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Research paper

Predicting physical stability in pressurized metered dose inhalers via dwell and instantaneous force colloidal probe microscopy

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ABSTRACT

Colloidal probe microscopy (CPM) is a quantitative predictive tool, which can offer insight into particle behavior in suspension pressurized metered dose inhalers (pMDIs). Although CPM instantaneous force measurements, which involve immediate retraction of the probe upon sample contact, can provide information on inter-particle attractive forces, they lack the ability to appropriately imitate all critical particle pMDI interactions (e.g., particle re-dispersion after prolonged pMDI storage).

In this paper, two novel dwell force techniques – indentation and deflection dwell – were employed to mimic long-term particle interactions present in pMDIs, using particles of various internal structures and a model liquid propellant (2H,3H perfluoropentane) as a model system. Dwell measurements involve particle contact for an extended period of time. In deflection dwell mode the probe is held at a specific position, while in indentation dwell mode the probe is forced into the sample with a constant force for the entirety of the contact time. To evaluate the applicability of CPM to predict actual pMDI physical stability, inter-particle force measurements were compared with qualitative and quantitative bulk pMDI measurement techniques (visual quality and light scattering).

Measured instantaneous attractive (snap-in) and adhesive (max-pull) forces decreased as a function of increasing surface area, while adhesive forces measured by indentation dwell decreased as a function of dwell contact time for particles containing voids.

Instantaneous force measurements provided information on the likelihood of floccule formation, which was predictive of partitioning rates, while indentation dwell force measurements were predictive of formulation re-dispersibility after prolonged storage. Dwell force measurements provide additional information on particle behavior within a pMDI not obtainable via instantaneous measurements.

1. Introduction

Most pressurized metered dose inhalers (pMDIs) are formulated as suspensions since the majority of inhalable drugs are practically insoluble in propellants (HFA 134a/HFA 227). Suspension-based pMDIs are typically robust to chemical degradation [1–3], but frequently suffer from various forms of physical instability, including flocculation, caking, partitioning (sedimentation or creaming) and wall losses [4], all of which stem from physical particle interactions. Developments in inhaler component design and materials have reduced wall drug losses, but lack of suspension uniformity continues to be a difficult problem to control since it is governed simultaneously by propellant-particle density differences and particle interactions [5]. Additionally, suspension re-dispersion is also a key factor in formulation development. Clinical studies have shown that many patients fail to properly shake pMDIs prior to administration [6,7], resulting in inconsistent dosing which suggests that an easily dispersible formulation (requiring minimal shaking) is favorable, to ensure accurate and uniform dosing.

Achievements in particle engineering [8] have resulted in more stable pMDI formulations. The attractive and cohesive forces present between these particles play a practical role in the stability of these suspensions; however, the use of some particle-interaction theories such as those developed by Derjaguin and Landau [9], and Verwey and Overbeek [10] (developed upon aqueous systems) is not widely accepted to predict and explain particle interactions in non-aqueous pMDI systems [11]. Thus, direct measurement of inter-particle forces or flocculation and partitioning rates is necessary to understand suspension stability mechanisms.

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Several techniques are available to evaluate pMDI suspension stability including: visual observation of partitioning [12], light scattering or reflecting particle sizing techniques for flocculation and partitioning (Turbiscan^{TM}) [13], or dose uniformity experiments for long term physical stability evaluations [14].

Turbiscan, an instrument that uses light backscattering to measure flocculation and particle concentration, is one such instrument that has been used to evaluate suspension stability [15,16]. The technique employs laser diffraction to provide quantitative information on the rate of partitioning; however, the technique does not provide any insights into the inter-particle forces that govern partitioning or re-dispersion.

To address the limitations of bulk measurement techniques such as the Turbiscan, an atomic force microscopic (AFM) technique, colloidal probe microscopy (CPM) can provide quantitative insight into the nano-scale particle interactions in pMDI formulations and can be an invaluable asset during formulation development. Previous studies have used CPM to evaluate inter-particle interactions of drugs with varying surface energies or polymeric stabilizing excipients, and particle-MDI component drug interactions in model propellants [2,4,17,18]. However, these previous studies focused only on instantaneous force measurements as a basis for comparison, which fails to represent all pMDI particle interactions. The adhesion forces that govern formulation re-dispersability form over extended periods of time and cannot be accurately measured using instantaneous force measurements. To address this issue, dwell force measurements, which involve prolonged particle contact during CPM, can be used to mimic such particle interactions.

The primary aim of this study was to use AFM–CPM techniques – instantaneous and dwell force measurements – to emulate various pMDI particle interactions that have not been previously studied by AFM (using particles of various internal structures as a model) and reveal how the techniques can provide important information to understand particle interactions vital to the development of pMDI formulations.

2. Experimental

2.1. Spray drying of particles

Spray dried particles of various surface areas were produced from an emulsified formulation containing diastearoyl-phosphatidylcholine (DSPC), calcium chloride ($CaCl_2$), and perflooctyl bromide (PFOB) using previously reported methods [19,20]. In brief, DSPC, $CaCl_2$, and PFOB were dispersed in deionized water using a high shear mixer. The emulsion was then homogenized using a high pressure homogenizer (Emulsiflex-C55; Avestin, Canada) before spray drying. Particles produced (denoted as A through E in this study) were of similar optical diameters (1.1–2.4 μm) with a wide spread of surface areas ranging from 4.2 m^2/g (solid) to 76.7 m^2/g (large internal void volume).

2.2. Particle characterization

Optical particle size distributions were measured using a Sympatec HELOS (Clausthal-Zellerfeld, Germany) equipped with an R1 lens and Aspiros unit. Surface area was measured using a 5-point nitrogen BET method (Micrometrics Tristar II Surface Area Analyzer, USA). Internal particle structures were imaged using a focused ion beam scanning electron microscope (Zeiss Auriga FIB-SEM, Carl Zeiss Microscopy, Jena, Germany); several sliced views were obtained with the following parameters: 30 kV and 250 pA at a depth of 3 μm. All SEM imaged samples were coated with 15 nm of gold.

2.3. Atomic force microscopy (AFM)

A Molecular Force Probe MFP-3D-Bio (Asylum Research, Santa Barbara CA, USA) was used for all colloidal probe measurements. After method optimization, a maximum loading force of 20 nm deflection and an approach/retract rate of 100 nm/s were set for all measurements. Prior to data acquisition, the spring constant of each probe was measured using the resonant frequency of the thermal noise spectrum and the sensitivity of each probe was calculated from the slope of the contact region in the acquired force vs. separation curves (Fig. 1). All measurements were conducted in a liquid medium using 2H, 3H perfluoropentane (HPFP) to mimic the inhaler propellant environment [21]. The liquid medium was temperature equilibrated to minimize baseline deviations caused by thermal drift.

A typical instantaneous AFM force vs. separation curve is shown in Fig. 1. The key measured values relatable to the study of pharmaceutical pMDI formulations discussed in this paper are: snap-in and max-pull force. The snap-in is a measure of the inter-particle attractive forces during approach, while max-pull conveys the force required to overcome the adhesion between two particles upon probe retraction.

Dwell measurements produce similar force curves with the exception of an extended probe contact region (Fig. 1). The two dwell types discussed in this paper to emulate expected pMDI inter-particle interactions include: deflection and indentation dwell.

In *deflection dwell* measurements the deflection of the cantilever was kept constant for a period of time once the probe contacts the sample. To ensure that particles were in contact and not separated by a thin liquid layer, the probe was pushed to a set value determined during method optimization (max-load of 20 nm deflection). Instantaneous force measurements are equivalent to zero second deflection dwell measurements.

In *indentation dwell* measurements the colloidal probe continuously indented the sample particle at a constant force throughout the dwell time.

2.4. Colloidal probe microscopy (CPM)

Three individually prepared functional colloidal probes were used for each particle type using a previously published method [22,23]. Briefly, particles were attached to Veeco tipless cantilevers (Model NP-O10 lever D with a nominal spring contact 0.06 N/m) (Veeco, Plainview, NY, USA) mounted on a custom designed 45° angle holder by using an optical microscope stage to manipulate cantilever movement to obtain a small amount of epoxy

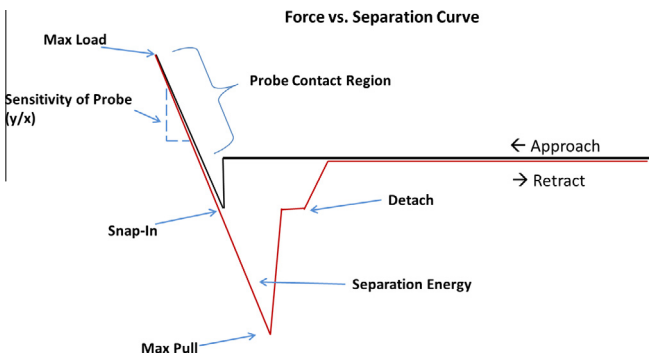

Fig. 1. Typical force curve obtained from an AFM/CPM measurement upon approach, contact and retraction of the probe. Key factors for pMDI applications are the snap-in and max-pull forces. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)