# Formulating Drugs for Inhalers and Stability Issues

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## Abstract

The main topic addressed in this paper is the formulation of pressurised metered dose inhalers and the problems associated with formulating medications for high pressure systems. Formulations for pressurised metered-dose inhalers (pMDIs) generally consist of two main types, solution and suspension-based systems. In the latter version, the active ingredient ideally remains mainly in the solid state, which can thus reduce problems with chemical degradation. However, the physical stability of such systems, in terms of aggregation, flocculation, creaming, sedimentation, propellant clathrates and bridging can then become problematic. The problems associated with solution-based systems revolve around the chemical stability of the active ingredients.

## Introduction

Formulations for pressurised metered-dose inhalers (pMDIs) generally consist of two main types, solution and suspension-based systems. In the latter version, the active ingredient ideally remains mainly in the solid state, which can thus reduce problems with chemical degradation. However, the physical stability of such systems, in terms of aggregation, flocculation, creaming, sedimentation, propellant clathrates and bridging can then become problematic. Particulates in suspension systems require an aerodynamic diameter of <6 um for pulmonary delivery [1] and tend to be naturally cohesive in their suspended state. The formulation variables for pMDIs typically include hydrofluoroalkane (HFA) propellants, active ingredients, surfactants and co-solvents. The HFA propellants currently comprise of either HFA 134a or 227ea. HFA 227ea has one and HFA 134a has two small, asymmetrically positioned, hydrogen atoms in their mantels. This creates a distinct dipole in the propellants, due to the electronegativity of fluorine, in comparison to hydrogen. Early determination of the solubility of an active ingredient in a propellant, or propellant blend, allows the formulator to decide on whether to use a suspension or solution-based pMDI. Suspension MDIs should ideally be formulated with drug substances which are insoluble in the propellant blends. If this is not the case, then the solubility of the drug, over a varying temperature range, should be minimised. If the solubility of a drug is high enough in a particular blend of a propellant and a co-solvent, then a solution formulation can be prepared. However, the use of ethanol to aid surfactant solubilisation can affect the vapour pressure of the mixture and the respirable fraction [2]. It can also affect the undesirable crystal growth of the drug particles [2].

### *Formulations for Pressurised Metered dose Inhalers*

A knowledge of the solubility of a drug and an excipient in a chosen propellant formulation is critical for the optimal performance of the inhaler. However, the study of pMDI formulations for pMDIs can be problematic, due to the difficulty in studying volatile formulation blends. However, a method has been published, which may address this issue, by directly actuating the metered dose inhaler into a HPLC system [3].

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Current techniques available for quantitative analysis of compounds in pMDI formulations either involve opening a chilled pMDI canister and emptying its contents into a suitable mobile phase, prior to analysis [4, 5] or by actuating the contents of a pMDI canister into a suitable diluent [6]. Compounds present in very low concentrations, whether the active ingredient, or degradation products, are often difficult to analyse, due to a lack of sensitivity [7, 8]. When necessary, compound levels can be increased by combining several different samples, in order to achieve an amount that enables quantitation or identification of the compound.

Gupta and Myrdal [3] have developed an on-line reversed-phase high-performance liquid chromatography for the quantitation of compounds in pMDI formulations. Their method involves a direct injection from the pMDI canister into the needle injector port of the manual injector. They validated the method using ethanol-HFA-134a- based pMDIs. Phase separation studies were conducted to investigate the miscibility of the ethanol-HFA-134a mixtures with different mobile phase solvent compositions. The simplicity of their technique could offer considerable advantages for the quantification of compounds in pMDIs. This would particularly be the case, if this method could be automated. This would allow direct analysis of the pMDI directly whilst being manufactured.

Dalby et al. [9] reported a method which can be used to determine the solubility of drug substance in pure propellant and propellant blends using a filtration method and crystal growth studies can be best observed using microscopy [10].

In addition, studies of the effect of different drug forms, compatibility studies and stability screens, are usually performed in parallel. In suspension systems, drugs should have no solubility in the propellant formulation and, consequently, have good chemical stability [11]. However, this benefit is often counterbalanced with the need for the addition of stabilising excipients, such as surfactants.

Williams [12] showed that the propellant system could affect the surface coating of a pMDI canister. Williams showed that the contact angle of an epoxy coating did not change over 3 months storage at 0, 25 and 40 C when exposed to either HFA 134a or HFA 227. However, topographical parameters obtained from AFM, showed slight differences in the surface properties of roughness and the magnitude was dependent on the propellant system.

The addition of surfactants to a pMDI formulation help to prevent agglomeration of micron-sized particles in suspension-based formulations. The choice of surfactant is dictated by its solubility in the propellant or propellant blends and is thus, somewhat limited. Furthermore, the need to avoid crystal growth and/or adhesion of micronised suspended drugs to internal container surfaces are problems that may be catalysed by some combinations of surfactant type/concentration, vehicles and physical forms of drug substance.

***Solution-Based Metered dose Inhalers***

Formulations for pMDIs usually comprise of either of two hydrofluoroalkanes, HFA 134a or HFA-227ea, along with an active ingredient and possibly a cosolvent and a surfactant. The presence of fluorine in both propellants and the fact that HFA 227ea has one and HFA 134a has two small, asymmetrically positioned, hydrogen atoms in their mantels, causes both molecules to have distinct dipoles, when compared to the presence of only hydrogen.

Unpublished work by Vervaet and Byron [13] apparently investigated the density and vapour pressure of the HFA propellants and their mixtures with anhydrous ethanol. They found that all three are completely miscible. Their results may imply that the intermolecular forces between ethanol (up to 30% by weight) and the HFA propellants, and between the two HFA propellants had similar orders of magnitude. However, vapour pressure studies did not mirror these results and they showed that HFA-ethanol admixtures have higher equilibrium vapour pressures than predicted. Although this data is not conclusive, it may indicate that the HFAs have a higher affinity for the gas-liquid interface than the ethanol, with ethanol being encased by a HFA molecular matrix [13].

If the solubility of a drug is high enough, using a blend of a propellant and co-solvent, then a solution formulation can be prepared. However, caution must be exercised to ensure that the drug remains in a solution form during the lifetime of the product. A typical example of such a case is beclomethasone dipropionate. BDP, which is freely soluble in ethanol and thus can be used in combination with a propellant to form a solution product.

Although issues with the physical stability of the formulation are not apparent with solution-based formulations, they can also offer some concerns for formulators. Such concerns include reduced chemical stability, [14] and possible loss of drug into gasket materials. A good selection of a valve and an overage, or excess, of the drug in the formulation

can counteract these issues. However, this can become expensive if the drug in question has a high intrinsic value.

The choice of surfactant can also influence the chemical stability of an active in some cases [15].

### *Formulations for Suspension-Based Metered dose Inhalers*

Ideally, drug substances in suspension pMDIs should be insoluble in the propellant blends. Alternatively, the solubility should be minimised over a varying temperature range. Ostwald ripening, where an entropic effect causes smaller particles of the drug to dissolve and recrysallise on flat faces of larger particles can be very problematic over the shelf-life of a pMDI. The larger particles tend to sphericalize, with a consequence that the particle size distribution will change. This could cause less reproducible dosing, which may alter the aerodynamic size distribution of the dose during, the shelf-life of the metered dose inhaler units [16]. Thus, the determination of drug solubility in a range of propellant formulations is a prerequisite and is usually best screened for during the early stages of development. The selections of optimum formulation types are usually then determined from these studies. Different physical forms of the compound could be chosen in order to avoid the possibility of polymorphism issues. However, it could present some difficulties if, for example, this is found during the stability trials and the consequences of such a change can have quite significant cost implications.

Recently there has been an increase in the number of combination drug products for the treatment and prevention of asthma. Combining two drugs within the same inhaler may have several therapeutic advantages, including increased patient compliance. Formulation challenges due to hetero-aggregate formation in suspension systems have been reported [17, 18].

In addition to the affect of the physicochemical properties in suspension formulations, the sampling mechanism can also be problematic. The sample, in a bulk suspension, is taken from the bulk reservoir, via a metering valve. This is exposed to atmospheric pressure, which results in rapid expansion of the propellant and subsequently, aerosolisation of suspended drug particulates. There is thus a requirement that the suspended formulations must be redispersible and remain stable between doses.

However, suspended drug particulates are known to destabilise over time via, flocculation, sedimentation or creaming [19, 2]. Variations in the suspension homogeneity and cohesive/adhesive nature may lead to changes in the performance of the resulting aerosol, not only within the bulk reservoir, but also via the sampling mechanism [20]. These problems could be amplified, if a low dose active pharmaceutical ingredient (API) is used.

Jinks et al. reported the use of lactose as a bulking excipient for low-dose suspension pMDIs [21, 22]. They showed that pMDI dosing performance was improved by the addition of a sub-micron-sized lactose excipient. These were found to flocculate and enhance the homogeneity of the suspension [21].

James et al. [23] compared the surface characteristics and surface energetics of two-potential bulking excipients, anhydrous sub-micron alpha-lactose and sub-micron sucrose, for use with low-dose, suspension formulations in pressurised metered dose inhalers. James found that both sub-micron sucrose and anhydrous sub-micron alpha-lactose exhibited a lower surface free energy than their respective parent materials. Both AFM and contact angle surface energy measurements also showed that sub-micron sucrose has a higher surface energy than anhydrous sub-micron alpha lactose. Theoretical work of adhesion values between anhydrous sub-micron alpha lactose and each API are considerably lower than those observed between micronised alpha lactose monohydrate and each API. It has been suggested that, during the micronisation process, the crystalline structure of alpha-lactose may be disrupted, thus exposing higher energy crystalline planes and potentially producing higher energy amorphous sites [24, 25].

James found that, with the exception of salmon calcitonin, sub-micron sucrose showed larger adhesive interactions to the selected APIs than anhydrous sub-micron alpha lactose. The consequence of exposed higher energy sites is complemented by the considerably rougher surface asperities of the micronised material, a correlation that has been previously reported by other laboratories in a range of studies using IGC and contact angle measurement [26-28] as well as AFM measurements [13].

Other methods have been studied in the search for stable suspension pMDI formulations. The density of the particulate drug can be matched to the density of the propellant blend [29]. Large porous or hollow particles have been utilised in an attempt to avoid creaming or caking of the particles in the

propellant [29, 30]. Techniques utilised in the formation of particulates include spray drying, freeze-drying, or co-grinding of the substance with or without surface active ingredients are established methods for the preparation of stable particulates [31, 32].

Other options such as choosing another more stable polymorph could be tried, but this is not always possible, if the material does not show polymorphism. An obvious, although perhaps unavoidable, scenario is to avoid the use of a co-solvent in such a system. However, commercial constraints, such as the need to avoid patent infringement may dictate that such co-solvents are required.

Another way of manipulating the rate of crystal growth involves the choice and concentration of surfactant used in suspension formulations. The effects of altering surfactants can only really be gauged by experimentation which investigate the kinetics of such systems. Philips et al have noted that the choice and concentration of such surfactants can be critical [25].

During sedimentation or creaming, depending on whether the drug density is higher or lower than the propellant density, the van der Waal's forces between drug particles can be large enough to cause irreversible caking. A technique called controlled flocculation seeks to control this by searching for a secondary minimum potential energy between the particulates. Thus, this would reduce the van der Waal's forces between the particulates [33].

Particulate charge, which may be present as a result of triboelectrification, may not be evenly distributed on surfaces and may even be bipolar in nature [34-36]. In addition, even if electrostatic charges are uniform across particles, electronic repulsive forces should be much smaller in these dielectric propellant media.

### *Active Ingredient Solvates*

Formulation screening studies can highlight interactions between the drug and other formulation components. A well known example of this is the clathrate formed between BDP and propellants. A fully characterised clathrate of BDP is that formed with a former pMDI propellant CFC-11.

Clathrates are a class of inclusion compounds, which generally consist of two molecular species. These arrange themselves in space so that one molecule (host) entraps the other (guest) [37] in polyhedral cavities [38]. Guest molecules can fully occupy (bonded to the host network) or partially occupy (occupying void spaces) the cages in the host framework [39].

The thermodynamic stability of the clathrate depends strongly on the size and shape of the guest molecules which must be small enough to fit into the cavity of the lattice, but large enough to lend stability to the structure [40]. Spontaneous crystal growth was found to occur rapidly when anhydrous BDP is dispersed in CFC-11, with the formation of the BDP CFC-11 clathrate. The structure is stabilised through hydrogen bonding. Since solid state chemistry can significantly alter the physical interactions within a suspension formulation, it is important to determine the most stable crystalline forms of BDP in the presence of the propellant. There is some evidence that a clathrate can also from between BDP and HFA-134a [41]. This forms much more slowly over time, than the well-known CFC-11 clathrate.

Bohroum (2010) [42] showed that the formation of the BDP-CFC-11 clathrate in the propellant was beneficial in terms of a reduction in the force of adhesion with different pMDI components.

Harris et al [43] studied the solid-state behaviour of BDP in 20% ethanol/hydrofluoroalkane (HFA-134a). They found that desolvation seemed to occur rapidly, once the crystal is removed from the pMDI formulation. Therefore, it is uncertain if any of the propellant is incorporated within the crystal structure, when grown in a mixture with 20% w/w absolute ethanol. However, the rapid desolvation at ambient conditions may suggest the possibility of a HFA-134a solvate. The resulting BDP crystal structure, has a large channel where the solvent moves freely. The channel is perpendicular to the BDP crystal layers. Some hydrogen bonding may occur within the channel, but it is not likely to be sufficient to order the solvent. This form seemed to differ from alternative crystal forms reported [43]. An interaction between BDP molecules and solvent molecules inside the MDI resulted in a conversion from the anhydrous form to a solvate/clathrate, which surprisingly, is the most stable form.

### *In Situ Precipitation*

Steckel et al. [44] investigated the precipitation of a drug substance with excipients in situ within the liquefied propellant system and this was found to form a physically stable suspension. Their rationale for using this route was to avoid the independent processing step, involving micronisation, required for the reduction of the particle size. Sur-

faces of mechanically micronised powders are not naturally grown as the crystal cleaves at the crystal face with the smallest attachment area [45]. The milling process is extremely inefficient [46] as the high energy used, decreases the crystallinity of the milled material, [47] and can cause chemical degradation due, in part, to an increase in solubility. The transformation of a crystalline surface, into partially amorphous material, and thus, the disordered structure in the material, can influence the performance of the formulations [48, 49].

### *Excipients for use in pMDIs*

Few excipients have been included in pMDI products that have successfully been marketed. Cosolvents (ethanol) and surfactants (oleic acid, sorbitan trioleate, and soya-derived lecithin) are currently the only excipients found in approved products in the US. Several alternative excipients have been suggested, but their use remains limited by an insufficient toxicological profile with respect to lung delivery. However, there are recent examples of new products with novel excipients achieving regulatory approval (Vfend®. Examples of novel pMDI excipients include: novel€ surfactants (e.g. 3 M oligolactic acid, acyl amide acids, monofunctionalised M-PEGS, [50-52] taste masking excipients [53] microemulsion excipients, [54, 55] microspheres and hollow porous microspheres [29, 56] cyclodextrins [57] nanoparticles [58] and alternative propellant systems (DME and propane) [59].

Williams et al [60] 150 showed that the use of HP-beta-C can be used to enhance the stability of a chemically labile drug. However, this depends on the specific way in which the inclusion complex is formed. A 1:1 lyophilised inclusion complex with HP-beta-CD showed an accelerated rate of degradation, which was assumed to be due to a partial inclusion of the molecule, aspirin in this case, within the HP-beta-CD cavity. However, when the inclusion complex was formed between aspirin and HP-beta-CD was formed in situ in the pMDI, then the degradation rate was retarded.

Steckel et al. [44] used modified cyclodextrins, which were freely soluble in a PEG/ethanol solvent system. The addition of the liquid propellant then gave a homogenous, milky suspension. This rapid precipitation in HFA-227 led to very fine particulates of both the drug and the derivatised cyclodextrin. The presence of the PEG rapidly covered the surface of the particulates and thus, reduced the interfacial energy between the propellant and the particulates. The cyclodextrin acted as a bulking agent where, they speculate that the cyclodextrin and the drug form a network-like structure of non-covalent bonding, probably hydrogen bonding. Formulations containing HP-beta-CD showed the most favourable suspension stability. Formulations containing PEG 300, ethanol and HP-beta-CD showed greater than 3 months of stability and no tendency for agglomeration or sedimentation was observed.

### *The use of Surfactants in Formulations for pMDIs*

Valve manufacturers have been attempting to improve valves for pMDIs since the gradual transition to HFA propellants began, in order to eliminate the need for lubrication, or to minimise this need. Formulations are now on the market, which possess little or no surfactant component. However, in HFA propellants, surfactants can still be utilised to aid the solubilsation of drugs, alter the temperature dependence of solubility and overcome valve sticking problems. In suspension formulations, they can serve to prevent irreversible caking, minimise drug particle adhesion to container walls and components, minimise cohesion of drug particulates and retard overly rapid separation between the solid and liquid phase. There is some uncertainty as to whether it is best to dissolve the surfactant in the propellant combination first or whether pre-coating the particles with surfactant can be equally effective. Byron et al, have shown that pre-coating salbutamol with oleic acid, prior to suspension in HFA-134a, was effective in retarding the drug's creaming from a suspension over a period of 30 seconds [2]. Blondino did not find any evidence of reverse micellization in HFA 134a using light scattering [61]. Dissolved surfactant molecules with high HLB values were present as monomers or very small molecular agglomerates.

Vervaet et al. were unable to make predictions about the behaviour of a large number of surfactants in 5% ethanol-HFA 134a blends [13]. They used polystyrene spheres in order to investigate the different surfactants at different concentrations and determined that the spheres tended to show an increased affinity for the container walls. They believed that this was due to repulsion by the propellants, via the predominant electronegative mantel of the HFA propellant. Suspended solids appeared to prefer to settle on HFA-depleted surfaces. This behaviour could create additional problems for the

formulator and indeed, the formulation and product stability. If the solid has any solubility in the propellant blend, then crystal bridges can form, which automatically stops any redispersion of the solids.

A repulsive force between particles is conferred sterically following the adsorption of surfactant to each particles surface. Manipulation of the steric forces between the particulates, is an obvious way to stabilise a suspension pMDI. This can be done by altering the type and concentration of the surfactant allowing optimisation of the suspension, sedimentation and redispersibility characteristics [62]. However, suspensions with ideal physical characteristics may not yield the best aerosols [32]. Ease of redispersibility does not automatically mean that the breakdown of agglomerates into primary particles.

The concentrations of surfactants required to elicit a stabilising effect with suspension formulations can be very low. Johnson claimed that a suspension of perfluoroalkanoic acids, potassium perfluoroalkyl sulphonates and ammonium perfluoroalkyl carboxylates showed some suspension stabilising effects [63]. Byron et al showed that the concentration required to do this was less than 0.1% by weight [2]. However, if the solubility of the surfactant in the formulation is too low, then this can cause problems with phase separation. This is particularly apparent if the storage temperature and humidity are not maintained within strict limits [64].

### *The use of Block Copolymers in pMDIs*

Ridder et al. investigated the solubility and aggregation orientation of a variety of surfactants in HFAs. HFA-soluble surfactants could mainly be found among the Brij and POE-PPO block copolymer surfactants [65]. Tween surfactants generally show little solubility in HFA and Span 20 is insoluble in all three HFAs. Liquid-pasty Brijs e.g. polyoxyethylene (10)-laurylether (POE10-C12), Brij 30, Brij 97 were found to be significantly more soluble in HFA 227ea than in HFA-134a and DFP. The solid Brijs, e.g. Brij 56, Brij 76 and Brij 35 exhibit low solubility in three HFAs.

Since the physical state of POE-based non-ionic surfactants is mainly determined by the length of the POE-chain, it appears that the hydrophilic POE moiety may significantly influence HFA-solubility. In HFAs, POE solubility decreases with increasing molecular weight (Mn) and becomes insoluble with Mn>1000. Similarly, Brij and POE-PPO block copolymer surfactant solubility decreases with increasing POE chain length to more than approximately 10 POE units. HFA solubility increases on increasing the POE moiety from 4-10 units, but a further increase to 23 units results in decreased HFA solubility in all three HFAs.

POE was found to change its conformation from a zigzag conformation to a meander configuration with increasing the degree of polymerisation to >10-12 units in aqueous solution and to >20-40 units in bulk. However, with the POE-based surfactants, the change in configuration starts at a degree of polymerisation of 15-20 [66, 67]. The meander configuration maximises hydrogen bonding, while minimizing the number of exposed hydrophobic groups, resulting in increased polarity and H-bonding capability.

The significant reduction of POE solubility in HFAs could possibly be caused by a similar change in configuration with increasing the level of polymerisation. The strong electron withdrawing effect of the fluorine atoms in HFAs, leaving a partial positive charge on the hydrogen substituents, allows a considerable charge separation and hence the possibility of H-bonding [2]. However, possible configurational changes of POE in HFAs, which may lead to increased polarity, as in water, may affect the interaction between the POE molecule and HFAs.

Furthermore, the introduction of a hydrophilic polyol structure, such as sorbitan in Span 20, results in HFA insolubility. HFA insolubility of Span 85 has previously been reported [2, 64]. Moreover, glucoside and thioglucoside surfactants were found to be insoluble, or only slightly soluble, in HFAs [68, 69].

All liquid POE-PPO block copolymers exhibit high solubility in HFA-227ea, whereas in HFA-134a and DFP only reverse pluronics show solubility >50% w/w. One explanation could be the confined middle position of the POE block in the triblock that might inhibit conformational changes. Pluronic 25-R4, however, shows significantly lower solubility compared to the other reverse Pluronics. This may be explained by the fact that Pluronic 25-R4 possesses the longest POE chain with a Mn of 1440.

Muller distinguishes two types of self-association behaviour in non-aqueous solvents, i.e. type I and type II association [70]. Type I is characterised by (1) small average aggregation numbers (2) not exhibiting a well-defined critical micelle concentration (cmc) (3) a progressively increase of

aggregation numbers without reaching a limiting constant value; and (4) a stepwise sequential multiple equilibrium model, i.e. reorganisation of small preaggregates to spherical aggregates at higher concentrations [71, 72]. On the other hand, type II association is characterised by: (1) a moderately defined critical micelle concentration (cmc): (2) relatively large aggregation numbers which reach a constant value at higher surfactant concentrations; and (3) aggregation can be described as a monomer-n-mer model for micellization, as in water.

### *The Effect of Water Ingress on Physical and Chemical Stability of a pMDI*

Ambient moisture diffuses into pMDIs through the valve gaskets [73-75] which can have an effect on the physical stability of pMDI formulations by promoting drug particle growth and aggregation [76, 77]. Water ingress has also been reported to affect HFA propellant charging [78]. Kulphaisal et al. found that the fine particle dose charge polarity of HFA-134a MDIs inverted from negative to positive, when the water content is greater than 300 ppm, after storing under ambient conditions for two weeks. HFA-227ea also charged negatively but the water content of these inhalers only increased slightly after storage and was found to not significantly alter the charge. Valve component materials may also affect aerosol charging because the pMDI formulation contacts these surfaces during the course of dispersion [79].

Thus, the control of the water content both during the manufacture and the storage of pMDIs is very important. The different dipolar nature of the two propellants has an effect on the solubility of the water in the two propellants. In HFA 134a, the water solubility is significantly greater than in HFA 227ea (mole fractions of 0.012 and 0.006 respectively) [13]. The water content of a propellant can also greatly influence the solubility of surfactants. This enhanced solubility has also been shown for solutes showing the possible importance of solute-solvent dipole-dipole interactions. Small amounts of competing dipolar molecules (e.g. water) can cause rapid, irreversible precipitation [64].

### *Particle Engineering Techniques*

Virtually all milling techniques cause partial disruption of crystalline materials and the formation of amorphous material. The presence of amorphous material and the subsequent potential for supersaturations should also not be overlooked. The formulator should chose a particle processing technique which minimises the possibility of amorphous material formation during particle size reduction. This should be assessed, using techniques such as, AFM, microscopic examination and calorimetry, preferably pre- and post-particle size reduction. The effects of long-term relaxation for the material should also be assessed.

Such particle size reduction techniques include spray drying [80] and SCF spray drying, [81]. Spray drying usually produces amorphous material with a consequent higher solubility, which could have detrimental effects, via Ostwald ripening. However, work using SCF spray drying has shown that this can produce high crystallinity products with predictable surface characteristics. This also shows some promise in reducing issues with batch variability in with micronised drugs, which remains an inherent problem [82].

### *ASES*

ASES has been used as an alternative to jet milling [83]. Jet milled drugs are often electrically charged and show a high agglomeration tendency [81]. The micronisation and a coating of drug with a surfactant is possible in a one process step in order to realise a better dispersibility of the active propellant [83]. The ASES process is one of the valid techniques for the precipitation of drugs in supercritical carbon dioxide. The RESS technique (rapid expansion from supercritical solutions) is a similar process which can be applied for drugs being soluble in supercritical gas phase: this is solution is then rapidly expanded to subcritical conditions and the drug precipitates [84, 10]. Another micronization process using supercritical carbon dioxide is the GAS (gas anti-solvent) recrystallisation which is similar to the ASES process [81].

Besides is non-invasive nature, pulmonary drug delivery has many advantages compared to alternative drug delivery strategies, including a large surface area for solute transport, fast drug uptake and improved drug bioavailability [85].

One natural step towards the development of the next generation of formulations is to take advantage of the high functionality that may be embedded into polymeric nanocarriers [86, 87].

### *High Pressure Homogenisation*

Crystalline particles have a number of favourable properties that make their use in pharmaceutical drug formulations very attractive. These are, for

instance, their intrinsic purity due to the strict regularity of the crystal lattice, their chemical stability against degradation such as oxidation. Conventional size reducing methods often have significant drawbacks. For example, it is known that milling can cause morphology changes, such as amorphisation [88] or polymorphic transformation [89]. These phenomena are often attributed to the high input of mechanical energy caused by milling. In some comminution techniques such as bead milling, heavy metal contamination stemming from eroded milling material can be a critical issue [90].

Another drawback intrinsically associated with wet milling techniques, is the necessity to recover the micronized material from the liquid dispersion medium in which the comminution is carried out. In the latter case, the application of dispersion media, such as liquid $CO_2$ could offer a significant advantage, as it is subject to residue-free evaporation upon pressure release, leaving behind a dry and homogenised product of high purity [91]. In this context, high pressure homogenisation represents a comminution technique where the application of a high pressure operating fluid, such as a liquefied gas, may be particularly advantageous. High pressure homogenisation (HPH) of crystalline pharmaceuticals is a comparably novel milling technique with especially promising scale-up properties [92].

The crystalline solid to be comminuted is dispersed in a suitable liquid, thus forming a suspension, which is delivered to the homogeniser using a high pressure pump. The homogeniser is essentially a bottleneck through which the suspension passes with high velocity, thus experiencing a significant pressure drop, turbulent flow conditions and cavitation phenomena. Comminution of particles is achieved by collisions of the particles with each other and with the homogeniser, respectively, and by cavitation [92]. There are only a few studies concerning the homogenisation of pharmaceutical solids that have looked at HPH from a process engineering perspective, e.g. by investigating the dynamics of the process, the performance of different homogenisers and the role of important process parameters such as the pressure drop and the number of passes across the homogeniser.

Johnson et al. [93] used HFA 134a as a dispersion media in HPH. The final product of the high pressure homogenisation, namely micronised drug particles suspended in liquid R134a, corresponds directly to the pharmaceutical dosage form, based on which the drug is bottled, marketed and administered.

Johnson demonstrated the viability of the process using a compound belonging to a group of macrolides and phenytoin. The particle size was found to mainly fall within the range 1-3 um, which is an important range for pulmonary drug delivery.

The performance of pMDIs is a result of the combination of the device and the design of the formulation. Formulation design should, therefore, proceed in parallel with device selection/design. In practice, formulation characteristics often drive selection decisions [94].

### *The Effects of Valve Lubrication on the Physical Stability of Pressurised Metered-dose Inhalers*

The operation of the pMDI device involves movement of the valve stem through a gasket. Upon actuation, this movement releases the dose from the metering chamber to the actuator. In order to prevent the stem from sticking (and thus delivering a sub-optimal dose) a lubricant is usually applied to the valve stem and/or valve seals that are in contact with the stem. For most commercial pMDI valves, food grade silicone oil is employed as the lubricant. There is thus a potential for the valve lubricant to enter into the product both during manufacture (i.e. pressure filling through the valve) and storage.

If silicone oil is in contact with the product contents, it is proposed that it could promote aggregation of the suspended drug particles. Silicone oil is insoluble in this suspension medium and may adhere to the surface of the drug particles modulating the aggregation kinetics of the suspended particles in such a way as to increase the aerodynamic particle size in the emitted aerosol plume.

It is plausible that the drug particles could be coated by silicone oil since both are of low polarity. The presence of surfactant in the formulation, a long chain fatty acid, may also aid in the stabilisation of the silicone oil at the drug surface. The adhesion of silicone oil to the drug surface could reduce the polarity of the drug, thus making it less likely to interact with the highly electronegative propellant medium and lead to an increase in interparticle attraction and aggregation of the suspended drug.

Factors that affect the surface energy of the particles (i.e. by steric or charge stabilisation), include the addition of additives such as surfactants, have been shown to control their aggregation behaviour [95-100, 32]. Furthermore, for a study involving a suspension pMDI, the larger aerodynamic size has

been linked to the high degree of interparticle attraction in the formulation [32].

It can also be hypothesised that silicone oil could alter the evaporation kinetics of drug-associated droplets, as observed in other pMDIs, by increasing the levels of surfactants or other non-volatile ingredients [101-104]. However, they found that silicone oil did not appear to affect the droplet evaporation rate when spiked into a placebo. However, packaging component related factors should also be considered during the product quality testing stage.

## Formulation Technologies for Biomolecules

### *Reverse Aqueous Aggregates*

Reverse aqueous aggregates have been suggested in order to be potentially utilised for the solubilisation and delivery of biomolecules to the lungs, via pMDIs [104]. A large number of surfactants have been screened for their ability to form and stabilize reverse aggregates of water in HFAs [58]. Some limited success was achieved with surfactants containing fluorinated moieties [105-107]. The results obtained indicated a mismatch between the investigated surfactant tail groups and the semi-fluorinated propellant.

Copolymers of propylene and ethylene oxide have high solubility in HFAs and tend to form reverse aggregates in the absence of water [63]. A patent also described such copolymers [108]. However, surfactant concentrations greater than 10% and cosolvent concentrations greater than 20% were required. Selvam et al have shown that surfactants with PO-based tails are shown to be more interfacially active at the HFA/water interface than those containing – $CH_2$ - based moieties [108]. Oleic acid is the most interfacially active of three FDA approved surfactants investigated by Selvam [108]. A typical model propellant for pMDI studies is HPFP. Selvam suggests caution with results obtained from such a model propellant, as the more polar HFA is expected to interact more strongly with polyethylene oxide than HPFP does [108].

The physical state of POE-based, non-ionic surfactants is mainly determined by the length of the POE-chain. The hydrophilic POE moiety significantly influences HFA-solubility. In HFAs, POE solubility decreases with increasing Mn and becomes insoluble when the Mn >1000. HFA solubility increases when the POE moiety increases from 4 to 10 units, but decreases in all HFAs if increased to 23 units.

Ridder et al. found that HFA-soluble surfactants could be mainly found among the Brij and POE-PPO block copolymer surfactants [65]. It appears that the hydrophilic POE moiety significantly influences HFA-solubility. They found that, POE changes its configuration from a zigzag configuration to a meander configuration with an increase in the degree of polymerisation to >10-12 units in aqueous solution and >20-40 units in bulk. However, with POE based surfactants, the change in configuration starts at a degree of polymerisation of 15-20 [66, 67]. This configuration maximises hydrogen bonding, whilst minimising the number of exposed hydrophobic groups resulting in increased polarity and H-bonding capability. The significant reduction in POE solubility in HFAs could be due to a similar change in configuration with increasing degree of polymerisation. The effect of the strongly electron withdrawing fluorine atoms in HFAs, leaves a partial positive charge on the hydrogen substituents. This may allow for H-bonding [2].

Ridder et al. conclude that their investigations into surfactant solubility, in HFA, suggest that there is a strong influence of the hydrophilic moiety on surfactant solubility [65]. Generally, non-ionic surfactants show higher solubility in HFA-227ea than compared to HFA-134a and DFP. They suggest that this is due to stronger interactions between the surfactant and HFA-227ea, for example, H-bonding interactions and dispersion forces. They also suggest that the solubility of surfactants in DFP only correlates with HFA-134a. Wu et al. [109] assessed the ability of biocompatible and biodegradable lactide (LA)-based amphphiles to screen the cohesive forces of salbutamol in HPFP. They noted that the cohesive forces between salbutamol crystals could be suppressed in the presence of ethanol and oleic acid, they could only be partially screened. The LA-based triblock copolymers are capable of reducing the cohesive forces down to zero, even in the absence of a cosolvent. They concluded that the LA moiety can be well-solvated by HPFP and is thus able to provide a steric barrier to flocculation

### *Micro and Nanoemulsions*

Usually drug nanoparticles for pulmonary drug delivery are created by milling down larger particles (top-down) process or precipitating out of liquid phase (bottom up process) Recently, Nyambura et al [110] reported that nanoparticles containing a model protein, lysozyme, had been produced, using microemulsion or nanoprecipitation methods coupled with freeze-drying so that they could be read-

ily dispersed in HFA-134a propellant. However, for both microemulsion and nanoprecipitation, the processes were relatively complex and the liquid containing protein was homogenised to reduce the size of the of the emulsion droplets by high-speed shearing force, which might destroy the molecular structure of the protein. Furthermore, because of the use of organic solvents with low freezing points such as chloroform, dichloromethane and ethanol during emulsification, the freeze drying of emulsion containing protein at -110C to -115C [112, 113] consumed much more electric energy than freeze-drying at conventional temperature (-50C to -55C).

Tan [112] overcome these disadvantages of microemulsion and nanoprecipitation methods to produce protein nanoparticles and simplify the process. They developed a novel bottom-up process to fabricate nanoparticles containing a bioactive model protein, lysozyme. They dissolved lysozyme in a TBA/water co-solvent system in the presence of lecithin as surfactant and lactose as cryoprotectant, followed by freeze-drying and a purification process. They optimised the formulation factors in order to produce nanoparticles with the desired characteristics (i.e. size and retained bioactivity of lysozyme). Furthermore, the dispersibility of the nanoparticles in HFA-134a and retention of lysozyme's bioactivity in HFA-134a suspension were evaluated. Their results indicated that surfactant lecithin concentration in the organic phase and water content in TBA/water co-solvent had a significant effect on the size and polydispersity index (PI) of nanoparticles containing the model protein lysozyme. An optimal formulation containing 24.0% (w/v) lecithin in organic phase, 37.5% (v/v) water in TBA/water co-solvent and 0.56% (w/v) lactose in water was determined to produce lysozyme-containing nanoparticles, with approximately 200 nm in mean size and 0.1 in the polydispersity index. Lactose concentration in the aqueous phase had significant effect on the retained bioactivity of lysozyme, but barely affected the hydrodynamic diameter and PI of the lysozyme nanoparticles. The results demonstrated that about 99% activity of lysozyme could be retained in nanoparticles when 0.56% (w/v) lactose was added in aqueous phase. The formulation with a high concentration of lactose and the production process used ensured the stability of lysozyme, while a minimum mass ratio of 0.25 for lactose-enzyme was previously reported [112].

Bharatwaj [113] Studied core-shell particles consisting of water-soluble, hydrofluoroalkane (HFA)-philic biodegradable copolymer of chitosan and poly(lactic acid) and a core of PLGA. The nanocarriers were prepared by a modified emulsification-diffusion methodology. Dispersions of the core-shell particles in HFA propellant revealed enhanced physical stability compared to polymeric nanocarriers

Polymeric nanocarriers were successfully formulated in propellant-based metered dose inhalers. Biodegradable polymeric nanocarriers were encapsulated within HFA-philic biodegradable and water soluble copolymer shells by emulsification diffusion. The core-shell particles thus formed provide for an opportunity to efficiently deliver nanocarriers to the lungs, by enhancing their dispersibility in the propellant and providing the appropriate geometry (aerodynamic size) so as to enhance the aerosol characteristics of the corresponding pMDIs. In vitro results reveal that nanocarriers from such core-shell formulations can be readily internalised by Calu-3 (airway epithelial) cells infected with C. pneumoniae and more importantly, they can gain access to chlamydial inclusions.

***Insulin Nanoparticles***

Application of ethanol as a co-solvent in pMDI formulations containing proteins has previously been reported [54]. However, the presence of ethanol may affect the stability of protein or the solubility of stabilizers present in protein pMDI formulations during storage.

Food grade natural flavours have been extracted using HFA 134a as the extraction solvent. This suggests that the active ingredients of these flavours that are mainly aldehydes and/or ketones are soluble in HFA-134a. However, the application of these molecules in pMDI formulations and particularly their influence on particle suspension characteristics has not been previously investigated [116]. In this work cinnamyldehyde, cineole and citral were investigated. The group of cinnamyl derivatives and citral are regarded as safe based on their sefl-limiting properties as flavouring substances in food. They are rapidly absorbed, detoxified and excreted in man. No adverse effects are reported and they lack a significant carcinogenicity, genotoxic and mutagenic potential [117].

Nyambura et al [114] produced insulin nanoparticles, via an emulsification process to produce insulin nanoparticles. They found that the emulsification stage in the manufacturing process required optimised parameters that would ensure reduction

of particle size as well as narrowing of the particle size distribution. Effective droplet disruption was achieved by exposing the droplets to high shear stress using a homogeniser. This may be detrimental to insulin, as it could have led to both physical and chemical degradation. However, their results indicated that insulin was not degraded after processing.

An altered composition of secondary structures of insulin has previously been reported when a water in oil emulsion was formed by homogenisation [118]. The authors suggested that exposure of insulin to the oil-water interface, followed by homogenisation, could lead to altered distribution in the secondary elements, e.g., alpha-helix and beta-sheet, measured by area overlap calculations of the infra-red spectra. Non-polar side chains of proteins are more soluble in organic solvents than in water, while hydrophobic interactions are weakened by organic solvents. This causes the protein to unfold and therefore there is loss of native structures. Considering the manufacturing process used, there was the potential for insulin to be denatured while forming the water in oil emulsion. However, their results indicate that insulin was not denatured after processing. The possible explanation for this might be the protection of the insulin from exposure to interfaces by incorporated lecithin in the emulsion. This is in agreement with Bam et al [119].

Removal of the functional water molecules during dehydration has been found to inactivate proteins [120]. As no aggregation or degradation was demonstrated by the results obtained, it can be deduced that insulin was protected from degradation by the lyoprotectant (lactose) during drying.

Their results have shown that a stable pMDI suspension was achieved with the aid of citral or cineole. The mechanism by which citral or cineole stabilised the pMDI suspension was not investigated. However, it is hypothesised that these molecules (citral or cineole) have surface active properties that enable them to stabilise a colloidal suspension. It is suggested that the non-polar end of these molecules orientate themselves towards HFA-134a, while their polar ends orientate towards the nanoparticle. This is thought to be possible because insulin-containing nanoparticles are made up of hydrophobic materials, while HFA-134a is hydrophobic.

**Spray-dried Microcrystals**

It has been hypothesised that hollow porous (Pulmosphere) particles provide advantages with respect to suspension stability and dose content uniformity in metered dose inhalers relative to traditional suspensions of micronized drug [29, 121-123]. The concept revolves around the formation of a “homodispersion”, wherein propellant is able to permeate within the core of the hollow porous particles such that the “dispersed” phase of propellant in the particle core, and the “continuous” phase of propellant which makes up the medium, are the same [122]. The formation of a homodispersion is expected to decrease sedimentation/ creaming and flocculation via a density matching and a reduction in interparticle attractive forces, respectively. Indeed, excellent physical stability of Pulmosphere suspensions have been noted in HFA-134a and this translated into good dose content uniformity, even for very potent drugs like formoterol [29].

Tarara et al. [122] prepared spray-dried porous particles from an emulsion-based feedstock with the active agent dissolved in the continuous phase of the emulsion. Hence, the process has been restricted to active agents that have sufficient solubility in water to enable formulation. They dispersed fine microcrystals of a water insoluble corticosteroid (budesonide) in the continuous phase of the emulsion. The spray-dried budesonide Pulmosphere particles exhibited a spheroidal shape and a high degree of surface roughness. The asperities were thought to impact on the agglomeration properties of the dispersed particles, as the asperities may prevent close approach of the particles to within van der Waals contact [123]. The low surface energy of the hydrophobic phosphatidylcholine also contributes to decreasing interparticle attractive forces [122]. These polymorphic features and surface characteristics likely contribute to the excellent suspension stability noted for these particles in HFA-134a.

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