Determination of Physical and Chemical Stability in Pressurised Metered Dose Inhalers (MDIs): Potential New Techniques

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Abstract

The pressurised metered dose inhaler (pMDI) is one of the oldest and most commonly prescribed therapeutic systems for drug delivery to the lung. pMDIs are subject to rigorous physical and chemical stability tests during formulation and prior to commercial approval. Due to the time and cost associated with formulation and product development studies, there is a need, especially within an industrial setting, for novel techniques that allow fast screening of new formulations in terms of physical and chemical (physico-chemical) stability. The key problem with achieving this goal is in the nature of pMDI formulations. While conventional intravenous, oral and topical formulations are in a solid-state at STP, pMDIs are by their definition, pressurised, making the direct observation of physico-chemical properties in situ, difficult.

Areas covered

This review highlights the state-of-the-art techniques and physico-chemical characterisation tools that can potentially enhance the formulation and product development process for pMDIs. Techniques investigated include: laser diffraction, Raman spectroscopy, isothermal ampoule calorimetry, titration calorimetry and gas perfusion calorimetry. These are discussed in the context of pharmaceutical development, with a focus on their use for the determination of the physical and chemical stability in pMDI delivery systems. The operational principles behind each technique are briefly discussed and complemented with examples from the literature. The strengths and weaknesses of the above techniques are highlighted with the purpose of guiding the reader to identify the most promising technique.

Expert opinion

Each technique has a unique set of advantages and disadvantages. Laser diffraction is theoretically well placed to analyse real-time physical stability as a function of particle size, however its use is restricted to suspension MDI formulations. Raman spectroscopy requires little sample preparation and can be potentially used to attain both suspension and solution pMDI spectra in real time, however the majority of experiments are ex-valve chemical composition mapping. The next logical step in the development of Raman spectroscopy for online pMDI monitoring is to begin in situ solid state analysis. Calorimetry is an effective technique in capturing both chemical and physical degradation of APIs in real time but requires redevelopment to withstand pressure for the purposes of pMDI screening. A combination of ampoule and gas perfusion calorimetry has the most potential to fast-screen for drug-propellant compatibility within a commercial environment.

Abbreviations

ACI	Anderson cascade impactor
ANDAs	Abbreviated new drug applications
Arcton-113	1,1,2-tri-chloro-tri-fluoro-ethane
CFC	Chlorofluorocarbon
CFC-11	Trichlorofluoromethane
DSC	Differential Scanning Calorimetry
EtOH	Ethanol
FDA	Food and Drug Administration
FPF	Fine particle fraction
FT	Fourier transform
He-Ne	Helium-neon
HFA	Hydrofluoroalkane
HFA-134a	1,1,1,2-tetrafluoroethane
HFA-227	1,1,1,2,3,3,3-heptafluoropropane
HPLC	High performance liquid chromatography
IR	Infrared
ITC	Isothermal titration calorimetry
MS	Mass spectroscopy
NDAs	New drug applications
O-H	Oxygen-hydrogen
RH	Relative humidity
pMDI	Pressurised metered dose inhaler
SI	International System of Units
STP	Standard Temperature and Pressure
TAM	Thermal Activity Monitor
UV	Ultraviolet

1. Introduction

The pressurised metered dose inhaler (pMDI) is one of the oldest and most commonly prescribed therapeutic systems for drug delivery to the lungs [1]. The popularity of this drug delivery system can be appreciated when observing its scale of growth in the global drug delivery market. The pMDI sector is expected to account for 59% of the global market, worth $22 billion, for drug delivery systems by 2017 [2]. The development of the first commercial pMDI began in 1955 by Riker Laboratories (now 3M Pharmaceuticals, St Paul, Minnesota) [3] and the original Medihaler design had key features (metering valve, canister, actuator orifice and mouthpiece) which are still present in modern devices [1]. Since the original introduction in 1955, pMDI formulations and inhaler designs have evolved to solve technical problems, notably patients' uncoordinated inhalation technique [4], high oropharangeal deposition, addition of dose counters [5] and the replacement of chlorofluorocarbon (CFC) with more 'ozone friendly' hydrofluoroalkane (HFA) propellants [6].

The phasing out of CFC propellants in medical devices actively began in 1998 and was a slow, gradual process. The last two CFC inhalers, Combivent Inhalation Aerosol (Boehringer Ingelheim) and the Maxair Autohaler (Medicis Pharmaceutical Corporation) were taken off the US market in July 2013 and December 2013, respectively [7]. Advancements in pMDI technology have focused on the delivery of drug with greater efficiency (including dosing reproducibility, delivering combination therapy and formulating proteins/peptides into pMDIs) and improving valve, elastomer and gasket design [8-10]. A list of Food and Drug Administration (FDA) approved products containing HFA, which are currently marketed in the US, is shown in Table 2.

Drugs are formulated in either solution or suspension (Table 2). Drugs that exhibit a small degree of solubility in HFA (i.e. have high enough solubility to be at risk from Ostwald ripening) are solubilised with co-solvents (usually ethanol) and formulated in solution. Drugs that are insoluble in HFA and ethanol co-solvent systems are prepared as a micron-sized suspension in HFA[11]. In order to reach the lung successfully, aerosols from pMDIs must generate particles of an appropriate size for their target. For conducting airway deposition this is generally agreed to be between 3-6 μm and for small airways < 3 μm [11, 12]. The reproducibility of emitted dose and aerodynamic endpoints of the product are the primary outcomes for successful formulation and are critically dependent upon the physical stability

of the active drug within the formulation. It is well known that suspension pMDI formulations have the propensity to undergo either size-specific particle growth (Ostwald ripening), especially if the drug has a small degree of solubility in HFA, or agglomeration upon settling (flocculation and sediment compaction). These phenomena lead to variation in emitted dose and aerosol size distribution, which will be detrimental to aerosol performance. An excellent discussion on particle growth mechanisms in pulmonary dispersion formulations is provided in a recent review article by O'Donnell *et al* [13].

The chemical stability of drugs in HFA is a further drug-specific issue, which must be thoroughly screened. For example, the phenylalkylamino derivatives (eg. formoterol, salbutamol) are prone to degradation due to their susceptibility to oxidative conditions [14]. Chemical instability is more likely to affect solution MDI products however [15], as degradation rates in the solid state are generally orders of magnitude slower than in solution. Furthermore, since water is likely to be present during the formulation process or during storage (via water ingression) [16], hydrolytic degradation is a possibility [15]. Polymorphic screening of the active pharmaceutical ingredient must be performed as different polymorphic forms have the potential to influence the performance and stability of the final product [17].

1.1 Existing Basic Testing Requirements for pMDI stability

The chemical and physical properties of the drug determined prior to submission to the FDA include density, particle size distribution, particle morphology, solvates and hydrates, polymorphs, amorphous form, solubility profiles, moisture and residual solvent content, microbial quality, dissociation constants and specific rotation of the drug. Assays to detect impurities or degradation products are commonly performed, with liquid or gas chromatography, and are either compared with products spiked with known impurities or samples stored under relevant stress conditions (light, heat, humidity, acid/base hydrolysis and oxidation) [18]. Peak purity tests (diode array, mass spectroscopy) are typically included in the latter case as impurity or degradation standards are not available in this situation [18]. For suspension formulations, tests to determine the particle size distribution and physical properties (shape, crystal habit, morphology, surface texture) of the active are also essential, as these parameters are fundamental for drug product reproducibility and performance. Assays for preservatives or stabilisers are also included in the submission documentation, as

well as associated microbiological tests to determine if the formulation is susceptible to microbial contamination and colonisation.

Stability testing is a crucial step to identify likely degradation products under the influence of common stresses (temperature, humidity and light) and so by extension to assess the intrinsic stability of the drug. A detailed stability protocol, based on ICH guidelines, is also required for a minimum of 6 months for intermediate and accelerated storage conditions, and 12 months at long term stability studies prior to the submission to the FDA. Ongoing re-tests intended for long-term ambient temperature and humidity studies are also conducted until the point of product expiry, or re-testing at the 12 month mark for products which exhibit instability during accelerated and intermediate studies [18]. Stress testing is often repeated when manufacturing processes and analytical processes are refined [19].

The analytical procedures to determine product acceptability for submission to the FDA and ongoing stability studies include ultraviolet (UV) and infrared (IR) spectroscopy studies, high performance liquid chromatography/mass spectroscopy (HPLC/MS), tests for identification of the active drug, concentration assays and chirality assays to detect racemization if applicable [20]. The preferred method to detect degradation products is through reverse-phase HPLC (with a UV detector or coupled to MS), as it is compatible with aqueous and organic solutions, highly precise and sensitive. Samples held under stress conditions are commonly screened with gradient method HPLC with varying mobile phase composition (usually low organic to high organic solvent) to assess the elution pattern of degradation products and/or related substances [21].

Arguably, several limitations exist for conventional stability testing. Conventional techniques for determining drug degradation rates rely on product storage at elevated temperatures to accelerate the process. In conducting these trials, it is assumed that the degradation process follows the Arrhenius relationship [22]. This may not be true as drug could undergo a phase transition [23], moisture redistribution or a change in reaction mechanism. Consequently there may be two or more reactions occurring simultaneously [24], or there may be alternative decomposition mechanisms that are activated only at high temperatures. For example, hydrate drugs or compounds that contain unbound water are prone to losing water at elevated temperatures and therefore will display different degradation mechanisms at these temperatures [25]. Extrapolation to determine rate constants would most likely result in error in calculating stability at ambient conditions. Other limitations include the sampling frequency;

if degradation plots have significant curvature or scatter, three or four data points may be insufficient to determine linear fit of data. Thus many time points are required.

In addition to the aforementioned limitations, current techniques are dependent upon the chosen sampling time within the ICH guidelines. The compatibility of the drug as a solution or suspension in HFA is virtually unknown until the time of testing. Real-time analysis of degradation and incompatibility would quicken product development and increase data contribution to the Quality by Design (QbD) approach. There is an industrial need for novel techniques that are capable of online monitoring and that could screen the compatibility of new compounds/formulations with HFA propellant rapidly. This would prevent potentially flawed products undergoing lengthy and costly stability studies. The subsequent sections of this review will discuss techniques used to detect solid-state stability, and stability of drug in both solution and suspension pMDIs.

2. Rapid screening approaches for MDI stability

2.1 Laser diffraction

2.1.1 Laser Diffraction – ex valve measurement

Laser diffraction is a standard method in particle sizing and is used commonly in the pharmaceutical industry due to its short analytical time, reproducibility and high precision. Briefly, laser diffraction measures particle size distributions by measuring the angular scattering of light as it passes through a dispersed particulate sample. The changes in laser projection after the particle interaction are complex, and vary as a function of the scattering angle, particle size and shape and refractive index. The resultant diffraction pattern is converted into a volume-based size, derived using mathematical algorithms, normally the Lorenz-Mie theory of light scattering or the simplified Fraunhofer approximation [26, 27]. The reader is directed to the ISO standard for further discussion on the operation principles of laser diffraction and information regarding the Mie theory and the Fraunhofer approximation [28].

Several research groups have investigated the *in situ* geometric growth of crystalline drugs suspended in HFA propellant and the relation to aerosolisation and product stability post actuation using laser diffraction systems. Smyth *et al.* investigated the effects of different ratios of EtOH:HFA-134a and the subsequent effects on geometric and aerodynamic particle size using both laser diffraction and an Anderson cascade impactor (ACI) [29]. The fine particle fractions (FPF) derived from both techniques were in striking agreement, although marked

differences in the raw values for mass median diameter were shown. Similarly, a study by Haynes *et al.* highlighted the need to develop further the existing Sympatec unit to achieve closer agreement with ACI data [27]. Berry *et al.* have previously established the link between increasing geometric particle size, measured as an aerosolised dry powder with a Sympatec HELOS compact laser diffraction system, with increased aerodynamic particle size distribution [30]. This study was conducted on different production batches of corticosteroid material and only the influence of geometric particle size upon aerodynamic particle size diameter was assessed. Furthermore, sample pMDI formulations containing larger geometric-sized particles were shown to be less stable under temperature cycling conditions, as particles shifted towards a larger aerodynamic particle size and decreased stage recovery.

These laser diffraction methods have usually been conducted 'ex valve'. However, to investigate particle size and stability inside the canister extensive modification of the particle size instrument is required to allow pressurized measurement.

2.1.2 In situ Laser Diffraction – in situ measurement in propellant

Jones *et al.* also manufactured two types of pressure cells designed to be used with a Mastersizer X laser diffraction instrument (Malvern Instruments, Inc., Southborough, MA) [31]. The first type of pressure cell was a single sealed unit consisting of a plastic cell with two optical borosilicate glass surfaces through which powder was first placed inside the unit and HFA 134a propellant later filled through a specially adapted valve. The second unit was a pressurized cell manufactured by Malvern instruments, which incorporated a re-circulatory system and filling apparatus. Two methods of recirculation were used in the measurement of the particle size distribution in HFA 134a suspension; continuous recirculation or stop flow circulation. The novel pressure cell sizing system showed a linear correlation with twin-stage impactor results ($r^2 = 0.8894$; n=10) for particles < 6.4 µm for 8 novel HFA based pMDIs and 2 commercial HFA pMDIs. The degree of shear applied to the re-circulation system was a source of potential measurement error; if the degree of shear supplied was insufficient there was a tendency to oversize the particles and likewise a process of over-shearing resulted in the under sizing of particles. The authors obtained a strong correlation between laser diffraction and impaction results with method optimization.

In summary the *in situ* laser diffraction method is well positioned to analyse the real-time physical stability of suspended micro particles in HFA as a function of particle size. Laser

diffraction is reliable and capable of ongoing and online monitoring. Of significant importance is the ability to use "real propellants" to mirror the drug stability in a commercial product and its use can be expanded to study propellant combinations, titrations of surfactant quantities or propellant-ethanol combinations. However laser light scattering also suffers from a number of disadvantages, limiting its more widespread use in stability studies. Ex-valve laser diffraction measurements are prone to beam steering as a result of propellant vapour evaporation during the measurement. Beam steering may lead to changes in the refractive index of the gas phase within the spray, which result in the overestimation of the particle size distribution. The laser diffraction method for in situ pMDI particle size measurements is restricted to suspension formulations, which accounts for just over half of all currently marketable products in the US (Table 2). Whilst the technique is suited to detect fast-occuring physical processes that happen internally in the pMDI canister in the analysis time-frame, it is unsuited to detection of chemical processes, except in the situation of particle size changes because of solvate formation. The mathematical calculation of the volume based size measurement also assumes all the particles are spherical, whereas in reality crystal growth will more commonly follow a needle-like or plate-like morphology and display high axial ratio (length to width ratio) growth [32]. The measurement of anisotropic particles or irregular-shaped particles can lead to inaccuracies in the particle size distribution [33]. Although the accuracy of laser diffraction measurement is absolutely necessary for the correlation of geometric data to aerodynamic particle size or for submission purposes to the FDA, the application of a fast-screening method only calls for the detection of significant deviation from time point zero, so as to signal whether to progress with the formulation. "It is important to note that any in situ laser diffraction measurement method must first be optimized to ensure that particles are completely de-agglomerated to fully assess any changes in the primary particle size distribution that may occur as a result of Ostwald ripening. However it is also important to note that excessive shear in a re-circulation laser diffraction system may prevent agglomeration that may have occurred as a result of formulation instability. Suitable method development which reflects the level of shear stress inflicted by patient on the canister prior to actuation would be a pre-requisite prior to using this technique as an online monitoring tool. It is envisioned that further time-dependent ex-valve particle size distribution measurements or direct APSD measurements can be made once a formulation passes the initial in-situ stability screen."

2.2 The mechanism and application of Raman spectroscopy to MDIs

Raman spectroscopy is an established technique for pharmaceutical and biopharmaceutical applications and can provide a characteristic "fingerprint" of intramolecular vibrations, which can be used to identify the molecular species, composition, the degree of order and disorder, structure and conformation [34]. Intermolecular interactions can also be identified through Raman scattering. Raman spectroscopy is most sensitive to symmetrical covalent bonds, as strong Raman scattering occurs with a larger change in the polarisability of the distribution of the electron cloud. By extension, Raman spectroscopy can provide data on phases and phase transitions, hydrogen bonding, polymorphs, hydrates, anhydrates, molecular conformation, polymer chain confirmation [35, 36] and foreign particulate contamination [37]. "Raman may also have utility in the measurement of amorphous content in formulations with high sensitivity. Vehring *et al* has previously used dispersive Raman technique to identify mixtures of amorphous mannitol with a limit of quantification of 5% w/w [38]. The use of dispersive Raman spectroscopy in particular may be more sensitive than FT-Raman, as Raman scattering cross sections and charge coupled device detector sensitivity increase with lower excitation wavelengths.

Raman spectroscopy is also ideal for aqueous systems as highly polar bonds (eg. O – H bonds) have a weak vibration and low Raman scattering due to their low polarisabilityRaman is less susceptible to convolution by water compared with IR methods. Further discussion of the theoretical aspects of Raman scattering is provided by Ozaki *et al.* [39] and an excellent overview of the different types of Raman spectroscopy with respect to drug delivery systems is given by Mansour *et al.* [35].

Raman scattering has been used to measure size, composition and temperature gradients of drying droplets [34, 40-42] such as the evaporation of water from mixed water/glycerol droplets [43], ethanol/water droplets [41, 44, 45], methanol/water droplets [41], 1-propanol/water droplets [41], coagulation of droplets [46], phase transitions of inorganic salts particles [47, 48] and chemical reactions between binary droplets [45, 47]. Raman spectroscopy can be used to elucidate intermolecular interactions in the condensed phase, such as the hydrogen bonding that occurs in liquid water [49, 50].

Quinn *et al.* were the first to use Fourier Transform Raman (FT – Raman) spectroscopy as an analytical tool to assess lysozyme protein conformation in suspension formulations in the HFA propellants tetrafluoroethane (HFA-134a) and heptafluoropropane (HFA-227) [51]. Additionally, this study was the first to capture the background Raman spectra of both HFA-

134a and HFA-227 with excellent signal-to-noise ratio. Descriptions of vibrational modes (e.g. stretch, deformation) were assigned to particular wavenumber bands. The structural integrity of lysozyme in HFA-134a and HFA-227 was elucidated by subtracting the spectra of the blank propellants from the Raman spectra of the HFA – lysozyme suspensions. FT – Raman spectroscopy provided structural information of the lysozyme backbone, disulfide bonds and C-C stretching vibrations.

Hot-stage Raman spectroscopy has been used by Bouhroum *et al.* to study beclomethasone dipropionate – CFC-11 clathrate stability as a function of temperature [52]. The release of propellant CFC-11 from the clathrate structure was successfully captured by examining the wavenumber shift corresponding to the carbonyl peak of the clathrate.
Raman chemical imaging has been used to map the chemical composition of combination pMDI deposition patterns [53, 54]. For example, *Steele et al.*, studied the Raman shift of salbutamol and beclomethasone from a Ventide combination formulation on stages 3 and 5 of an Anderson Cascade impactor and correlated the data with conventional analytical methodologies [53]. Similarly, Raman chemical imaging of salmeterol xinafoate and fluticasone propionate from combination inhalers suggested interparticulate agglomeration and chemical synergy whilst budesonide/formoterol formulation spectra showed the distribution of the two drugs on the ACI to be separate [54].

Raman characterisation is also applicable to the detection of bioaerosols and could contribute to future screening techniques for microbial contamination of MDIs. Aerosolised aqueous suspensions of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium* have been examined using Surface-enhanced Raman spectroscopy (SERS) at concentrations as low as 10^3 cfu/mL [55]. SERS suppresses fluorescence, which originates from biomaterial fluorophores, and amplifies the Raman signal [56]. Recently Schwarzmeier *et al.* developed a label-free microarray readout based on SERS which was capable of detecting an *Escherichia coli* laced nebulizer suspension at a limit of detection of 144 organisms per cubic centimetre [57]. This detection method is beneficial and time saving, since the standard array methods are based on incubation on agar plates, impaction and filtration.

Theoretically, the use of Raman spectroscopy for the online monitoring of drug stability in inhalers confers a number of advantages. The Raman analysis sampling time is short and therefore would expedite formulation development and the QbD approach. Spectra can be

obtained non-invasively with little sample preparation, thus avoiding costs and errors due to preparation (eg. evaporation of propellant and grinding of drug sample which could lead to changes in solid state). The major disadvantage of Raman scattering is that it is a weak effect, as the incident photon is not absorbed and therefore is much smaller than the molecular disturbance for fluorescence or infrared [58]. Shifting the laser wavelength to the near infrared spectral region (800 – 2500 nm or 12500 to 4000 cm^{-1} wavenumber) can avoid the fluorescence overlap in most cases. The measurement container must also be transparent to visible or near-infrared laser light to minimize spectra interference.

2.3 Isothermal calorimetry

Calorimetry operates on the principle of detecting heat (a universal accompaniment to chemical and physical change). A material may change its physical form or chemical structure through the interaction with another species or as a result of environmental conditions. The measurement of this change is crucial to determining the time frame in which the transformation would occur. Measurement of the heat output from a process *(q,* SI units Joules*)* will give thermodynamic information, while the measurement of heat output with time (*dq*/*dt*) conveys kinetic information. Thermodynamics, which is the study and quantification of transformations of energy, is a core principle on which calorimetry is based. Thermodynamics is concerned with heat (*q*) and its relation to the two process-dependent terms: energy (*U*) and work (*w*), and macroscopic variables of volume, pressure and temperature associated with those systems. Further explanation of thermodynamics in relation to calorimetry is given by Gaisford and O'Neill [59, 60].

As heat is a universal accompaniment to chemical and physical change, calorimetry is well placed to study non-destructively long-term chemical and physical events such chemical degradation, ageing, recrystallisation or the formation of hydrates/solvates. The technique itself is sufficiently sensitive to allow detection of degradation directly under storage conditions, which is a major advantage when checking product viability. The sensitivity of isothermal calorimetry has been proven to be a useful method to confirm solid-state degredation rates. Its precision exceeds standard methods such as determination of degredation through time course chemical degredation studies followed by HPLC to determine activation energies [23, 25] and overcome limitations such as the need for a suitable chromophore and the requirement to measure in solution form [59]. Three types of isothermal

calorimetry instrumentation are discussed in the sections below: ampoule calorimetry, titration calorimetry and gas perfusion calorimetry.

2.3.1 Ampoule calorimetry

Ampoule calorimetry is the most common type of isothermal experiment. The setup involves sealing the material or test subject in an ampoule, typically constructed from glass or metal. Ampoules typically contain up to 20 mL of sample [61]. Pharmaceutically, this is the most direct method to study heterogeneous systems (e.g. creams and emulsions) as well as solids and liquids [60].

Several studies have compared ampoule calorimetry with the established HPLC technique to assess its potential to measure long-term solid-state stability [23, 25, 62, 63]. Koenigbauer utilized the Arrhenius equation (Equation 5) to estimate the degradation rate using data obtained by both HPLC and ampoule isothermal calorimetry.

$$k = Ae^{-E_a/RT}$$

$$\ln k = \ln A - \frac{E_a}{RT} \qquad \text{(Equation 5)}$$

Where k = initial rate constant

A = pre-exponential factor

R = gas constant

T = absolute temperature

E_a = activation energy

The heat output (q) was recorded for drugs sealed in calorimetric ampoules at varying measuring temperatures ranging from 40 °C to 80 °C. Assuming a zero-order reaction rate, the relationship between heat output and rate constant is as such: (Equation 6).

$$\frac{q}{D_0} = -\Delta H \beta k = C = constant$$

q = rate of heat output (Joules)

D_0 = initial amount of drug present

ΔH = enthalpy change for the reaction

β = reactive portion of the sample

k = initial rate constant (Equation 6)

If enthalpy (ΔH) and the reactive portion of the sample (β) are assumed not to be a function of temperature, it is apparent that the rate constant (k) is proportional to heat output (q) (Equation 7).

$$q = Ck \qquad \text{(Equation 7)}$$

By plotting ln k against the reciprocal of temperature it is possible to extrapolate back to ambient temperature and determine the rate constant at that temperature. The findings by Koenigbauer demonstrated that isothermal calorimetry was proven to be more precise than conventional HPLC and could determine slow degradation rates that could not be determined with HPLC (e.g. phenytoin, triamterene, diltiazem, theophylline) [25]. Otsuka *et al.* applied ampoule isothermal calorimetry to study two different mechanisms of degradation: oxidation and hydrolysis [62]. Meclofexonate hydrochloride was the drug of choice to study degradation by hydrolysis and dl-α-tocopherol was chosen to model the oxidation process. Ampoule calorimetry was compared with the traditional HPLC method. The degradation rate constant for meclofexonate hydrocholoride was shown to follow first order rate kinetics in an aqueous environment, and was calculated to be $1.29 \times 10^{-4}\ s^{-1}$ by HPLC and $1.14 \times 10^{-4}\ s^{-1}$ by the calorimetric method. This indicates that calorimetry is well placed to accurately determine degradation constants.

Dl-α-tocopherol, a viscous liquid, was exposed to varying intervals of oxygen before sealing and measuring with the calorimeter. An Arrhenius plot was derived from a combination of both HPLC and isothermal calorimetry methods, with HPLC used to determine degradation rates from temperatures 80 - 50°C and isothermal calorimetry 40 – 23 °C. No marked discrepancy was observed in the linear regression drawn between the two methods. The study further concluded that the HPLC method would require a period of one year or more to determine the dl-α-tocopherol rate constant at lower temperatures (23 °C) and thus isothermal calorimetry was suggested to be highly advantageous in this circumstance. Tan *et al.* used microcalorimetry to investigate the solid state stability of 13-*cis*-retinoic acid and 13-*trans*-retinoic acid in the presence and absence of oxygen [63]. Rates of degradation of both compounds were determined through microcalorimetry and confirmed with HPLC. Both half-life and shelf lives at room temperature could be extrapolated from rates of degradation. Samples in the absence of oxygen displayed first order reaction kinetics from microcalorimetry, but HPLC analysis showed no degradation, which suggests that microcalorimetry was able to detect a physical change.

Similarly, an ampoule isothermal calorimetry study was conducted by Pikal *et al.* on cephalosporins in the solid state form which concluded that the technique was capable of detecting decomposition rates as low as 1% per year with an overnight experiment [23]. All of the studied amorphous compounds and the crystalline non-stoichiometric hydrate of ceftazidime showed decreased stability at higher water content, which was expected as water is a likely reactant in the decomposition of cephalosporins. Pikal also theorized that the annealing process, which is the decrease in energy of an amorphous solid as a function of storage time, contributes to the thermal activity of amorphous solids but its contribution decreases as a sample approaches equilibrium.

Recently, a study by D'sa *et al.,* sought to utilise ampoule isothermal calorimetry to expedite the detection of instability of MDI formulations [64]. The authors cold-filled aluminium ampoules with HFA-134a and later added model drugs, spray-dried beclomethasone dipropionate and spray-dried formoterol fumarate, to assess the viability of the isothermal calorimetry method. Three parameters were identified to be crucial in obtaining an accurate signal: the preconditioning of the ampoule O-ring, the hermetic sealing of the ampoule and the HFA-134a fill volume. The authors provided an advance in utilizing the 'real' propellant intended for MDI formulation and, provided solutions for issues with propellant leakages. The method presented is currently the most direct method to study thermal interactions arising from drug-propellant interactions. However the equilibration period (up to 30 min) limits the method from fully capturing crystallization events, especially those that occur rapidly. The addition of amorphous drug to a cooled HFA environment may also lower the rate of crystallization, and therefore artificially lower the heat output (q) (Equation 6) [65].

2.3.2 Titration calorimetry

In isothermal titration calorimetry (ITC), small volumes of titrant solution are added sequentially to a solution of substrate held within the calorimetric vessel (Figure 1). The number of titrations is calculated to ensure the reaction progresses past its end point. Integration of the data allows construction of a binding isotherm, from which the binding constant can be determined. Several control experiments are needed to take into account dilution of the substrate and dilution of the ligand. An example of ITC used to study pMDI formulations is provided in Figure 2; the surfactant polyethylene glycol was titrated into a

suspension containing spray dried salbutamol sulphate in decafluoropentane to ascertain the ideal ratio of surfactant coverage to surface area of drug.

As heat is ubiquitous, isothermal calorimetric data are limited in specificity, but conversely the technique is ideally suited to study complex, heterogeneous samples. Isothermal titration calorimetry has been extensively employed to academically study the self-assembly of micellar systems [66-69], drug – surfactant interactions [70-72], polymer – surfactant interactions [73, 74], ligand binding [75-77], enzyme activity [78, 79], protein – protein reactions [80, 81], lipid – lipid systems [82] and lipid – small molecule interactions [83]. A broader summary of isothermal titration calorimetry applications from 2010 can be found in Ghai *et al.* [84]. Multiple studies have utilised isothermal titration calorimetry to investigate the energetics of surfactant-based systems [71, 85, 86]. The majority of these studies have been conducted in an aqueous medium; however there is one key study that is of relevance to pMDI systems. Blackett *et al.* investigated the absorption of oleic acid and Span 85 onto amorphous and crystalline salbutamol sulphate in an attempt to assess the effect of surface energy properties on surfactant – drug interactions in Arcton-113, a model propellant (1,1,2-tri-chloro-tri-fluoro-ethane) [86]. Three batches of salbutamol sulphate were used; freshly milled, milled and stored at 78% relative humidity (RH) for 72 hours to encourage re-crystallisation, or stored at 78% RH for 72 hours and returned to 0% RH for at least 24 hours to reproduce an "aged dry sample". Samples (50 mg) of each batch of salbutamol sulphate were suspended in Arcton-113. It was established that the freshly milled sample was partially amorphous, whilst the crystalline samples were shown to be > 99.7% crystalline (the detection limit for crystalline materials using ampoule isothermal calorimetry). Two solutions, oleic acid and Span 85 in Arcton-113, were titrated separately into the calorimeter ampoule containing the suspension of drug in the non-polar model propellant. Calorimetric data revealed that adsorption of both surfactants onto partially amorphous surfaces produced a net endotherm as surfactant concentration increased, which was a result of a combination of exothermic (surfactant adsorption) and endothermic (particle deaggregation) events. Crystalline samples did not disperse when surfactant was added, which therefore resulted in a net exothermic event with respect to surfactant concentration. Titration calorimetry was a suitable technique to probe liquid-surfactant-drug interactions in the model pMDI system in real time.

Despite the promise for the detection of key events associated with pMDI stability, ITC suffers from a lack of ability to use commercial propellants, which are only liquid under

elevated pressure, and requires redevelopment to withstand pressures up to and beyond 6.7 Bar.

2.3.3 Gas perfusion calorimetry

Gas perfusion calorimetry involves the controlled flow of vapour over a solid sample in the measuring ampoule of the calorimeter. The vapour is commonly an organic solvent or humidified air and is specifically chosen to interact with the sample in some way. The vapour is transported using a carrier gas, usually nitrogen, which passes through two solvent reservoirs prior to the measuring ampoule. A second gas line is used to carry a dry gas, also usually nitrogen. Mass flow controllers are used to regulate gas flow along the two line; defined partial pressures of vapour can be created by proportional control of the flow rates. Partial pressure can be altered either in steps or a linear ramp. Typical examples of gas perfusion calorimetry include the use of vapour to probe specific binding sites on the sample surface, wetting the sample to induce the formation of hydrates or solvates and inducing recrystallisation of an amorphous material [60].

Originally, the determination of amorphous content in powders was conducted by introducing a glass hydrostat containing either a reservoir of pure water or salt solution into an ampoule calorimeter [87]. The first published study, conducted by Briggner *et al.,* utilised isothermal calorimetry to investigate the crystallisation of spray-dried and micronised lactose monohydrate [87]. It was found that the amount of amorphous material produced by micronisation was directly proportional to the microcalorimetric observations correlated to the recrystallisation of lactose in response to humidity exposure. It was proved that the detection limit of the percentage of amorphous material was 1% or less, which far exceeded the 5-10% limit of detection for differential scanning calorimetry (DSC) and X-ray Powder Diffraction (XPRD) [22]. Ahmed *et al.* investigated the use of gas isothermal calorimetry using organic vapours to characterize the amorphous content of drugs with poor aqueous tolerability. The study agreed with previous literature, and identified a detection limit of 1% amorphicity [88]. With the advent of the gas perfusion device, the exact RH or relative vapour pressure experienced by the sample can be controlled by adjusting the flow rates of the purge gas through the dual lines [59].

The isothermal gas perfusion apparatus has been used in a recent study by Ooi *et al.* to investigate the interaction of model propellant 2H,3H-decafluoropentane with beclomethasone dipropionate and salbutamol sulphate [89]. The early stages of beclomethasone dipropionate-decafluoropentane clathrate formation were successfully captured. The stability of salbutamol sulphate in HFA-227 was also predicted using microcalorimetry coupled with multiple analytical methods. The disadvantage of this technique is the inability to currently utilize pressurized liquids at STP. The substitution of HFA with the higher molecular weight HPFP model propellant gives an indication of API stability in HFA but a shift towards utilizing true HFA propellants is necessary for the further development of this technique. The replacement of the internal perfusion shaft O-rings and the redesign of the mass flow controllers are factors to consider when developing gas perfusion calorimetry to withstand fluorinated propellants.Broadening the selection of powders to include commercially manufactured active pharmaceutical ingredients for gas perfusion testing is necessary to compare this method with traditional long-term studies.

3. Conclusions

This review has examined the use of analytical techniques, such as Raman spectroscopy, isothermal ampoule microcalorimetry, titration calorimetry and gas perfusion calorimetry, in the context of their potential use by the pharmaceutical industry in pharmaceutical development of pMDI formulations. The main focus was to determine their potential use for screening the compatibility of active pharmaceutical ingredients, excipients and propellant in metered dose inhalers, particularly as fast-screening technologies for accelerating the development time of pMDI formulations.

Each technique has a set of unique advantages and disadvantages. The *in situ* laser diffraction method is well positioned to analyze real-time physical stability as a function of particle size. It is simple to operate and cost effective, however the analysis is restricted to only pMDI suspension formulations and more suited to fast-occurring physical processes, which happen in the analysis time-frame. Theoretically it may be possible to correlate particle growth rate to the shelf-life of the product, if the API undergoes a prolonged rate of degradation.

Raman spectroscopy is effective as it is non-invasive, requires little sample preparation and can potentially be used to attain chemical stability data from both suspension and solution pMDIs as the spectra for hydrofluoroalkanes has already been documented. . Whilst component-specific Raman chemical imaging have been recently made available, current techniques rely on assessing the particle size distribution and chemical composition after propellant evaporation and particle impaction onto an ACI stage. This current technique is suited for indirectly assessing drug-drug and drug-excipient aggregation, after the evaporation of the propellant vehicle. The next logical step in the development of Raman characterization for drugs in propellant vehicles is to begin *in situ* solid state analysis. It has already been shown that FT-Raman is suitable for analysis of *in situ* protein stability in HFA suspensions as it is cost effective, reproducible, efficient and requires little sample pretreatment. Opportunities to develop an online Raman characterization method rely on further organic drug compound studies in HFAs to assess the method's sensitivity in determining chemical changes in real time and *in situ.*

Calorimetry is effective as it is able to detect both chemical and physical degradation of an API as a function of heat; however it is unfortunately unable to distinguish between the two

processes. Whilst this is not absolutely necessary if calorimetry is to be used as a fast-screen method for API-propellant compatibility, additional tests will be required to determine the reason for drug instability. Ampoule calorimetry, whilst it can be adapted to be filled with pressurized HFA, is unable to detect rapidly occurring degradation events, as an initial calibration and temperature stabilization of the sample ampoule is required prior to measurement. It is thus challenging to determine accurately the overall rate of degradation. Loss of the initial data does not occur when gas perfusion calorimetry or titration calorimetry are used. Both gas perfusion and titration calorimetry are currently well placed to measure solid-gas or solid-liquid interactions and have been used with model HFA propellants, but require re-development to withstand pressures of up to and beyond 6.7 Bar to mirror pressures in a typical liquid HFA-134a environment. The correlation of enthalpy to particular formulation parameters, namely the EtOH-HFA ratio, drug-surfactant ratio, drug degradation rates, canister pressure and excipient compatibility are areas that could be assessed with this dual system and quicken formulation development. The possibility of studying elastomer swelling or valve degradation in pressurized hydrofluoroalkane is another possibility and can be achieved by substituting the powdered sample for the elastomer or valve material in the TAM ampoule. Future TAM studies will need to utilize high percentage crystallinity particles with clean, predictable surface characteristics to mirror more closely an industrial micronized drug product. The "whole system approach" of ampoule calorimetry combined with the investigations of the initial propellant-drug reaction with gas perfusion calorimetry is best placed to fast-screen for drug-propellant compatibility and has the most extensive application set for pMDI testing within a commercial lab. The study of the phase transformations of organic compounds under pressure is another area of development that is complementary to thermal analysis. Further developments on the kinetics of propellant mediated phase transformation of organic compounds with synchrotron SAXS/WAXS radiation will be beneficial in identifying intermediate organic phases and contributing to the a high throughput for formulation development.

Expert Opinion

Understanding the factors that affect API stability in hydrofluoroalkanes has been the focus of much empirical research within the last 20 years, since the switch from the chlorofluorocarbons to hydrofluoroalkane propellant vehicle. Previous pMDI research has focused on separate failure modes for pMDIs, including solubilisation properties of the hydrofluoroalkanes and propellants, the interaction of propellant and active pharmaceutical ingredients, the chemical interaction between multiple pharmaceutical ingredients in a propellant environment, the interaction of ingredients with device components and the effects of water and leachables.

The achievement of a functional online monitoring tool seeks to detect formulation defects, which may involve more than one mode of failure. The detection of issues with formulation quality prior to stability testing will benefit the pharmaceutical industry immensely, in terms of improving manufacturing efficiency, reducing waste and shortening the development time for new products. There are also immediate, practical and financial benefits associated with pulling products which are deemed unsuccessful prior to undergoing lengthy stability studies.

Substantial progress has been made towards developing online monitoring tools for pMDI production; the key chemical and physical properties of propellants have been documented along with compatibility studies involving elastomers and plastics and solubility studies with APIs. Background infra-red and raman spectra of propellants have been documented by academic groups. *In situ* experiments involving characterisation of APIs in a pressurised propellant environment have been developed using laser diffraction, isothermal calorimetry, and Raman spectroscopy. Key findings in the literature indicate that there is practical application of the aforementioned methods to stability testing as these methods are relatively fast, easy to operate, require little sample preparation and are capable of being integrated into pMDI formulation development or batch testing.

However, several challenges are apparent in above approaches for stability testing. Firstly, they are not designed to detect pMDI failures associated with the device, which includes batch to batch variations on pMDI seals and gaskets, slight leakages, leachables from both sealing and canister materials, canister material properties and coatings or actuator malfunctions. The possibility of studying elastomer swelling or valve degradation in pressurized HFAs can be achieved by substituting the powdered sample for the elastomer or valve material in the calorimetric ampoule. It is also possible to mimic the canister surface in

calorimetric experiments by coating the internal surfaces of sample and reference ampoules with polymeric materials. If the latter is chosen, recalibration of the instruments will be required to take the different heat conduction properties into account. Calorimetric methods may be the most sensitive method to determine both chemical and physical stability, however further developmental work must be performed to define the enthalpy limits for *in situ* crystallisation, agglomeration and/or dissolution. Another disadvantage for the screening methods is that the reason for pMDI failure may not be readily apparent. The inclusion of ethanol in metered dose inhaler formulation is another dimension which must be considered; solubilisation of the API via ethanol will transform the suspension into a solution, thus causing *in situ* laser diffraction measurements to be ineffective.

Future work in this field of study must be directed to determine the limit of detection for the above methods, and attempt to establish a relationship between the residual aerodynamic particle size distribution and the rate of *in situ* API crystallisation, sedimentation or agglomeration. Despite the technical challenges encountered for predicting formulation stability in pMDIs, pre-emptive screening approaches are highly sought after due to the immediate benefits for manufacturing efficiency, waste reduction strategies and pMDI formulation.

Technique	Typical outcome measured	Special equipment needed	Advantages	Disadvantages
Laser diffraction	Size by volume diameter	• Modification to allow pressurized measurement (in-situ measurement) • Transparent portal to allow for measurement	• Real time measurement and ability to extract kinetic data • Correlation between laser diffraction data (ex-valve) measurements and aerodynamic particle diameter in some circumstances [29, 30]	• Methodology, in particular levels of shear, needs to be assessed in a recirculation *in situ* system • Unable to characterize solution MDIs • Prone to beam steering • Measurement of anisotropic particles can be inaccurate • Unsuited to detect chemical processes, except processes involving change in geometric diameter
Raman spectroscopy	Solid state characterization: phase transformations, polymorphs, hydrates, anhydrates, molecular conformation, polymer chain confirmation	• Transparent measurement container	• Non invasive • Little sample preparation • Applicable to bioaerosols and can be potentially used for microbial screening • Potential for online monitoring of solid state changes	• Raman scattering is a weak effect. May require method development to reduce spectra interference
Isothermal calorimetry	Thermodynamic and kinetic information, and probing of liquid-surface-drug interactions in pMDI systems	• Modification to allow pressurized measurement (in-situ measurement) [64]	• Determination of degradation through time for both chemical and physical degradation studies • Ability to extrapolate long term stability data	• Equilibration period for ampoule calorimetry required, during which the material may have undergone change

Table 1. Advantages, disadvantages and special equipment required for the discussed techniques.

Approval Date	Year Approved	Drug Name	Active Ingredient	Formulation	Company	Strength	Route
15-Aug	1996	Proventil-HFA	Albuterol Sulfate	Suspension	3M	Eq 0.09mg Base/Inh	Inhalation
24-Dec	1997	Sclerosol	Talc	Suspension	Bryan	400mg/Spray	Intrapleural
15-Sep	2000	Qvar	Beclomethasone Dipropionate	Solution	Teva Branded Pharm	0.04mg/Inh 0.08mg/Inh	Inhalation
19-Apr	2001	Ventolin HFA	Albuterol Sulfate	Suspension	Glaxo Grp Ltd	Eq 0.09mg Base/Inh	Inhalation
17-Nov	2004	Atrovent HFA	Ipratropium Bromide	Solution	Boehringer Ingelheim	0.021mg/Inh	Inhalation
14-May	2004	Flovent HFA	Fluticasone Propionate	Suspension	Glaxo Grp Ltd	0.22mg/Inh 0.11mg/Inh 0.044mg/Inh	Inhalation
29-Oct	2004	Proair HFA	Albuterol Sulfate	Suspension	Teva Branded Pharm	Eq 0.09mg Base/Inh	Inhalation
11-Mar	2005	Xopenex HFA	Levalbuterol Tartrate	Suspension	Sunovion	Eq 0.045mg Base/Inh	Inhalation
8-Jun	2006	Advair HFA	Fluticasone Propionate & Salmeterol Xinafoate	Suspension	Glaxo Grp Ltd	0.045mg/Inh & Eq 0.021mg Base/Inh 0.115mg/Inh & Eq	Inhalation

						0.021mg Base/Inh 0.23mg/Inh & Eq 0.021mg Base/Inh	
27-Jan	2006	Aerospan HFA	Flunisolide	Solution	Acton Pharms	Eq 78mg Base/Inh	Inhalation
21-Jul	2006	Symbicort	Budesonide & Formoterol Fumarate Dihydrate	Suspension	Astrazeneca	0.08mg/Inh & 0.0045mg/Inh 0.16mg/Inh &0.0045mg/Inh	Inhalation
10-Jan	2008	Alvesco	Ciclesonide	Solution	Takeda Gmbh	0.08mg/Inh 0.16mg/Inh	Inhalation
22-Jun	2010	Dulera	Formoterol Fumarate Mometasone Furoate	Suspension	Merck Sharp Dohme	0.005mg/Inh & 0.1mg/Inh 0.005mg/Inh & 0.2mg/Inh	Inhalation
23-Mar	2012	Qnasl	Beclomethasone Dipropionate	Solution	Teva Branded Pharm	0.08mg/Actuation	Nasal
20-Jan	2012	Zetonna	Ciclesonide	Solution	Takeda Gmbh	0.037mg/Inh	Nasal

Table 2. FDA approved products containing HFA which are currently marketed in the US

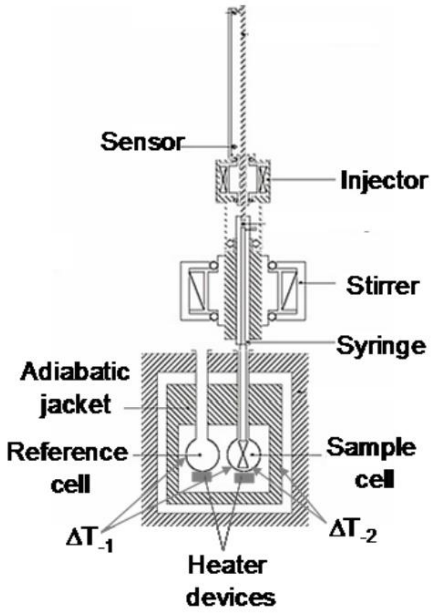

Figure 1. Schematic of a titration unit comprised of an external syringe housing the titrant solution and cannula leading into the calorimetric ampoule in the measuring position in the Thermal Activity Monitor (TAM) (illustration taken from Martinez *et* [90])

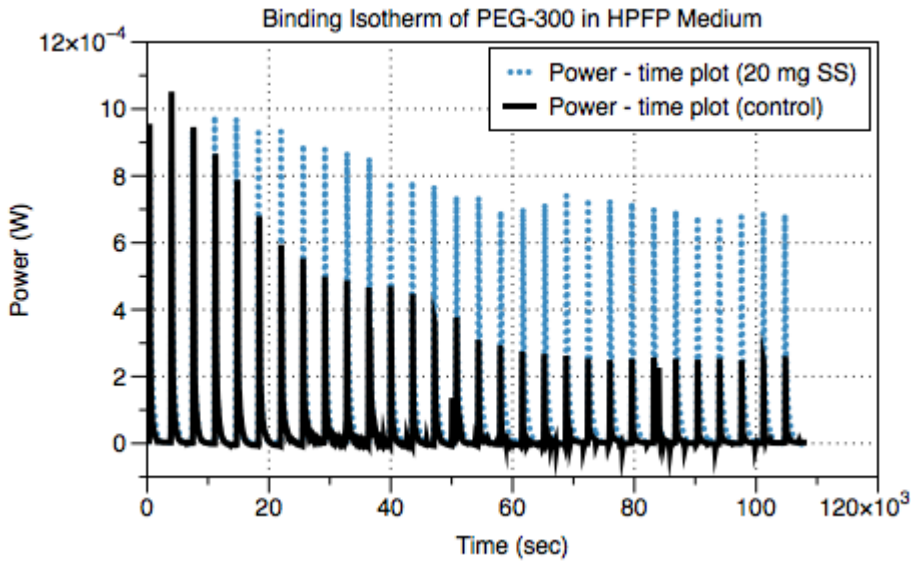

Figure 2. Titration thermogram of polyethylene glycol 300 into model propellant decafluoropentane.

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