Chapter 6

Development of colloidal probe microscopy technique to investigate the interaction between colloids.

In the last 20 years several new devices for measuring surface and intermolecular forces have been developed: the surface forces apparatus of Israelachvili [1, 2] and Adams, the force balance of Derjaguin et.al. [3] and osmotic stress device of Parsegan et.al. [4] these new devices have allowed accurate measurement of surface forces and have led to improved understanding of these forces, and phenomenon such as wetting, tribology, macromolecular self assembly and colloid structural stability. The invention of Atomic Force Microscopy (AFM) allowed molecular and surface forces to be measured on a near molecular scale for the first time. The phenomenon of molecular recognition has been extensively studied to provide a concrete understanding of the lock-and-key concept for drug design involving forces between the donor-receptor complex.
6.1 Introduction

The pull-off forces in AFM measurements are the actual observed adhesive forces. This phenomenon occurs in the contact region and constitutes the total interaction forces. This includes long range forces such as van der Waals and electrostatic; capillary forces, solvation forces, hydrophobic interactions and steric interactions and chemical bonding between groups present on the surface. Adhesion of active pharmaceutical ingredient (API) to other particles and container surfaces has been investigated and reported in numerous studies in the literature [5–10]. The most common technique is to use the centrifuge or ultracentrifuge method to study the particle-substrate adhesion [11]. But quantifying the adhesion force for individual particle and theoretical treatment of the contact between particles and surfaces is difficult when this technique is used. The colloidal probe technique using AFM has the advantage to solve these problems [12]. The advantage with this technique is the ability to make measurements on single particle level and to measure forces and hence interaction energies, with respect to intersurface distance. Depending upon the experimental setup being employed, a number of different interactions may be measured, either separately or simultaneously, including long range forces such van der Waal and electrostatic forces hydrophobic interaction as well as adhesion forces.

In an interactive mixture of API, the drug particles are considered to firmly adhere to the carrier particles [13]. It is desirable to have selective attachment-detachment of drug particles such that it is not detached from its excipient material during manufacturing process, storage or transportation. Hence measurement of adhesion forces between drug and excipient enables one to choose the right kind of storage or transportation container in an industrial setup. As AFM colloid probe technique enables the direct measurement of single particles and surfaces, it is becoming the method of choice to characterize particle adhesion in pharmaceutical systems [14].
Several factors can influence the adhesion between particles and surfaces viz. surface roughness, surface free energy and mechanical properties such as hardness and elasticity etc. This chapter describes the adhesive interactions between the pharmaceutical drug particles. Here we developed a colloid probe technique to study the adhesive interaction between API (Salmeterol Xinafoate and Fluticasone Propionate) with different processing (JET milled processed, SCF processed and unprocessed respectively) and lactose carriers with different blend numbers (Respitose SV001 and Respitose SV003). The pull-off forces between carriers and drug particles have been determined which corresponds to adhesion force. Also the surface topography of representative particle is characterized with AFM. This allows for a quantitative assessment of the role of 'surface roughness' plays in the adhesion of micrometer size particles to the substrate. Apart from adhesion force measurement and surface roughness analysis, calculation for surface energy for both lactose carriers has been carried out along with the measurement of deformation.

6.2 Initial study with the understanding of adhesion using force-distance curves.

AFM consists of a minute cantilever with a sharp tip at its end that is brought into close proximity to a sample. The tip-sample system in AFM is shown schematically in Fig. 6.1. The sample is assumed to be at ground level, positioned at \( z = 0 \). The base of the cantilever is at a height \( z \) as shown in Fig. 6.1. The cantilever is approached towards the sample by using a stepper motor. Now the presence of a tip-sample force will bend the cantilever, displacing the position of the tip to \( s \) from its position \( z \) (when it is free). The deflection of the cantilever, \( d \), will
6.2 Initial study with the understanding of adhesion using force-distance curves.

Figure 6.1: A schematic diagram of AFM tip and sample assembly (a) and deflection-distance curves (b). The dotted line in (a) marks the equilibrium position of the cantilever in the absence of an external force. $d$ is positive when measured upwards. The arrows in (b) show the direction of motion of the cantilever.

therefore be given by $d = s - z$. In a real experiment the tip-sample force may involve contributions from atomic, adhesion, and indentation forces, to name only a few. However, the salient features of an AFM can be obtained quite accurately by modeling the attractive tip-sample interaction using a van der Waals force and the repulsive interaction by simple Hertzian elastic contact model. In contact mode, initially the cantilever placed far enough from the sample that the tip does not feel the force from tip-sample interaction. As the tip is brought very close to the surface, jump-into-contact occurs (JIC) (denoted by $z_i$) if it feels sufficient attractive force from the sample. After loading the cantilever to a desired force value, the process is reversed. As the cantilever is withdrawn, adhesion or bonds formed during contact with the surface may cause the cantilever to adhere to the sample some distance past the initial contact point on the approach curve. This point is referred as jump-off-contact (JOC) is also called as snap back point (denoted by $z_o$). The concept of JOC has been used extensively in the past as a
6.2 Initial study with the understanding of adhesion using force-distance curves.

Prior to the experiments described below, force curve study has been carried out on standard sample (Si, HOPG, mica) to understand the behavior of adhesion mechanics. Fig. 6.2 shows contact mode force curves taken on Si, HOPG and mica. Contact mode cantilever (from Mikromasch) of force constant of 0.35 N/m and resonant frequency of 28 KHz is used. For Si and HOPG surface, the distance at which the tip breaks away from the substrate is slightly less than that measured quantitatively.
6.2 Initial study with the understanding of adhesion using force-distance curves.

Figure 6.3: Force-distance curve in presence of electric field, (a): HOPG, (b): Mica mica surface. The difference indicates that the adhesion force for the mica surface is larger than that for Si and HOPG surface. The area under the curve is a measure of adhesion energy. From the Fig. 6.2 it can be seen that the adhesion energy for HOPG and mica is greater than Si. Also, the gradient value of the repulsive region for both HOPG and mica is less than for Si suggesting that Si is much harder than HOPG and mica.

Electrostatic interactions between colloid particles at liquid/liquid interfaces play a crucial role for the stability and the properties of solid stabilized emulsions, which are currently used in a number of industrial products like paints, surface coatings, cosmetics, pharmaceutical formulations and agrochemicals. Similar interactions take part between integral proteins adsorbed or integrated within cellular membranes and organelles. The behavior of charged particles at liquid-liquid and liquid-air interfaces has been a subject of great interest over the last 50 years but is still far from being well studied and fully understood.

In many cases the tip-sample interaction arises from the van der Waals interaction. In such cases, the use of an electrostatic force, by application of a small voltage between the sample and tip, is an interesting option to change the effective tip-sample interaction in a controlled way. This is because both van der Waals and electrostatic interactions have $1/s^2$ dependence. The tipsample force is mod-
eled by a combination of the van der Waals force at large tip-sample distances (z) which is essentially attractive and by the Derjaguin-Muller-Toporov (DMT) force which is a combination of the attractive van der Waals like force and the repulsive forces arising due to elastic interaction between the tip and the sample. Thus, formally, the force is given by [19]

\[ f(x) = \begin{cases} 
-\frac{HR}{6s^2} & s < a_0 \\
-\frac{HR}{6a_0^2} + \frac{4}{3}E^*\sqrt{R(a_0 - s)^{3/2}} & s > a_0 
\end{cases} \]  

(6.0)

where H and R are the Hamaker constant and the radius of curvature of the tip respectively. The attractive force is the only force present when s > the intermolecular distance (a_0), whereas when s < a_0 the force has a repulsive component, which increases with reducing z. In presence of electric field, the electrostatic force can be written by adding the term of force due to applied electric field to the force term due to van der Waals force.

\[ f(x) = \begin{cases} 
-\frac{HR}{6s^2} - \pi\varepsilon_0 V^2 R^2 s & s < a_0 \\
-\frac{HR}{6a_0^2} - \pi\varepsilon_0 \frac{R}{a_0^2} + \frac{4}{3}E^*\sqrt{R(a_0 - s)^{3/2}} & s > a_0 
\end{cases} \]  

(6.0)

To study the effect of electric field, force-distance have been measured on both conductor as well as insulator. We used HOPG as a conductor and mica as an insulator for this study. Fig. 6.3 shows the contact mode force-distance curves taken on HOPG and mica. From the figures it is clear that on application of electric field JIC and JOC positions shift to higher tip-sample separations and also the deflection of the cantilever increases as marked in circles [19].

6.3 Materials and Methods

In medicinal dry powder inhalers (DPI), particle interaction is one of the important factor where the redispersion of drug particles from excipient carrier particles (usually lactose) is critical for lung deposition. The delivery of drug to the lungs
is achieved in DPI through the use of micronized drug particle with a particle size smaller than 5 µm, which is dispersed during inhalation to ensure it reaches the patient’s lungs [20]. Unfortunately, these particles are intrinsically cohesive and have poor flowability leading to the manufacturing problems [21]. The common solution is to blend fine respirable drug particles with carrier particles. This leads to adhesion between drug particles and carrier particles. This adhesion between drug and carrier control the powder flow, which is important for dosage form manufacturing and dose sampling.

In this work, two blends of lactose carriers have been used to study the interaction between drug particles and lactose carriers, 1) Respitose SV001 and Respitose SV003 respectively. The drug samples are in the powder form with different types of processing. All the samples were provided by Sun Pharmaceutical Advanced Research Centre (SPARC). These samples are:

1. Salmeterol Xinafoate (SX)
   - Air Jet Milled processed
   - Unprocessed

2. Fluticasone Propionate (FP)
   - Air Jet Milled Processed
   - Super Critical Fluid Technology (SCF)
   - Unprocessed

**Salmeterol Xinafoate**

Salmeterol is a long-acting $\beta_2$-adrenergic receptor agonist drug that is currently prescribed for the treatment of asthma and chronic obstructive pulmonary disease (COPD). It is currently available as a proprietary "disk-styled" inhaler that releases a powdered form of the drug (DPI). Salmeterol has an aryl alkyl group with
a chain length of 11-atoms from the amine. This bulkiness makes the compound more lipophilic and it also makes it $\beta$ receptor selective.

**Fluticasone Propionate**

FP is a synthetic corticosteroid derived from fluticasone used to treat asthma and allergic rhinitis. It is also used to treat eosinophilic esophagitis. FP is a medium-potency synthetic corticosteroid. It is used topically to relieve the inflammatory and pruritic manifestations of corticosteroid-responsive dermatoses and psoriasis, intranasally for symptoms of allergic and non-allergic rhinitis, and by oral inhalation for asthma. FP inhalations are also used clinically in certain patients with chronic obstructive pulmonary disease (COPD).

### 6.3.1 Powder micronization

In order to obtain the optimal particle size distribution for pharmaceutical powders in dry powder inhalers the particles have to be micronised. Micronization is
a term used to describe the size reduction where the resulting particle size distribution is less than 10 $\mu$m. In most cases the process of micronisation is connected with a high input of energy which induces disorder and defects on the surface of the drug particles and as a result changes in the crystallinity. Consequently, changes in the physical stability of the powders may occur. In this study two main processing methods are used: 1) Air Jet Milling and 2) SCF

**Air Jet Milled Process**

Air jet micronization is a well proven technique that consistently produces particles in the 1-30 micron range. The advantages of air jet micronizers are; particle reduction occurs due to particle to particle collisions with limited reduction from metal to product contact and no generation of heat. Other advantages include no moving parts and easy to clean surfaces. Micronization size reduction involves acceleration of particles so that grinding occurs by particle to particle impact or impact against the solid surface. Fluid energy mills used for micronization because of the high impact velocities possible as result of particle acceleration in a fast gas stream. Particle velocities in jet mill are in the range of 300 to 500 meters per second compared to 50 to 150 meters per second in a mechanical impact mill.

**SCF Process**

The use of SCF for the preparation and control of the specific physical form of pharmaceutical substances relevant to those fluids used for drug delivery systems have been described [22–24]. A substance whose temperature and pressure are simultaneously higher than at the critical point is referred to as a supercritical fluid. The typical operating temperature and pressures for supercritical fluids are 1.01(Tc) to 1.1(Tc) and 1.01(Pc) to 1.1(Pc), respectively. SCF have been proposed as solvents, solutes, antisolvents and reaction media and SCF technology is very attractive for manufacturing innovative therapeutic particles, either
of pure active compounds or mixtures of excipient and active compounds. Certainly, pharmaceutical companies can develop pharmaceutical products of much higher yields greater purity, and quality than possible by conventional chemical engineering unit operations using SCF. The most commonly used SCF for a variety of applications include supercritical fluid carbon dioxide (SC-CO$_2$), nitrous oxide, water, methanol, ethanol, ethane, propane, n-hexane and ammonia [25]. SC-CO$_2$ is an attractive solvent or anti-solvent as it is safe, inexpensive, readily available, and an ideal substitute for many hazardous and toxic solvents. SC-CO$_2$ exists when both the temperature and pressure equal or exceed the critical point of 31°C and 73 atm and has both gas-like and liquid-like qualities, and it is this dual characteristic of SCF that provides the ideal conditions for extracting compounds with a high degree of recovery in a short period of time. By controlling the level of pressure/temperature modifier, SC-CO$_2$ can dissolve a broad range of compounds, both polar and non-polar.

Particle size has been measured for both Jet milled and SCF process using Malverns Patcile Size Analyzer working on the principle of laser diffraction. Size distribution and summary statistics are the average of three determinations. The particle size distributions of the drugs each approximated to a log-normal distribution, and were therefore summarized using d10, d50, d90 values. Here in a typical specification, a D50 size refers to the equivalent diameter greater than 50% of the particles by mass. In other words, a required size of 10 microns should be further defined in reference to a D50 or D90 of 10 microns. Since a resultant distribution with a D50 of 10 micron actually requires a top end of 15-20 micron, less energy required to produce a D90 of 10 micron. The summary of particle size distribution of drug sample with different processing is tabulated in Table 6.1.
Table 6.1: Particle size distribution using Malvern Laser Particle size analyzer.

<table>
<thead>
<tr>
<th>Material</th>
<th>PSD (µ)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d10</td>
</tr>
<tr>
<td>Respitos SV001</td>
<td>26.81</td>
</tr>
<tr>
<td>respitose SV003</td>
<td>30.60</td>
</tr>
<tr>
<td>Fluticasone Propionate (Unprocessed)</td>
<td>5.79</td>
</tr>
<tr>
<td>Fluticasone Propionate (Jet milled)</td>
<td>0.37</td>
</tr>
<tr>
<td>Fluticasone Propionate (SCF processed)</td>
<td>2.29</td>
</tr>
<tr>
<td>Salmeterol Xinafoate (Unprocessed)</td>
<td>5.60</td>
</tr>
<tr>
<td>Salmeterol Xinafoate (Jet milled)</td>
<td>1.96</td>
</tr>
</tbody>
</table>
6.3 Materials and Methods

6.3.2 Scanning Electron Microscopy

Particle size and shape were qualitatively assessed using scanning electron microscopy. Samples were fixed to sticky carbon tape mounted on a aluminium stubs and excess powder removed with a puff of air. They were then coated with platinum and examined under SEM (JEOL 6360A). Following figures show SEM microphotographs of both drug samples with different processing and lactose carrier of different blend No. Fig. 6.5 shows the scanning electron microscope (SEM) image of the Respitose SV001 and SV003. The inspection of SEM images of Respitose SV001 and Respitose SV003 correlates with the particle size data as shown in fig. The particle size of Respitose SV001 and Respitose SV003 differed considerably. The particle shape of both lactose was related to their underlaying crystal habits. From SEM microphotograph, it can be seen that the particle size of the SV001 is 2 times greater than SV003.

Fig. 6.6 and Fig. 6.7 shows SEM microphotographs of SX and FP with different processing. The size of drug particles observed from SEM images correlates with the particle size data taken from Malverns Patcile Size Analyzer.
Figure 6.6: (a) and (b): SEM image of SX (jet milled processed), and SX (Unprocessed); (c) and (d) Corresponding EDS
Figure 6.7: (a), (b) & (c): SEM image of FP (jet milled processed), FP (SCF processed), FP (Unprocessed); (d), (e) & (f) Corresponding EDS
6.4 Characterization of lactose carriers

6.4.1 Roughness analysis

Measurements of the pull-off forces between pharmaceutical particles with irregular morphologies and varying degree of surface roughness have previously been reported [26]. Although the effects of surface roughness on variations in adhesion forces are acknowledged, direct quantification of true contact area between two contiguous surfaces remains difficult. In DPI formulation, the dispersion of drug from interactive mixture of drug and carrier is affected by not only the magnitude of adhesion force but also its distribution which depends on surface roughness. Due to surface irregularities on both the carrier and drug particle changes the effective area of contact. For many years mechanical and optical surface profilers were used for analyzing surface texture of various types of materials [26–28]. However, such profilers are limited in their horizontal resolution to approximately one micron. Thus, they can readily measure at a length scale of a few millimeters. Traditional quantitative surface roughness parameters such as roughness average ($S_a$), root mean square roughness ($S_q$) and mean roughness ($S_{mean}$) can be directly calculated from AFM images.

Force curve studies, as a function of variety of parameters including the particle size, substrate material and surrounding gas environment were carried out. Because the surface roughness is known to play an important role in determining the adhesion of particles with substrate, further studies to elucidate this aspect of adhesion would be worthwhile. Roughness measurements was carried out using Scanning Probe Image Processor (SPIP) software via the following equations,

$$S_a = \frac{1}{M N} \sum_{k=0}^{M-1} \sum_{l=0}^{N-1} |z(x_k, y_l)|$$

(6.0)
### Table 6.2: Roughness parameters for lactose carriers.

<table>
<thead>
<tr>
<th>Material</th>
<th>$S_q$</th>
<th>$S_q/S_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respitos SV001</td>
<td>0.322 µm</td>
<td>1.28 (0.04)</td>
</tr>
<tr>
<td>Respitos SV003</td>
<td>0.119 µm</td>
<td>1.26 (0.02)</td>
</tr>
</tbody>
</table>

and

$$S_q = \sqrt{\frac{1}{MN} \sum_{k=0}^{M-1} \sum_{l=0}^{N-1} [z(x_k, y_l)]^2}$$  \hspace{1cm} (6.0)

where,

- $M$ and $N$ are equally spaced points along X and Y direction and
- $Z$ is the vertical distance from the mean line to the $k^{th}$ and $l^{th}$ data points in X and Y direction respectively.

For AFM measurements lactose particles were sprinkled on sticky carbon tape mounted on an aluminium stubs and excess powder removed with a puff of air. True non-contact mode surface scans were performed in air at ambient temperature and humidity using Burleigh Metris 2000. Non-contact mode cantilever from Mikro Masch (NSC35-CrAu) with force constant 4.5 N/m and resonant frequency of 150 KHz was used for imaging. A defined scan size of 4µm × 4µm with 256 × 256 points in image was used. The set point was adjusted in such a way that no damage to the sample surface was caused by the AFM tip. Due to the restricted movement of the cantilever on the very rough sections of the lactose surface, we were unable to scan large area (above 4µm). The surface roughness for different blends of lactose carriers was calculated. Atleast 4-5 sample areas, each of a 4µm × 4µm, from 2-3 different particles were scanned. The number of 4µm × 4µm sample area on each particle depended on the carrier size. Small particle size carriers (Respitose SV003) utilized only 1 or 2 sample area per particle, while for larger carrier (Respitose SV001) up to 4-5 sample area per particle were able
to be used. Roughness parameters measured from AFM for lactose carriers are tabulated in Table 6.2.

The values of $S_q$ for both the sample suggests that Respitose SV001 is more rough than Respitose SV003. The ratio of $S_q/S_a$ for both Respitose SV001 and Rspitose SV003 lactose carriers is greater than unity, which indicated a high surface irregularity.

### 6.4.2 Adhesion force measurement

Adhesion of a particle to a substrate surface involves the following essential steps:

1. Initially the area of contact between particle and substrate is of atomic dimensions.

2. By long-range attraction forces between the two, the particle generally is subject to a moment of force so that several contacts are formed, at least between non-perfectly smooth adherents.

3. By the interaction forces the contact area at these contacts increases until the attractive forces and the forces resisting the further deformation at the interface are in equilibrium. An adhesive area of finite size is formed between the adherents.

The roughness of the surface has a large effect on the adhesion force since it reduces the contact area between the bodies leading to significantly reduced interaction [29–31]. Surfaces may posses roughness in several length scales, but due to the short range of the van der Waals interaction, roughness in nanoscale ultimately determines the strength of adhesion. In case of large particle, It will often have surface asperities whose effective radius of curvature is much smaller than the particle radius. The interaction of the large particle with the substrate
may therefore be reduced largely to that of a much smaller particle whose radius is that of the radius of curvature of the surface asperity facing the substrate.

Adhesion force measurements were carried out using silicon tip from Mikro-Masch with force constant of 1.75 N/m and resonant frequency of 155 KHz respectively. Contribution from capillary forces was avoided by placing the silica gel near tip-sample assembly throughout the experiment. Fig. 6.8 shows the representative force versus distance curves taken for both lactose carriers. The averaged adhesion force is 9.03 nN ± 2.7 for SV001 and 13.3 nN ± 2.8 for SV003 respectively. The observed adhesion force for SV003 is greater than SV001. From the values obtained we can say that the adhesion force is depends on surface roughness which increases with decreasing surface roughness.

### 6.4.3 Calculation of Surface Energy

Surface energy and strength of adhesion between two bodies are clearly related through the action of surface forces. In order to separate bodies in intimate contact, mechanical work must be expanded to overcome the adhesive forces. The energy required to create unit area of new surface can be defined as the free surface
6.4 Characterization of lactose carriers

energy of the solid. Although this is a straight forward definition it is difficult to find direct experimental evidence in support of it [32]. From the Hertz approximation, the two most popular models have been developed to determine the van der Waals force of interaction, JKR and the DMT respectively. Considering JKR approximation, the resulting force of adhesion between two spheres is given by [33]

$$F_{adh} = n\pi R W_{adh} \quad (6.0)$$

where $R$ is the radius of the sphere (radius of the probe) ($\sim$10 nm), $n$ is a predetermined constant depending on the selected model ($n = 3/2$ for JKR and $n = 2$ for the DMT). $W_{adh}$ is the work of adhesion which is given by $W_{adh} = 2\sqrt{\gamma_1\gamma_2}$. Where $\gamma_1$ and $\gamma_2$ are the dispersive components of the surface energy of the tip and sample. $\gamma_1$ was taken to be 42 mJ ($m^{-2}$) for the silica tip (18,19). The radius of curvature for silicon tip was assumed to be 10 nm (manufacturer species 10 nm). From Eq. 6.4.3 the surface energy of the sample (Respitose SV001 and Respitose SV003) is calculated using following equation

$$\gamma_2 = \left( \frac{F}{3\pi R} \right)^2 \frac{1}{\gamma_1} \quad (6.0)$$

The adhesion force is calculated as discussed above. 10 to 15 reproducible force-distance curves were obtained for both lactose carriers. The averaged adhesion force is 9.03 nN $\pm$ 2.7 for SV001 and 13.3 nN $\pm$ 2.8 for SV003 respectively. The observed adhesion force for SV003 is greater than SV001. Considering all the parameters mentioned above, the calculated surface energy is 218.7 mJ/$m^2$ for SV001 and 473.6 mJ/$m^2$ for SV003 which higher than SV001.

6.4.4 Calculation of Deformation

Apart from adhesion force and surface energy measurement, deformation is obtained for both lactose carriers. Deformation ($\delta$) induced in the sample surface
Figure 6.9: Cantilever deflection versus piezo displacement curve obtained for Si (reference) and sample to calculate deformation.

when a load is applied through the cantilever tip. δ is measured by collecting an AFM force-distance curve on a stiff silicon wafer surface under the same conditions (Fig. 6.9) [33].

In AFM force measurement, when the probe is in contact with a hard surface without deformation, the cantilever Deflection is equal to the displacement of the Z scanner. Therefore, if the force curve is plotted ascantilever deflection vs. z displacement (D vs z), the slope would be 1. On the other hand, when the probe is in contact with a (relatively softer) sample surface with a non-zero deformation, the cantilever Deflection would no longer be equal to the z displacement because of the sample surface deformation. Actually, the relation \( z = D + \delta \) holds for all force measurements, where \( z \) is the scanner displacement, \( D \) is the cantilever deflection and \( \delta \) is the sample surface deformation (which is zero when measured on a hard reference surface). This is how the surface deformation is calculated out. That’s also why the D vs. z slope is less than 1 for force curves measured on sample surfaces (with non-zero deformation). In AFM, the force is measured by the probe cantilever deflection. Since the linear relationship between the deflection and the
force (The Hooke’s Law: force \( F = k \times D \), where \( k \) is the spring constant of the cantilever, and \( D \) is the cantilever Deflection), when the D vs z curves are presented as F vs z curves, the unit of the slope will become N/m (or nN/nm) while profiles of all curves don’t change at all. (Note that the slope in the D vs z presentation has the unit nm/nm, or 1.) That’s why we simply say that the deformation can be determined from the contact part of the force curve in comparison with the force curve measured on a hard reference surface. The deformation is determined by the ”deflection curves” instead of ”force curves”. The observed mean values of deformation are 0.71 for SV003 and 0.9 for SV001 respectively which is slightly greater than deformation obtained for SV003.

### 6.5 Development of colloidal probe technique and experimental details

In the last years, different techniques have been developed for attaching smaller particles including nanoparticles and nanotubes to the cantilever used for force measurement, chemistry and biochemistry applications [34–36]. Duel wire technique is one of the most commonly used technique for attaching the microparticle to the cantilever. This technique is first introduced by Ducker et al. [12] and the other by Butt [37]. Here we used two techniques for particle attachment,

1. Dual wire technique [38].

2. Cantilever moving technique in Z direction while taking the force curve.

#### 6.5.1 Dual Wire Technique

Since a particle size of the drug particles bigger than 1 \( \mu \)m, it can easily be observed with an optical microscope. An optical microscope used for mounting of the
Figure 6.10: Schematic of the attachment procedure of the particle to the cantilever. In (A) a small drop of glue is picked up with the copper wire, and in (B) the glue is applied to the cantilever. In the next step (C) the particle is picked up by another clean copper wire, simply relying on intermolecular surface forces between the sphere and pipette as the adhesive. In the last step (D) the particle is positioned on the glue spot on the cantilever.

The particle is from WESCO which is provided by Burleigh itself. The specifications of an optical microscope is given below.

1. Magnification: 60X minimum - 270X maximum

2. Working distance: 38 minimum

To pickup the particle copper wire is used. To get the diameter of the wire as thin as possible (\(\sim 10-15 \, \mu m\)), the wire has been etched using \(\text{FeCl}_3\) for 3-5 minutes. To remove contamination from the wire, it is rinsed with methanol and then distilled water. Fig. 6.10 illustrates the technique. While observed under an optical microscope, a probe is fixed on a probe holder with cantilevers tip facing up. To begin the work, standard cantilever with tip radius of 10 nm has been used. Further, in order to provide a well-defined smooth surface for the
6.5 Development of colloidal probe technique and experimental details

Figure 6.11: A particle of Respotope SV003 mounted on a normal cantilever, k: 0.03 N/m.

particle to be attached, contact mode tipless cantilevers have been used. The first step is to put a drop of glue as small as possible onto the cantilever by using the copper wire. This is achieved by picking up the glue with a piece of wire. The glue is barely touched with the copper wire Fig. 6.10A. Then the glue is deposited on the cantilever as depicted in Fig. 6.10B. The critical part, the transfer of the glue to the brittle cantilever, is simplified by the softness of the wire. Initially the tungsten wire was used which is too hard. Due to hardness of the tungsten wire, the cantilever will remain damaged while mounting the glue/particle. To avoid this, copper wire has been used because of its softness instead of tungsten. If this wire touches the cantilever accidentally too hard, the wire is slightly bent, and the cantilever is thus not damaged. This can be directly observed in the optical microscope. In the next step the desired particle of few micron, is picked up with another clean copper wire Fig. 6.10C. The particle is simply touched by the end of the copper wire. We observed that the particle we mounted, adhered to the copper wire without using additional adhesives. This effect can be due to intermolecular surface forces (such as the van der Waals or electrostatic interactions) or might be mediated by a thin adsorbed water film. The other wire is then used to pick up a particle via capillary force and attach it to the glue spot on the cantilever.
6.5 Development of colloidal probe technique and experimental details

6.5.2 Cantilever Moving Technique

using the method mentioned above, epoxy resin was applied to a cantilever tip. the epoxy-coated cantilever was mounted in the AFM setup. It was positioned directly above a small agglomerate of drug particles, and lowered microscopically until contact occurred. Retraction of the tip enabled initial confirmation of particle attachment. After drying overnight the probe was examined under an optical microscope to ensure successful attachment. Fig 6.12 shows the SEM microphotograph of SX (unprocessed) particles mounted on a tipless cantilever using cantilever moving technique. Initially the cantilevers with tip were used for trial experiments, but were not used in actual measurements to avoid perturbation from the tip. To achieve uniform particle mounting tipless cantilevers were used. After depositing the drug particles, the force constant of the cantilever would have been modified, but this change was not factored into the measurements.
6.6 Results and discussion

6.6.1 Measurement of adhesion force between SX and Lactose Carrier

Colloidal probe technique (discussed in above section) was used to study the inter particle interaction. Force curve measurements were performed by challenging the modified cantilever as described in previous section. Initial study of SX particles on lactose carrier has paved the way for systematic studies of adhesion. Particles of SX (both jetmilled and unprocessed) were used to probe lactose carriers. The tipless cantilevers of force constant 0.02 and 0.01 N/m were used for these experiments to avoid the contribution of the sharp tip. Fig. 6.13 shows the SEM of the probe prepared with SX drug particles with different processing. Typical force-
6.6 Results and discussion

Figure 6.14: Force-distance curves taken on same area for (a) SX (Jet Milled Processed) and Respitose SV001, (b) SX (Unprocessed) and Respitose SV001.

Table 6.3: Adhesion forces between SX probes and lactose carriers.

<table>
<thead>
<tr>
<th>Material</th>
<th>Processing</th>
<th>No. of curves</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respitos SV001</td>
<td>Jet Milled</td>
<td>85</td>
<td>0.49</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>Unprocessed</td>
<td>90</td>
<td>0.66</td>
<td>0.19</td>
</tr>
<tr>
<td>Respitos SV003</td>
<td>Jet Milled</td>
<td>85</td>
<td>0.50</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Unprocessed</td>
<td>85</td>
<td>0.59</td>
<td>0.18</td>
</tr>
</tbody>
</table>

distance curves were obtained from these experiments are shown in Fig. 6.14. The size of the well in the force-distance curve, observed as the tip is withdrawn from the sample, reflects the strength of adhesion between drug and carrier. At least 100 measurements were recorded using each cantilever modified with the drug particle and averaged to provide an adhesion measurement. In a force versus piezo displacement curve, the attractive forces are negative. In Table 6.3, the mean of absolute values of adhesion force and their standard deviation are shown. The standard deviations (up to 25-30 % of the force value) are partially attributed to the inherent fluctuations of pull-off forces, partially to the variation of the contact areas between probe and sample because of roughness and to inhomogeneities on
the film surface, as mentioned above. The adhesion force observed in individual approach-retract curve were plotted in histogram as shown in Fig. 6.15. From the mean value and the histogram, the observed adhesion force for SX (Jet Milled processed) is considerably larger than SX (unprocessed). Also the magnitude of adhesion force between SX and SV001 is slightly larger than that of adhesion force between SX and SV003.

Figure 6.15: Adhesion force distribution histogram for (a), (b): SX probe and Respitone SV001, (c), (d): SX probe and Respitone SV003.
6.6.2 Measurement of adhesion force between FP and Lactose Carrier

Fig. 6.16 shows the SEM micrograph and corresponding EDS of colloid probes of Fluticasone propionate particles with different processing. Tipless cantilevers of force constant 0.01 and 0.02 N/m were used for force measurements. Fig shows the force-distance curve taken on one area for each of the drug particles. In these measurements two types of features are observed. As labeled in Fig. 6.17 d, e and f, different color curves show force curves grabbed for different run on the same area. Each curve shows some systematic trend. The value of the snap back point gradually decreases or increases as the number of runs increases. This behavior predict mainly two possibility,

1. When the probe and substrate come into contact, the probe may be landing over different areas due to the surface roughness of the lactose carrier which lead to different contact area. By the interaction forces the contact area at these contacts increases until the attractive forces and the forces resisting the further deformation at the interface are in equilibrium.

2. Electrostatic charging of the particles generally modifies the interaction in the jump-of-contact regime. In the absence of any significant electrostatic force or upon complete dissipation of the build-up of electrostatic charges from interacting surfaces, the principal forces contributing to particle adhesion are the van der Waals and capillary forces.

The mean of absolute values of adhesion force and their standard deviation for FP with different processing are tabulated in Table 5.3.1.

One observes that the forces obtained with the colloidal FP (SCF processed) and FP(jetmilled) probe are higher than the forces obtained with the FP (Unprocessed). This may be attributed to the contact area between FP (with jetmilled
Figure 6.16: SEM micrograph and corresponding EDS of particle mounted on a tipless cantilever: (a) FP (Jet Milled Processed), (b) FP (Unprocessed), (c) FP (SCF Processed).
Figure 6.17: Force-distance curves taken on same area for (a), (b) FP (Jet Milled processed) and Respitose SV001, (c), (d) FP (SCF processed) and Respitose SV001 and (a), (b) FP (Unprocessed) and Respitose SV001.
### Table 6.4: Adhesion forces between FP probes and lactose carriers.

<table>
<thead>
<tr>
<th>Material</th>
<th>Processing</th>
<th>No. of curves</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respitos SV001</td>
<td>Jet Milled</td>
<td>116</td>
<td>0.53</td>
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<tr>
<td></td>
<td>SCF processed</td>
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<td>0.17</td>
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<td>Respitos SV003</td>
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<td>0.19</td>
</tr>
<tr>
<td></td>
<td>SCF processed</td>
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<td>0.54</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Unprocessed</td>
<td>110</td>
<td>0.44</td>
<td>0.14</td>
</tr>
</tbody>
</table>

### Figure 6.18: Adhesion force distribution histogram for (a), (b) & (c): FP probe and Respitos SV001, (c), (d) & (f): FP probe and Respitos SV003.
and SCF processed) probe and lactose carrier is larger, which leads to an increased magnitude in force. Also the magnitude of adhesion force between FP-sv001 is comparatively smaller than the force between FP-SV003. The frequency distribution of adhesion force between FP particles and lactose carriers are shown in Fig. 6.18.

Separation energy for each drug and carrier was also measured from the multiple force-distance curves. The area under force curve as shown in Fig. 6.19 is a function of separation energy between two particles. The area under each curve was integrated using Origin 8.0 software. The mean values of separation energies and their corresponding standard deviation are tabulated in Table 5.1. From the mean values it can be seen that the separation energy between FP (SCF and Jet milled processed) and lactose carrier is higher than the separation energy between FP (unprocessed) and lactose carrier. Also the the mean values of separation energy between FP and SV003 are greater in magnitude than FP and SV001. This shows that the separation energy is a measure of adhesion force. Larger the separation energy, greater is the adhesion force. The separation energy histogram is shown in Fig. 6.20 Histogram analysis for adhesion force and separation energy shows the energy values are spread in an asymmetrical and skewed distribution.
Table 6.5: Separation energies between FP probes and lactose carriers.

<table>
<thead>
<tr>
<th>Material</th>
<th>Processing</th>
<th>No. of curves</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
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<td>SCF processed</td>
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<td>16.7</td>
<td>12.5</td>
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<tr>
<td></td>
<td>Unprocessed</td>
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<td>11.8</td>
<td>9</td>
</tr>
<tr>
<td>Respitos SV003</td>
<td>Jet Milled</td>
<td>107</td>
<td>24.1</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>SCF processed</td>
<td>117</td>
<td>19.5</td>
<td>10.9</td>
</tr>
<tr>
<td></td>
<td>Unprocessed</td>
<td>110</td>
<td>10.6</td>
<td>6.7</td>
</tr>
</tbody>
</table>

Figure 6.20: Separation energy distribution histogram for (a), (b) & (c): FP probe and Respitoze SV001, (c), (d) & (f): FP probe and Respitoze SV003.
6.7 Conclusion

The study of forces between active pharmaceutical ingredients and their carriers is cumbersome due to random particle size and geometry, inhomogeneity in surface morphology of carrier particles and mounting of drug particles on the cantilever. To study the interaction between drug and carrier, colloidal probe technique has been developed in the laboratory. Protocols of attaching drug particles to the cantilever have been described in detail. The probes were prepared successfully by mounting the particle on the cantilever manually. With the prepared colloid probes, statistical force curve analysis of Salmeterol Xinafoate (SX) and Fluticasone Propionate (FP) (drug particles) with lactose (Respitose SV001 and Respitose SV003) as carrier has been performed. Comparison of the force curves between two drug particles using different processing strategies suggest that adhesive for the Jet Milled SX drug is greater than the unprocessed SX drug with all the lactose carriers. The analysis also suggests that SV001 lactose carrier binds stronger than SV003 to both the jet milled and unprocessed drugs. Similar observation was made for FP drug particles, that the processed drug particles have a higher adhesive force than the unprocessed one. A very important role of surface roughness on adhesion could have been seen is that the surface roughness reduces the adhesion force. Also it could be shown that larger the separation energy, stronger the adhesion force between drug and carrier. We could also calculate the surface roughness parameters, surface energy and deformation for both the lactose carrier Respitose SV001 and Respitose SV003 which can be used as experimentally observed values in the theory of contact mechanics.

Delivering of the drug carrier complex to the active site (lung tissue) critically depend on the efficiency of detachment of the said complex during the process called aerosolization. This critical balance between cohesive and adhesive forces of the complex is still an unsolved problem in the mechanism of dry powder inhaler formulation.
Bibliography


