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International Journal of Pharmaceutics 320 (2006) 58–63
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Investigation into the influence of polymeric stabilizing excipients on inter-particulate forces in pressurised metered dose inhalers

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Received 27 December 2005; received in revised form 8 March 2006; accepted 1 April 2006
Available online 5 May 2006

Abstract

Colloid probe atomic force microscopy (AFM) was utilised to quantify the cohesive forces of salbutamol sulphate in a model non-pressurised fluorinated liquid (mHFA), in the presence of increasing concentrations of poly(ethylene glycol) (PEG; molecular weight (MW) 200, 400 and 600). In addition, samples of PEG 400 (0.05–0.5%, v/w), were analysed in the presence of 0.001% (w/w) of poly(vinyl pyrrolidone) (PVP). In the absence of any stabilizing agents, strong attractive forces were present between particles. Increasing the concentration of the different MW PEG solutions in the mHFA system (up to 0.5%, v/w), significantly decreased the force of interaction (ANOVA, $p < 0.05$). The decrease in cohesion was particularly evident at very low concentrations of PEG (0.05–0.1%, v/w). Further data analysis ($p < 0.05$) suggested that the reduction in the force of cohesion was dependent on the concentration and molecular weight of PEG. The addition of low concentration of PVP to the PEG 400-mHFA system had the most significant influence on drug particle cohesion. In the presence of PVP, increasing addition of PEG 400 (0.05–0.5%, v/w) to the mHFA, resulted in no significant reduction in the force of cohesion ($p > 0.05$). Clearly, an understanding of the conformation of polymer molecules at interfaces is of vital importance when controlling the stability/flocculation behaviour of sterically stabilized pMDI suspensions. In this context, the use of the colloid probe AFM technique has provided a quantitative insight into the interactions of these complex systems and may be an invaluable asset during the early phase of formulation product development.

Keywords: pMDI; Suspension; PEG; PVP; AFM

1. Introduction

The transition from chlorofluorocarbon (CFCs) to hydrofluoralkane (HFAs) propellant-driven metered dose inhalers (pMDIs) has been the motivation for further evaluation of pMDI technologies over the last 20 years. In the majority of cases, the transition to HFAs has not been seamless. This has been mainly attributed to differences in the physicochemical characteristics of the propellants (Vervaet and Byron, 1999). While HFA liquid environments have many similar proprieties to the old CFC's, their physical properties impart poor suspension stability to many pMDI formulations. Often surfactants are required to assist with the dispersion of medicaments and with lubrication of the valve in a pMDI canister. The stabilizers commonly used to disperse micronised drugs in CFC-driven pMDIs (*e.g.* oleic acid, sorbitane trioleate and lecithin), are effectively insoluble in HFAs propellants (Blondino and Byron, 1998; Vervaet and Byron, 1999). Thus, whole new range of materials have generally been required to stabilize suspension formulations. Previous studies have investigated the specific role of these stabilizing excipients on suspension behaviour of HFA-driven pMDIs (Vervaet and Byron, 1999).

To date, only a limited number of studies have been undertaken to quantitatively investigate the specific influence of stabilizers on the interactions between drug particulates within pMDI systems (Clarke et al., 1993; Michael et al., 2000). With the development of the colloid probe technique for *in situ* atomic force microscopy (AFM) (Binnig and Quate, 1986) measurements of particulate interactions (Ducker et al., 1991), direct quantification of the cohesive and adhesive interactions within

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doi:10.1016/j.ijpharm.2006.04.016

model pMDI systems is feasible (Ashayer et al., 2003; Young et al., 2003; Traini et al., 2005).

Suspension stability can be facilitated through steric repulsive forces (arising from colliding particles with adsorbed layers) and/or electrostatic repulsive forces (that originates when the electrostatic double layer of interacting particles overlap) (Pugh et al., 1983). There is still a debate in this area and recent investigations (Hsu et al., 2005; Yu et al., 2003; Kosmulski, 1999) on electrostatic stabilization within non-aqueous vehicles have shown that charge can play an important role in non-polar media. However, previous work has shown that, electrostatic stabilization is only effective in non-aqueous suspensions of carbon black particles when the zeta (ζ)-potential was >100 mV (Pugh et al., 1983). The ζ-potential required for electrostatic stabilization is far greater than that reported for a model drug commonly used in pMDI systems (*i.e.* salbutamol sulphate) (Clarke et al., 1993). These observations were further substantiated by Kitahara (Kitahara, 1974), which stated that unless particles in suspension are large and posses a substantial ζ-potential, the electrostatic mechanism of stabilization in a low dielectric constant would be ineffective. Furthermore, previous studies (Osmond and Waite, 1975) suggested that employing electrostatic effects for the stabilization of drug particle in pMDI should be discounted (Miller et al., 1991; Ashayer et al., 2003). Consequently, the repulsive electrostatic double layer interactions have been assumed to have negligible influence on the stabilization of drug particulates within a non-polar system.

Steric stabilization is associated with the use of surface active polymers to reduce particulate interactions, due primarily to a sufficient reduction in the "effective" van der Waals interaction energy between colliding particulates (Heller and Tanaka, 1951; Heller and Pugh, 1960). The polymers must interact with both the particles and the medium in a way that the polymer is adsorbed to the particle substrate yet freely soluble in the medium. Such a combination of properties is generally obtained through the use of macromolecular stabilizing agents. These are usually block copolymers with a lypophobic "head", which attach strongly to the particle surfaces, and a lyophilic "tail", which moves freely in the dispersive medium (Heller and Pugh, 1960). The lyophilic tail extends from the surface giving a layer thickness (d) that can be several nanometers in dimensions. As a result, when two particles come into contact the polymeric chains may overlap and/or become compressed preventing particle contact by limiting the short-range van der Waals energy of interaction between particles (Heller and Pugh, 1960). Optimal steric stabilization is achieved when the polymer chains are well solvated and properly unfurled. In this case, overlap is energetically unfavourable and leads to a repulsion that increases sharply as the separation distance between the particles decreases.

It is well established that the efficient stabilization of particles in suspension depends on various factors related to the surface chemistry of the particles as well as on the ionic nature, molecular weight, charge density and bulk properties of the polymers in solution (Heller and Tanaka, 1951; Heller and Pugh, 1960; Lyklema, 1968; Wyatt and Vincent, 1989). However, one of the most key parameters affecting the extent and mechanism of steric stabilization is the nature and conformation of the polymer used (Heller and Tanaka, 1951).

A recent study has shown that the addition of poly(vinyl pyrrolidone) (PVP) in partially fluorinated solvent like mHFA, was found to adsorb more strongly than poly(ethylene glycol) (PEG). Furthermore, no competitive adsorption effects were observed between PVP and PEGs in mHFA, with the amount of each polymer absorbed being unaffected by the presence of the other (Paul et al., 2005).

In this study, PEGs (hydrophilic polymers), of three different molecular weights (MW 200, 400 and 600), and PVP (MW 30–75) were used to investigate their behaviour in providing steric stabilization within a model pMDI systems. Their influence on suspension stabilization was investigated by *in situ* colloid probe AFM measurements of the forces of interaction between β_2 agonist (salbutamol sulphate) particles within a model pMDI formulation.

2. Materials and methods

Micronised salbutamol sulphate was supplied by AstraZeneca (R&D Charnwood, Loughborough, UK). Water was produced by reverse osmosis (Millipore, Molsheim, France). Solvents were supplied by BDH (Poole, UK) and were of analytical grade. The model propellant, 2H, 3H decafluoropentane (mHFA), was supplied by Apollo Scientific (Derbyshire, UK). Poly(ethylene glycol) (PEG (MW) 200, 400 and 600, polymers of industrial grade suitable for pharmaceutical applications) were supplied by Acros Organics (NJ, USA). Poly(vinyl pyrrolidone) (Povidone K25, PVP, non-ionic water soluble polymer) was supplied by AstraZeneca (R&D Charnwood).

2.1. Preparation and characterisation of drug crystals

Single crystals of salbutamol sulphate were nucleated and grown on a glass substrate using a sitting drop technique, described elsewhere (Rhodes, 1993). This process produced planar crystals with large areas of sub-nanometre smooth surfaces. Using mesoscopically smooth and pristine crystals, problems related with the heterogeneity of surface materials and the inadequate knowledge of the contact area is eliminated. To avoid problems related to potential contamination (due to polymer residues in the AFM *in situ* cell) between experiments, freshly nucleated crystals were used for each set of experiments (12 crystals in total). The surface topography of the drug crystal surfaces was investigated using the AFM in conventional *ex situ* imaging mode prior to force measurement.

2.2. Preparation of polymeric solutions

Commercially available mHFA was purified before use following the method devised by Goebel and Lunkenheimer, 1997. Increasing concentration of PEGs in mHFA, 0.05, 0.1, 0.25 and 0.5% (v/w), were prepared for PEG 200, 400 and 600. In addition, samples of PEG 400 and PVP in mHFA were prepared in a similar manner, where the concentration of PEG 400 was

0.05, 0.1, 0.25 and 0.5% (v/w) while concentration of PVP was kept constant at 0.001% (w/w) for all samples. All solutions were stored in tightly sealed containers under ambient conditions prior to use. For the solubility of PEGs and PVP in chlorine-free liquefied gas propellants the authors refer to previously published data by Blondino and Byron (1998) and Paul et al. (2005), respectively.

2.3. AFM topographical measurements of drug crystals

Previous investigations have suggested that substrate morphology significantly influences adhesion (Young et al., 2003). The surface topography of the drug crystal surfaces was investigated using the AFM in a conventional imaging mode. Topographical data were produced using Tapping Mode™ with a high-aspect-ratio silicon probe (OTESP, Digital Instruments (DI), Cambridge, UK), at a scan rate of 0.7 Hz. All AFM studies were performed using a commercially available Multi Mode AFM with a Nanoscope III controller (DI). The root-mean-squared surface roughness (rms) of each sample was calculated from the AFM height data over a 5 μm × 5 μm area as follows:

$$R_{\mathrm{rms}} = \sqrt{\frac{1}{n}\sum_{i=1}^{n} y_i^2} \quad (1)$$

where n is the number of points in topography profile and y_i is the distance of asperities (i) from the centreline.

2.4. In situ AFM colloid probe measurements of salbutamol drug probe on salbutamol crystal substrate with three different molecular weight non-ionic polymers

The interaction of drug particles with drug crystal surfaces in a mHFA system filled with saturated mHFA solution of the respective drug under investigation was conducted using the atomic force microscope equipped with an *in situ* cell (DI, Veeco Instruments Ltd., Cambridge, UK). Colloid probes were prepared by mounting an individual drug particle (approximate diameter 5 μm) onto a V-shaped tipless cantilever (spring constant $k = 0.32$ N/m, DNP-020; DI) using a quick-setting epoxy resin (Ducker et al., 1991). Prepared drug probe tips were stored in tightly sealed containers for 24 h prior to use. Possible variations in spring constant were minimized by obtaining a wafer of tipless cantilevers (>500 tips), so batch-to-batch tip thickness concerns were eliminated. Randomly chosen tips ($n = 5$) from across the wafer indicated <14% variance in spring constant using the thermal method (Hutter and Bechhoefer, 1993).

Extreme care was taken during drug probe preparation to limit the amount of drug–glue contact. The micromanipulation technique is described in detail elsewhere (Young, 2002). The integrity of all drug probes were investigated prior to and post-measurement using a high-magnification 500× long-working-distance optical microscope. All salbutamol sulphate drug probes appeared proud of the cantilever surface, and no visible differences between the start and end of the experimental procedure were observed (indicating no macro/microscopic change in drug probe morphology). As the AFM cannot be used in a pressurised environment, the forces of cohesion between individual micronised salbutamol sulphate, colloidal probe particles and respective crystal surfaces were conducted using the colloid probe approach under *in situ* conditions with a model propellant (Rogueda, 2003; Young et al., 2003) and *in situ* liquid AFM cell (Muster and Prestidge, 2002). Force separation measurements were collected using force–volume mode to produce multiple force distance curves ($n = 512$) between each drug probe and crystal surface over a 5 μm × 5 μm area with the following settings: approach-retraction cycle 500 nm, cycle rate 4.07 Hz and a loading force of 20 nN. A disadvantage in the use of the colloid probe technique in cohesion studies is the fact that the actual size and geometry of the AFM probe is unknown and, therefore cannot be normalised. Consequently, variability in probe contact radius geometry was expected. Accordingly, each study was performed in triplicate ($n = 3$ salbutamol sulphate probes for each set of PEG or PEG + PVP studies), with a new drug particle attached on the AFM cantilever and a new crystal substrate used for each set of experiments so as to minimize the effects of cross contamination and that of the environment on the particle and substrate. Furthermore, a great deal of care was taken to maintain the integrity of the colloid probe throughout each study to avoid inter–intra probe variation. To avoid any drug solubility issues, the mHFA solution was pre-saturated with salbutamol sulphate and filtered before use. Twenty minutes were allowed to elapse after the addition in the *in situ* cell of each different concentration of the polymeric solution to allow system stabilization.

3. Results and discussion

The main objective of the study was to investigate the influence of the concentration and molecular weight of PEG polymers, with and without the presence of PVP, on the physical interactions between salbutamol sulphate drug particles in a model pMDI system. Atomic force microscopy (AFM) was used for the assessment of the cohesion of salbutamol sulphate with salbutamol sulphate crystals in a mHFA in the presence of different concentrations of these excipients. In addition, the influence of PVP on the force of interaction of the drug was evaluated.

3.1. Analysis of crystal substrates

Prior to AFM cohesion measurements, the surface roughness on the dominant crystal face of each drug substrate was investigated, avoiding problems related with face-specific crystal chemistry (Muster and Prestidge, 2002). Roughness analysis ($n = 25$, 5 × 5 μm area) of the surface topography for each drug crystal indicated are presented in Table 1. The data suggested extremely smooth surface morphologies of the salbutamol sulphate dominant drug crystal face under investigation (Face {1 1 1}), with each of the crystals exhibiting a root-mean-squared roughness below 5 nm over a 5 μm^2 area. It is important to note, that significant differences in roughness between crystals were observed (ANOVA, $p < 0.05$). However, each PEG concentration study was conducted with a single specific crystal

Table 1
Roughness analysis ($n = 25$, 5 μm × 5 μm area, ±S.D.) of the surface topography for each salbutamol sulphate drug crystal dominant face

Salbutamol sulphate crystal samples	Roughness (nm ± S.D.)
1	0.96 (±0.04)
2	1.20 (±0.01)
3	1.68 (±0.01)
4	0.80 (±0.03)
5	0.41 (±0.04)
6	1.72 (±0.05)
7	2.93 (±0.01)
8	1.87 (±0.02)
9	1.08 (±0.03)
10	1.66 (±0.06)
11	1.95 (±0.03)
12	1.82 (±0.05)

thus allowing comparison. Furthermore, in all cases, the salbutamol sulphate crystalline substrates possessed an absence of micrometre scale roughness, thus making them highly suitable for quantitative colloidal probe AFM analysis (Buckton, 1995).

3.2. *In situ* AFM force of cohesion measurement: the stabilizing effect of surfactants and stabilizers and its variation with concentrations

The *in situ* AFM probe technique allowed direct measurement of the cohesion between single salbutamol sulphate drug particulates and salbutamol sulphate crystal surfaces in the presence of surfactants and/or stabilizers. As previously discussed, the measurement of the force of adhesion between each drug probe and substrate was conducted using force–volume imaging over 5 μm × 5 μm areas ($n = 512$ measurements per probe). Integration of each data set indicated normal, Gaussian distributions in forces of adhesion. This is expected however, since the contact area between each probe and 'atomically smooth' substrate will remain constant. Subsequently, mean adhesion values and standard deviations were used to describe the interactions between drug probe and substrate.

The force of adhesion between separate drug probes and drug crystal substrates are shown in Fig. 1. As previously discussed, three AFM probes were used for each molecular weight polymer study (probes 1–3 were used for PEG 200, probes 4–6 for PEG 400 and probes 7–9 for PEG 600). New probes and substrate were utilised for each set of measurements to avoid polymer contamination issues. Furthermore, each study was conducted using sequentially increased concentrated PEG solutions (*i.e.* lowest concentration first).

Analysis of the results suggested that strong attractive forces were present in the systems in the absence of any stabilizing agents. In general, cohesion forces ranged from 11.84 ± 1.62 to 111.07 ± 29.79 nN in pure mHFA alone ($n = 12$ probes). Although, in previous works, electrostatic interactions between powders and non-polar solvents have been reported (Yu et al., 2003; Hsu et al., 2005) and even in very inert solvents the counter charge in solution has been shown to exist (Kosmulski, 1999), as discussed in the introduction, the attractive forces observed in

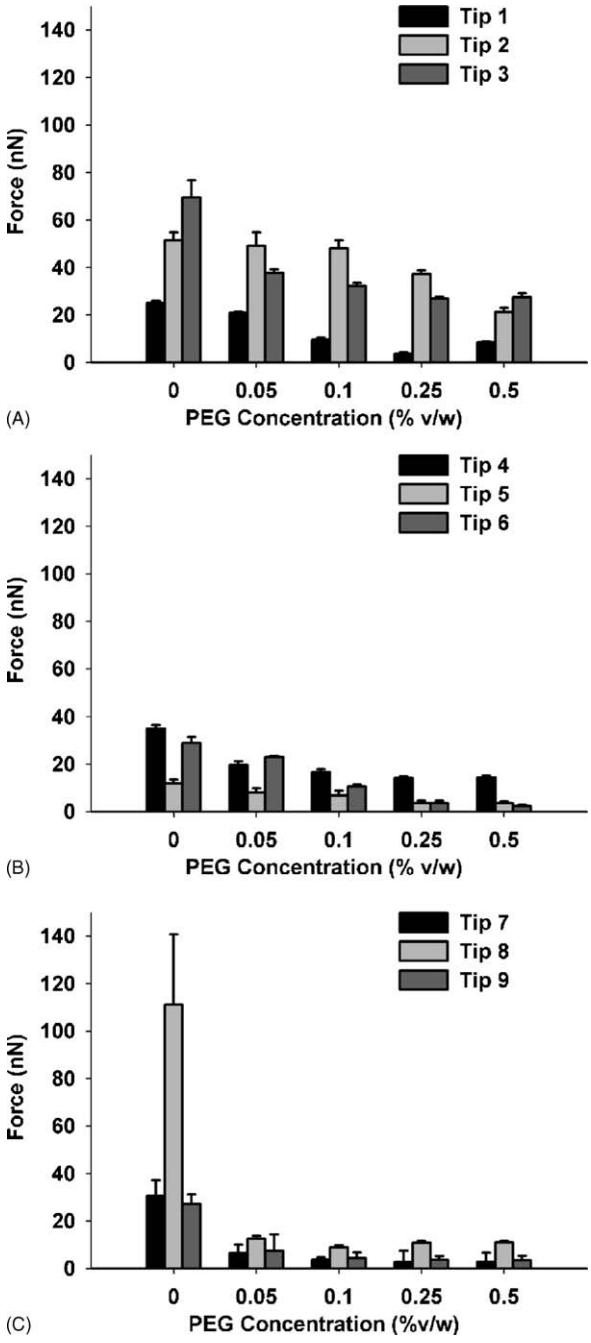

Fig. 1. Force of cohesion between salbutamol sulphate drug probes ($n = 3$) on salbutamol sulphate crystals in mHFA with a range of concentrations of: (A) PEG 200, (B) PEG 400 and (C) PEG 600 (±S.D.).

the pure mHFA most probably have their origin in the London dispersive component (van der Waals interactions) as neither the salbutamol sulphate micronised drug particle or drug crystal substrate are likely to be highly charged in a non-aqueous environment (Romo, 1963; Wyatt and Vincent, 1989).

Sequential increase in concentration of each of the PEG solutions, up to a concentration of 0.5% (v/w), resulted in a statistically significant decrease in cohesion (ANOVA, $p < 0.05$). In general, the decrease in cohesion was particularly evident at low concentrations of PEG (0.05–0.1%, v/w). In comparison,

Table 2
Normalised mean values and standard deviation for the cohesive interaction between salbutamol sulphate drug probe–substrate ($n = 3$) with increasing concentrations of PEG (MW 200, 400 and 600) in mHFA (±S.D.)

PEGs	PEGs concentration (±S.D.)			
	0.05% (v/w)	0.1% (v/w)	0.25% (v/w)	0.5% (v/w)
MW 200	0.78 (±0.21)	0.59 (±0.30)	0.42 (±0.29)	0.38 (±0.04)
MW 400	0.68 (±0.11)	0.47 (±0.11)	0.28 (±0.14)	0.26 (±0.17)
MW 600	0.20 (±0.08)	0.12 (±0.04)	0.11 (±0.02)	0.11 (±0.02)

relatively high concentrations (0.25–0.5%, v/w) resulted in little cohesion decrease. Interestingly, such data correlated well with previous reports (Kerkar and Feke, 1991). Kerkar and Feke (1991) reported that the adsorption of polymers onto solid surfaces showed adsorption isotherms with a rapid adsorption rate at low concentrations followed by a plateau value. For pharmaceutical applications, the ideal maximum absorbed layer thickness should be achieved at a lower surfactant concentration to avoid adverse effects on propellant evaporation and product performance.

Further data analysis (Fishers pairwise, $p < 0.05$), suggested that variation in cohesion was dependent on the concentration and the MW of PEG. For example, analysis of the highest molecular weight data (PEG 600), suggested that no variation in cohesion was observed above concentrations of 0.25% (v/w). In comparison, data for PEG 200 suggested variations across the entire concentration range. It is envisaged that such observations may be due to increased steric hindrance with the higher molecular weight polymers.

In order to compare the relative decrease in cohesion with respect to polymer concentration and molecular weight, the mean values for each drug probe were normalised to the cohesion values obtained in mHFA only. The mean ± S.D. for the normalised mean data values are presented in Table 2 and Fig. 2. In general, analysis of the salbutamol sulphate cohesion force curves in the presence of PEG solutions suggest a rank decrease in nominal mean separation force of the order: PEG 200 > PEG 400 > PEG 600 ($n = 3$ probes for each polymer analysed). Equally, variability of the results decreases from PEG 600 < PEG 400 < PEG 200. Such observations may be due to both an increase in particulate surface coverage and increased polymer protrusion into the mHFA media with increase in molecular weight. As previously discussed it has been reported that the degree of the steric stabilization is dependent, in part, on the adsorbed layer thickness (Kerkar and Feke, 1991) and accordingly we should expect PEG 600 to be more effective at reducing cohesion due to a higher molecular weight and consequently longer polymeric chain.

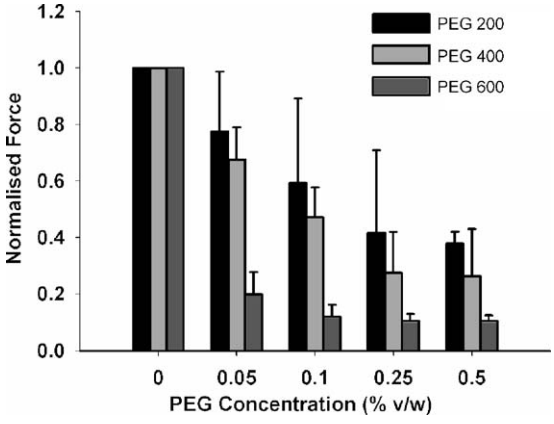

Fig. 2. Normalised force of cohesion between salbutamol sulphate drug probes ($n = 3$) on salbutamol sulphate crystals in mHFA with a range of concentrations of PEG (MW) 200, 400 and 600. Data represent mean ± S.D. of the mean for three probes with each PEG.

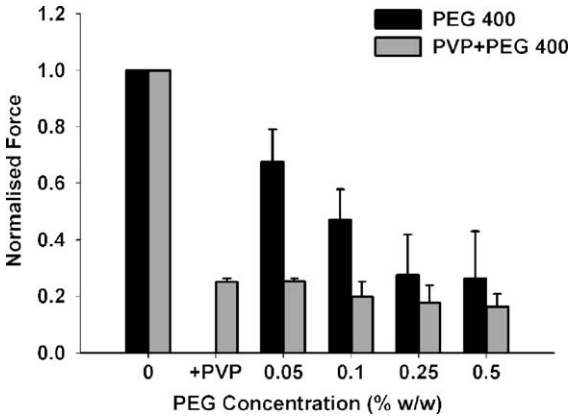

Fig. 3. Normalised force of cohesion between salbutamol sulphate drug probes ($n = 3$) on salbutamol sulphate crystals in mHFA with PEG 400 (0.05, 0.1, 0.25 and 0.5%, v/w) with and without 0.001% (w/w) PVP. Data represent mean ± S.D. of the mean for three probes with each PEG concentration.

In comparison, a different cohesion profile was observed with the addition of 0.001% (w/w) PVP to different concentrations of PEG 400. In the presence of only PVP and mHFA, a large statistical decrease (approximately 70%) in force of cohesion was observed when compared to mHFA only (ANOVA Fishers, $p < 0.05$). Interestingly, any further addition of different concentrations of PEG 400 (0.05, 0.1, 0.25 and 0.5%, v/w) to the mHFA–PVP system did not result in a significant reduction of the force of cohesion (ANOVA Fishers, $p < 0.05$). As with the PEG studies, the data were normalised to allow comparison of PVP systems to PEG. Data are presented graphically in Fig. 3. In general, such observations are in good agreement with the proposed mechanism of PVP adsorption. It is proposed that once PVP is added to the mHFA its large planar rigid molecules spread across the surface of the drug particles, achieving a greater surface coverage, preventing any further stabilizing effect due to the added concentration of PEG.

4. Conclusions

The concept of steric stabilization has been tested through direct particle adhesion measurements in a model propellant system. Results are believed to be quantitative and representative for this kind of model suspension system, where drug particulates have been proven to have a certain degree of instability in the presence and/or absence of polymers. It was found that an increase in chain length, of low molecular weight polymers, added to drug particulate suspensions in a low polarity media like mHFA, led to a decrease in inter-particulate cohesion. In addi-

tion, the use of homopolymers had a positive effect in decreasing particle–particle interactions.

There is no clear evidence of direct PVP–PEG binding: it is proposed that the changes observed in suspension stability may be due to the change in the thermodynamics of the system. The presence of PVP in a suspension system will dominate the suspension stability and further addition of a second polymer does not destabilize the suspension (Paul et al., 2005). The results of the present study tend to support the hypothesis that hydrogen bonding is primarily responsible for the stabilization of a suspension. With regards to the applicability of the AFM to investigate pMDI suspensions, it can be concluded that such techniques can allow a better insight into the interactions of such a complex system and it could prove invaluable during the early phases of formulation product development. Clearly, an understanding of the conformation of polymer molecules at interfaces is of vital importance when controlling the stability/flocculation behaviour of sterically stabilized suspensions.

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