin (Sigma Aldrich, Castle Hill, NSW) particles were spray dried from solution.

Isothermal Microcalorimetry

Isothermal microcalorimetry measurements were conducted using a Thermal Activity Monitor III (TAM III) (TA Instruments, Sollentuna, Sweden). This non-destructive technique allows accurate measurement of temperature changes in the sample through ultra-sensitive thermoelectric elements, which convert temperature differences to electric voltage differences. One side of the sensor contacts the sample ampoule, while an empty reference ampoule is maintained at a constant temperature by a controlled heat sink [22]. Precise temperature control (±0.0001°C), ensures that any change in heat from the sample due to physical or chemical reactions will produce a temperature gradient across the sensor resulting in a proportional measurable voltage. This change in voltage is then reported as heat flow. The technique is non-specific, therefore prior knowledge of the expected reaction(s) or post-analysis of the samples can aid interpretation of the reactions/changes occurring within the formulation.

TAM samples were prepared using a previously published procedure [21]. Briefly, pMDI canisters (20 mL Bespak Cans, Norfolk, UK) were cleaned with ethanol and DI water, fitted with metering valves (Valois DF30plus, Aptar Pharma, Tokyo, Japan), and pressure filled with HFA 134a (Fluor Ineos United, Cheshire, UK) using a hand pressure filler (Pamasol Willi Mäder AG, Pfäffikon Switzerland). Filled canisters and 5 mL transfer syringes (protected from moisture) were chilled for 30 min, while ampoules were chilled for 10 min. After the chilling step, particle samples were added to the ampoules followed by a known amount of deionized water when applicable, and finally filled with ~4 ml of HFA 134a. Ampoules were prepared in a dry environment (RH of 0–5%) to limit moisture exposure. The filled ampoules were sealed and equilibrated for 5 min in a water bath at 25°C, equilibrated in the TAM III for 15 min and lowered into the measurement position. Samples were measured for > 24 h at 25°C and all ampoules were weighed upon completion of the experiment to determine propellant and particle sample weights.

The 15 min equilibration step in the TAM III for measurement of spray dried glycopyrrolate particles was bypassed due to the rapid crystallization of glycopyrrolate when formulated in HFA 134a. Due to the abnormal measurement conditions, blank HFA 134a and raw crystalline glycopyrrolate

Table I API Spray Drying Parameters

Excipient	GP Mass fraction	Solid loading	Inlet temperature	Outlet temperature	Atomizer flow	Liquid feed rate	Aspiration flow
None	100%	20 mg/ml	88°C	58–60°C	360 NL/h	2.5 ml/min	35 m^3/h
DSPC-$CaCl_2$	0%	10 mg/ml	85°C	58–60°C	360 NL/h	2.5 ml/min	35 m^3/h
DSPC-$CaCl_2$	20%	10 mg/ml	85°C	58–60°C	360 NL/h	2.5 ml/min	35 m^3/h
βCD	0%	10 mg/ml	105°C	60–61°C	470–540 NL/h	3 ml/min	35 m^3/h
βCD	20%	10 mg/ml	105°C	60–61°C	470–540 NL/h	3 ml/min	35 m^3/h

samples were measured concurrently and exposed to the same equilibration parameters for proper baseline subtraction and comparison.

X-Ray Powder Diffraction

X-ray Powder diffraction (XRPD) 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. Amorphous glycopyrrolate was evaluated using a Skyscan 6000 (Siemens, VIC Australia) fitted with a nitrogen stage. This setup was used to limit moisture exposure during measurement, which can greatly affect the sample. Samples of measured TAM formulations were recovered by allowing the propellant to evaporate from the ampoule (after carefully chilling and opening ampoules) in a dry box kept below 10% RH. Samples were stored desiccated using silica gel (Chem-Supply, SA Australia) until measurement.

Scanning Electron Microscopy

All SEM samples were prepared in a dry environment (RH < 10%). Sample stubs were coated with 15 nm of gold for 2 min and imaged using a Zeiss Evo Quemscan microscope (Zeiss, Carl Zeiss Microscopy, Jena, Germany). Samples were evaluated using a beam current of 10 μA and voltage 15 kV. During the transfer of samples from preparation to coating, dry ice in a secondary container and desiccating agents were used to keep samples chilled and protected from environmental moisture. SEM samples were recovered similarly to those obtained for XRPD analysis.

Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FTIR) spectroscopy samples were prepared using an attenuated total reflectance (ATR) crystal method on a Nicolet 6700 FTIR (ThermoFisher Scientific, MA, USA). The ATR crystal was maintained in an enclosed glove bag and purged with nitrogen to ensure samples were kept below 5% RH during the measurement. This was done to prevent crystallization of the amorphous glycopyrrolate during measurement.

RESULTS AND DISCUSSIONS

Amorphous Glycopyrrolate

Spray dried glycopyrrolate particles were spherical, 2–5 μm in diameter (Fig. 1b) and amorphous (Fig. 2b). The loss of sphericity in some samples may suggest that amorphous glycopyrrolate particles were unstable and started to coalesce (Fig. 1b inset). This likely occurs from exposure to ambient

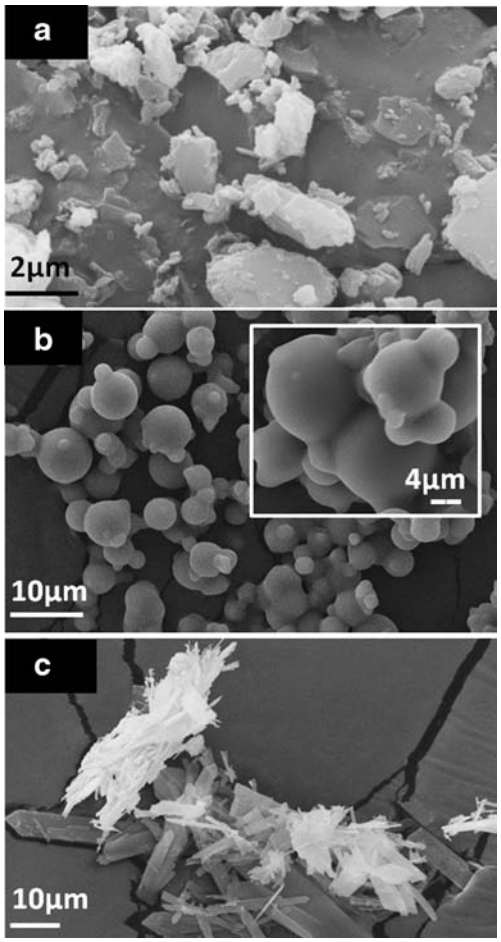

Fig. 1 SEM images of (**a**) micronized crystalline glycopyrrolate source, (**b**) spherical amorphous spray dried glycopyrrolate, and (**c**) crystalline glycopyrrolate recovered from measured amorphous GP-HFA 134a formulations.

conditions during SEM sample preparation. Nonetheless, when adequately protected from moisture the glycopyrrolate remains amorphous, conveyed by XRPD analysis (Fig. 2b).

Amorphous glycopyrrolate experienced a large exothermic change within the first couple hours when formulated in HFA 134a (Fig. 3b). The exotherm was confirmed by XRPD analysis to be the effect of glycopyrrolate crystallization (Fig. 2c). When exposed to the propellant, the spherical amorphous glycopyrrolate particles transformed into elongated crystals (Fig. 1c), while crystalline glycopyrrolate remained stable in the propellant, evident by the absence of a change in heat flow (Fig. 3a). In the amorphous formulation, the minima and maxima of the heat profiles (Fig. 3b) represent the dissolution (endothermic signal, indicated by a solid arrow) and crystallization (exothermic signal, indicated by dashed arrows) of the spray dried particles in HFA 134a. Multiple large peaks were present within the first 3 h because glycopyrrolate was added in excess (related to the solubility), resulting in overlapped cycles of dissolution and crystallization.

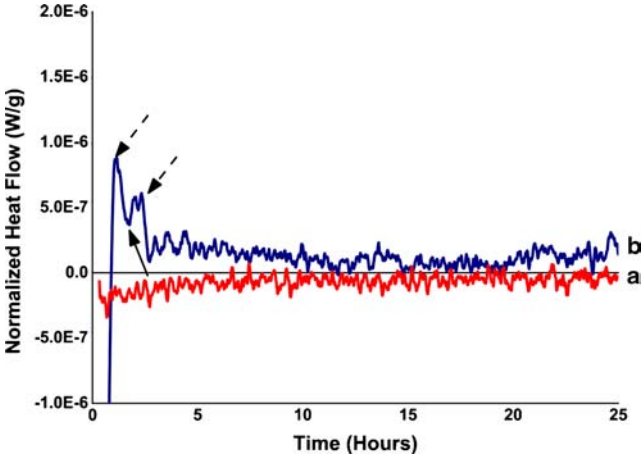

Fig. 3 TAM III normalized heat flow signal for various formulations. (**a**) Crystalline glycopyrrolate and HFA 134a. (**b**) Amorphous glycopyrrolate and HFA 134a. Note: The heat flow for crystalline glycopyrrolate/HFA 134a is identical to the baseline HFA 134a only.

Effect of Excipients

Co-spray dried DSPC/$CaCl_2$-GP particles were unstable in propellant as indicated by the large exothermic peak (Fig. 4). Conversely, DSPC/$CaCl_2$ particles without glycopyrrolate were stable in propellant as shown by the steady heat flow (Fig. 4) and SEM images of the recovered particles (Fig. 5a and b). The instability of the co-spray dried particles was thus due to the amorphous glycopyrrolate. Analysis of the DSPC/$CaCl_2$-GP particles recovered from the measured propellant formulations showed that glycopyrrolate crystallized out of the particles and formed elongated crystals joining several particles (Fig. 5d). XRPD analysis confirms the presence of crystalline glycopyrrolate post propellant exposure (Fig. 6c). The lack of large peaks in the XRPD pattern for the crystallized particles is likely attributed to the possibly incomplete recrystallization and the minimal mass of crystalline glycopyrrolate relative to DSPC in the particles.

Unlike the DSPC/$CaCl_2$-GP particles, measured heat flows for βCD alone and co-spray dried βCD-GP particles were identical to the expected baseline reading, indicating that βCD based formulations were stable in propellant (Fig. 7). SEM images of the recovered particles confirmed that βCD-GP and βCD particles remained unchanged after exposure to HFA 134a (Fig. 8). Cyclodextrins have been shown to prevent crystallization and polymeric transitions, for several solid state amorphous spray-dried drug formulations [23, 24], which may translate to particles formulated in HFA 134a.

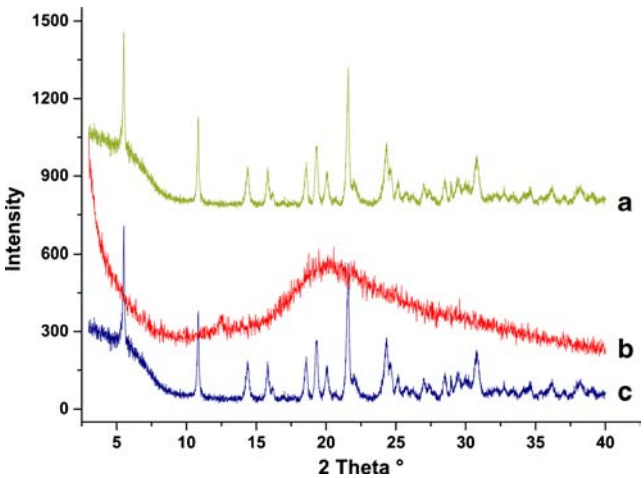

Fig. 2 XRPD patterns of (**a**) micronized crystalline glycopyrrolate source, (**b**) amorphous spray dried glycopyrrolate, and (**c**) crystalline glycopyrrolate recovered from measured amorphous GP-HFA 134a formulations.

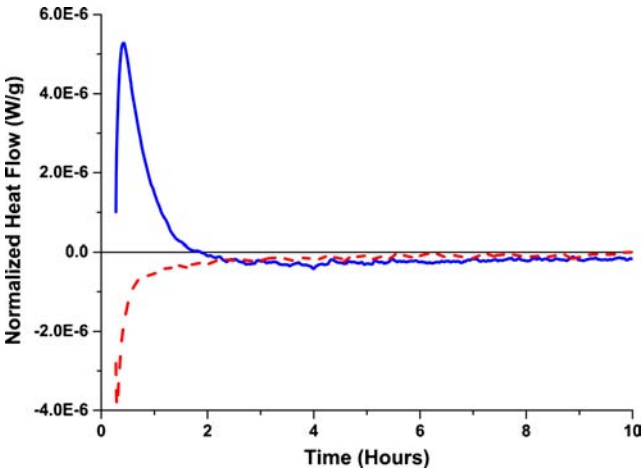

Fig. 4 TAM III normalized heat flow signal for HFA 134a-based formulations. Stable DSPC/$CaCl_2$ particles (*dashed line*) and unstable co-spray dried DSPC/$CaCl_2$-GP particles (*solid line*).

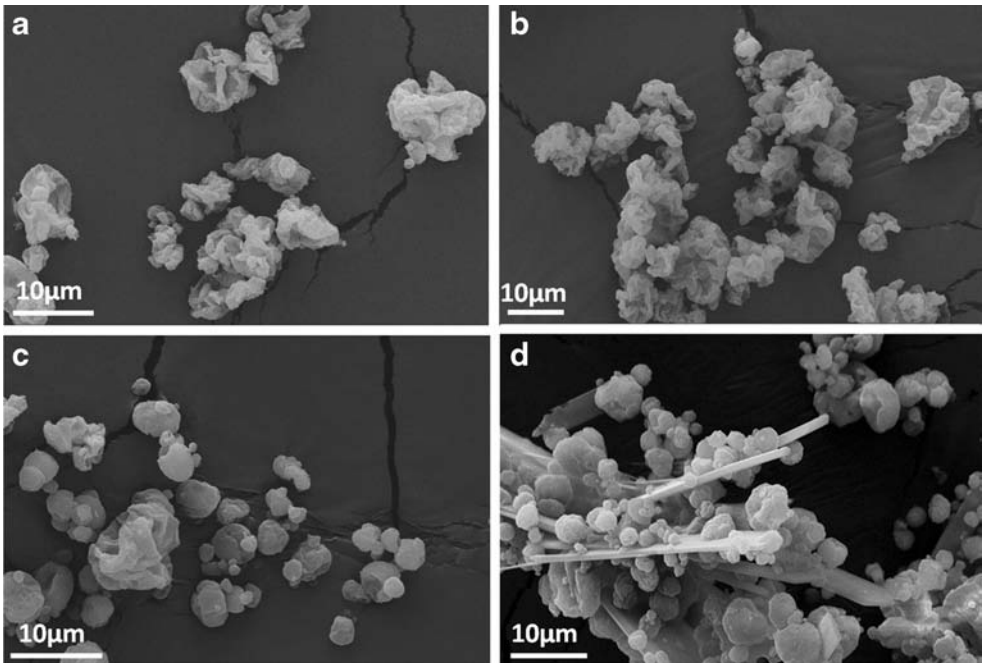

Fig. 5 SEM images of (**a**) spray dried DSPC/$CaCl_2$, (**b**) spray dried DSPC/$CaCl_2$ recovered from the measured HFA 134a formulation, (**c**) co-spray dried DSPC/$CaCl_2$-GP and (**d**) co-spray dried DSPC/$CaCl_2$-GP recovered from the measured HFA 134a formulation.

The stability afforded by cyclodextrins is due to the formation of drug-inclusion complexes [25, 26]. FTIR spectral analysis (1,200–1,900 cm^{-1} region) of a physical mixture and spray dried βCD-GP support the formation of an inclusion complex (Fig. 9). The bands corresponding to the carbonyl group of glycopyrrolate in the amorphous sample and physical mixture are at 1,730 cm^{-1}, but in the spray dried material is shifted to 1,736 cm^{-1}. This shift suggests intermolecular hydrogen bonding between the encapsulated glycopyrrolate and β-cyclodextrin [27, 28]. Previous literature also supports the formation of intermolecular hydrogen bonding between the multiple components [29] and between the secondary OH groups of β-cyclodextrin [30] in spray dried formulations. One mechanism for formation of this complex is the release of enthalpy-rich water molecules from the cyclodextrin cavity, which allows replacement with a less polar guest/drug molecule [31, 32]. During spray drying of βCD-GP particles, water molecules are likely to be forcibly displaced from the cyclodextrin molecules, providing a significant driving force for the formation of cyclodextrin-drug complexes. Additionally several other forces may be important in the possible complex formation including van der Waal interactions, hydrophobic

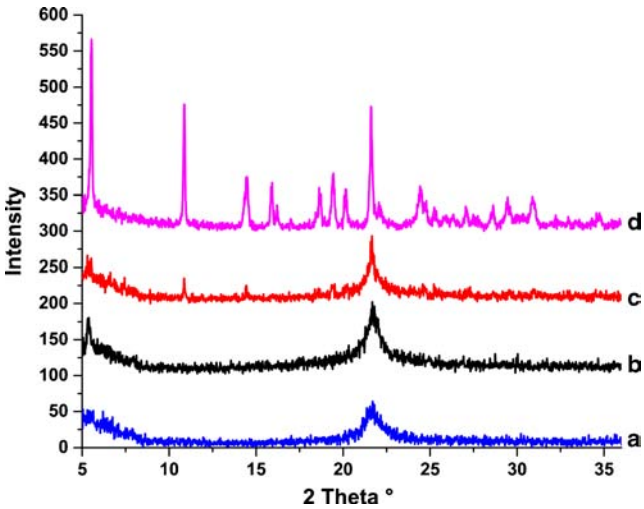

Fig. 6 XRPD patterns of (**a**) spray dried DSPC/$CaCl_2$, (**b**) co-spray dried DSPC/$CaCl_2$-GP, (**c**) co-spray dried DSPC/$CaCl_2$-GP recovered from the measured HFA 134a formulation, and (**d**) crystalline glycopyrrolate source material.

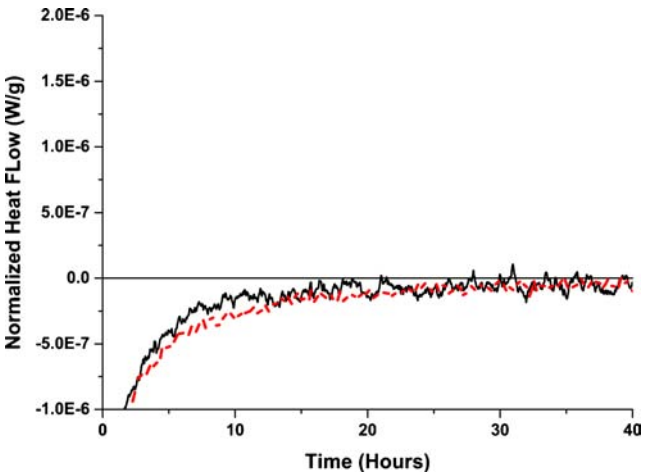

Fig. 7 Normalized heat flow of spray dried particles in HFA 134a. β-cyclodextrin only (*solid line*) and β-cyclodextrin with glycopyrrolate (*dashed line*).

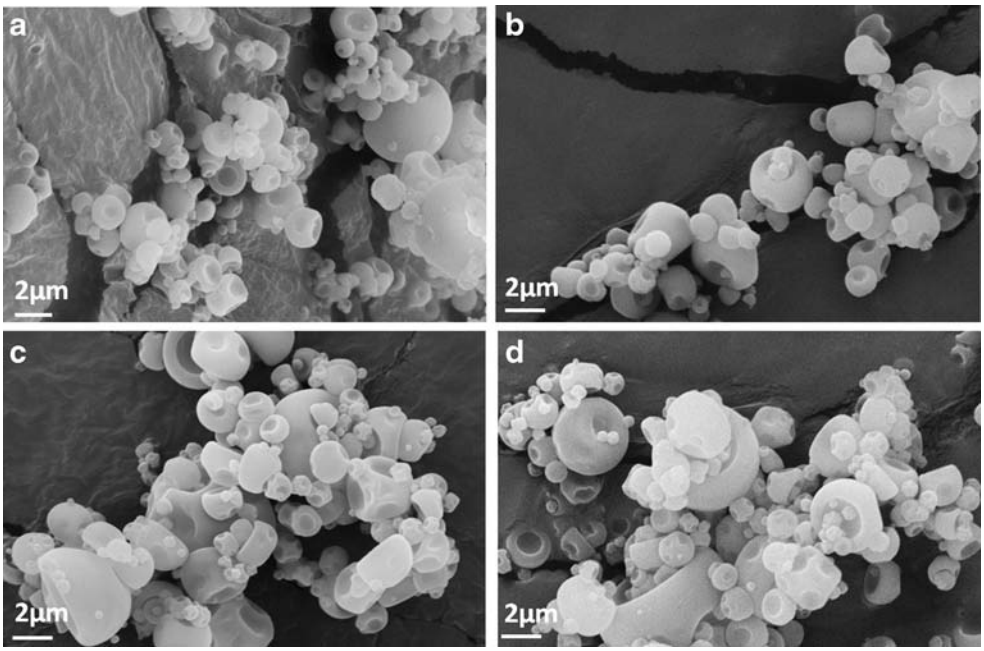

Fig. 8 SEM Images of (**a**) spray dried βCD, (**b**) spray dried βCD recovered from measured HFA 134a formulation, (**c**) co-spray dried βCD-GP, and (**d**) co-spray dried βCD-GP recovered from measured HFA 134a formulation.

interactions, ring strain release, and changes in solvent-surface tensions either in solution or during spray drying. The inclusion complex formation protects the amorphous glycopyrrolate, effectively inhibiting its dissolution into the propellant and subsequent crystallization.

Effect of Water Content

Although formulations may initially appear stable, product shelf life and the effect of storage factors such as water ingress are major concerns for product development. pMDI formulations containing HFA 134a can absorb up to 700 ppm of water, depending on storage conditions [18, 33]. Initial testing

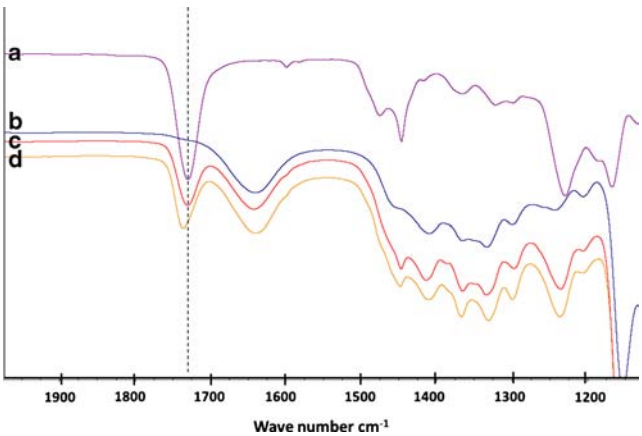

Fig. 9 FTIR spectra of (**a**) amorphous GP, (**b**) spray dried βCD, (**c**) physical mixture βCD-GP, (**d**) spray dried βCD-GP.

with 400 ppm of moisture added to the sample resulted in exothermic heat flows for both initially stable βCD and co-spray dried βCD-GP formulations (Fig. 10a). The different heat profiles of the two formulations are likely due to varying process kinetics. The βCD-only particles rapidly absorb water molecules and coalesce (Fig. 10b), which produces a nearly symmetrical exotherm (minimal tailing) (Fig. 10a). However, the heat changes in the co-spray dried βCD-GP formulation occur more gradually, resulting in a positively skewed exotherm (large tailing) (Fig. 10a). The skewed profile is likely due to the overlap of multiple reactions occurring within the formulation since two components (βCD and amorphous glycopyrrolate) are affected by water. The water molecules absorbed by the particles will first affect the protective structure of the particle, which may expose the encapsulated amorphous glycopyrrolate to the propellant. Once exposed to the propellant, glycopyrrolate is expected to rapidly crystallize (Fig. 3a). However, XRPD analysis of the recovered co-spray dried βCD-GP particles fails to convey that glycopyrrolate crystallized out of the particles; instead measured diffraction peaks are more consistent with crystalline β-cyclodextrin (Fig. 11). Furthermore, glycopyrrolate crystals [like those found in samples recovered from the unstable amorphous GP-HFA 134a (Fig. 1c) and DSPC/$CaCl_2$-GP (Fig. 5d) formulations] were not present in the recovered co-spray dried βCD-GP samples (Fig. 11c), suggesting that the amorphous glycopyrrolate did not crystallize in the formulation. This occurs because βCDs may prevent nucleation of the drug by altering bulk properties such as saturation

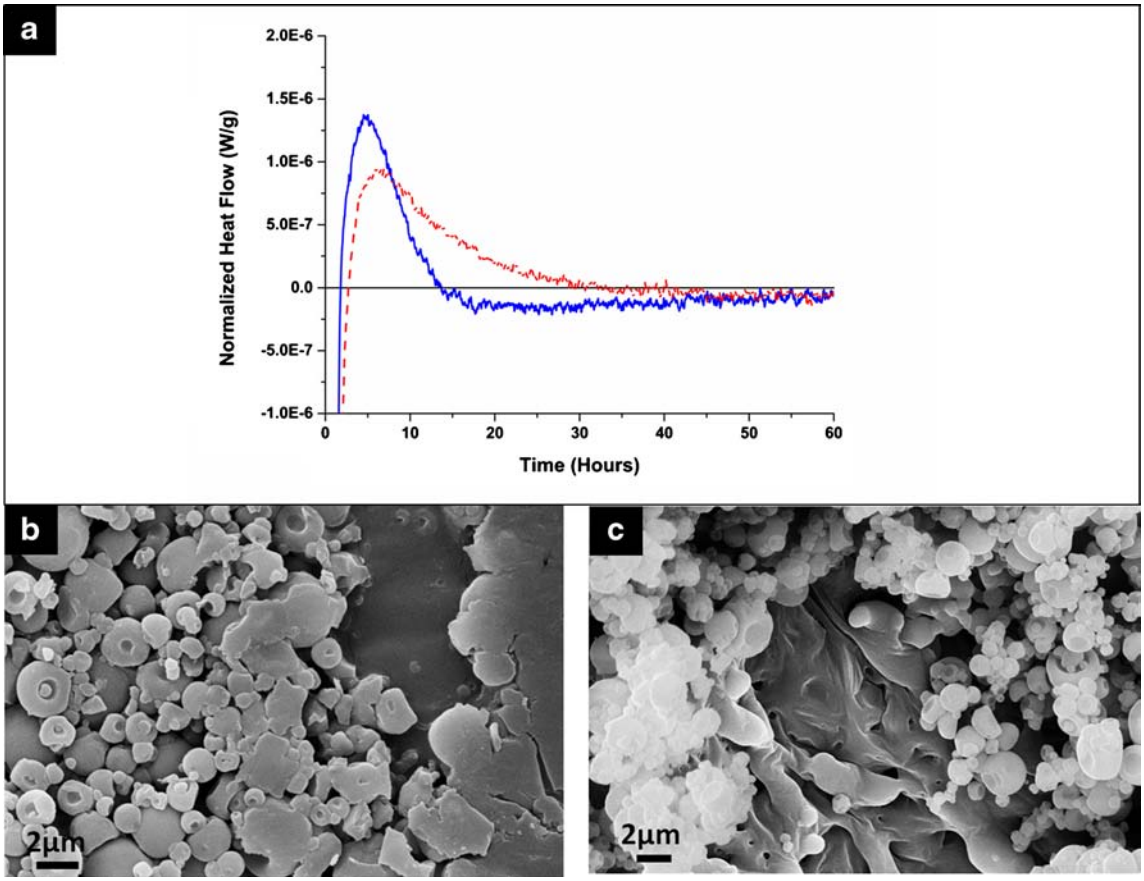

Fig. 10 Effect of water content on formulation stability. (**a**) Normalized heat flow of HFA 134a formulations with 400 ppm water; spray dried βCD [*solid line*] and co-spray dried βCD-GP [*dashed line*]. (**b**) SEM image of spray dried βCD particles exposed to HFA 134a containing 400 ppm water. (**c**) SEM image of co-spray dried βCD-GP particles exposed to HFA 134a containing 400 ppm water.

solubilities, surface tension and cohesion [34]. Additionally, they can prevent crystallization even in a super-saturated solution by selectively adsorbing into growth layers and surface imperfections, disrupting and blocking crystal growth [35]. Thus, the exothermic heat flow was not an effect of amorphous glycopyrrolate crystallization but rather βCD crystallization, morphological changes, and various other water-particle interactions. Nonetheless, the formulation is not stable when exposed to propellant containing 400 ppm of water; and thus actions should be taken to prevent such moisture exposure.

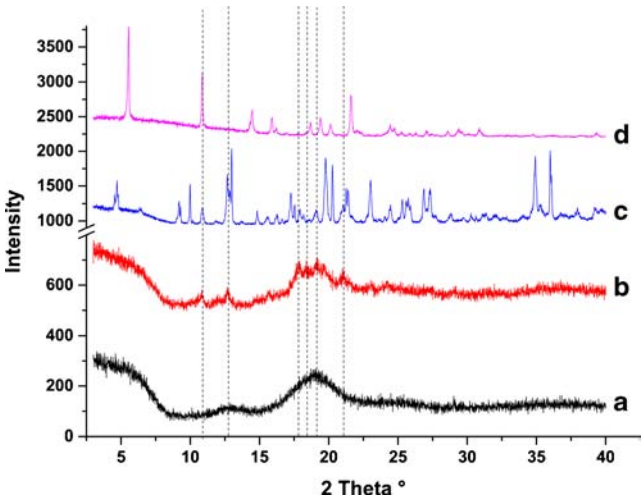

Fig. 11 XRPD patterns of (**a**) co-spray dried amorphous βCD-GP particles, (**b**) βCD-GP particles recovered after exposure to HFA 134a containing 400 ppm water, (**c**) crystalline βCD source material, and (**d**) crystalline glycopyrrolate.

To limit moisture exposure, several preventative storage measures (desiccants) and appropriate packaging can be used. As such, the effects of various water contents on β-cyclodextrin formulations were also studied to determine the maximum water content in which the particles remained stable. Above 100 ppm, the maximum heat flows increased as a function of water content while below 100 ppm the heat flows remained at the baseline (Fig. 12a). The larger exothermic outputs correspond to greater morphological changes in the particles (Fig. 12b and c). Thus, a formulation containing 800 ppm of water experienced significantly greater particle agglomeration and morphological changes

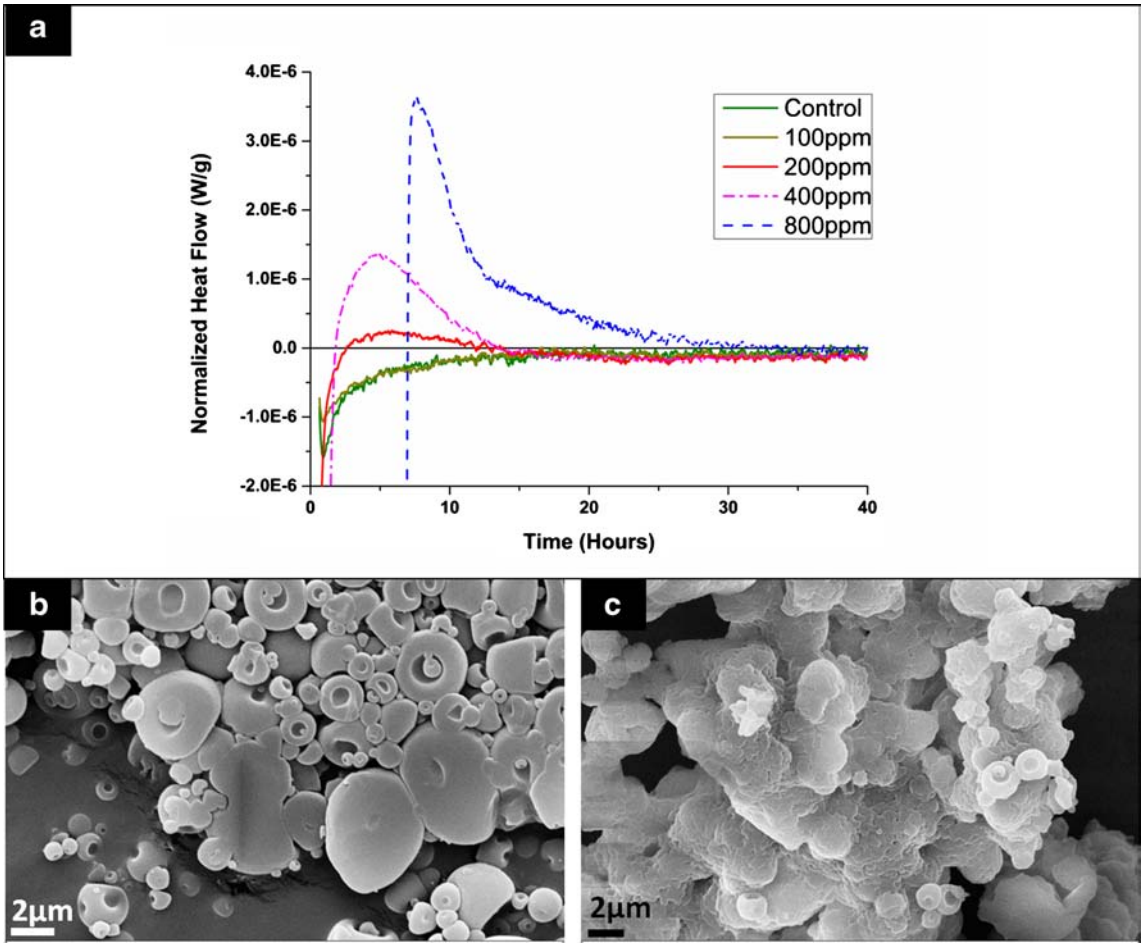

Fig. 12 (**a**) Normalized heat flow for formulations with an added 800 ppm, 400 ppm, 200 ppm, and 100 ppm of water compared to a control. [Depicted top down in decreasing amounts of water]. (**b**) SEM image of spray dried βCD particles exposed to HFA 134a containing 400 ppm water. (**c**) SEM image of spray dried βCD particles exposed to HFA 134a containing 800 ppm water.

compared to 400 ppm, while a formulation containing 100 ppm remained stable.

CONCLUSION

We have demonstrated the effectiveness of isothermal microcalorimetry as a rapid screening tool to evaluate formulation parameters such as amorphous form, excipient composition and water content, all of which are important to assess during pMDI product development. We determined that amorphous glycopyrrolate crystallized when formulated alone or co-spray dried with DSPC/$CaCl_2$ in HFA 134a. However, when co-spray dried with βCD the particles remained physically stable at low water contents (≤100 ppm). Furthermore, increasing the water content above 100 ppm resulted in particle agglomeration and morphological changes, which suggest that such formulations require appropriate packaging to protect from moisture ingress. The quick measurement time and high sensitivity of isothermal microcalorimetry make it a useful technique for formulation scientists to rapidly develop pMDI formulations.

ACKNOWLEDGEMENTS AND DISCLOSURES

The authors gratefully acknowledge [1] the financial support of the Australian Research Council (DP120102778) and [2] 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 a recipient of the Australian IPRS and APA scholarships.

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