Applications of the Atomic Force Microscope in the Development of Propellant-based Inhalation Formulations[†]

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Abstract

As the atomic force microscope (AFM) becomes a more accessible tool in the laboratory, researchers involved in the development of pharmaceutical formulations are taking advantage of its unique capabilities. In this work we review the uses of the AFM in the development of hydrofluoroalkane-based pressurized metered-dose inhaler formulations. Perspectives and limitations of the AFM and related techniques in this area also discussed.

Keywords: AFM, pMDIs, force measurements, propellant-based inhalation formulations, hydrofluoroalkanes, HFAs

1. Introduction

It is now accepted that aerosol inhalation therapy is not simply the preferred route for the treatment of respiratory disorders¹⁾, but it may also offer significant advantages in targeting the systemic circulation¹⁻⁴⁾. The large surface area⁵⁾, sluggish clearance⁶⁾, and low proteolytic activity⁷⁾ of the alveolar region provide for the opportunity of fast drug uptake, second only to the intravenous route⁸⁾, and (typically) enhanced drug bioavailability^{5, 9)}. The lungs are also now seen as much more resilient and robust than first thought¹⁾. Another advantage of oral inhalation is that, in contrast to oral and nasal routes, therapeutic biomolecules delivered to the lungs can cross into the systemic circulation without the need of penetration enhancers⁵⁾. All these advantages have stirred a lot of interest in the development of novel oral inhalation formulations⁷⁾.

One can, thus, ask why such potential has not been fully realized yet. While the pulmonary drug delivery technology market is no doubt large, and expected to grow to \$9 billion by 2009, it still represents less than 20% of the overall drug delivery market¹⁰⁾. Perhaps one of the most notable challenges has been with respect to formulation of product development. For

example, protein and gene delivery to the lungs hold great promise in the treatment of clinically important diseases such as cystic fibrosis¹¹⁾ and lung cancer¹²⁾. However, early attempts to formulate genes in nebulizers proved to be quite challenging¹³⁾. Difficulties in translating the CFC-based pressurized metered-dose inhaler (pMDI) formulations (strictly small molecular weight molecules) to hydrofluoroalkane (HFA) propellants¹⁴⁾, as mandated by the Montreal Protocol¹⁵⁾, serves as another illustration of the hurdles in the development of oral inhalation formulations.

Based in part on the typically poor performance of existing pMDI formulations, little work has been done to formulate therapeutic biomolecules in propellant-based inhalers⁷⁾. It is also surprising that while dry powder inhalers (DPIs) are expected to soon occupy 75% of the pulmonary drug delivery market¹⁰⁾, it was not until 2006 that the first DPI product containing a large therapeutic biomolecule (insulin) was launched in the market - this is not only the first DPI, but also the first oral inhalation formulation with a therapeutic biomolecule¹⁶⁾. These problems are expected to be addressed, at least in part, by carefully considering the drug delivery devices and formulation strategies during the pre-formulation stage of product development, and by improved characterization of the physical behavior of the formulations.

The Atomic Force Microscope (AFM) is uniquely suited to probe the interaction between single particles, and particle-surface interactions in both air and liquid, and can thus provide microscopic information that can help guide the design of devices, excipients

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and novel particle technologies to develop systems of relevance to both DPIs and pMDIs. This contrasts with traditional approaches that measure dispersion stability and particle interactions from a bulk perspective as in sedimentation rate experiments¹⁷, centrifugal technique¹⁸, laser scattering¹⁹, and inverse gas chromatography (IGC)²⁰. In this work we focus on the application of AFM and its related techniques to systems of relevance to Hydrofluoroalkane (HFA)-based pMDIs. For detailed information on the applications of AFM for systems of interest to DPIs, the reader is directed to another recently published review²¹.

In what follows (part 2 of the manuscript), we will briefly discuss different oral inhalation devices, focusing on the formulation challenges that pMDIs are currently facing. A description of some of the recently proposed alternative propellant-based inhalation formulations is provided. This section will serve to demonstrate the relevance of AFM in the development of novel propellant-based inhalation formulations. The fundamentals of AFM and related techniques will be presented in detail in part 3. In the last part of this work (part 4), we review the studies that have focused on systems of relevance to Hydrofluoroalkane (HFA)-based pMDIs - mostly those systems where forces were measured in liquid HFAs. Current challenges and opportunities in the application of AFM and its related techniques to systems of relevance to pMDIs are also discussed.

2. pMDIs: The Formulation and Challenges in Improving their Market Share

2.1. The pMDI formulation

A typical pMDI consists of a canister sealed with a metering valve, and lodged upside down in an actuator, as schematically shown in **Fig. 1**.

The compressed liquid propellant, which consists of more than 98% of the formulation²², is in equilib-

Fig. 1 Schematic diagram of a pressurized metered-dose inhaler (pMDI).

rium with its vapor (under saturation pressure) in the canister. The system is purposely kept in equilibrium with its vapor phase (vs. being in a compressed liquid phase) so that the solvent quality and the pressure inside the canister remain the same from the beginning to end of the formulation, thus helping maintain dose uniformity²². Commercial pMDI formulations contain small solutes either in solution (drugs dissolved in the propellant, usually with the help of a co-solvent) or in suspension (micronized drug crystals with or without the help of other excipients)²³. The approximately 20 HFA-based pMDIs in the market today are somewhat evenly distributed between solution and dispersion formulations²³. The propellant generates a pressure of 50-80 psig inside the sealed unit¹⁴. Upon pressing the actuator, the propellant and active drug (with potentially other excipients) that have been previously stored in the metering chamber are exposed to atmospheric pressure. The propellant flash evaporates to form an aerosol cloud containing the drug (along with any other excipients), which is inhaled by the patient²⁴.

2.2. Challenges in the development of pMDI formulations

The advantages and disadvantages associated with the different aerosol inhalation devices have been extensively discussed in the past^{22, 25, 26}. Nebulizers require that the aqueous medication is atomized into fine droplets that are inhaled by patients through a mouthpiece or mask. The use of nebulizers is generally limited to in-house treatment since the devices are not portable²⁷. pMDIs are the most widely used inhalation devices because they are portable, easy to use, and have high patient compliance. They also typically generate reproducible dosages, and the drugs are protected from the elements, as they remain in a sealed environment¹⁴. One of the major drawbacks of pMDIs is the substantial oropharyngeal deposition typically observed, which may be somewhat mitigated by the use of spacers²⁸. Other considerations with pMDIs are the limited concentration of the delivery dosage²⁹, and limited range of drugs that can at this time be formulated²². DPIs are breath-activated devices. Patient coordination, therefore, is not as important, resulting in typically better deposition in the deep lungs compared to pMDIs. The deposition efficiency of DPIs is, however, still dependent on the patient's respiratory flow³⁰. While motor-driven DPIs have been introduced to overcome such deficiencies³¹, the devices are costly. It is worth pointing out, however, that the device selection depends highly on

the medical indication, patient compliance, the formulation, and the characteristics of the agent being administered²⁷. The ability to formulate in both DPIs and pMDIs is, therefore, of importance from the patient compliance and commercial stand points.

Besides the inherent difficulties (discussed above) in formulating with pMDIs, the development of propellant-based inhalers has also been challenged by the phase-out of CFCs, as mandated by the Montreal Protocol¹⁵. The more environmentally friendly and non-ozone depleting³²* HFA propellants, more specifically, 1,1,1,2-tetrafluoroethane (HFA134a) and 1,1,1,2,3,3,3-heptafluoropropane (HFA227), are currently used in pMDIs³³. In spite of the fact that the operation of pMDIs with HFAs is similar to those with CFCs, previous CFC formulations are generally not compatible with HFAs due to the significantly different solvent properties between these two classes of fluids¹⁵. For example, the excipients used in FDA-approved CFC formulations have extremely low solubility in HFAs⁸. The design of HFA-philic excipients is, therefore, of great relevance. However, the lack of fundamental understanding on the solvation forces in the semi-fluorinated HFAs is hindering the resolution of the challenges in improving existing formulations, and also from developing novel HFA-based pMDIs³⁴.

While the market share of HFA-based pMDIs by 2009 (out of the whole inhalation delivery market) is estimated to be only 5%, losing ground especially to DPIs, this trend may be reversed if existing reformulation issues can be overcome^{10, 22}. As new technologies become available in propellant-based inhalation formulations, especially with therapeutics that target the systemic circulation and/or therapeutic biomolecules, it is expected that the market share of pMDIs will grow within the inhalation aerosol market, and may also take some of the market share currently not being targeted by oral inhalation products.

2.3. Novel HFA-based pMDI formulations

With the phase-out of CFC-based formulations, and challenges in reformulating in HFA propellants, research groups in academia and industry are taking the opportunity to innovate²³. Efforts in the development of novel propellant-based inhalation formulations[†] can be classified with respect to the nature of the dispersed phase: aqueous- (emulsions/

microemulsions) and nonaqueous(solid)-based systems^{23, 35}.

Aqueous-based dispersions can be stabilized in the form of emulsions (micron size and kinetically stable)²² or microemulsions (nanometer in size - transparent, and thermodynamically stable)^{36, 37} provided a suitable amphiphile is identified. Poly(lactide)-³⁸, poly(propylene oxide)-³⁹ and fluorine-based⁴⁰ non-ionic amphiphiles have been shown to be effective in stabilizing reverse water-in-HFA (W/HFA) emulsions. The emulsion-based formulations can be potentially used for the local or systemic delivery of therapeutics to and through the lungs. W/HFA microemulsions have also been suggested as a potential formulation for the pulmonary delivery of therapeutics (both small molecules and large biomolecules)³⁶. Fluorinated ionic and non-ionic amphiphiles can form microemulsions of water in propellant HFA^{41, 42}. However, the range of suitable fluorinated surfactants is limited due to their potential toxicity^{40, 43}. Water-in-HFA227 microemulsions have been recently reported in the presence of an ethoxylated triblock copolymer and ethanol³⁶. The uptake of biomolecules and anti-cancer therapeutics, and the aerosol characteristics of the corresponding formulations have been investigated³⁶. Microemulsions may serve as an alternative to traditional solution/dispersion-based formulations of high-potency systems as the active component remains solubilized within the core of the aggregate, preventing its interaction with the container walls and other parts of the device that may significantly reduce the dosage^{41, 42}. Some results of the attempts associated with the formulation of aqueous emulsions and microemulsion in propellant HFAs are shown in **Fig.2**.

One of the challenges in the development of such formulations is the fact that a large concentration of surfactants and cosolvents are usually required to stabilize the aqueous domains.³⁶ The presence of such low-volatility excipients can reduce the vapor pressure of the aerosol mixture, thus negatively affecting the efficiency of the aerosol³⁶. A significant body of work from AFM-related techniques provided the insight for the design of amphiphiles containing highly HFA-philic groups, which are required for the formation and stabilization of such aqueous aggregates^{34, 35}. We will discuss (below) in detail how AFM can be used to probe solvation forces in systems of relevance to pMDIs.

Traditional non-aqueous pMDI formulations are usually composed of pharmaceutical particles in suspension or drugs in solution in a propellant HFA²³.

* Although HFAs are not ozone-depleting, they are green house gases and are also known to produce tri-fluoroacetic acid (TFA, CF_3COOH) in the troposphere³².

† Solution-based formulations are not being directly considered here.

Fig.2 Results from some attempts to develop emulsion and microemulsion-based pMDI formulations. (a) Schematic diagram and digital images of the water-in-HFA227 emulsion formed at 30:70 v/v water/HFA227 and 1 w/w % $EO_{13}PO_{30}EO_{13}$; adapted from ref. [39]; (b) physical stability of pMDI formulations containing reverse aqueous microemulsions of (left) a yellow probe, and (right) 3mM irinotecan hydrochloride (anti-cancer drug) in HFA134a, in the presence of 10 % w/w ethanol, stabilized by 5.65 % w/w (21.5 mM) $PO_{22}EO_{14}PO_{22}$; adapted from ref. [37]; (c) Small angle scattering spectra of reverse aggregates of water in HFA134a in the presence and absence of alcohol. Curves as a function of the corrected water-to-surfactant molar ratio (W_o). The curve with highest scattering demonstrates the uptake of a model biomolecule within the core of the aggregate. Reprinted from ref. [36], with permission from RSC Publishing.

Cosolvents (notably ethanol) are generally required in the solution-based formulations to help solubilize the active pharmaceutical ingredients. There are several limitations associated with solution formulations. The drug of interest must be soluble in the propellant or propellant-excipient mixture, thus significantly restricting the range of therapeutics (certainly of biomolecules) that can be formulated. The presence of less volatile components (such as alcohol) may alter

the aerosol performance³⁷, and the chemical stability of the formulation⁴⁴. In dispersion-based formulations, colloidal stability of the pharmaceutical particles is key in controlling aerosol efficiency^{23, 35, 45}. Stability of colloids in HFA propellants can be achieved either through the addition of stabilizing excipients⁴⁵ or by altering the particle surface chemistry or its morphology⁴⁶⁻⁵⁰. There have been several studies focusing on the development of novel HFA-philic excipients capable of imparting stability to micronized drug dispersions in HFA propellants^{23, 45, 51-53}. Efforts have also been made to modify the surface of particles. For example, poly(ethylene glycol) (PEG), poly(vinyl pyrrolidone) (PVP), and poly(vinyl acetate) (PVA) have been employed to coat the particles surface to reduce the cohesive forces⁴⁷⁻⁴⁹. Drugs have also been formulated as cores within polymeric shells designed with HFA-philic (biodegradable and biocompatible) moieties, which were used to reduce inter-particulate forces in HFA propellants⁴⁶. The advantage of particle surface modification compared with surfactant-stabilized colloids is that no / little free stabilizers remain in solution, thus decreasing the potentially toxic effects of excipients⁴⁷. Challenges associated with the synthesis of well-balanced amphiphiles are also circumvented. Engineering particles with certain morphological architectures can also help enhance colloidal stability^{50, 54, 55}. For instance, porous particles possess excellent stability in HFAs, as the propellant can penetrate inside the particles, leading to improved density matching of the particles with the propellant, and also a reduction of the van der Waals forces between the particles⁵⁰. Particles with a high degree of surface roughness can also be used to improve physical stability of the formulation by reducing the area of contact between particles⁵⁶. Some results related to efforts in the development of novel (solid) suspension-based pMDI formulations are shown in Fig.3.

Fig.3 (a) SEM micrograph of PEG-trapped salbutamol sulfate (SS) spheres prepared by emulsification diffusion; long term stability in HFA227 was achieved; Reprinted from ref. [47] with permission from Elsevier. (b) SEM and (Inset) Optical image of salbutamol suspension with 0.1 mM $LA_{53}EO_{22}LA_{53}$ HPFP solution. Reprinted from ref. [45] with permission from American Chemical Society. (c) SEM image of core-shell particles with SS as the drug core and oligo(lactide)-g-chitosan as the HFA insoluble shell, (Inset) TEM image. Reprinted from ref. [46] with permission from Springer. Enhanced stability in HFA227 was achieved due to the good solvation of the outermost part of the particle shell by HFA.

Knowledge of the influence of the modification of the chemistry of the particle surface, or the particle morphology, or the presence of excipients affect particle cohesive/ adhesive interactions have contributed significantly to the development of novel excipients and particle engineering technologies relevant to pMDI formulations, some of which were discussed above. The applicability of AFM to the measurement of forces between particles and particle-surface forces of relevance to pMDIs will be discussed in detail below. First, however, it is necessary to understand the basics of AFM hardware, related theory and AFM-derived techniques.

3. The AFM and Related Techniques

3.1. The AFM and its hardware

The AFM was invented in the mid 80's⁵⁷⁾. It was a welcome addition to the family of scanning probe techniques as previous microscopes could not image the topography of non-conductive samples. With the resolution of nanometers (nm) or higher (lateral of order of 10 nm and vertical as high as 1 Å), and force sensitivity as low as 10^{-12} N (1 pN)⁵⁸⁾, the AFM has key components of (i) a cantilever, usually made of Si or Si₃N₄, which can be integrated with a pyramidal sharp tip with radii of 5~50 nm; (ii) a piezoelectric scanner, which controls the movement of the AFM tip or sample precisely in the x, y and z directions; and (iii) a photo diode sensor, which is used to detect the laser reflected from the backside of the cantilever (gold plated to enhance the reflection of the laser). A schematic diagram of the main components of an AFM is shown in **Fig.4**. There are many variants to

Fig.4 Schematic diagram of the main components of an AFM. Adapted from ref. [60].

this scheme. The reader is directed to earlier reviews for more detail on this topic.⁵⁹⁻⁶²⁾

3.2. Imaging with AFM

There are two basic types of operation modes for AFM imaging: contact mode and tapping mode⁶⁰⁾. In the contact mode regime, the system is connected, via a feedback loop, to a piezo motor, which controls the position of either the sample or AFM tip. The force between the tip and the sample surface is usually kept repulsive and constant during scanning so that any changes in the surface topography will cause a deflection on the cantilever⁶²⁾. This deflection is detected by the photodiode signal via the reflected laser, thus leading to an adjustment in the height of the piezo-electric scanner to compensate for the deflection. The force is, thus, maintained at the set point. Based on the force measurements, a topographic image of the surface is generated⁶³⁾. Tapping mode is the other commonly used imaging mode⁶⁰⁾. In this case, the cantilever is oscillated at or close to its resonant frequency. Since the cantilever is oscillating and tapping on the sample surface, it contacts the sample in a very short time scale so that the lateral forces applied to the cantilever during scanning are reduced significantly. Tapping mode is especially suited for soft surfaces⁶⁰⁾. AFM imaging is an important technique in the pharmaceutical industry⁵⁹⁾. The applicability of AFM imaging to pMDI related formulations includes the investigation of the effect of propellant on the aerosol canister lining⁶⁴⁾, roughness measurements for drug crystal substrates²⁰⁾, and determination of contact radii for colloidal AFM probes⁶⁵⁾. AFM can also be used to evaluate fluid viscosity according to the resonant frequency shift of the cantilever when immersed in a medium⁶⁶⁾. This may be an attractive way to determine the effect of more expensive excipients on the viscosity of systems relevant to pMDIs.

3.3. Force measurements with AFM

AFM is not only capable of generating images with high resolution⁶⁰⁾, but also (and perhaps most importantly) to directly probe forces between surfaces^{59, 62)}. There have been several excellent reviews on the principles and applications of force measurements by AFM^{59, 62)}, and the reader is directed to those references for more details on the technique. Here we provide the minimum amount of information on the basics of force measurements by AFM, which are required to appreciate the discussion that follow on solvation forces in HFAs, and colloid stability in pMDI-related systems.

Fig. 5 Schematic diagram of a typical AFM force-curve.

A typical force-distance AFM curve with the complete approach-retract cycle is shown in **Fig. 5**. At large separation distances (1) there is no detectable force between tip and substrate, so that the AFM cantilever is not deflected; the difference between the approach and retract cycles in this region is usually caused by the hydrodynamic drag due to the presence of the medium⁵⁹; (2) as the tip approaches the substrate, it jumps into contact due to Van der Waals (VDW) attractive forces; (3) after the contact, a linear cantilever deflection is observed as the tip moves further into contact with the substrate; (4) when the cantilever deflection reaches the force set point, the tip starts retracting from the substrate; in this contact regime, the discrepancy between approach and retract line is originated from the plastic or viscoelastic deformation of the sample; (5) the force needed to pull the tip off from contact with the substrate as the tip retracts from the substrate is defined as the force of adhesion (F_{ad})[‡] between the tip and substrate. F_{ad} is determined from the normal displacement (Δz) and spring constant (k) of the cantilever ($F_{ad} = k \cdot \Delta z$)⁵⁹.

There are two types of modifications to a typical AFM force measurement (modifications to the tip and/or substrate) that can be extremely valuable in the studies of colloid stability and surface science, namely: chemical force microscopy (CFM)⁶¹, and colloidal probe microscopy (CPM)⁵⁹.

3.3.1. The chemical force microscopy (CFM) technique

CFM is an area of great interest as it can be utilized to probe forces in a variety of systems of relevance in engineering and science^{61, 67}. Several excellent reviews have been published on CFM, and the reader is directed to those for more details on

[‡] Loosely called adhesion; can represent either cohesive or adhesive forces.

Fig. 6 Schematic diagram of a CFM probe and substrate modified with a monolayer containing terminal groups having a particular chemistry, in a solvent environment.

the technique^{59, 61, 62, 67}. To perform CFM, a sample surface and AFM tip are both chemically modified with functionalities of interest⁶¹. The functionalities typically have terminal groups with some specific chemistry, and points of attachment to the substrate - either silanes or thiols. The functionalities can be deposited by self-assembly (vapor phase or solution)³⁴. CFM can in principle be used to measure molecular interactions ranging from weak van der Waals (VDW) ($< 10^{-12}$ N) to strong covalent bonds (10^{-7} N)^{59, 62}. A schematic diagram of a chemically modified tip and substrate for a CFM experiment is shown in **Fig. 6**.

Thiols with appropriate alkyl chain lengths have been shown to form compact monolayers when deposited onto solid substrates⁶⁸. The reliability in depositing thiols may prove valuable, as microscopic information on the monolayer is often required to interpret the CFM results⁵⁹. Under appropriate circumstances, compact silane monolayers can be also formed^{68, 69}. This is relevant because it may circumvent the need to generate (the sometimes cumbersome step) smooth gold layers on both the substrate and the AFM tip, which are required for the deposition of thiols⁷⁰. The use of silanes also simplifies the functionalization of colloidal probes⁶¹. That flexibility may prove valuable as higher sensitivity may be achieved compared to the modification of the tip alone. Colloidal probes also provide the opportunity to determine the geometry of the interacting surfaces more quantitatively³⁴.

Typical force curves as those shown in **Fig. 5** are also observed in CFM experiments with small mole-

cules⁶²). The complexity of the force curves increases significantly, however, for systems involving polymers and/or those with specific interactions (e.g. ligand-receptor)⁵⁹. One of the key pieces of information that can be extracted from CFM experiments is contained in the F_{ad} obtained in the retract stage of the force curve measurement⁶². Different types of interactions can be studied quantitatively by varying the moieties attached to the AFM tips, including the solvent medium effects^{61, 67}. The solvent environment plays a crucial role in the force measurements^{59, 61}. When the solvent is chemically similar to the functional groups on the tip and sample, the force required to pull the tip off the sample is expected to be small as the enthalpic penalty for breaking the contact (creating the functionalized tip-medium and functionalized substrate-medium interfaces) is small. On the other hand, the incompatibility of the solvent with the functional groups on the tip and sample would render a large F_{ad} .⁵⁹

CFM can serve, therefore, to understand solvation forces, and thus to identify/design/screen chemistries that could have enhanced solubility and capable of working as stabilizers of drug dispersions³⁵. Some of the limitations associated with the CFM technique include the fact that some chemistries of interest may not be commercially available in the form of a silane or thiol, thus requiring the synthesis of those moieties - which may not be trivial. In certain circumstances, degradation of the monolayers may occur⁵⁹. Because the region of contact between the AFM probe and the substrate may not be probed directly, reproducibility in the experiments (in many independent repeats) is thus key to CFM^{61, 62}.

3.3.1.1. Single molecule (pair) force from CFM

CFM results are typically normalized by the radius of curvature of the AFM tip (R) to allow for comparison between different systems⁶¹. While such information is quantitative, and generally sufficient to understand trends in solvation forces⁶², theories describing elastic deformation that exists during the contact between sample and substrate may be employed to further normalize the F_{ad} ⁵⁹, and can thus be used to corroborate experimental findings or in tandem with theoretical studies⁵⁹.

The F_{ad} of an elastically deformed tip and substrate can be described by the Johnson-Kendall-Roberts (JKR)⁵⁹ or the Derjaguin-Muller-Toporov (DMT)⁶² model, as shown in the equations grouped in **Fig. 7**.

The difference between these two models is that the JKR model considers the interaction only within

(a)	$F_{ad} = 2\pi RW_{tms}$	(DMT Model)
(b)	$F_{ad} = 1.5\pi RW_{tms}$	(JKR Model)
(c)	$\phi_0 = \left(\frac{W_{tms}^2 R}{E^* z_0^3} \right)^{\frac{1}{3}}$	
(d)	$W_{tms} = \gamma_{tm} + \gamma_{sm} - \gamma_{ts}$	
(e)	$\gamma_{sm} = \gamma_s - \gamma_m \cos \theta$	
(f)	$a = \left(\frac{1.5\pi R^2 W_{tip-medium-substrate}}{K} \right)^{\frac{1}{3}}$	
(g)	$\frac{1}{K} = \frac{3}{4} \left(\frac{1 - \nu_{tip}^2}{E_{tip}} + \frac{1 - \nu_{substrate}^2}{E_{substrate}} \right)$	
(h)	$n = \pi a^2 / A$	
(i)	$F_s = F_{ad} / n$	

Fig. 7 (a) DMT Model for F_{ad} , where R is the radius of curvature of the AFM tip. W_{tms} is the work per unit area required to separate the modified tip and substrate in the solvent medium. (b) JKR Model for F_{ad} . (c) This equation may be used to select which model is more appropriate (DMT, JKR), where z_0 is the equilibrium size of the atoms at contact. E^* equals to $3K/4$, K is the reduced elastic modulus shown in equation (g). JKR is suggested if ϕ_0 exceeds 0.3, otherwise DMT model should be employed⁷¹. (d) W_{tms} is given by the interfacial energy, where γ_{tm} and γ_{sm} are interfacial free energy of the tip and substrate with the medium respectively, γ_{ts} is the interfacial free energy between the tip and substrate. For the same functional groups on the surface of the tip and substrate, γ_{ts} is zero, and $W_{tms} = 2\gamma_{tm} = 2\gamma_{sm}$.⁵⁹ (e) Young's equation from which γ_{sm} and γ_{tm} can be calculated, where γ_s and γ_m are the surface free energy of substrate and medium respectively and θ is the contact angle of the medium on the substrate. Note that according to the equations given above, F_{ad} can be estimated from θ measurements, provided θ is large enough. (f) Contact radius a according to the JKR Model, where K is the reduced elastic modulus for the tip-substrate combination and given by equation (g), ν is the Poisson ratio, and E is the Young's modulus. The number of contact groups n at rupture can be determined as shown in equation (h), where A is the area per molecule. (i) F_{ad} can be further normalized to single molecule (pair) force F_s .

the contact region while DMT model assumes that adhesion forces are originated from interaction outside the contact region^{59, 65, 71}. The area of contact between tip and substrate (as the contact ruptures during the retraction stage of the CFM) is given by equation (f) shown in **Fig. 7**. One of the challenges related to the evaluation of single molecule (pair) force from CFM is to find out the area per molecule

(A) on the highly curved AFM tip - less of an issue on the flat substrate, necessary to find ' n ' see **Fig. 7(h)**. There is also some potential variability in the value selected for the Young's modulus and Poisson ratio of the modified AFM tip⁵⁹. Moreover, both DMT and JKR models are just theoretical approximations. Therefore, a combination of repeated experiments, different approaches to surface modification, and corroboration with theoretical results all should help improve confidence on CFM results.

3.3.2. The colloidal probe microscopy (CPM) technique

The principle of operation of CPM is very similar to CFM⁵⁹. Instead of measuring interaction forces between functional groups, however, the adhesive/cohesive interactions between a single particle attached to an AFM tip with another particle or a planar substrate are evaluated. A schematic diagram and real (optical and SEM) images of the tip and substrate assembly for a CPM experiment are shown in **Fig. 8**.

The single particle is usually glued to an AFM tip using epoxy resin or another adhesive⁵⁹. CPM can be used to understand the forces between particles, or particles with surfaces, which dominate the colloidal behavior of a variety of materials and processes of technological relevance⁵⁹. When retracting the tip from the substrate, the total force (F_{ad}) required to detach the colloidal probe from sample surface is a sum of components, which depend on the conditions of the medium (air/solvent; polar/nonpolar) and the nature of the particle and substrate⁵⁹. The lowest common denominator (forces that are always present) are the van der Waals forces (F_{vdw}). When F_{vdw} dominates F_{ad} , as it is expected in most systems of relevance to pMDIs, then F_{ad} can be used to estimate

the Hamaker constant of drugs of interest⁵⁹. This will be the topic of a section to be presented later on the manuscript.

F_{ad} from CPM has been successfully used to understand the effect of the solvent medium and excipients on the stability of colloidal dispersions⁵⁹, including those of relevance to pMDI formulations^{45, 51, 72}. A low F_{ad} correlates with reduced inter-particle forces (and thus enhanced physical stability), and vice versa. The variation of F_{ad} is dependent on the roughness of substrate surface. F_{ad} histograms generally have a Gaussian distribution of forces for smooth substrates^{20, 73}. The size of the particles are typically not important. Even for large particles, gravitational forces can be usually neglected during CPM measurements - the gravitational forces on a 50 μm particle is $\sim 1 \text{ pN}$ ²¹.

The magnitude of the force obtained from CPM is highly dependent on the contact area, whose precise determination is especially difficult in systems where the probe is a micronized crystal, as it is typically the case in pMDI formulations^{45, 51}. Hence, normalization of forces with respect to the contact area and the subsequent comparison for different types of probes in the case of CPM is a much harder proposition when compared to CFM⁵⁹. Despite availability of established methodologies in the literature, as for example contact area deconvolution by a tip characterization grating substrate⁶⁵ and AFM imaging of lithography indentation⁷⁴, it is still very challenging and demanding to precisely determine the contact area and geometry of an irregular drug crystal attached to an AFM tip^{20, 65}. This limitation may restrict comparison of data collected between different groups, and even within the same group, when using different probes²⁰. While careful experimentation using a single tip (and then repeated experiments to confirm trends) may be used to screen excipients, the comparison between different particle preparation methodologies, for example, is restricted if a precise contact is not known²⁰. Readers are directed to ref. [59] (review article) for more details on the CPM technique.

3.4. The measurement medium

The magnitude of the forces between surfaces (such as those measured by AFM) is highly dependent on the measurement medium^{59, 61, 62}. DPIs are composed of particles that come in contact with (humid) air upon device actuation²⁶. AFM experiments for DPI-related systems are, therefore, usually conducted in air^{75, 76}, and preferably with rigorous

Fig. 8 (a) Schematic diagram of the elements in CPM microscopy in a liquid solvent; (b) SEM micrograph of a CPM probe. Reprinted from ref. [47] with permission from Elsevier; (c) Optical micrograph of the modified AFM cantilever and the smooth single crystal during an F_{ad} measurement in HPFP. Reprinted from ref. [45] with permission from American Chemical Society.

control of the humidity⁷⁷), as capillary forces (due to the presence of water in the environment) dominate particle-particle interaction^{21, 76}). On the other hand, the drugs in pMDIs are either solubilized or dispersed in the propellant²³). Therefore, force measurements for pMDI-related formulations are typically performed in liquid.⁸ However, due to the low boiling point of HFA227 (246.88 K)¹⁵ and HFA134a (256.65 K)¹⁵, the HFA propellants are in the gaseous state under ambient conditions. HFA propellants cannot, therefore, be used directly as the force measurement medium in existing commercial AFM systems. Model propellants, which are liquid at ambient conditions and are suitable for use in conventional AFMs, have, therefore, been proposed⁷⁸). The model solvent should have a molecular structure and physicochemical properties similar to the propellant HFAs. Because of its physicochemical properties, the hydrofluoroalkane 2H,3H-perfluoropentane (HPFP)^{**} has emerged as the model liquid propellant of choice. Some of the properties of HPFP more closely resemble those of HFA134a such as dipole moment and dielectric constant, while others more closely match those of HFA227, such as molecular size and boiling point⁷⁸). While the literature is still somewhat limited in terms of directly probing the ability of HPFP to mimic propellant HFAs⁷⁸), at least in terms of the physical stability of dispersion formulations, the results in HPFP seem to better correlate to those in HFA227⁷⁸). Our group has recently demonstrated that the magnitude of the enthalpic interactions (dispersion+electrostatic) between propellant HFAs and polar fragments increased significantly on going from HFA134a to HFA227 due to the acidity of *H* and the larger size of the HFA227 molecule⁷⁹). In this aspect, we would expect HPFP to be a closer match to HFA227. Currently, the measurement medium of AFM is limited to air or liquid, and there is still no AFM available in the market that has accommodated a high-pressure environment. Although HPFP is widely accepted as being similar to HFAs, and it is commonly used as an alternative propellant for HFAs (not only for AFM experiments)⁷⁸), these solvents have different physicochemical properties. Caution is

thus suggested when extrapolating the results from AFM to propellant-based formulations.

4. The AFM and the Development of Propellant-based Inhalation Formulations

Force measurements by AFM have been widely used in the development of novel inhalation formulations, especially for DPIs²¹), and more recently for systems of relevance to pMDIs²³). DPI formulations usually contain micronized drug particles by themselves or drug particles mixed with a larger particle carrier (e.g. lactose particles) that work as a flowing agent^{26, 80}). CPM is especially suited to assess the adhesive/cohesive balance between the drug and carrier particles, which affects the blending and efficiency of the aerosol generated²¹). AFM is also of great relevance in the design of alternative propellant-based pMDI formulations²³). CFM can be used to screen potential candidate chemistries with enhanced solubility and ability to stabilize dispersions in HFAs^{34, 35}). CPM is also well-suited for the development of suspension-based pMDI formulations, which account for approximately 50% of currently marketed pMDIs^{23, 45}). The effects of the solvent environment and additives, such as cosolvents and amphiphiles, on traditional dispersion formulations can be investigated by CPM⁴⁵). A review^{† †} of all AFM, CFM and CPM work reported to date on pMDI-related systems is discussed below. A summary of the studies is given in **Table 1**. We also address other measurements that can be made with the AFM and that are of potential interest to pMDIs such as determination of particle density^{81, 82}) and Hamaker constant^{59, 83}).

4.1 Solvation forces by CFM

Understanding solvation in hydrofluoroalkane propellants is of great importance for the development of novel pMDI formulations. Steric stabilization is the predominant mechanism for imparting stability to colloidal particles in low dielectric media such as HFA propellants⁸⁴). The ability of the propellant in solvating the stabilizing moieties govern particle-particle interaction^{35, 45}). When two colloidal particles approach each other, the stabilizing tails on the particles' surfaces may interpenetrate. If the medium is a good solvent for the stabilizing moiety, the interpenetration is thermodynamically disfavored, leading to stable colloidal suspensions⁸⁴).

CFM studies are relevant not only in the context of the traditional solution and dispersion pMDI formulations, which generally require surfactants as

[§] Some initial force measurements related to pMDI formulations were conducted in air⁷⁵).

^{**} Vendors for HPFP include Synquest Labs, Inc; TMC Industries, Inc; Apollo Scientific, Inc; and Dupont.

^{† †} An exhaustive search was performed on SciFinder[®]. Works published by April 2008 (which was the deadline for submission of this manuscript) have been included in this review.

Table 1 A summary of the literature work (journal articles) on AFM, CFM and CPM related to pMDI formulations

AFM	AFM probe	Substrate	Excipient	Medium	Purpose	Year
Imaging	^a silicon tapping-mode tip	Epoxy-coated aluminum	None	Air	Characterization of the properties of the wall lining after exposure to propellants, up to 40 °C	1997 ⁶⁴
	Silicon tapping-mode tip	SS, BUD and FF crystal	None	Air	Determination of drug crystal roughness	2005 ²⁰
	Silicon tapping-mode tip	Aluminum coated with various materials	None	Air	Determination of canister surface roughness coated with various materials	2006 ⁷³
CFM	AFM probe	Substrate	Excipient	Medium	Purpose	Year
Solvation forces	^b Silane-modified AFM tip	Silane-modified substrate	None	HPFP	Investigation of HFA-philicity of hydrogenated and fluorinated moieties	2007 ³⁴
	Silane-modified AFM tip	Silane-modified substrate	None	HPFP	Investigation of HFA-philicity of an ester-based moiety	2007 ⁸⁵
	Silane-modified AFM tip	Silane-modified substrate	None	HPFP	Investigation of HFA-philicity of an ether-based moiety	2007 ³⁵
CPM	Drug probe	Substrate	Excipient	Medium	Purpose	Year
Design of pMDI hardware	^c Single micronized Salbutamol sulfate (SS) particle	Borosilicate glass, aluminum, PTFE-coated aluminum	None	HPFP	Evaluation of forces between a single SS particle with different pMDI canister materials	2003 ⁷²
	Formoterol fumarate (FF) particle cluster	Aluminum PEG, PVP and FF coated aluminum	PEG, PVP	HPFP	Evaluation of the effect of excipients on the interaction between drug particles and canister wall	2004 ⁹⁰
	Single micronized SS, budesonide (BUD) and FF particles	Aluminum coated with various materials	None	HPFP	Evaluation of the interaction of various drugs with a series of pMDI canister lining materials	2006 ⁷³
Formulation design without excipients	Single micronized SS particles	Pyrolytic graphite	None	HPFP	^d Determination of the effect of particle geometry on forces	2003 ⁶⁵
	Single micronized SS, BUD and FF particles	SS, BUD and FF crystals	None	HPFP	Determination of cohesive/adhesive balance for various drugs in excipient-free medium	2005 ^{20, 89}
Excipient Design	Single micronized (SS)	SS crystal	PEG, PVP	HPFP	Investigation of the effect of polymeric excipients on inter-particulate forces for SS	2007 ⁵¹
	Single micronized Salbutamol base (SB)	SB crystal	Ethanol, PLA-PEG-PLA, Oleic acid, Pluronic [®] surfactant	HPFP	Evaluation of the effect of various excipients on the inter-particulate forces for SB	2007 ⁴⁵
Particle Surface Engineering	Single SS sphere, Single PEG-coated SS sphere	SS spheres, PEG-coated SS spheres	PEG 300	HPFP	Evaluation of effect of PEG-coating on inter-particulates forces	2008 ⁴⁷
	Single SS core-shell particle, Single BSA core-shell particle	SS core-shell particles, BSA core-shell particles	Oligo(lactide)-grafted-chitosan	HPFP	Evaluation of the effect of an HFA-philic shell on the inter-particle forces for both small drug solutes and biomolecules	2008 ⁴⁶

^a first AFM; ^b First CFM; and ^c first CPM study involving a system of relevance to pMDIs. ^d the geometrical profile of the drug probe (and area of contact) was evaluated, and the work of cohesion quantitatively determined by CPM.

excipients, but also for the development of novel HFA-based formulations that can be potentially used for the delivery of drugs (small molecule and large therapeutic biomolecules) to and through the lungs, such as reverse aqueous microemulsions and emulsions in HFAs, and other particle-based approaches that require the presence of well-solvated stabilizing moieties²³⁾.

Our group has utilized CFM to evaluate the forces of interaction between a chemically modified AFM tip and substrate in HPFP^{34, 35, 85)}. The tip and substrate were modified by silane chemistry, using both solution and vapor phase approaches^{34, 35, 85)}. Contact angle measurements and extensive force measurements were conducted to confirm quality of the silane monolayer at each step during the surface modification procedure³⁴⁾. Several different types of chemistries were investigated, including a methyl-based (*CH*) moiety (octyltrichlorosilane, $\text{CH}_3(\text{CH}_2)_7\text{SiCl}_3$), and a more polar ether- (*COC*) (3-methoxypropyltrimethoxysilane, $\text{CH}_3\text{O}(\text{CH}_2)_3\text{Si}(\text{OCH}_3)_3$) and ester- (*C(O)OC*) (2-acetoxyethyltrichlorosilane, $\text{CH}_3\text{COO}(\text{CH}_2)_2\text{SiCl}_3$) terminated silanes^{34, 35, 85)}. The *CH* moiety represents the tails of surfactants in FDA-approved pMDI formulations, which have low solubility in HFAs³³⁾. It thus represents the baseline moiety. *COC* and *C(O)OC* contain polar sites that may provide for an opportunity for enhanced interactions with the propellant HFAs.

The CFM results, plotted as the normalized adhesion forced F_{ad}/R (where R is the radius of curvature of the AFM tip) are shown in **Fig.9**. For a methyl-modified tip and substrate in HPFP F_{ad}/R was found to be $68.2 \text{ mN} \cdot \text{m}^{-1}$.³⁴⁾ The F_{ad}/R for the ether and ester-based moieties is 24.8 and $4.8 \text{ mN} \cdot \text{m}^{-1}$, respectively. A lower F_{ad} indicates a smaller enthalpic penalty for creating an interface between the chemical groups of interest (on the tip and substrate) and the liquid HFA; i.e., the ester-based moiety is the most HFA-philic, followed by the ether group. The moiety that is least solvated, as expected, is the *CH*. Such favorable enthalpic interactions between HFA and the ester and (to a less extent) ether groups is important for the development of novel pMDI formulations as those moieties are biodegradable and biocompatible, and have been used as excipients in several (other routes) formulations^{45, 48, 49, 51)}. It is worth mentioning, however, that the solvation of the ester-based moiety is still far away from ideal; i.e., there is still room for improvement in terms of an optimal candidate chemistry. The ideal solvation is represented by $F_{ad}/R \sim 0 \text{ mN} \cdot \text{m}^{-1}$, as is the adhesive force of methyl-based

Fig.9 Normalized F_{ad} with respect to the radius (R) of the AFM tip. Candidate chemistries (*CH*, *COC* and *C(O)OC*) in HPFP and isooctane.

modified tip and substrate in an alkane (iso-octane) solvent³⁴⁾.

We have also evaluated the F_{ad} of a fluorinated moiety (*CF*) (1H,1H,2H,2H-perfluorooctyltrichlorosilane, $\text{CF}_3(\text{CF}_2)_8\text{CH}_2\text{CH}_2\text{SiCl}_3$) in HPFP. The F_{ad}/R of $6.4 \text{ mN} \cdot \text{m}^{-1}$ was comparable to that observed for the ester group³⁴⁾, indicating that the fluorinated moiety can be solvated very well by HPFP. The application of fluorinated tail in pMDI formulations is limited owing to its potential toxicity^{40, 43)}. However, it is a good model system as there is detailed information on the nature and characteristics of fluorinated self-assembled monolayers^{69, 86, 87)} - and for methyl-based silanes as well, thus allowing a direct comparison between those two systems. Using the JKR theory, the F_{ad} was further normalized for the *CF* and *CH* systems in HPFP down to the single molecule (pair) force (F_s). F_s for the fluorinated tail in HPFP was found to be 86 pN, which is significantly less than that observed between a pair of equivalent methyl-based molecules (156 pN)³⁴⁾.

The discussion above illustrates how F_{ad} results from CFM can provide an absolute scale for HFA-philicity, and are thus of great relevance for the rational design of HFA-philic that can be utilized in both traditional pMDIs and also in the development of novel pMDI formulations as will be demonstrated below. Such scale has been successfully utilized to identify and develop novel amphiphiles capable of forming stable reverse aggregates of water in HFA^{36, 37)}, and to aid in the stabilization of solid-based dispersion in the form of traditional dispersion formulations^{45, 85)} and

novel particle engineering technologies^{46, 47}. It is also worth pointing out that the CFM results discussed above have been corroborated and complemented by computational studies where the binding energy between the HFA propellants and fragments representing the chemistries above have been determined by *ab initio* calculations^{34, 35, 85}. The limitation associated with single molecule force calculation is that it is hard to obtain precise parameters regarding the properties of the silane monolayer, such as Poisson ratio, modulus and the area per molecule.

4.2. CPM in the development of pMDI formulations

There have been several studies that have highlighted the applicability of CPM to the different aspects of the formulation in pMDIs, from the hardware design, to the screening of new excipients, to the development of novel particle engineering approaches.

4.2.1. CPM and the pMDI hardware design

pMDI containers are generally manufactured with glass or aluminum, with or without an inert organic coating¹⁴. The interaction between drug particles and the walls of pMDI canisters should be taken into account in the pre-formulation stage of formulation development⁷². The adhesive forces between drug particles and the internal walls give rise to irreversible adsorption of the particles, thus leading to the so-called “wall loss”⁷², and a subsequent reduction (and/or non-reproducibility) in the emitted dose⁷³. Such losses can be detrimental to the aerosol performance of pMDIs, especially for those formulations with low drug dosages, as it is the case for high potency drugs, as the total surface area of drug may be less than that of the canister walls⁷². It is also important to observe that the internal coating of the canister should be chemically stable to the propellant-excipient mixture during the shelf life of the pMDI, so that no undesirable chemicals are extracted⁶⁴. This (and the stability of the coating) may be a challenge in certain HFA-based pMDIs, where ethanol is typically a required excipient³³. The adsorption of drugs onto valve gaskets and plastic valves has also been reported⁸⁸, and should thus be considered during pre-formulation as well. Several AFM studies have addressed the adhesive forces between therapeutics of interest in pMDI formulations and the walls of pMDI canisters^{72, 73}, and also the stability of the lining of canisters in the presence of propellant HFAs⁶⁴. Those studies are reviewed next.

The first application of AFM in the formulation of pMDIs was in 1997, with a study on the compatibility of an epoxy lining (of aluminum cans) with propellant HFAs (HFA134a and HFA227)⁶⁴. The effect of the propellants on the chemical stability of the lining was assessed by measuring the topography of the coating after exposure to HFAs at different temperatures (0 to 40 °C) by contact mode AFM⁶⁴. The results showed that the surface roughness of the lining was affected by the storage temperature and propellant type. At higher temperatures, the ‘jaggedness’ of the coating decreased, thus decreasing the sites for drug nucleation⁶⁴. At the same time, it was demonstrated that alternative techniques, including contact angle, SEM and reflection-absorption FTIR, were not able to identify any changes in the morphology of the coating. These results illustrate the uniqueness of the AFM in the study of the compatibility of the coating in the presence of propellant HFAs.

CPM studies have been performed to directly probe the adhesive interactions between drugs and the walls of pMDI containers, which was previously possible only by indirect measurements⁷². The adhesion of salbutamol sulfate (SS, a short acting β_2 -agonists) and various pMDI canister materials including borosilicate glass, aluminum, and poly(tetrafluoroethylene) (PTFE) coated aluminum was measured⁷². A single micronized SS particle was mounted onto the AFM tip using an epoxy resin. The force measurements were conducted in the model propellant HPFP. The separation energy was determined by integration of the area under the force-distance curve. The surface topography of each material was also measured with tapping mode AFM in air⁷². The force-distance curves and the AFM images are shown in **Fig.10**.

The CPM results showed that glass has the highest separation energy with SS particles, followed by aluminum, and PTFE coated aluminum, suggesting that low surface-energy PTFE is the best canister coating⁷². It is important to note here that the roughness of the materials in question is significantly different from each other, as indicated by the AFM micrographs. This is important as a smooth substrate is typically desired so that the contact area between probe and substrate remains approximately constant in all measurements (for the same colloidal probe tip), thus allowing the results for different substrates to be directly compared⁷².

More recently, a similar CPM study from the same group extended the range of drugs and surface materials⁷³. More importantly, they developed a combined

Fig.10 Representative topographical AFM images of (A) borosilicate glass, (B) aluminum, and (C) PTFE-coated aluminum canisters. Note different degrees of roughness. (Inset): Representative force–distance curves for salbutamol sulfate (drug probe) with the corresponding substrates. The order of decreasing interaction energy with SS is: glass > aluminum > PTFE-coated aluminum. Reprinted from ref. [72], with permission from Elsevier.

AFM/surface component approach (SCA) to study the nature of the drug-substrate interaction. The study helps to partially address one serious shortcoming of the CPM technique, which is the fact that the contact area between probe and substrate is typically unknown, thus preventing normalization of the force, and consequently preventing the results from different probes from being compared directly. The SCA allows one to estimate the interaction energy between drug and substrate indirectly, by using the surface tension components of both substrate and drug to determine the work of adhesion⁷³. Because the work of adhesion is directly proportional to the adhesive force as measured by CPM via DMT or JKR theories - see **Fig.6**, a theory that describes well the cohesive forces is expected to correlate with the CPM results.

The adhesion of micronized SS, budesonide (BUD) and formoterol fumarate (FF) to various canister materials including aluminum, anodize aluminum and aluminum coated with perfluoroalkoxy, fluorinated ethylene propylene-polyether sulphone and PTFE was investigated⁷³. The separation energy between each drug and various substrates was evaluated by CPM. The CPM results indicate that anodized aluminum has the highest adhesive force with SS, while PTFE has the lowest. The results had excellent correlation with the SCA only when the polar surface tension components of the substrate and drug particles were used, thus highlighting the relevance of polar forces on the (attractive) adhesive interactions between drug and substrate. Here again it is worth mentioning that differences in the roughness of the substrates exist. The authors point out, however, that they do not expect those to impact significantly the observed trends, as roughness less than 100 nm have been shown not to impact the interaction energy values⁷³. Another important aspect of the work is that the surface tension of HPFP was assumed to be all

dispersive, while HPFP is known to have a significant dipole moment⁷⁸.

For completeness, it is worth mentioning yet another CPM work where the adhesive interactions between SS and glass and PTFE (model coating for aluminum canisters) have been studied⁷⁵. However, the CPM experiments in that work were performed in air, and are thus of little relevance to pMDIs.

Due to the challenges in the reformulation of pMDIs with HFAs, ethanol is generally used in the formulations to help solubilize excipients or active pharmaceutical ingredients³³. The presence of ethanol may affect the physical and/or chemical stability of the canister lining and/or the interaction between the drug particles and the canister wall. CPM is uniquely suited to address those issues, and can thus be used to guide the design of pMDI hardware, including the valve stem, metering chamber, actuator orifice, and the effect of different excipients on drug-surface interactions. The studies above also indicate that AFM imaging may at times be the only tool (sensitive enough) to capture changes in the morphology of the lining in contact with the propellant-excipient mixtures. It may prove to be, therefore, an important characterization tool to investigate the effect of ethanol and new excipients as they are introduced in the market.

Perhaps one of the biggest challenges in the CPM studies lies in the inability to accurately determine the area of contact between micronized drug crystals (typically used as inhalants) and the substrates of interest, which prevents the comparison of the results between any two probes (between different labs and even within the same lab). That in turn makes it hard to compile a large body of understanding from work published in the literature. While some success has been achieved in terms of deconvoluting the contact area from a grid template⁶⁵, this is still an area where improvements would be welcome. The combination

of complementary tools as CPM and SCA may prove to be very useful, as intrinsic material properties can be obtained, and thus extrapolated to other systems of interest.

4.2.2. CPM and adhesive/cohesive forces in excipient free environment

Currently there are some marketed pMDI products where no excipients are used in the formulations such as Ventolin HFA[®] (micronized SS in HFA134a) and Advair HFA[®] (combination of micronized fluticasone propionate and salmeterol xinafoate in HFA134a). The aerosol efficiency of these formulations is governed by the adhesive or cohesive interparticulate / particle-device surface forces, mediated by the propellant medium. The quantitative understanding of the interaction between drug particles is, therefore, of importance for the development of excipient-free formulations.

The effect of particle manufacturing techniques on the work of adhesion (W_{ad}) of SS was determined by CPM⁶⁵. This is the only report attempting to quantitatively relate the F_{ad} to the work of adhesion, as determination of the area of contact (demanding proposition) is required. The F_{ad} between SS and a smooth (HOPG) substrate was related to W_{ad} by means of the JKR theory. It was observed that micronized SS is more cohesive ($13 \text{ mN} \cdot \mu\text{m}^{-2}$) than SS particles made via solution-enhanced dispersion by supercritical fluid (SEDS) ($3.0 \text{ mN} \cdot \mu\text{m}^{-2}$). The CPM results were noted to be in qualitative agreement with IGC trends. The authors rigorously characterized the area of contact between the (irregular) probes and the (atomically smooth) substrate utilizing a grid template deconvolution approach⁶⁵. The results suggest sub-micron asperities on the SS particles. Challenges arise in such determinations as damage during the imaging processing of the particle against the calibration grating may occur. Furthermore, the contact radius is still an estimate (a fitted value) to the dimensions (X-Y) of the area believed to be in contact with the particle. The roughness at the nanoscale, which can also affect the F_{ad} ⁵⁹, cannot be resolved either.

Inter-particulate forces between SS, BUD and FF have been determined by CPM²⁰. The drug probes were prepared for all the three drugs on tipless cantilevers, and large crystal substrates were obtained by a sitting drop crystallization technique. The surface topography of the large drug crystals was examined by tapping mode AFM in air, and shown to be smooth, with the root-mean-squared roughness less than 2 nm over a $25 \mu\text{m}$ area, thus providing

a uniform contact area for the CPM experiments. The forces of adhesion and cohesion between the drug probes and crystal substrates were measured in HPFP, and the cohesion/adhesion ratios were obtained. A single probe was utilized to measure the cohesive/adhesive forces - the results can thus be directly compared for that same probe. The experiments were done in triplicate to guarantee that consistent trends were observed. The results demonstrate that cohesive forces between SS particles are much stronger than the adhesive interactions with BUD and FF. BUD and FF are shown to be more adhesive with SS than cohesive. Finally, CPM results indicate that the cohesive and adhesive forces involving BUD and FF are very close. The experimental CPM results correlated very well with cohesive/adhesive ratios that were calculated from contact angle and IGC, only when the polar surface components were included²⁰. The results again suggest that polar forces are important contributions in HFA solvents. Although the calculations based on the contact angle results correlated with the experimental CPM observations in terms of the cohesive/adhesive balance trend, there was still a significant quantitative discrepancy between the two methods²⁰.

A later study by the same group focused on correlating the adhesive/cohesive forces from contact angle and CPM with the physical stability of the formulations and the corresponding aerosol characteristics⁸⁹. The results indicate that a strong cohesive force correlated well with poor physical stability and a small sedimentation volume (strong cohesion) of the flocculated micronized crystals. The emitted dose also decreased for the more cohesive particles, as did the fine particle fraction (concentration of drug expected to reach the lower airways). For systems where the determined adhesive forces were lower than cohesive, higher emitted doses were observed with the combination drugs⁸⁹.

As a microscopic characterization tool, CPM can be thus successfully used to determine the work of cohesion/adhesion and to evaluate adhesive/cohesive balance. In spite of the fact that the contact area between probe and substrate may not be known, the absolute value of the ratio of work of adhesion and cohesion can still be determined (as that is independent of the contact area for the same probe). Another important result is that there seems to be a strong correlation between the CPM results (in HPFP), physical stability (in propellant HFA) and the aerosol characteristics of the corresponding formulations.

4.2.3. Excipient design by CPM

While chlorofluorocarbons (CFCs) were employed as the propellants in pMDI formulation for decades, concerns about their ozone depletion potential prompted the search for more environmentally friendly alternatives¹⁵⁾. Due to certain favorable properties, particularly low toxicity, low flammability, and zero ozone depletion potential, hydrofluoroalkanes were selected as acceptable alternatives to CFCs⁸⁾. However, the transition from CFCs to the more environmentally friendly HFAs has been difficult due to the significantly different solvent properties between these classes of fluids¹⁵⁾. For instance, the hydrogenated excipients in FDA-approved CFC-based formulations have very limited solubility in the semi-fluorinated HFA propellants³³⁾, thus restricting their direct application in HFA-based pMDIs³³⁾. Such challenges have sparked significant interest on the design of HFA-philic excipients for pMDI formulations³³⁾. Due to its sensitivity and unique force detection capability, CPM has been successfully employed in the design of excipients for pMDI-based formulations. These results are summarized below.

The ability of polyethylene glycol (PEG) and polyvinylpyrrolidone (PVP) to screen adhesive/cohesive forces in systems containing FF was investigated by CPM.⁹⁰⁾ These two excipients were chosen since they have been reported as acceptable excipients for use in HFA-based pMDIs⁹¹⁾, and are readily available. The drug particle probe was prepared by attaching a cluster of drug particles with the size of around 50 μm to an AFM tip. CPM was used to investigate the interaction between FF particles and various substrates including bare aluminum and aluminum spin-coated with FF (in that case, the drug-drug cohesive forces would be determined), PEG, PVP, or a mixture of PEG and PVP in HPFP solution.⁹⁰⁾ The CPM results demonstrate that the presence of PEG homopolymer in solution can only slightly reduce the interaction forces between FF particles or FF with the canister aluminum wall. PVP or the mixture of PEG and PVP was shown to be more effective than PEG alone in decreasing the adhesive forces, but not greatly.⁹⁰⁾ However, a new probe was prepared in each experiment, making direct comparison between the substrates difficult (even though several probes were measured for each system), as the forces can vary drastically from one probe to the next^{20, 51, 65)}, and averaging is not necessarily representative.

The inability of PEG and PVP homopolymers to completely screen the cohesive/adhesive contact in the systems described above are not necessarily

related to the (in)ability of HPFP to solvate the homopolymers. As a matter of fact, PEG interacts well with HFA134a and HFA227^{79, 91)}. Moreover, when tethered to the surface of drug particles, PEGs have been shown capable of imparting stability to dispersions in propellant HFAs⁴⁷⁾. Perhaps the poor performance of the homopolymers in those system can be more closely related to an inadequate balance/partition between HFA and the drug / lining surface⁹⁰⁾. The authors also suggest the poor performance of the excipients to bridging flocculation, as indicated by the jagged nature (snap-outs) of the force profile (retraction) in the presence of PVP. This is an important result that points out to the relevance of AFM in the design of excipients with appropriate balance and stabilizing moieties.

The influence of PEG (concentration and molecular weight) in the presence and absence of PVP homopolymers on the interaction forces between micronized SS particles and a smooth SS substrate was also studied by CPM⁵¹⁾. Individual micronized SS particles were attached to AFM tips using a quick-setting epoxy resin. A large SS crystal substrate was prepared by recrystallization in ethanol⁵¹⁾. The force measurements were conducted in purified HPFP. The concentration and molecular weight of PEG was shown to have a significant impact on inter-particulate forces⁵¹⁾. Increased PEG concentration resulted in a reduction in inter-particulate forces, with the most pronounced effect in the concentration range between 0.05~0.1 v/w % for the PEGs investigated (MW of 200, 400 and 600).

The screening of cohesive forces was reported to be more effective with higher molecular weight PEGs. PEG 600 at the concentration of 0.5 v/w % can reduce the cohesive forces in SS by 89 %. This was attributed to the fact that higher molecular weight PEG is expected to provide a longer steric barrier, thus reducing the effect of the van der Waals forces⁵¹⁾. However, the cohesive forces between the SS particles were not completely screened.⁵¹⁾ Similar reasons as those discussed above (for FF) can be attributed to the inability of the PEG homopolymer in completely shielding the interactions between SS particles. It is important to note, however, that in this study, the results from different probes were compared, and some variability is expected as F_{ad} may be greatly affected by changes in contact area. The effect of PEG on the F_{ad} was not correlated with the physical stability in propellant HFAs nor the aerosol characteristic of the corresponding formulations. More recently, our group investigated the ability of a series of bio-

compatible and biodegradable lactide-based copolymers in screening the cohesive forces of salbutamol base (SB) particles⁴⁵⁾. Copolymers are designed such that one of the blocks contains an ‘anchor’ segment that strongly interacts with the dispersed phase, and a well-solvated ‘tail’ segment that extends into the bulk/dispersing medium thus proving for the steric barrier required for screening cohesive forces that arise due to van der Waals interactions⁴⁵⁾.

A series of triblock copolymers of the type poly(lactide)-poly(ethylene glycol)-poly(lactide) ($LA_mEO_nLA_m$) with varying molecular weight (MW) and % *EO* were synthesized. CPM was used to quantitatively examine the effectiveness of the amphiphiles to screen the cohesive forces between SB particles. The effect of cosolvent (ethanol), oleic acid (surfactant present in FDA-approved pMDI formulations), and a nonionic triblock copolymer with the propylene oxide moiety as the HFA-phile were also investigated.⁴⁵⁾

These results are of relevance as they are the first to directly assess the effectiveness of excipients typically used in FDA-approved pMDI formulations. While cohesive forces between SB crystals were shown to be somewhat suppressed upon the addition of ethanol and oleic acid to HPFP, the forces are only partially screened. In sharp contrast, the presence of $LA_mEO_nLA_m$ is capable of reducing the cohesive forces between the bare SB particles down to approximately zero, even in the absence of co-solvent. The results indicate that the *LA* moiety can be well solvated by HPFP, and is thus able to provide a steric barrier to flocculation⁴⁵⁾. This is in direct agreement with the CFM results discussed earlier where the *C(O)OC* moiety was shown to be well-solvated by HPFP⁸⁵⁾, and also to binding energy results from ab initio calculations that studied the interaction between HFA134a and HFA227 and a *C(O)OC* fragment⁸⁵⁾. These results directly validate the CFM experiments and the theoretical results from ab initio calculations, indicating that those may indeed be appropriate tools for screening moieties for HFA-based pMDI formulations.

The effect of the concentration of $LA_mEO_nLA_m$ amphiphiles on drug-drug interaction was also investigated. A minimization of the surfactant concentration is important to reduce potential toxicity of the formulation, and its impact on the physical properties of the propellant-excipient mixture, which in turn may affect the aerosol characteristics⁴⁵⁾. CPM results also allowed the evaluation of the effect of the *LA* tail length on the adhesion force between SB drug particles.

This is relevant as colloidal stabilization is dependent on the thickness of the barrier provided by the tail (*LA* moiety), which is directly related to the number of *LA* repeat units and the solvent environment⁴⁵⁾. The length of the *LA* is also relevant to its biodegradability⁵²⁾. The results revealed that amphiphiles with very short tails (less than about 2×4 repeat units) could not completely screen cohesive forces between SB (at least at moderate to low concentrations)⁴⁵⁾.

In the study discussed above, the CPM results (performed in HPFP) were directly correlated to the physical stability of the formulation in a propellant HFA (HFA227). The results are shown in **Fig. 11**. The physical stability of the formulations in HFA227 was in excellent agreement to the F_{ad} results from CPM; i.e., low F_{ad} in HPFP correlate well with good suspension stability of the formulations in HFA227.

While the use of the (above mentioned) particular amphiphiles is limited to drugs where the PEO anchor is expected to interact with, the results regarding the applicability of *LA* as a stabilizing moiety are fairly general. It was, thus, proposed that *LA*-based amphiphiles could be extended to other suspension-based formulations, provided a suitable head-group could be identified. This hypothesis was demonstrated on a study with BUD (hydrophobic drug). The PEG group was replaced with the more hydrophobic

Fig. 11 Dispersion stability of micronized salbutamol base particles in HFA227 ($2 \text{ mg}\cdot\text{ml}^{-1}$) at 298 K and saturation pressure. (a) Pure HFA227; (b) HFA227 in presence of $0.1 \text{ mM } LA_{53}EO_{22}LA_{53}$. Note the enhanced stability in the presence of the amphiphiles. The enhanced stability correlates well with low F_{ad} from CPM experiments. Reprinted from ref. [45] with permission from American Chemical Society.

polycaprolactone (PCL) group. A random copolymer of PCL-PLA provided an effective steric barrier capable of imparting stability to micronized BUD in HFA227, while the PEG-based amphiphile does not⁸⁵. With the addition of the synthesized PCL-PLA copolymer, the interaction force between BUD particles was reduced nearly 10 fold, indicating the presence of a steric barrier on the surface of the drug particles. There was also a significant improvement in terms of the physical stability of the BUD formulation in HFA227 in presence of PCL-PLA compared to that without the copolymer. The PCL-PLA copolymer is not applicable to HFA134a due to its limited solubility.

Although macroscopic information regarding the colloidal stability of suspension-based pMDI formulations can be obtained by traditional bulk sedimentation rate experiments, such techniques cannot provide quantitative microscopic information on inter-particulate forces. CPM is especially suited for that task, and in the design of excipients for pMDIs. Moreover, CPM allows decoupling of the confounding information regarding the physical stability of the dispersion and the aerosol characteristics of the corresponding formulation, which may also be affected by the device components and other formulation parameters. One important issue that is still left unresolved is the inability to directly compare the results from different groups or to use them to generate a broad knowledge on the effect of excipients on cohesive/adhesive interactions as the probes used in those studies do not have a regular geometry and the results are generally not normalized.

4.2.4. Particle surface engineering by CPM

The stabilization of solid dispersions in propellant HFAs can be also achieved via modification of the particle surface or particle morphology^{23, 35}. Such approaches may offer the possibility of formulating (besides small molecular weight drugs) large therapeutic biomolecules to either target the lungs locally or for systemic delivery in the form of dispersion pMDIs³⁵. Compared to surfactant-stabilized colloids, the surface and morphological modification of particles may reduce (or avoid altogether) the presence of free excipients in solution, which may serve to reduce the toxicity of the formulations. Moreover, the challenges associated with the synthesis of well-balanced amphiphiles are circumvented⁴⁷. The study of the forces between particles with engineered surfaces and morphology is thus of relevance to the development of novel pMDIs.

One important breakthrough in particle engineer-

ing for pMDIs was the introduction of the concept of porous particles⁵⁵. The propellant is allowed to penetrate into the pores of the particles, thus decreasing the density mismatch between the propellant and the particles. This results in an improved dispersion, as the sedimentation (creaming) velocity decreases. The van der Waals attractive interactions are also expected to be reduced, thus minimizing cohesive forces between particles⁵⁰. The physical stability of such formulations in pMDIs and the resulting aerosol characteristics point out to the fact that indeed porous particles impart those characteristics to the dispersions⁵⁰. An opportunity for CPM studies here would be to demonstrate how much each effect (density matching and decrease in cohesive forces) is contributing to the overall stability of the system, and how similar approaches could be used in the future to enhance the aerosol characteristics of pMDIs.

Our group has recently proposed a novel methodology for engineering polar drug particles with enhanced stability and aerosol characteristics in propellant HFAs by 'trapping' HFA-philic moieties (in that case PEG) onto the surface of drug particles using a modified emulsification-diffusion method⁴⁷. The trapped moieties act as stabilizing agents, thus preventing flocculation of the otherwise unstable drug particles in propellant HFA. The interaction between single SS particles was evaluated in HPFP using CPM. A single particle was directly attached onto an AFM tip (epoxy), while the other particle was physically (and strongly) adsorbed on the substrate.

The CPM results demonstrated that the surface of SS particles can be densely covered by PEG 300 molecules, as indicated by a reduction in the cohesive force from 1.36 nN for bare SS spheres, down to approximately zero (0.07 nN) for PEG-coated SS particles. The results are summarized in **Fig.12**.

Thus, the cohesive forces between PEG-coated SS particles are almost completely screened upon trapping the HFA-philic groups (PEG) at the surface⁴⁷. The excellent physical stability of PEG-coated SS in HFA propellant (both HFA134a and HFA227) also corroborated the CPM force results. Another important piece of information from this work was the establishment of a correlation between the CPM, the physical stability of the formulation and the corresponding aerosol characteristics. It was demonstrated that the CPM results correlate very well not only with the physical stability of the formulations, but also with the aerosol properties. A large fine particle fraction (FPF) was achieved for the PEG-coated SS formulations (65.1%) that had small F_{ad} and good

Fig.12 Adhesion force (F_{ad}) histogram between bare- (distribution to the right of the diagram) and PEG-coated SS spheres (distribution to the left of the diagram) in HPFP. The black lines represent the Gaussian fit of the histograms. (Inset I): average force curves for bare- and PEG300-modified-SS particles. (Inset II): SEM image of PEG300-coated SS spheres. As PEG is trapped onto the surface of the particles, it is capable of reducing the cohesive forces down to about zero, indicating that it can be well solvated by HPFP. Reprinted from ref. [47] with permission from Elsevier.

physical stability. These results can be contrasted with those for the unmodified SS formulation with a PPF of 39.1%, which had large F_{ad} and poor physical stability.

CPM was also used in the development of particles with a core-shell morphology⁴⁶⁾. In this modified emulsification-diffusion approach, particles were formed with the active component (water soluble or dispersible) in the core, and with a shell (biodegradable and biocompatible) designed so as to impart stability to the particles when dispersed in propellant HFAs⁴⁶⁾. Studied cores include a model small polar drug (SS) and a model biomolecule (bovine serum albumin, BSA). The particle shell consisted of oligo(lactide) (LA) grafts attached onto a short (degraded) chitosan (CS) backbone. LA was selected based on the CFM studies and its biocompatible and biodegradable nature. CS was utilized to impart interfacial activity to the polymer at the water-ethyl acetate interface, required in the selected particle engineering approach⁴⁶⁾. CPM was utilized to quantitatively evaluate the effectiveness of the shell in screening the attractive forces by measuring the F_{ad} between two single particles in HPFP⁴⁶⁾. The CPM results demonstrate that the cohesive forces between drug particles are significantly reduced (to nearly zero) upon the coating of the shell around the drug particle, again demonstrating the fact that HFAs are capable of solvating LA moieties.

The CPM results also correlated well with the bulk physical stability in HFA277. However, the core-shell particles cannot be well dispersed in HFA134a, which seems to indicate that that HPFP is a better mimic of HFA227 than HFA134a. This is an important particle engineering approach as it may create the opportunity of using pMDIs for the delivery of nanotherapeutics to and through the lungs⁹²⁾. In the studies discussed above, CPM results were shown to be to provide a novel predictor of the suspension stability of pMDI formulations and offer an opportunity to decouple the effect of particle-particle interactions from the other formulation variables on the performance of the aerosol. Because smooth spherical particles can be made using the emulsification-diffusion technique, this could perhaps be utilized as a means of normalizing the force of interaction, and thus allowing the comparison from experiments done with different CPM probes. It remains to resolve, however, whether such particles are sufficiently smooth⁵⁹⁾ and whether the morphology (amorphous) of the particles has a significant effect on the cohesive forces as measured by CPM⁹³⁾.

4.2.5. Particle density measurements by CPM

A novel method based on the CPM technique has been suggested to determine the density of particles^{81, 82)}. A single spherical particle is initially glued onto the AFM cantilever as in a typical CPM experiment. The mass of the single particle on the cantilever can be determined based on the shift in the resonant frequency of the cantilever, as is typically done in the determination of the spring constant⁹⁴⁾. The density is then obtained based on the particle volume, measured by a microscope. Using this technique, the effective density of spray-dried carbohydrate particles was determined and compared to the result from nitrogen pycnometry⁸¹⁾. The CPM together with various microscopic techniques revealed that the particles with an effective particle density close to the true density showed a solid appearance while hollow particles gave a lower effective particle density. The results from nitrogen pycnometry, however, could not detect the density difference for solid and highly gas permeable particles⁸¹⁾. It may be concluded that AFM can provide valuable information on both particle structure and density, which is not possible to be acquired by pycnometry. The limitation of this method is the requirement for regular shaped particles so that the volume can be easily determined from microscopy⁸¹⁾. There is also a limitation associated with the size of particles since the attachment of

the particles to the AFM tip becomes more challenging as particle decreases to less than 10 μm .

4.2.6. Hamaker constant by CPM

Interactions between colloidal particles in the low-dielectric HFAs are dominated by the attractive van de Waals (VDW) forces as electrostatic interactions have little or no relevance in non-aqueous media. VDW forces are thus responsible for colloidal instability in HFAs. The determination of Hamaker constant is, therefore, of great relevance for the understanding of fundamental colloidal phenomena associated with pMDIs⁹⁵.

The Hamaker constant (A_H) can be calculated from spectral or optical properties of materials through various approaches⁹⁶⁻⁹⁷, and also using AFM^{59, 83}. For the interaction between particles with a spherical geometry and a flat surface, the Hamaker constant can in principle be obtained by fitting the attractive part of the force-distance AFM curve to equation $F = -A_H R / 6D^2$, where R is the radius of curvature of the tip and D is the separation distance.

The applicability of this method is somewhat limited due to the jump-to-contact, which occurs when the force gradient exceeds the spring constant of the cantilever, as described in section 3.3. An alternative method to determine A_H is based on the measurement of adhesion force as the AFM tip is pulled out of contact with sample surface. This approach takes into account various parameters such as surface roughness, particle geometry, and elastic properties of the interacting materials^{59, 83}. There have been several theories that have addressed the determination of A_H from CPM experiments⁹⁸⁻¹⁰⁰. The challenges associated with this approach include difficulties in estimating the contact geometry, and in determining the surface roughness of the probe. Readers are directed to excellent reviews on this subject for more details^{59, 62}.

5. Concluding Remarks

In this review we have highlighted the applicability of the AFM and related techniques to systems of relevance to pMDIs. Despite the limited number of publications in the area and the recent nature of the studies (most articles having been published in the last 5 years), it is clear that the AFM can provide valuable information to address a range of formulation issues from hardware design, to excipient screening, to particle engineering. Other areas where the AFM could be potentially used to gain relevant information

to the formulation of pMDIs have been identified, including viscosity measurements, true particle density measurements, and the determination of Hamaker constants. The capability of the AFM to provide unique microscopic information with a degree of sensitivity often not achievable with alternative / bulk techniques has also been discussed. However, certain challenges, most notably the difficulty in determining the area of contact in CPM, and the structure and properties of adsorbed monolayers on the AFM probes in CFM, are clear constraints that need to be addressed. While experimental AFM results under pressure (propellant HFAs) are not available, further validation of the model solvent (HPFP) used in the AFM experiments is also granted. A combination of results from AFM and bulk techniques (e.g. contact angle) or computational studies (e.g. ab initio calculations) seem to be a powerful approach to address the many challenges in the formulation of pMDIs. It is the expectation that such fundamental information could provide for an opportunity for pMDIs to rebound on the oral inhalation market, and perhaps achieve new grounds on the overall drug delivery market, as for example in the local and systemic delivery of nanotherapeutics and (large) therapeutic biomolecules.

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Author's short biography

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