The influence of particle engineering on drug delivery by dry powder aerosols.

Zeng, Xian Ming

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The Influence of Particle Engineering on Drug Delivery by Dry Powder Aerosols

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(B.Sc, M.Sc)

曾宪明

A thesis submitted for the degree of

Doctor of Philosophy

of

the University of London

Department of Pharmacy
King’s College London

May 1997
In the fond memories of my grandmother and Xianqun

To my parents, parents-in-law and especially
my wife, lili
my daughter, Jingqiu

长风破浪会有时
直挂云帆济沧海
Acknowledgements

I am greatly indebted to my supervisors, Dr Gary P. Martin and Prof. Christopher Marriott for their continual guidance, expert supervision and constant encouragement throughout the entire duration of my project. I regard them as not only supervisors but friends as well. Thank you for everything! Gary and Chris.

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ABSTRACT

Dry powder aerosols for inhalation are usually comprised of an ordered mixture of the drug with carrier particles, such as lactose. In this study, blends of micronised salbutamol sulphate and coarse lactose particles were employed to investigate the effects of some formulation factors on drug delivery by dry powder aerosols. Lactose particles with different morphological properties were prepared by crystallisation using various different conditions. The particles were then blended with salbutamol sulphate in a ratio of 67.5 : 1 (w/w). The drug deposition was shown to be affected by many factors such as the surface texture, particle size and particle shape of the carrier, as well as the components of the drug and carrier mixtures. For example, the fine particle fraction (FPF) of the drug (particles < 6.4 μm) was increased from about 10% to more than 20% of the administered dose when the rugosity value of the carrier particles was reduced from about 2.7 to 1.5. The use of elongated lactose crystals in the formulation resulted in significantly higher (p < 0.01) FPF (> 20%) than most of the other more isometric particles. Particle size of the carrier also affected the deposition of the drug, larger carrier particles producing lower drug FPF.

Crystallisation of lactose from Carbopol 934 gels produced lactose crystals of a regular, elongated shape with a perfectly smooth surface. These gels did not change the crystal form of lactose since the lactose crystals prepared from Carbopol 934 gels exhibited a crystal form of α-monohydrate. However, the crystals prepared from Carbopol gels possessed higher crystallinity than those prepared under constant stirring. When used as the carrier for salbutamol sulphate, such crystals were able to produce drug FPF significantly higher (p < 0.01) than lactose particles prepared under constant stirring.

Addition of a small amount (5% w/w) of smaller sized lactose (5-10 μm) to powder formulations substantially increased the delivery of salbutamol sulphate. The use of needle shaped lactose crystals was found to be superior to micronised lactose in terms of the delivery efficiency of the drug. The formulations, composed of coarse lactose (63-90 μm), needle shaped lactose crystals (5.9 μm) and salbutamol sulphate (1.9 μm) in a ratio of 64.125 : 3.375 : 1 (w/w), produced an FPF of salbutamol sulphate 1.64, 1.60, 1.54, 1.64, 2.77, 5.50 and 5.67 times those of Ventolin Rotacap® calculated as drug particles less than 6.18, 4.00, 3.20, 2.30, 1.40, 0.76 and 0.45 μm, respectively. Therefore, such a formulation would provide an important means of improving the delivery of drug to the lower airways.
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<th>Description</th>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>$\mu$</td>
<td>coefficient of friction</td>
<td>$C_L$</td>
<td>lactose concentration</td>
</tr>
<tr>
<td>$\chi$</td>
<td>dynamic shape factor</td>
<td>$D$</td>
<td>diffusion coefficient</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>mean free path of a particle</td>
<td>$d$</td>
<td>particle diameter</td>
</tr>
<tr>
<td>$\Delta$</td>
<td>root mean square displacement</td>
<td>$d_{air}$</td>
<td>diameter measured by air permeation</td>
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<td>$\psi$</td>
<td>shape index</td>
<td>$\rho_a$</td>
<td>air density</td>
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<td>$\gamma$</td>
<td>surface tension</td>
<td>$\eta_{app}$</td>
<td>apparent viscosity</td>
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<td>$\eta$</td>
<td>viscosity</td>
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<td>$\rho_a$</td>
<td>air density</td>
<td>$\Delta H_c$</td>
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<td>enthalpy of dehydration</td>
<td>$\Delta H_v$</td>
<td>enthalpy of vaporization</td>
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<td>enthalpy of vaporization</td>
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<td>particle density</td>
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<td>$\Theta_s$</td>
<td>angle of slide</td>
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<td>Hamaker constant</td>
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<td>$A_{air}$</td>
<td>specific surface area by air permeation</td>
<td>$A_{micr}$</td>
<td>specific surface area by microscopy</td>
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<tr>
<td>ACI</td>
<td>Andersen cascade impactor</td>
<td>$A_{micr}$</td>
<td>apparent viscosity</td>
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<td>DPI</td>
<td>dry powder inhaler</td>
<td>$d$</td>
<td>diameter measured by microscopy</td>
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<td>surface-number mean diameter</td>
<td>$d_{sv}$</td>
<td>surface-volume mean diameter</td>
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<td>volume-number mean diameter</td>
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<td>elongation ratio</td>
<td>$ECD$</td>
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<td>emitted dose</td>
<td>$f_{50}$</td>
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<td>Van der Waals force</td>
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<td>fine particle fraction</td>
<td>$GMD$</td>
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<td>$GSD$</td>
<td>geometric standard deviation</td>
<td>$HPLC$</td>
<td>high performance liquid chromatography</td>
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<td>$I$</td>
<td>airway generation</td>
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<tr>
<td>Symbol</td>
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<tr>
<td>K</td>
<td>Boltzmann constant</td>
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<tr>
<td>$K_n$</td>
<td>Knudsen number</td>
<td></td>
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<tr>
<td>m</td>
<td>particle mass</td>
<td></td>
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<td>MDIs</td>
<td>metered dose inhalers</td>
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<td>MLI</td>
<td>multistage liquid impinger</td>
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<td>MMD</td>
<td>mass median diameter</td>
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<tr>
<td>$N_a$</td>
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<tr>
<td>$N_d$</td>
<td>concentrations of electron donor centres</td>
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<tr>
<td>q</td>
<td>electrical charge</td>
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<tr>
<td>r</td>
<td>separation distance</td>
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<td>$r_c$</td>
<td>radius of contact area</td>
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<td>RD</td>
<td>recovered dose</td>
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<td>RDC</td>
<td>relative degree of crystallinity</td>
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<td>$R_e$</td>
<td>Reynolds number</td>
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<tr>
<td>RH</td>
<td>relative humidity</td>
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<tr>
<td>$r_{\text{max}}$</td>
<td>Maximum Martin’s radius</td>
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<td>$r_{\text{min}}$</td>
<td>Minimum Martin’s radius</td>
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<tr>
<td>S</td>
<td>surface area</td>
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<tr>
<td>SCF</td>
<td>supercritical fluid</td>
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<tr>
<td>$S_{\text{cir}}$</td>
<td>shape factor</td>
<td></td>
<td></td>
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<tr>
<td>SD</td>
<td>standard deviation</td>
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<td></td>
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<tr>
<td>SE</td>
<td>scanning electron</td>
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<tr>
<td>SEM</td>
<td>scanning electron microscopy</td>
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<tr>
<td>$S_{\text{rec}}$</td>
<td>'surface factor'</td>
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<tr>
<td>SSA</td>
<td>specific surface area</td>
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<td>Stk</td>
<td>Stokes' number</td>
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<tr>
<td>T</td>
<td>absolute temperature</td>
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<tr>
<td>$T_c$</td>
<td>crystallisation temperature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$t_c$</td>
<td>time period of crystallisation</td>
<td></td>
<td></td>
</tr>
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<td>TGA</td>
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<td></td>
<td></td>
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<tr>
<td>V</td>
<td>air velocity</td>
<td></td>
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<tr>
<td>VC</td>
<td>vital capacity</td>
<td></td>
<td></td>
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<tr>
<td>$W_c$</td>
<td>weight of a carrier particle</td>
<td></td>
<td></td>
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<tr>
<td>$W_f$</td>
<td>weight of a fine particle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XRPD</td>
<td>X-ray powder diffraction</td>
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CHAPTER ONE

INTRODUCTION
1.1 GENERAL INTRODUCTION

Drug delivery to the airways has received considerable attention over many years since it can be used for the delivery of drugs for both localised and systemic effects. For many drugs used in the treatment of disease such as bronchodilators, antiasthmatic drugs, antibiotic and antiviral agents, this route of administration provides a means of delivering drugs to their sites of action and hence, maximised pharmacological effects can be obtained with minimised associated side effects. The direct delivery of drugs such as these to their target sites also produces a rapid and predictable onset of drug action. Furthermore, the human lung possesses a relatively large surface area in the order of 126 m$^2$ for drug absorption (Wall, 1995), lower metabolic activities when compared with the liver or gastrointestinal tract, desirable permeability of alveolar epithelium to many molecules as well as a rich blood supply. The lung may thus provide an ideal portal for drug delivery to the systemic circulation and as such, has attracted special interest for the possible systemic delivery of peptides, polypeptides and other macromolecules (Byron and Patton, 1994).

Although metered dose inhalers (MDIs) have represented the major means of drug delivery to the lung for over two decades, the use of these devices might become more restricted in the future due to the ozone depletion by the currently employed chlorofluorocarbon (CFC) propellants in MDIs. Nebulisers, which do not use such propellants, have been limited to hospital or domiciliary environments even though some portable, pocket nebulisers are currently being developed. Therefore, over recent years dry powder inhalers (DPIs) have become more and more attractive since they possess many advantages over MDIs and nebulisers. Without the use of any propellants, the dispersion and entrainment of drug particles in DPIs are caused by the inhalation efforts of the patient alone. Thus, DPIs are environmentally friendly and very easy to use and handle, overcoming the problems associated with the synchronisation of actuation and inhalation during the operation of MDIs. DPIs are highly portable and relatively inexpensive in comparison with nebulisers. The drugs are kept in the solid state in DPIs and thus, would be expected to have the high physico-chemical stability. This is of particular relevance when considering the formulation of peptides for pulmonary delivery.
In most DPIs, drug particles are micronised and adhered to inert carrier particles. A number of different carriers have been used although lactose has been employed most frequently, only because it has a history as a widely used and safe excipient in solid dosage forms (Timsina et al, 1994). The carrier particles are designed to be of such a size that after inhalation, most of them remain in the inhaler or deposit in the mouth and upper airways. In order to reach the lower airways, drug particles must therefore dissociate from the carrier particles and become redispersed in the air flow. The redispersed drug particles may then undergo inertial impaction in the mouth, on the back of the throat and the upper airways. Some particles (2-5 μm) are likely to reach and deposit in the lower airways through gravitational sedimentation, interception and Brownian diffusion. Therefore, three major processes are involved in the delivery of drugs from DPIs, namely, the detachment of drug particles from the carrier, dispersion of drug particles in the air flow and deposition in the respiratory tract. Any factor that affects any of these processes could ultimately influence the deposition properties of the inhaled particles. These include the design of inhaler devices, airway physiology, inhalation manoeuvre and powder formulation (Table 1.1). For example, the drug-carrier particle interaction (adhesion) will greatly influence the detachment of drug particles from their carrier whilst the drug-drug interaction (cohesion) will affect the deaggregation of drug particles into single particles suitable for deep lung penetration. In addition, the design of the inhaler device, inhalation flow rate and turbulence of the inhaled air stream also determine the efficiency of particle-particle separation. Furthermore, the particle size, shape, density and hygroscopicity have been shown to affect the aerodynamic properties of the particles and these factors will eventually influence their dispersion and deposition of the drug in the respiratory tract. The physiology of the airways and breathing patterns including tidal volume, breathing frequency and breathing hold, etc., are also factors in determining the deposition profiles of the inhaled particles.
Table 1.1 Some of the major factors affecting drug delivery from DPIs

<table>
<thead>
<tr>
<th>Design of Inhaler Devices</th>
<th>Rotahaler®, Spinhaler®, Inhalator®, Turbuhaler®, Diskhaler®, Diskus®, etc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powder Formulation</td>
<td>Drug-drug interaction, Drug-carrier interaction, Particle size and shape, Surface texture, Crystallinity, Hygroscopicity, etc.</td>
</tr>
<tr>
<td>Inhalation Manoeuvre</td>
<td>Flow rate, Tidal volume, Breath-holding, Breathing frequency, Air acceleration, etc.</td>
</tr>
<tr>
<td>Physiology of Respiratory Tract</td>
<td>Geometry, Lung function, Pulmonary ventilation, Mucus secretion, etc.</td>
</tr>
</tbody>
</table>

Numerous reports and review articles have been published describing the influence of these factors on the overall delivery efficiency (often determined by the fraction of total dose that can reach the lower airways) of DPIs (Hickey, 1992). However, most currently used DPIs fail to possess a high delivery efficiency (Timsina et al., 1994) since only approximately 10% of the total administered dose reaches the lower airways. About 10% total dose is retained in the inhaler devices and over 80% is found to deposit in the upper airways and most of this is eventually swallowed (Gupta and Hickey, 1991). Thus, the optimisation of drug delivery from DPIs to the lower airways so as to increase therapeutic effect and decrease any possible side effects has been a major concern in the development of DPIs. However, the bulk of the previous work has focused on the inhaler device design and surprisingly, little has been published on approaches to improve the delivery efficiency of drugs utilising dry powder formulations. The formulation factors of dry powders are equally, if not more, important than the design of inhaler devices in the optimisation of drug delivery to the lung. For example, drug particles are usually present in low concentrations in the powder formulation, with a drug to carrier ratio of 1 : 67.5 being typical. A large portion of drug particles may therefore be expected to be adhered to the active binding sites of the carrier particles or entrapped in any surface crevices existing on the carrier surfaces. The resultant strong interaction of drug with carrier particles impedes drug detachment from carrier particles and dispersion in the inhalation air-stream and consequently, reduces the overall deposition of drug particles in the respiratory tract. Insufficient detachment of drug from the carrier due to strong interparticulate forces is the major cause of low delivery efficiency encountered for most DPIs. Therefore, increasing drug detachment and dispersion...
by means of reducing interparticulate forces in powder formulations is one of the major strategies to improve drug delivery to the lower airways. Since particle-particle interaction is primarily a surface phenomenon, such an interaction is mainly dependent upon the surface texture, particle size and shape of the interactive particles. For example, increasing surface smoothness of lactose carrier particles was shown to increase the deposition of salbutamol sulphate from dry powder inhalers, the fraction of fine drug particles < 5 µm being increased from 4.1% to 23%, after inhalation at 60 l min⁻¹ via a Rotahaler®, by smoothing the surface of lactose particles (Ganderton and Kassem, 1992). This was attributed to the reduced interaction of the drug with the carrier particles due to the increased surface smoothness of the carrier. However, adhesion of fine particles to coarse particles is also determined by other factors such as particle size, shape of both components, their relative concentrations, existence of amorphous regions and active binding sites on the particle surface, addition of ternary components, etc. The control of any individual factor such as surface smoothness would appear to be insufficient with a view to minimising drug-carrier interaction and maximising detachment of drug particles from carrier particles on inhalation. The combination of the factors mentioned previously would provide an ideal strategy to optimise drug delivery from DPIs.
1.2 Interaction between drug and carrier particles

Powders that are composed of a small amount (usually < 10% w/w) of fine particles adhered to large particles are defined as ordered mixtures (Hersey, 1979). The interaction of a smaller particle with a larger particle is often termed adhesion and hence, the interaction forces are called adhesion forces. Higher forces of adhesion between fine particles of drug and coarser carrier particles are usually desirable to produce ordered mixtures in pharmaceutical preparations. This ensures that during manufacturing processes, such as those in tabletting for example, the stability of the ordered mixes is improved towards any vibration during handling and storage. However, in the case of dry powders for inhalation, the situation is different. Due to the intrinsic cohesive forces between the fine particles which are required for deep lung penetration, then drug particles for inhalation are often mixed with coarser carrier particles, such as lactose, in order to improve the flowability. The carrier particles are designed such that after inhalation, they will be retained in the inhaler device or deposit in the upper airways, mostly in the mouth and on the back of throat. In order for the drug particles to reach the lower airways, they must therefore dissociate from the carriers and become dispersed in the inhaled air stream (Fig. 1.1).

The extent of the dissociation of drug from carrier particles is largely dependent upon the interactions between the two components in question. Stronger adhesion results in lower amounts of drug detaching from carrier particles at the same drag forces of a fluid and it is the major cause of low delivery efficiency of drugs from most DPIs. Adhesion forces are mainly composed of van der Waals', electrostatic and capillary forces (Zimon, 1982). Since adhesion involves the interaction between drug particles (2-5 μm) and coarse carrier particles (63-90 μm) in a typical aerosol dry powder, mechanical interlocking of fine particles within the surface asperities which exist on coarse particles may also play an important role in determining the overall adhesion forces.
(A) Fine drug particles and coarse carrier particle before mixing

(B) The adhesion of fine drug particles to coarse carrier particle after mixing

(C) Dissociation of fine drug particles from carrier particle after inhalation

Figure 1.1 Schematic representation of adhesion and dissociation of fine particles.

1.2.1 Van der Waals forces

1.2.1.1 Theoretical consideration

If a particle with a diameter $d_1$ is separated by a distance $r$ \emph{in vacuo} from a plane surface, the adhesion forces ($F$) of a sphere to a plane composed of the same molecules due to Van der Waals' forces can be given as

$$ F = \frac{A_{11}}{6r^2} d_1 \quad (1-1) $$

The adhesion of a sphere (1) to a plane surface (2) composed of different molecules can be calculated as:
where $A_{11}$ and $A_{22}$ are Hamaker constants for the particle and plane surface, respectively; $d_1$ is the diameter of the particle and $r$ is the separation distance between the particle and the plane surface. In aerosol powders, the surface of carrier particles can be considered an adhesive plane to drug particles since the former particles are usually more than 10 times larger than the latter particles.

### 1.2.1.2 Factors affecting adhesion due to van der Waals' forces

#### 1.2.1.2a Particle size and shape

The adhesion forces due to van der Waals' forces are a function of both the adhered particle $(d_1, A_{11})$ and the interaction between the particle and the surface $(\sqrt{A_{11} A_{22}})$ as well as the distance of separation $(r)$. Van der Waals' forces increase with particle diameter but such forces would become less significant in comparison with gravitational forces in the case of large particles. For example, gravitational forces may be dominant for particles having diameters of millimetre order whilst Van der Waals' forces may be dominant for particles of the order of micrometre. Equations (1-1) & (1-2) were generated for smooth, perfect spheres. However, the adhesion of irregularly shaped particles is far more complicated, depending not only on the macroscopic shape of the particles, such as whether they are cylindrical, plate-like, spherical, etc, but also on the microscopic shape factors, such as surface-smoothness and existence of sharp angles, as well as on the procedures used to adhere these particles to the substrates.

Thus, reports about the influence of particle shape on adhesion are often contradictory. Theoretically, irregular particles should have higher adhesion forces than more spherical particles of a similar mass under the same conditions, if the microscopic factors are negligible and the particles seek the most stable orientation on the substrate surface. This is because of a higher contact area with, and a shorter separation distance from the substrate surface for the irregular particles. For example, the adhesion of glass particles to a glass substrate, measured with an ultrasonic vibration technique in a dry, static free environment,
was found to be influenced by the shape of the particle, decreasing in the order of plates > cylinders > spheres for particles of a given mass (Mullins et al., 1992). Similarly, calcium carbonate particles, of more macroscopically elongated shape, produced more stable ordered mixes toward mechanical vibration, corresponding to the pressure of higher adhesion forces, after mixing with lactose particles (500-710 μm) at a concentration of 0.5% w/w than when spherical particles of calcium carbonate were used (Wong and Pilpel 1988). Thus, irregularity of particle shape appears to improve the adhesion of the particle. However, the reverse trend has also been reported. For example, the average adhesion force, $f_{50}$, for calcium carbonate P-70, sulfadimethoxine and silica sand particles on a glass substrate, measured by an impact separation method, increased linearly with the shape index, $\Psi$, which is an indicator of sphericity of the particles (Otsuka et al., 1988). For smooth-surfaced particles, there was a linear relationship between $\log f_{50}$ and $\Psi$ for particles within the size range of $25 \, \mu m < d < 60 \, \mu m$, and $0.255 < \Psi < 0.734$, regardless of the material. This was attributed to the more angular shape of particles having lower $\Psi$ values. The angular particles may contact the substrate with the tip or short edges of the particles and thus, the effective areas of contact may be lower as compared with those of spherical particles. This hypothesis was further confirmed by the fact that after the treatment of calcium carbonate particles with hydrochloric acid to obtain a smoother particle surface, the $f_{50}$ values were found to increase about ten-fold, as increased surface smoothness may have resulted in an increase in contact area and a decrease in separation distance between particles and substrate. Interestingly, the addition of a small amount of fine particles (0.3-2.0% w/w) to the smooth-surfaced particles before mixing with the substrate was shown to reduce the adhesion forces, producing adhesion forces similar to those of the particles not treated with hydrochloric acid. The effect of fine particles was thought to have the similar mechanism to that of the surface asperities, reducing the area of contact between the larger particles and substrate.

1.2.1.2b Blending process

The dependence of adhesion forces of irregular particles on the processing procedures, and more specifically mixing, is obvious, since most of pharmaceutical drug particles are irregular in shape. Under extensive blending, particles may seek the orientation of smallest potential energy, which involves the shortest separation and largest contact area, especially
Chapter one: Introduction

for elongated and plate-like particles. Thus, a higher adhesive force can be expected under these conditions. Kulvanich and Stewart (1988) showed that the adhesive tendency of drug particles in a model drug-carrier system increased with blending time. These effects may be partly due to the fact that under extensive blending, drug particles would progressively move to positions of greatest stability (Ganderton and Kassem, 1992), although an increased triboelectrification of the particles may also partly contribute to the increased adhesion.

1.2.1.2c Carrier surface energy

Solids with a high surface energy have a high tendency to adsorb other materials on their surfaces and form stronger bonds with adhered particles (Sutton, 1976). A material shows its highest surface energy when its surface is completely clean. Thus, any contamination of particle surfaces by gas, liquid or solid particles would reduce the adhesion forces between adhered particles as long as liquid and/or solid bridging is not involved. This may have significant implications for the theory of ordered mixes. Hersey (1975) suggested that some areas of carrier surfaces are devoid of binding sites whilst other areas possess strong binding sites. On clean surfaces, these strong binding sites are available for adhesion and would induce strong adhesion forces to adhered particles. However, on contaminated surfaces, these strong binding sites may be more likely to be saturated by the contaminants and thus, more particles may adhere to less strong binding sites and lower adhesion forces between the particles and carrier surface may be expected.

All the currently used carriers for DPIs are carbohydrate crystals, such as lactose and glucose. Under normal conditions, these crystals will take certain crystal shapes as a result of the different rates of growth on different crystal faces. Lactose crystals, for example, usually take on a tomahawk shape after slow crystallisation from aqueous solution (Nickerson, 1974). The crystals grow with the highest rates in a longitudinal direction and therefore, the tips of the tomahawk will have the highest surface energy when compared with the other faces on the same crystal. Furthermore, some crystal defects due to the misarrangement of molecules in the crystal lattice, may be formed during crystallisation. These defects may also possess higher energy than other sites. Thus, the surface energy of the crystals will not be evenly distributed. Therefore, clean carrier crystals would be expected to produce stronger adhesion forces of drug particles than if the carrier was
contaminated by other materials such as solid fine particles. For example, it was shown that coarse lactose (63-90 μm) particles could be stripped of adhered fine particles (< 5 μm) using a compressed air stream to obtain particles of cleaner surface (Tee, 1996). When used as the carrier for salbutamol sulphate for inhalation, these lactose particles produced significantly lower (ANOVA, p < 0.05) respirable fraction (6.8%) of the drug than that (11.8%) of the same batch of lactose before removal of fine particles. Since the respirable fraction of a drug is inversely related to the adhesion force between drug and carrier particles, the decreased respirable fraction might be attributed to an improved adhesion force between the drug and lactose carrier particles after removal of the fine particles from carrier surfaces.

1.2.1.2d Drug to carrier ratio

Since the number of active binding sites on a carrier surface is limited, increasing the number of adhered fine particles would increase the likelihood of drug particles adhering to weaker binding sites and consequently, the overall adhesion forces would be reduced. For example, the adhesion profiles of salicylic particles of mean radius 2.5 μm to different carriers including recrystallised lactose, were largely dependent upon the quantity of drug powder (Staniforth et al., 1981). Increasing the amount of drug powder generally decreased the median adhesion forces. The median adhesion forces between drug and recrystallised lactose decreased from 7.8x10⁻³ N for 2 % w/w drug powder to 2x10⁻³ N for 5 % drug powder. Similar results were reported in the case of ordered mixes composed of potassium chloride powder (median diameter 5 μm) and carriers including Emdex® (a spray-crystallised maltose-dextrose), Dipac® (a direct compacting sugar) and recrystallised lactose (Staniforth and Rees, 1983). The stability of all the ordered mixes towards vibration decreased with an increase in the concentration of potassium chloride and this was attributed to a reduction in the adhesion forces with a increase in the concentrations of the adhered particles.

A similar relationship has also been reported previously for aerosol dry powders (Kassem, 1990). The respirable fraction of salbutamol sulphate aerosolised from blends of the drug and lactose via a Rotahaler®, measured with a cascade impactor, was found to increase significantly as the amounts of drug increased and the effect was more pronounced at higher
flow rates (Kassem 1990). Electron micrographs showed that multilayers of drug particles were formed despite the availability of free space on the carrier surface. These results were indicative of active binding sites on lactose crystals. The increased respirable fractions at higher drug concentrations was attributed to the relatively low percentages of drug powders adhered to these sites (Ganderton and Kassem, 1992).

1.2.1.2e Fine particles
The use of an increased amount of drug particles to decrease the adhesion forces and hence increase the respirable fraction is not always practical since the dose of drug administered is primarily dependent upon its therapeutic index. An alternative means of increasing respirable fraction may be through the use of a ternary component such as magnesium stearate to saturate the active binding sites of the carrier particles before blending with drug powders. For example, the addition of a small amount of magnesium stearate to the ordered mixes of sucrose and salicylic acid was found to reduce the homogeneity of the mixes and this was attributed to the action of magnesium stearate stripping the drug particles from the ordered units (Lai and Hersey, 1979). Magnesium stearate (Kassem, 1990) and l-leucine (Staniforth, 1996) was also shown to increase the fine particle fraction of salbutamol sulphate from lactose carrier due to the similar mechanisms.

1.2.1.2f Deformation of particle or surface
Any deformation of either the particle or surface will greatly increase the contact area which will concomitantly increase the adhesion forces. After deformation, the total adhesion force will consist of two additive components, namely, the forces between the adherents before deformation at the instant of first contact and the forces acting on the contact area due to the deformation. According to Zimon (1982), the total adhesion forces \( F \) can be calculated as:

\[
F = \frac{\sqrt{A_{11}A_{22}}}{6r^2} d_1 (1 + \frac{r_c}{3rd_1})
\]  

(1-3)

where \( r_c \) is the radius of the contact area between the particle and surface.
In elastic contact, when a sphere with a radius $d_1$ is pressed against an ideally smooth surface with a force $F_p$, the radius of contact area ($r_c$) is calculated by Hertz formula:

$$r_c = \sqrt{0.75d_1 F_p \left( \frac{1-\mu_1^2}{E_1} + \frac{1-\mu_2^2}{E_2} \right)}$$

where $\mu_1, \mu_2$ are Poisson ratio for the particle and surface, respectively. $E_1, E_2$ are the moduli of elasticity for the two materials.

It can be seen from equation (1-3) that the larger the contact area, the higher the adhesion forces. The particle deformation may be decisive in determining the adhesion forces. Easily deformable particles will have higher adhesion forces than harder particles under similar conditions. This can be seen in the case of diamond and wax. The extremely hard diamond particles are not adhesive. On the other hand, wax is readily adhesive, due to its high deformability on contact. Many pharmaceutical compounds are deformable either plastically or elastically. Whether a particle will undergo elastic (reversible after withdrawal of the external forces) or plastic (irreversible) deformation is largely dependent upon material properties of the particle and the extent and duration of external forces (Rimai and Busnaina, 1995). For most particles, the contact area could be divided into two coaxial regions of stress. The inner zone, being subjected to greater stresses, would undergo plastic deformation whereas the outer zone, experiencing the lower stress, would deform elastically (Krishnan et al., 1994).

Adhesion of easily deformable materials can be expected to be more sensitive than harder materials to powder preparation and handling procedures such as the applied pressure, mixing and storage conditions. For example, the median adhesion forces of particles of different materials to a stainless steel substrate had different sensitivities to applied pressure although adhesion forces of all the particles increased linearly as a function of compression force (Figure 1.2).

In Fig. 1.2, the slope of each line expresses the change of the adhesion force per unit increase in compression force, the order being PEG 4000 > starch 1500 > spray-dried
lactose > heavy calcium carbonate. This difference in the sensitivity to the change of compression forces was attributed by the authors (Lam and Newton, 1991) to the different hardness of these materials. PEG 4000 was the softest and thus, produced the highest adhesion force, which was the most sensitive to the change in external pressure. Heavy calcium carbonate on the other hand was the hardest and hence, it showed the smallest adhesion force, which was the least sensitive to applied pressure.

![Graph showing the relationship between geometric median adhesion to a stainless steel substrate and compression forces.](image)

Figure 1.2 Relationship between the geometric median adhesion to a stainless steel substrate measured by a centrifugation method and the compression forces (adapted from Lam and Newton, 1991)

The effect of applied pressures on the adhesion of micronised particles suitable for inhalation has also been investigated using salmeterol xinafoate and lactose as model samples (Podczek et al., 1995a). Although the adhesion of either micronised lactose to compact salmeterol xinafoate or micronised salmeterol xinafoate to a compacted lactose disc increased with applied pressures, lactose particles were found to adhere more strongly to salmeterol xinafoate surfaces than were salmeterol xinafoate particles to the lactose surface. Theoretically, the adhesion forces should have remained the same whichever micronised particle was adhered to the opposing surface. This deviation from theory was
attributed by the authors to the rougher surface of the compacted salmeterol xinafoate disc in comparison to the lactose disc (Podczek et al., 1995a). However, the use of compacted discs may not reflect the true situation existant within ordered mixes and the compacting process may also change the surface properties with regard to other parameters such as free energy of the substrate surface. Thus, the adhesion of salmeterol xinafoate particles to lactose particles (200-250 μm) was compared with that of the same drug particle to a compacted lactose disc (Podczeck et al., 1995b). For smaller press-on forces (i.e. ≤0.22 ×10^8 N), adhesion of salmeterol xinafoate to lactose particles was shown to be similar to that of the drug to lactose particles, but at higher press-on forces the adhesion force to compacted lactose surfaces was shown to be nearly twice as high as the adhesion to lactose particles.

The hardness of a material is largely dependent upon its crystalline state and crystals are known to be harder than amorphous forms at similar temperatures. Thus, a crystalline drug can be expected to have lower adhesion to the same surface than its amorphous forms. For example, spray dried salbutamol sulphate was found to be softer and more sticky than the micronised drug which was crystalline (Chawla et al., 1994). After mixing with lactose carrier particles, the spray dried drug particles would be expected to adhere more strongly to the carrier than micronised salbutamol sulphate. This is confirmed by the lower fine particle fraction of spray-dried salbutamol sulphate than micronised salbutamol sulphate obtained from drug-carrier blends, even though the spray dried drug particles had a lower mass median diameter than the micronised particles (Venthoye et al., 1995).

1.2.1.2g Contact time

Deformation of particles on contact is affected not only by the applied pressure and particle hardness but also by the duration of contact between the adherents. Deformation manifests itself particularly for polymer-like materials as the time of adhesion is increased. The longer the time that pressure is applied to deformable particles, the stronger the adhesion will be. In the absence of applied force, the increase in the adhesive interaction between particles and a substrate surface with increasing time of contact is commonly termed as ‘ageing’. ‘Ageing’ may be an important factor in the generation of adhesion forces and eventually, this phenomenon may affect the removal efficiency of particles from a surface. For example, Krishnan et al. (1994) deposited submicrometre (0.5 μm) diameter polystyrene latex
particles on a silicone wafer and measured the contact radius and particle removal as a function of time (Figure 1.3).

Figure 1.3 Contact area and removal efficiency of submicrometer polystyrene spheres from a silicone substrate as a function of time (adapted from Krishnan et al., 1994).

It can be seen that the contact area between polystyrene particles and the silicone substrate increased with time. The removal efficiency showed an opposite dependence as contact was extended. This decreased removal efficiency was attributed to an increased adhesion at higher contact area due to the prolonged period of contact.

The influence of duration of applied pressure on particle adhesion was also investigated in a study where particles of different hardness were initially pressed onto a steel surface by centrifugation and then, adhesion forces determined by re-centrifugation (Lam and Newton, 1993). The results indicated that all the particles including those of PEG 4000, starch 1500, spray-dried lactose and heavy calcium carbonate showed an increase in the adhesion force to the substrate as the duration of compression was increased.

The effect of applied pressure and ‘ageing’ on adhesion force may also bear significant importance in the formulation of drug powders for inhalation. For example, during powder mixing processes, increasing the mixing time and/or force (e.g. by increasing the speed of
rotation of a mixer) will blend drug and carrier particles more vigorously. An increased applied pressure results which in turn would be expected to lead to an increased adhesion of drug particles to the carrier. Current work being carried out at King’s College London (KCL) has shown that extending mixing time and/or increasing mixing speed generally results in a reduction in the fine particle mass of salbutamol sulphate particles from lactose carriers (unpublished data). This phenomenon may be partly due to the increased force of adhesion generated between drug particles and the carrier under the more vigorous mixing, although other factors such as possible increases in triboelectrification may also partly contribute to the increased force. Although there are no reported studies examining the effect of ‘ageing’ of ordered mixes, on drug respirable fraction, some unpublished data, obtained at KCL indicates that after storage in a desiccator for prolonged period of time (approximately 4 months), the fine particle mass of salbutamol base from different carriers such as lactose decreased significantly as compared to that of the freshly prepared powders. This reduction in the respirable mass may have resulted from the increased adhesion force between drug and carrier particles after the prolonged period of contact. Consequently, the drug particles would have been less likely to detach from the carrier under similar inhalation conditions, resulting in a reduction in the number of respirable particles.

1.2.1. 2h Surface smoothness of the substrate

As van der Waals’ forces increase with contact area and decrease rapidly with separation distance, any means of increasing contact area (as discussed above) and/or decreasing the distance of separation between the adherents will theoretically increase the overall adhesion force. Apart from hardness, external pressure and contact duration, the particle shape of the adhered fine particles and the surface smoothness of the substrate surface can largely affect both contact area and separation distance. Providing the adhered particles have diameters larger than any existent gap on a surface, that is to say the adhered particles will not be entrapped into any of the surface asperities, then increasing surface roughness of the substrate will generally reduce contact area and increase the separation distance between the adhered particles and substrate surface. Increasing surface roughness should therefore decrease the adhesion forces. For example, the adhesion of glass spheres to a glass plate in atmospheres of 100% relative humidity decreased from 100% for highly polished glass plate to 0% for highly rough surfaces (Zimon 1982). However, the use of increased surface
roughness to reduce the overall adhesion forces may prove to be extremely difficult for the ordered mixes used for inhalation since fine powders (< 6 \mu m in diameter) are often adhered to larger particles (63-90 \mu m). The dimension of the asperities or 'hills and valleys' on the substrate surfaces has to be much smaller than the diameter of the adhered particles. Otherwise, adhesion due to mechanical interlocking, which will be discussed later, will be greatly increased.

1.2.2 Electrostatic forces

1.2.2.1 Theoretical background

If a particle carries a charge of q and induces an equal image of opposite charge on a neighbouring plane surface which is separated from the particle by a distance, d, the attractive forces due to electrostatic interaction can be calculated as

\[ F_e = \frac{q^2}{16\pi\varepsilon_0 d^2} \]

and \[ q = e(N_d - N_a)S \]

where e is the charge of an electron; \( N_d \) and \( N_a \) are the surface concentrations of donor centres and acceptor centres, respectively; \( \varepsilon_0 \) is the permittivity of the environment and S is the contact area between the particle and surface. Hence, the electrostatic forces may be increased or reduced by changing the electrical properties of either the particle or surface, and these are largely dependent upon the chemical structures and physical state of the materials concerned. Some of the possible molecular groups have been ranked as a donor-acceptor series (Zimon, 1982):

Donor -\( \text{NH}_4 \)-\( \text{OH} \)-\( \text{OR} \)-\( \text{COOR} \)-\( \text{CH}_3 \)-\( \text{C}_6\text{H}_5 \)-\( \text{halogen} \)=\( \text{CO} \)-\( \text{CN} \) Acceptor

Each successive member is an acceptor of electron with respect to the preceding member, which acts a donor. Therefore, a material will possess different electrostatic charges when in contact with different materials.
1.2.2.2 Factors affecting electrostatic forces of adhesion

1.2.2.2a Fine particle preparation
Generally, every process during powder handling may induce triboelectric charges on drug particles. The major processes employed in the production of dry powders for inhalation involve the preparation of fine particles (micronisation or spray drying), mixing as well as aerosolisation in a turbulent air stream. During mechanical micronisation, particle size is usually reduced by means of collisions between coarse particles so that the larger particles are broken up and fine particles are produced. The violent collisions between drug particles and between drug particles and the inner walls of the microniser chamber will undoubtedly induce high electrical charges on micronised particles. Spray-dried particles may also be highly electrically charged since solutions are aerosolised into small droplets which are then dried in a hot air cyclone. Although the electrical charges of the fine particles after preparation may play an important role in the subsequent handling, studies of the influence of this charging process on the behaviour of the final powders are still awaited.

1.2.2.2b Electrical properties
Electrical charge will also arise from the collision and friction among the particles during powder mixing and other handling processes. As mentioned above, the charge sign of each individual group of particles will depend largely upon their relative electron-donor or acceptor properties and work functions. For particles prepared from the same material, electron transfer from large particles to small particles is more favourable than the reverse, since large particles have lower work functions than small particles (Bailey, 1984). Electrical charges not only arise from collision among particles but also result from interaction between particles and containers. Most pharmaceutical excipients, including lactose, become electronegative following contact with a glass surface, which usually acts as an electron-donor (Staniforth and Rees, 1982). In contrast, such excipients become electropositive after contact with a polyethylene surface. Salbutamol sulphate and lactose particles, however, both become positively charged after contact with plastic containers, whereas following the contact with a metal surface, lactose particles become charged electronegatively but salbutamol sulphate positively. Powders with like-charges formed less
stable ordered mixes than those in which drug and excipient particles carried opposite charges (Staniforth and Rees, 1982). Therefore, it is possible, in principle, to obtain desirable electrostatic forces in ordered mixes by choosing processing containers made from materials of suitable electron-donor or -acceptor properties.

1.2.2.2c Turbulent air flow

Particle-particle collisions and particle-wall interactions will be greatly increased with a consequent increase in electrostatic charge, if turbulent air flow conditions are imposed on the powder mix as in the case of aerolisation or fluidisation of particles in an airstream. Some pharmaceutical powders were found to have charges at least 100 times greater after triboelectrification in an air cyclone (Staniforth and Rees, 1982). Salbutamol sulphate and beclomethasone dipropionate particles carried charges of opposite signs after fluidisation in steel and brass containers (Carter et al., 1992) whereas micronised lactose particles became negatively charged in both containers (Figure 1.4)

Figure 1.4 The charge to mass ratio of salbutamol sulphate, micronised beclomethasone dipropionate and lactose after fluidisation in brass or steel containers using a feeder pressure of 8.68 psi (adapted from Carter et al., 1992).
1.2.2.3 Significance of electrostatic forces in dry powder formulation

The contribution of electrostatic forces to the adhesion forces is usually thought to be less important than that of van der Waals' forces. However, electrostatic forces will become more significant and even dominate over other forces when the particle size is diminished. For example, the adhesion forces of some model drug particles, namely, sulphapyridine (27.2 μm), sulphamerazine (17.7 μm) and succinylsulphathiazole (23.4 μm) to hydroxypropylmethylcellulose coated glass beads were found to increase linearly (coefficient > 0.90) with the square of the average charge to mass ratio (Kulvanich and Stewart, 1987). All interactive systems showed a decrease in the extent of interaction during storage over 23 d due to charge decay in the systems.

In aerosol dry powders, the practical significance of electrostatic interactions is considered to be critical in every aspect of powder formulation and more importantly, the timely disruption of drug-carrier agglomerates, allowing for the reproducible delivery of respirable particles (Bailey, 1996). In order to maintain a stable ordered mix, then a suitable magnitude of opposite charges of drug and carrier particles is desirable (Staniforth, 1994). However, excessive opposite charges may result in strong adhesion forces, leading to poor deaggregation of drug powders on aerosolisation especially by children and patients with severe bronchoconstriction. Excessive forces may be overcome by the addition of a ternary material. For example, magnesium stearate was found to cause 'stripping' of drug particles from carrier particles in ordered mixes of salicylic acid and sucrose (Lai and Hersey, 1979). This phenomenon may be partly attributable to the saturation of active binding sites on sucrose by magnesium stearate and partly due to the change in the electrostatic properties of the constituent powders. Both salicylic acid and sucrose were found to develop electronegative charges when in contact with glass whereas magnesium stearate was strongly electropositive (Lai and Hersey, 1979). Thus, magnesium stearate would be strongly attracted to the electronegative sucrose, competing for the active binding sites on the carrier particles and consequently, salicylic acid would be expected to become dislodged or relocated at weaker adherence sites on the sucrose surface.
The charge accumulated on powder surface will be dissipated over a certain period of time (Malave-Lopez and Peleg, 1985). The rate of charge decay is mainly determined by the conductivity of the materials, contact with other conductive material and the relative humidity of ambient air surrounding the particles. A material that has lower electron conductivity will need a longer period of time to dissipate its charge. Most pharmaceutical powders are insulators and hence, charge decays usually at slow rates. If powders are in contact with highly conductive materials, such as metals, charge will decay more quickly than when the same powders are in contact with insulators such as polymers. Relative humidity is a very important factor in determining the rate of charge decay. At high humidities (> 65%), water vapour may condense on particle surfaces producing a conductive layer and thus, the triboelectrification will greatly be reduced or even eliminated. Any charge formed will dissipate so quickly at high humidities that its influence on the overall adhesion forces may become negligible.

The electrical charges on a lactose surface were reported to be dissipated within a few minutes (Staniforth and Rees, 1982). Thus, the triboelectrification which occurs during a preparation process may appear to be a minor factor affecting the adhesion of drug particles to carrier particles when the DPI is used by patients since the device will be used over several months in practice. However, the strength of the van der Waals’ forces between macroscopic bodies depends critically on the contact area and separation distance between them. The initial electrostatic forces may be one of the major forces to bring the separate particles closer to a surface, producing an increased dispersion force which would connect the particle much more tightly to the surface even though the electrical charges may have long been dissipated.

The electrification of particles on aerosolisation is also an important concern. As stated previously electrical charges resulting from turbulent air flow were shown to be more than 100 times stronger than their contact charges (Staniforth and Rees, 1982). The highly charged particles may coagulate with each other in the air stream or impact on the surface of the inhalers due to electrical precipitation. This may be partly attributable to the loss of drug in the inhalers (> 10% total dose). If bipolar charging (particles being induced to carry opposite charge) occurs during aerosolisation, the charged, deaggregated drug particles may
coagulate with each other to form large agglomerates in the air stream. Individual charged particles may induce opposite image charges in the upper airways and accelerate the deposition of drug particles in this region. Powder characteristics, design and composition of inhaler devices may influence the triboelectrification of drug particles during aerosolisation. For example, the relative electron-donor or electron acceptor properties of drug and carrier and the composition of the inhalers may be decisive in the signs of charges induced on the drug and carrier particles. It would be advantageous for drug and carrier particles to carry the same charge sign on aerosolisation so that more complete detachment of the drug particles may be obtained.

Excessive charging should be avoided in order to minimise electrical precipitation of drug particles in the upper airways. Turbulence is normally induced by increasing resistance to air flow by the design of inhaler device (Timsina et al., 1994). This may lead to an increased triboelectrification of the deaggregated particles, since the probability of the particles to hit or collide with a surface is increased. The triboelectrification of insulators is a very complicated process. More work needs to be done with regard to the charging properties of drug and carrier particles and the coagulation and deposition profiles of charged particles should also be the subject of further investigation.

1.2.3 Capillary forces

If water vapour condenses on the interface between a particle and a surface (Figure 6), the liquid bridge or meniscus thereby formed, will add to the overall adhesion force by means of surface tension.

\[ F_C = 2 \pi \gamma r (\cos a + \cos b) \]  

(1-7)

where \( r \) is the radius of the particle, \( \gamma \) is the surface tension of the liquid and \( a \) and \( b \) are the contact angles of the liquid with the surface and particle, respectively (Figure 1.5).
According to equation 1-7, capillary forces have their greatest effect on adhesion in the case of hydrophilic particles and surfaces, when $a$ and $b \to 0^\circ$, and have their least effect on the adhesion between hydrophobic particles and surfaces, when $a$ and $b \to 90^\circ$. Therefore, rendering the surface and/or particle more hydrophobic would result in a reduction in the capillary forces. The effect of capillary condensation on the overall adhesion depends upon the extent of water vapour condensation on the particle surface. At low relative humidities ($< 50\%$), capillary forces usually do not contribute to the adhesive interaction. With air relative humidities between 50 and 60\%, capillary forces only begin to be manifest; with air humidities between 65 and 100\%, they may prevail over other forces and are likely to be the main factor in adhesive interaction (Zimon, 1982). However, excessive low humidities may increase the electrostatic charges of powders during handling. This may in turn increase the adhesion of particles to surfaces (Karra and Fuerstenau, 1977). High ambient humidities (> 40\%) were shown to decrease the degree of interaction of three sulphonamide powders with model hydroxylpropylmethylcellulose phthalate-coated glass beads during blending (Kulvanich and Stewart, 1987). The reduction in adhesion was attributed to the decrease in electrical charges due to the increase in electrical conductivity of materials and surrounding atmospheres when the relative humidities were over 40-50\%. Substantial
capillary interaction was not observed at relative humidities between 65 and 80%. However, since the adhesion measurements in the report were performed immediately after inertactive system preparation such results may not represent the situation when the liquid bridges were fully developed.

Capillary interaction takes place over a period of time, depending upon the physicochemical properties of the interactive systems and the environmental conditions, such as humidity and temperature. High humidities and temperatures should accelerate capillary condensation and the hygroscopicity of the interactive systems would have the similar effects. Therefore, capillary interaction would be expected to increase with storage time until it reaches a constant level, when the partial pressure of the liquid produced by the system is in equilibrium with that of the vapour in the surrounding atmosphere.

The influence of relative humidity on the performance of dry powders for inhalation has been a matter of concern for many years (Kontny et al., 1994) since most drugs and carriers employed are hygroscopic. Exposure of these mixtures to high relative humidity may result in not only an increase in capillary interaction but also possible drug degradation as well as growth of microorganisms. A powder formulation of micronised sodium salts of xanthine carboxylic acid, after equilibration at high humidity, gave virtually no respirable material in a fluidization test. However, the acid was much less influenced by increased humidities as compared with its salt (Chowhan and Amaro, 1977).

Apart from the physicochemical properties of dry powders, other formulation factors of DPIs such as the use of gelatin capsule shells, multi- or unit dose packaging and designs of inhaler devices can also affect the sensitivity of a dry powder system to environmental moistures. Dry powders for inhalation are filled either directly into the packaging system as a unit dose (e.g. Diskhaler®) or multiple doses (e.g. Turbuhaler®) or encapsulated in gelatin capsules prior to presentation in unit dose (e.g. Inhalets®) or multi-dose (e.g. Rotacaps®) packaging. Since gelatin has a much higher moisture sorption capacity than many drug powders, encapsulation in gelatin capsules should reduce the rate of increase in the relative humidity inside the capsule shells and thus provide a protective barrier to external water vapour condensing on the encapsulated drug powders. For a multidose system, increasing
the moisture sorption capacity inside the package by addition of dosage units or a desiccant would reduce the rate of increase in the relative humidity inside the packaging chamber (Kontny et al., 1994). On the other hand, unencapsulated, unit dose drug powders (e.g. Diskhaler®) have the lowest moisture sorption capacity, and the relative humidity in the dose chamber would rapidly reach an equilibrium with the surrounding environments. Thus, encapsulated, multidose drug powders (Rotacaps®) would be more stable with regard to the influence of the environmental humidities than unencapsulated, unit dose drug powders, such as those employed in the Diskhaler®.

1.2.4 Mechanical interlocking

Interaction due to mechanical interlocking is often encountered. When two particles come into contact, only relatively small parts of their total surface area are actually touching. The pressure imposed on the contact area may be sufficient to cause elastic and plastic deformation, which will increase the interaction not only due to an increase in van der Waals’ forces but also promote mechanical interlocking. Mechanical interlocking becomes more significant as the surfaces become rougher. Particles having rougher surfaces will have a higher tendency to interlock mechanically than particles having smoother surfaces. In ordered mixes, small particles are mixed with larger particles. If the dimension of the asperities on the surface of the large particles are larger than the diameter of the smaller particles, then these particles may be entrapped in these asperities and the interaction may be much more significant than those due to intermolecular and electrostatic interactions.

The entrapped drug particles may not be available for the deaggregation caused by inhalation, leading to a decreased portion of drug that can reach the lower airways. For example, the respirable fractions of salbutamol sulphate, delivered from three kinds of lactose particles having different surfaces smoothness, were compared (Ganderton and Kassem, 1992). Recrystallised lactose particles were prepared such that they possessed the smooth surfaces. The surface roughness was assessed by rugosity, a ratio of the surface area derived from air permeability and the theoretical surface assuming the particle was spherical. The fraction of the delivered dose that was less than 5 μm was reported (Ganderton, 1992) to be higher for the formulation employing lactose particles with a lower
value of rugosity as carrier than in the case of lactose particles with higher value of rugosity (Table 1.2).

Table 1.2 Effects of surface roughness (rugosity) of lactose carriers on the respirable fraction of salbutamol sulphate after inhalation through a Rotahaler® (data from Ganderton, 1992).

<table>
<thead>
<tr>
<th>Lactose carrier</th>
<th>Rugosity</th>
<th>% respirable fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>60 l min⁻¹</td>
</tr>
<tr>
<td>Crytalline</td>
<td>2.3</td>
<td>4.1</td>
</tr>
<tr>
<td>Recrystallised</td>
<td>1.2</td>
<td>23.0</td>
</tr>
<tr>
<td>Spray-dried</td>
<td>2.6</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Increasing surface smoothness would be expected to decrease mechanical entrapment of fine drug particles into any asperities on the surface of the carrier. However, when the mechanically entrapped drug particles are available for deaggregation, further improvement in the surface smoothness may have a very complicated impact on the overall interaction between drug and carrier particles. For example, increasing the surface smoothness may increase van der Waals’ forces and increase the sensitivity of the interactive systems to liquid bridging. Thus, increasing surface smoothness under these conditions may not result in any further increase in the deaggregation of fine powders.
1.3 Particle entrainment and detachment after inhalation

Drug particles must be detached from their carrier and dispersed in the air flow in order to obtain suitable particles which will penetrate deep into the lung. An ideal formulation of drug powder for inhalation should ensure that all the drug particles are detached and deaggregated into single particles and the detached particles are dispersed in a laminar air flow whilst the carrier particles should have the minimum dispersion in the inhaled air stream so as to minimise any side effects due to their inhalation.

During inhalation, dry powders may undergo two major processes, namely, entrainment of the ordered mixes of drug and carrier particles in the airstream and detachment of drug particles from the air-borne carrier particles. The first step involves the fluidisation of dry powders from the surface of the inhaler chamber where the powders were originally packaged or released. This process resembles particle detachment from a stationary surface. After dispersion, drug particles will be detached from their carrier particles, due to the turbulence of the air stream and different aerodynamic properties between the large carrier and fine drug particles.

1.3.1 Theoretical considerations

1.3.1.1 Drag forces of air streams

The entrainment of dry powders for inhalation is mainly dependent upon the forces acting on the particles from the inhaled air stream, which are usually called drag forces. If the drag forces can overcome particle-particle and particle-surface interaction as well as the gravitational forces of the particles, the latter will be detached from surfaces and entrained into the air stream. The drag forces of an air stream on an individual particle are very difficult, if not impossible, to measure accurately. However, insight into the fluid-particle interaction in particle entrainment may be gained from a treatment of a particle suspended in a steady air stream.
Chapter one: Introduction

A large aerosol particle is constantly bombarded from all directions by a large number of gas molecules. The gaseous flow can then be considered to be continuous for the particles. If a particle is less than the mean free path, \( \lambda \), which is the mean distance a gas molecule travels before colliding with another gas molecule, then the motion of the particle is no longer determined by continuum flow considerations. Whether a particle is subject to a continuous or discontinuous flow depends upon the Knudsen number, \( K_n \), which relate the gas molecular mean free path to the physical dimension of the particle.

\[
K_n = \frac{2\lambda}{d} \tag{1-8}
\]

where \( d \) is the diameter of the particle in question and \( \lambda \), of an air stream at normal temperature and pressure is 0.0665 \( \mu \)m (Rader, 1990). \( K_n \ll 1 \) indicates continuum flow whilst \( K_n \gg 1 \) indicates free molecular flow. The intermediate range, around 0.4-20, is usually referred to as the transition or slip flow regime.

If a spherical particle is submerged into a steady air stream that can be considered to be continuous with the particle, then the aerodynamic drag force on the particle can be calculated as (Baron and Willeke, 1993):

\[
F_{drag} = \frac{\pi}{4} C_d \rho_g V^2 d_p^2 \tag{1-9}
\]

where \( d_p \) is the particle diameter; \( V \) is the velocity of the free stream, \( \rho_g \) is the gas density and \( C_d \) is the drag coefficient.

Drug particles for inhalation usually have a particle size between 2-5 \( \mu \)m, which correspond to the values of Knudsen number between 0.0665-0.0266 at normal conditions. Therefore, the air stream can be regarded to be continuous for these particles. However, most of inhaled drug particles are irregularly-shaped and hence, the equation (1-9) must be modified to give:
\[ F_{\text{drag}} = \frac{\pi}{8} C_d \chi \rho g V^2 d_p^2 \] (1-10)

where \( \chi \) is the dynamic shape factor of a particle. It usually has a value > 1 for non-spherical particles and more anisometric particles usually have a higher shape factor than more isometric particles.

Thus, the drag force of an air stream on a particle is a function of both particle size, shape and flow properties. Increasing the viscosity of the medium will increase drag force of the stream on particles. This is very easy to accept as water exerts a much higher drag force on solid objects than an air stream. Increasing the velocity can also increase drag forces, which is equally apparent. It should be noted however that the relevant particle diameter in equations (1-9) & (1-10) is the diameter of the cross-sectional area of the particle vertical to the stream direction, which may not be the true diameter of the particles.

1.3.1.2 Detachment of particles from stationary surfaces

Detachment of particles from stationary surfaces has attracted much attention by research workers in different scientific fields, such as those associated with the semiconductor industry, and numerous studies concerning particle detachment mechanisms have been reported in the literature (Mittal, 1995). This process is also important in the delivery of dry powders for inhalation because of its involvement at two stages in the inhalation procedure. First, the drug-carrier particles have to be detached from the inhaler chamber, which can be regarded as a stationary surface, and then become dispersed into the inhaled airstream before the drug particles can be further detached from the air-borne carrier particles. Second, the basic theory generated from the detachment of particles from stationary surfaces would provide a useful guide to predicting the detachment of fine particles from air-borne carrier particles, since they are likely to follow similar mechanisms although some quantitative differences would be involved in the detachment under the two sets of conditions.

The detachment of particles from their adhered surfaces usually follows three mechanisms, rolling, sliding and lifting (Soltani et al., 1995).
A particle will be removed or dislodged from its adhered surface if the drag force overcomes the adhesion forces and the weight of the particle (Figure 1.6).

![Figure 1.6 A diagram showing the relationship between adhesion force, drag force and geometry of the particle.](image)

A particle will start to roll along the surface, if

\[
F_{dr} \geq (F_{ad} + W) \frac{r - r_c}{r - r_c}
\]  

(1-11)

where \( F_{dr} \) is the drag force, \( F_{ad} \) is the overall adhesion force, \( W \) is the particle weight, \( r \) and \( r_c \) is the radius of the particle and contact area, respectively.

A particle will slide or be lifted, if the following conditions are realised.

\[
F_{dr} \geq \mu (F_{ad} + W)
\]

(1-12)

where \( \mu \) is the coefficient of friction.

For fine particles, when \( F_{ad} \gg W \), then,
Thus, apart from the drag forces, lifting or sliding detachment is mainly dependent upon the overall adhesion forces and the friction between the particle and the surface.

### 1.3.1.3 Detachment of fine particles from air-borne coarse particles

Numerous reports have been published reporting the detachment of dust and powders from stationary surfaces (Corn, 1966; Zimon, 1982) but very little work has been carried out to investigate the dislodgement of adhered fine particles from air-borne coarse particles. Particle detachment from air-borne carriers is more complicated than that from a stationary surface, but the processes involved may still be assessed using the similar principles.

![Diagram](image)

**Figure 1.7** A diagram depicting the detachment of fine particles from an air-borne carrier particle.

A fine particle will detach from a coarse particle, if

\[
\frac{F_{dr2} - \mu F_{ad}}{W_f} > \frac{F_{dr1}}{W_C}
\]  

(1-14)

If \(W_C \gg W_f\), then equation (1-14) can be simplified as

\[
F_{dr2} > \mu F_{ad}
\]

(1-15)
where $F_{d1}$ and $F_{d2}$ are the drag forces for the coarse and fine particles, respectively. $W_f$ and $W_c$ are the weight of the fine and coarse particles, respectively and $F_{ad}$ is the adhesion force between the fine and coarse particles. These equations only apply to the fine particles that are positioned at the top or the bottom of carrier particles. These fine particles are more likely to detach from the carrier than particles positioned at the back or front of the carrier particle (Zimon, 1982). However, air-borne particles are known to rotate constantly in the air stream and hence, most of the adhered particles may be, at some point in time, oriented in a position favourable for detachment. Further, this spin generates centrifugal forces on adhered particles, which also assists particle detachment, but in a much more complicated manner.

Particle detachment may also be carried out by the turbulence of the air stream. Local turbulence at the sites of particle adherence will be induced due to the change in the direction of air stream in these areas. Such a change in direction leads to lifting forces being generated, which if they exceed the adhesion forces, will lead to the fine particles being detached from the carrier.

1.3.2 Factors affecting particles entrainment and detachment

As discussed above, the detachment of particles from both stationary surfaces and air-borne carriers is determined by fluid factors (e.g. flow rates) and particle size as well as particle-carrier interaction (adhesion forces)

1.3.2.1. Fluid properties

One of the most important fluid properties determining detachment efficiency is the rate of flow experienced by the particle, since the drag force on a particle increases with the velocity of the flow rate of the stream. Higher flow velocities produce higher drag forces and increase both the degree of entrainment of drug-carrier mixes and the detachment of fine particles from air-borne carrier particles. The practice of increasing the flow rate of a stream to improve particle removal from a stationary surface is well-known in powder technology. For example, the removal efficiency of standard latex (styrene/divinylbenzene)
particles of 1-10 μm in diameter from a glass surface was found to be directly related to the dynamic pressures, which in turn was proportional to the square of the linear flow rate of the air stream from a high-speed air jet (Gotoh et al., 1994a), regardless of particle size. Similarly, increasing the flow rate of an inhaled air stream was also shown to increase the detachment of dry powders for inhalation from the inhalers, producing a higher emitted dose, which may eventually result in a higher respirable fraction of drug particles. For example, the doses emitted from most marketed dry powder inhalers were found to be higher after inhalation at 100 l min⁻¹ for 2.4 s than those after inhalation at 60 l min⁻¹ for 4 s despite the inhalation volume being maintained at 4 l (Hindle et al., 1994) (Figure 1.8).

![Figure 1.8](image)

Figure 1.8 The relationship between mean % emitted dose of the loaded dose and inhalation flow rate (adapted from Hindle et al., 1994).

The role of flow rate in particle aerosolisation can be better seen in a report employing laser light scattering to measure particle size of micronised salbutamol base from different carrier systems under different flow rates (MacRitchie et al., 1995).

Table 1.3 shows that as the flow rate increased, the fine particle fraction was increased regardless of the properties of the carrier used. Increasing the flow rate from 28.3 to 80 l min⁻¹, resulted in the fine particle fraction being increased by over 2-3 times for all the carriers. The difference in the fine particle fraction for different carriers may be due to
different adhesion forces of the drug particles to these carriers, although no explanation was
given in the report.

Table 1.3. The fraction of fine particles (<5 μm) of salbutamol base from different carriers
at four flow rates [Mean % (sd)] (after MacRitchie et al., 1995).

<table>
<thead>
<tr>
<th>Carriers</th>
<th>Flow rates (l min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>28.3</td>
</tr>
<tr>
<td>lactose</td>
<td>5.35(1.3)</td>
</tr>
<tr>
<td>SD lactose*</td>
<td>1.00(0.5)</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>6.45(1.8)</td>
</tr>
<tr>
<td>Dextrose</td>
<td>2.72(0.9)</td>
</tr>
<tr>
<td>Maltose</td>
<td>6.93(1.4)</td>
</tr>
</tbody>
</table>

*Spray dried lactose

Apart from the velocity of air flow, the turbulence generated by air stream is also important
for the entrainment and detachment of drug particles. Turbulent airflow is more effective
than laminar airflow in dispersing the powder mixture (Moren et al., 1985). Turbulence
generated within the inhaler device is particularly important for aerosol dispersion.
Increasing flow rate will increase the turbulence of the inhaled air stream but the latter is
often modified by the design of inhaler devices (Timsina et al., 1994).

1.3.2.2 Particle properties

As both drag and adhesion forces are dependent upon the size of adhered particles, particles
of different size should have different properties of detachment in similar fluid conditions.
According to equations (1-9) & (1-10), drag force is proportional to d_p^2 whereas, the mass of
a particle is proportional to d_p^3. Thus, the ratio of drag force to mass should increase with
decreasing particle size. Smaller particles will have larger ratios of drag force to mass than
larger particles and smaller particles would therefore have a higher acceleration under the
same fluid conditions. It would appear that the smaller particles are the more likely to
detach. However, this is only true when the gravitional forces (W) of the particles exceed
their adhesion forces (F_ad). For fine particles, when F_ad >> W, the trend is reversed, since
drag forces decrease faster ($d^2$) than van der Waals forces ($d$). Thus, for fine particles, the critical velocity needed to detach a particle increases as particle diameter decreases (Soltani et al., 1995). For example, particle size was shown to be the dominant factor in determining removal efficiency of latex particles from a stationary surface by an air stream (Gotoh et al., 1994a). The dynamic pressures ($P_{d50}$) required to dislodge 50% of the adhered particles from a surface increased greatly with decreasing particle size, regardless of the surface characteristics. $P_{d50}$ for particles of 2.02 μm was more than 10 times higher than that of particles with a diameter of 6.4 μm and $P_{d50}$ increased more markedly with decreasing particle size for smaller particles than for larger particles.

The removal of pharmaceutical powders by an air stream from a stationary surface such as the inner walls of capsule shells and inhaler devices may also be dictated by the size of particles to be removed. For example, the entrainment efficiency of particles of different sizes from a Spinhaler® capsule was found to be dependent upon particle size (Bell et al., 1971). At the same flow rate, entrainment efficiency increased with particle size until it reached a maximum of about 60% at a size of 100 μm. Then, entrainment efficiency decreased drastically with increased particle size and virtually no entrainment was detected when particles were over 120 μm under the experimental conditions. The linear velocity ($V_i$) of an air stream required to initiate entrainment of lactose particles of two large size fractions (90-125 and 125-180 μm) was about 8 m s$^{-1}$, which was less than half of $V_i$ (more than 16 m s$^{-1}$) for two small size fractions (63-75 and 75-90 μm) (Staniforth, 1995). The air velocity required to achieve complete entrainment was also lower for the large particles (about 18 m s$^{-1}$) than for the small particles (about 25 m s$^{-1}$). The coarser particles became entrained in a smooth continuous manner as discrete particles, whereas the smaller particles exhibited an erratic plug entrainment behaviour, with agglomerates of particles entrained intermittently. Although the entrainment of relatively larger particles is easier than that of smaller particles, it does not necessarily indicate that the larger carrier particles are always more favourable than smaller carrier particles for drug particles for inhalation. According to equations 1-11 to 1-15, the detachment of fine particles from coarser carrier particles depends upon the adhesion forces and the mobility of the entrained carrier particles. Large carrier particles were shown to exert stronger adhesion forces on drug particles than smaller carrier particles (Staniforth et al., 1982). Further, the mobility of air-borne smaller particles
would be higher than that of the coarser particles. Thus, in terms of drug detachment from air-borne carrier particles, smaller carrier particles may be more favourable than larger carrier particles. For example, the *in vitro* respirable fractions of salbutamol sulphate from smaller lactose particles were higher than those from larger lactose particles at all flow rates from 60 to 200 l min\(^{-1}\) (Ganderton and Kassem, 1992). The increased respirable fraction was indicative of an improved detachment of drug particles from the smaller carrier particles. However, smaller particles have poorer flowability than larger particles, which may result in other problems during powder handling processes. Therefore, the size of carrier particles has to be optimised according to the entrainment and dispersion properties of drug and carrier as well as the flowability of the ordered mixes.

1.3.2.3 Particle-surface interaction

According to equations from 1-11 to 1-15, the detachment of particles from both a surface and a carrier particle is determined by adhesion forces. Particles having weaker adhesion forces would be more easily detached than particles having stronger adhesion forces under similar flow conditions. In other words, higher adhesion forces would require stronger drag forces to detach a particle from either a stationary surface or an air-borne particle. The critical velocity of air flow necessary to detach particles having lower adhesion forces should be lower than that needed for particles having higher adhesion forces. Thus, any factors that affect the interparticulate forces would ultimately influence particle detachment. These factors include the contact area between interacting particles, surface smoothness of the carrier, relative humidity, etc.

1.3.2.3a Relative humidity

Environmental relative humidity is an important factor governing particle-particle and particle-surface interaction. Properties and behaviours of solid particulate systems can be substantially controlled by capillary forces (Schubert, 1984). As mentioned above, relative humidity affects interparticulate forces by means of inducing capillary forces and reducing electrostatic forces. At low relative humidities (< 60 %), when water vapour condensation on the particles is negligible, increasing relative humidity would reduce interparticulate forces by decreasing electrostatic forces, as a result of improving electrical charge decay. At
higher relative humidities when water uptake by the particle is substantial, increasing relative humidity would increase interparticulate forces through capillary forces. Therefore, there may exist an optimum relative humidity where interparticulate forces are at a minimum and particle performance is optimised. For example, increasing the ambient relative humidity increased the removal efficiency of latex (styrene/divinylbenzene) particles of 3.7 μm in diameter from a glass surface by airstreams at relative humidities up to 67%, when particle removal reached a peak value (Gotoh et al., 1994b). As the relative humidity was increased further so the removal efficiency of particles was decreased. A similar tendency was observed for particle removal from other surfaces, such as metals and plastics (Gotoh et al., 1994c). The humidity for maximum removal efficiency was shown by these authors to be dependent on the surface roughness in that the higher the latter was, there was usually required a higher relative humidity for maximum removal of particles.

Although capillary forces appear to be decisive in determining the removal efficiency of some particles, especially those of hydrophilic materials, condensation of water vapour to the particles is a function of duration of exposure of the particles to environmental humidities. If the particles are exposed to the environment for such a short period of time that water vapour condensation does not become manifest, even highly hygroscopic particles may not be substantially influenced by high humidities. This may be the main reason that the performance of dry powders composed of drug and carrier particles from the Ventolin Rotahaler®, was largely unaffected by the extreme test conditions (i.e. equilibrated at 45°C and 80% relative humidity for 30 min), with respect to both fine particle fraction and emitted dose when tested by a twin stage liquid impinger (Hindle et al., 1994). However, if water vapour is allowed to condense on particle surfaces for a prolonged period of time, especially at elevated temperatures, water vapour will not only increase interparticulate forces but also induce growth of drug particles due to migration of adsorbed water molecules within drug particles. Both effects will reduce the delivery efficiency of drugs. For example, both the emitted dose and fine particle fraction of salbutamol base (1.36 μm) and sulphate (2.5 μm) particles from a model dry powder inhaler were found to decrease with increasing relative humidity at any given temperature, with the difference being more marked at higher temperatures (Jashnani et al., 1995). The mobility of water
molecules is increased at higher temperatures and this will accelerate the condensation of water vapour on the particles.

1.3.2.3b Contact area
A smaller contact area (r_c) between particles and surface will lead to an easier detachment of particles from the surface. According to a calculation (Soltani and Ahmadi, 1995), the critical shear velocity required to detach rubber particles from both smooth and rough surfaces was at least 10 times larger than that for graphite particles because rubber is more easily deformed on contact than graphite particles and hence, there exists a larger contact area and frictional coefficient for rubber particles than for graphite particles under similar adhesive conditions. Further, as mentioned above, since adhesion forces are a function of contact area, a higher contact area leads to higher adhesion forces. Increased adhesion forces due to a larger contact area will further increase the velocity needed to dislodge the particles.

1.3.2.3c Surface smoothness of carrier
Increasing surface smoothness usually increases adhesion forces between particles as a result of an increased contact area between the interacting particles. Unless mechanical interlocking is implicated in adhesion, decreasing carrier surface smoothness will decrease the adhesion forces and this will lead to easier detachment of adhered particles under similar conditions. For example, apart from the size of the adhered particles, surface smoothness of the substrate was also shown to affect the detachment of latex particles by an air stream (Gotoh et al., 1994a). The dynamic pressure of a compressed air stream required to detach 50% adhered latex particles (P_d50) decreased with increasing surface roughness, indicating that particles adhered to a rougher surface were easier to detach than the same particles adhered to a smoother surface. This was attributed by the authors to decreased van der Waals’ forces between particles and rougher surfaces. However, the change in the near-wall fluid properties due to change in the surface smoothness may also contribute partly to the effects. The particle size had more pronounced effects on detachment than surface smoothness under the specific conditions. Smaller particles were more difficult (higher P_d50) to detach than larger particles regardless of the surface materials used. The detachment of particles having different hardness from surfaces of different smoothness was modelled
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theoretically, based upon particle rolling, sliding and lifting mechanisms (Soltani et al., 1995). Increasing the surface roughness of substrate was shown to reduce the critical acceleration required to detach all the particles investigated. This was attributed to reduced adhesion forces of the particles to the surface due to the decreased contact area at higher surface roughness.

Increasing surface roughness may further improve particle detachment by changing the fluid properties in the vicinity of adhered particles to be detached. The near-wall flow behaviour is expected to have a profound effect on the particle detachment and the near-wall turbulent flow is thought to be dominated by vortical coherent structures and occasional bursts (Hinze, 1975). Increasing surface roughness would increase the turbulence of flow near the particles to be detached and consequently, would improve particle detachment at a similar velocity.

1.3.2.3d Frictional coefficient

Although increasing surface roughness may improve particle detachment, this effect may be reduced by an increased frictional coefficient between particles and surfaces of higher roughness. According to equations 1-11 to 1-15, decreasing the frictional coefficient (μ) between fine particles and coarser carrier particles would favour particle detachment. The flowability of lactose particles was found to be greatly increased by the coating of such particles with polymers (Fernandez-Arevalo et al., 1990) due to a reduction in the interparticulate friction as a result of improved surface smoothness. The coating of carrier particles with other biodegradable and non-toxic polymers may be a reasonable means of improving dispersion of drug particles, especially for highly expensive drugs. An optimised coating material would have to be able to provide desirable electrostatic charging properties, a reduction in interparticulate forces through the modification of surface texture, electrical charge and moisture absorption. These effects combined should modify not only the adhesion forces but also frictional coefficient and consequently, alter the dispersion of drug particles on inhalation.
1.4 Deposition of particles in the airways

1.4.1 Deposition mechanisms

Deposition is a process by which inhaled particles separate from the flow streamlines and contact a respiratory surface from which there is no rebound or resuspension. Particles that remain airborne throughout the respiratory cycle are exhaled. The deposition of particles in the airways is a very complicated process. Inhaled particles may be deposited at or captured by any sites in the respiratory tract by different mechanisms, depending on the physiological factors of the respiratory tract, inhalation manner and physico-chemical properties of particles. There are five possible mechanisms by which significant deposition may occur, namely, impaction, sedimentation, diffusion, interception and electrostatic precipitation.

1.4.1.1 Impaction

Impaction is the most important mechanism of particle deposition. The inhaled air follows a tortuous path through the branching airways. Each time, the air stream changes its direction, the airborne particles tend to keep their established trajectories. If the particles possess sufficient momentum, they will not follow the gas streamlines and will impact on any obstacles in their trajectories.

If a particle of mass, \( m \), is moving with velocity \( V \), through still air, the particle will stop after travelling a distance, \( S \), as a result of frictional forces, in accordance with equation 1-16.

\[
S = B \times m \times V 
\]  

(1-16)

where \( B \) is the mobility of the particle, i.e. the velocity per unit of force. Thus, the greater the particle mobility, mass and velocity; the longer it will persist flying in its original trajectory and hence, it is more likely for the particle to hit any obstacle in the airways. That is to say the chances of deposition by impaction are increased.
For a particle travelling in an airway, the probability of its deposition by impaction is a function of a dimensionless parameter involving the air velocity, known as Stokes’ number (Stk), which can be obtained, using equation 1-17.

\[ \text{Stk} = \frac{\rho_p d^2 V}{18 \eta R} \]  

(1-17)

where \( \rho_p \) is the particle density, \( d \) is the particle diameter, \( V \) is the air velocity, \( \eta \) is the air viscosity and \( R \) is the airway radius.

The higher the value of Stokes’ number, the more readily particles will deposit by impaction. Therefore, increasing either the particle size or air flow rate can increase deposition by impaction. This is usually undesirable for most inhaled drug particles as impaction occurs most frequently in the upper airways which are not the sites of action for most drugs. Therefore, with large particles whenever the convective airflow is fast or turbulent as in the upper airways or changing direction at bifurcations between successive airways generations, impaction is the most likely mechanism of particle deposition and hence, ‘hot spots’ are found particularly at bifurcations and on the carinal ridges (Martonen and Yang, 1996).

1.4.1.2 Sedimentation

An airborne particle with a density \( (\rho_p) \) greater than that of air \( (\rho_a) \), will experience a downward force \( (F) \) which can be expressed as:

\[ F = \frac{\pi}{6} d^3 g (\rho_p - \rho_a) \]  

(1-18)

The particle accelerates downwards and reaches a constant terminal velocity when the resistant force due to interaction with air molecules balances its weight. For spherical particles, Stokes’ law can be used to predict the resistant force \( F_r \).
When $F = F_r$, the terminal velocity is given as:

$$V_t = \frac{(\rho_p - \rho_g)d^2g}{18\eta} \tag{1-20}$$

Stokes' law is valid for unit density particles of 1 to 40 $\mu$m in diameter settling in air. For particles of larger diameter, the resistant force is more than that predicted by Stokes' law as a result of the inertial effects of accelerating a mass of gas to push it aside. When particle size decreases, the resistance force is less than that predicted by Stokes' law since the particle size is comparable in size to the mean free path of air ($\lambda$, normally 0.0665 $\mu$m for air) molecules and can slip between molecules. A correction factor, known as the Cuningham slip correction, must be applied to the calculation to obtain the true value (Allen and Raabe, 1985). Further, equation (1-20) assumes a laminar flow within the airways. However, as the air flow increases, the stream will become turbulent, characterized by large and irregular velocity fluctuation. The flow pattern is expressed by the dimensionless Reynolds number, $R_e$ (equation 1-21).

$$R_e = \frac{\rho_g V d'}{\eta} \tag{1-21}$$

where $V$ is the velocity of the gas, $\eta$ the dynamic gas viscosity, $\rho_g$ the gas density and $d'$ a characteristic dimension of the object. Laminar flow occurs in a circular duct when the flow $R_e$ is less than about 2000, whilst turbulent flow occurs for $R_e$ above 4000 (Baron and Willeke, 1993). Turbulent flow will increase the contact of particles with the airways and hence, increase the deposition by impaction,

When the particle has such a small size that the Knudsen number (equation 1-8) is more than 10, the motion of the particle becomes primarily diffusive, due to the molecular bombardment, known as Brownian motion.
1.4.1.3 Diffusion

Particles less than 1 μm in diameter, will undergo a random motion in the air stream due to the bombardment of the gas molecules. The Brownian motion increases with decreasing particle size and becomes an important mechanism for particle deposition in the lung. The root mean square displacement, $\Delta$, with time, $t$, for Brownian motion, is expressed as:

$$\Delta = \sqrt{6Dt} \quad (1-22)$$

where $D$ is the diffusion coefficient of the particle:

$$D = \frac{KT}{3\eta d} \quad (1-23)$$

where $K$ is the Boltzmann constant, $T$ is the absolute temperature, $\eta$ is the gas viscosity and $d$ is the particle diameter.

Thus, particle size is the key factor in determining the mechanism of particle deposition whether this be due to sedimentation or diffusion. The displacement in one second of a unit density sphere due to gravitational sedimentation and Brownian motion, for particles from 0.05 to 50 μm is given in Table 1.4.

Thus, Brownian motion increases with decreasing particle size whilst sedimentation exhibits the inverse trend. The total displacement decreases with decreasing particle size until it reaches a minimum value at about 0.5 μm, after which the displacement increases with decreasing particle size. These results suggest that the chances of deposition for large particles are higher than those for smaller particles and particles of 0.5 μm in diameter are the least likely to deposit in the airways.
Table 1.4 Particle displacement (µm) in one second under conditions similar to the respiratory tract (adapted from Brain et al., 1985)

<table>
<thead>
<tr>
<th>Particle diameter (µm)</th>
<th>Displacement (µm) by Brownian motion</th>
<th>Sedimentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>1.7</td>
<td>70,000</td>
</tr>
<tr>
<td>20</td>
<td>2.7</td>
<td>11,500</td>
</tr>
<tr>
<td>10</td>
<td>3.8</td>
<td>2,900</td>
</tr>
<tr>
<td>5</td>
<td>5.5</td>
<td>740</td>
</tr>
<tr>
<td>2</td>
<td>8.8</td>
<td>125</td>
</tr>
<tr>
<td>1</td>
<td>13</td>
<td>33</td>
</tr>
<tr>
<td>0.5</td>
<td>20</td>
<td>9.5</td>
</tr>
<tr>
<td>0.2</td>
<td>37</td>
<td>2.1</td>
</tr>
<tr>
<td>0.1</td>
<td>60</td>
<td>0.81</td>
</tr>
<tr>
<td>0.05</td>
<td>120</td>
<td>0.35</td>
</tr>
</tbody>
</table>

1.4.1.4 Interception

Interception is the mechanism of deposition of a particle when the centre of gravity is within the streamlines of the gas phase but a distal end of the particle has touched a surface. Deposition by interception in the airways is important when the dimension of the airway radius is comparable to the dimensions of the particles. Thus, elongated particles are more likely to deposit by interception than spherical particles of the similar volume (Timbrel, 1965; Johnson and Martonen, 1994).

1.4.1.5 Electrostatic precipitation

Significant electrostatic charges may be imparted at the generation stage to the droplets and particles of an aerosol (John, 1980). Drug particles are usually immediately inhaled after generation without the charging being neutralised and thus, such charges will influence the overall deposition of the particles in the airways. A charged particle may induce an image
charge on the surfaces of the airways and subsequently deposit by electrostatic precipitation (Chan and Yu, 1982). The repulsion between like-charged inhaled particles (space charge forces) may also direct the particles toward airway walls, resulting in deposition in those regions (Chen, 1978). However, the deposition of charged particles in the airways has not been extensively studies and still requires more investigation.

1.4.2 Factors governing the deposition of particles in the airways

The factors affecting the deposition of inhaled particles have been investigated extensively by both experimental work and mathematical modelling (Heyder et al., 1986; Martonen, 1993). Many factors have been shown to affect the deposition of inhaled particles in the airways. These include physiological factors, inhalation patterns and formulation factors of aerosol dry powders.

1.4.2.1 Physiological factors

1.4.2.1a Lung Morphology

Based on experimental data, a morphological model of the human lung has been proposed by Weibel (1963) and it has become the most widely accepted model in the investigation of particle deposition in lung. The Weibel model is a symmetrical, dichotomously branching network of cylindrical tubes. From trachea, through terminal bronchioles, to alveolar sacs the airways are divided into 24 generations. Each airway divides to form two smaller daughter airways and thereby, the number of airways at each generation is double that of the previous generation and there are $2^i$ (I number of generation) airways in each generation. The Task Group on Lung Dynamics (1966) proposed that the respiratory tract be divided into three compartments, namely, nasopharynx, tracheobronchial and pulmonary. As most dry powders are administered via oral inhalation, it is more relevant to consider the oropharynx region rather than the nasopharynx region. The tracheobronchial region was defined as the non-alveolated bronchial airways including the trachea and terminal bronchioles (generations 0-16), its function being mainly air-conducting. The pulmonary
region was the gas-exchange compartment inclusive of respiratory bronchioles and alveolar ducts, corresponding to the generations 17 to 23 of the Weibel model.

In passing from the mouth through trachea to the alevolar sac, several physiological changes occur in the airways that are important in determining particle deposition (Table 1.5). First, the airway diameter decreases with increasing generations whereas the number of airways for each generation increases at a much higher rate (double the previous generation). Thus, whilst the calibre for the individual airway decreases the total area for that generation increases drastically. This change has a great impact on the air flow dynamics and eventually, influences the deposition of particles. Second, the upper respiratory tract (all airways external to the trachea) is composed of the mouth, pharynx and larynx. Whilst moving through this region, the air stream is subject to a sharp change in direction especially from pharynx to larynx (which is a short cavity containing a slit-like opening in its central portion). This structure apparently induces instability of the air stream (Pedley et al., 1979) and increases the chances of deposition by impaction. Third, the tracheobronchial region contains large numbers of ciliated cells, which sweep a sheet of mucus towards the pharynx, where it is swallowed. Thus, particles deposited in this region will be rapidly removed. Deposition in the region is not uniform with most drug particles deposited at the bifurcations and on the bulged cartilagenous rings (Martonen, 1993). In the lower airways, the air stream becomes more stable (Sudlow et al., 1976) and the majority of the remaining air-borne particles will deposit mainly by sedimentation and diffusion mechanisms.

Thus, large particles, such as those > 10 μm, will most probably impact in the mouth and on the back of the throat (Table 1.5), and these will eventually be swallowed and be delivered to the gastrointestinal tract. The pharynx, larynx and the entrance to treachea provide a major region for deposition of airborne particles, especially by impaction. The transfer through the larynx provides significant resistance to airflow and induces considerable turbulence downstream. The flow disturbance may persist for several generations of the bronchial airways before becoming attenuated. A portion of remaining airborne particles can be expected to deposit in this region by impaction. Deposition in this region is often considered undesirable since it is rarely the target site for many drugs and deposited particles will eventually be removed by the mucociliary escalator and swallowed.
Table 1.5 Some characteristics of the respiratory tract related to drug deposition in certain pulmonary regions. Calculations assume a steady inspiratory flow of 60 l min$^{-1}$: $D_{\text{sed}}$ is the distance that a 1 $\mu$m particle will sediment during transit of that region; Stk is the Stokes' number for a 1 $\mu$m particle in that region; Re is the Reynolds' number for that region. (adapted from Davies, 1961)

<table>
<thead>
<tr>
<th>Region</th>
<th>Number</th>
<th>Diameter (cm)</th>
<th>Length (cm)</th>
<th>Residence time (s)</th>
<th>$D_{\text{sed}}$ ($\times 10^4$ cm)</th>
<th>Stk ($\times 10^5$)</th>
<th>Re</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouth</td>
<td>1</td>
<td>2</td>
<td>7</td>
<td>0.022</td>
<td>0.7</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>Pharynx</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>0.021</td>
<td>0.7</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Trachea</td>
<td>1</td>
<td>1.7</td>
<td>12</td>
<td>0.027</td>
<td>0.9</td>
<td>0.78</td>
<td>5010</td>
</tr>
<tr>
<td>Main bronchi</td>
<td>2</td>
<td>1.3</td>
<td>3.7</td>
<td>0.010</td>
<td>0.3</td>
<td>0.88</td>
<td>3300</td>
</tr>
<tr>
<td>Lobar bronchi</td>
<td>5</td>
<td>0.8</td>
<td>2.8</td>
<td>0.007</td>
<td>0.2</td>
<td>1.50</td>
<td>2220</td>
</tr>
<tr>
<td>Segmental bronchi</td>
<td>18</td>
<td>0.5</td>
<td>6</td>
<td>0.021</td>
<td>0.7</td>
<td>1.71</td>
<td>942</td>
</tr>
<tr>
<td>Intrasegmental bronchi</td>
<td>252</td>
<td>0.3</td>
<td>2.5</td>
<td>0.045</td>
<td>1.5</td>
<td>0.57</td>
<td>225</td>
</tr>
<tr>
<td>Bronchioles</td>
<td>504</td>
<td>0.2</td>
<td>2.0</td>
<td>0.032</td>
<td>1.1</td>
<td>0.95</td>
<td>84</td>
</tr>
<tr>
<td>Secondary bronchioles</td>
<td>3024</td>
<td>0.1</td>
<td>1.5</td>
<td>0.036</td>
<td>1.2</td>
<td>1.27</td>
<td>28</td>
</tr>
<tr>
<td>Terminal bronchioles</td>
<td>12,100</td>
<td>0.07</td>
<td>0.5</td>
<td>0.023</td>
<td>0.7</td>
<td>0.93</td>
<td>10</td>
</tr>
<tr>
<td>Respiratory bronchioles</td>
<td>$1.7 \times 10^5$</td>
<td>0.05</td>
<td>0.2</td>
<td>0.067</td>
<td>2.2</td>
<td>0.18</td>
<td>1</td>
</tr>
<tr>
<td>Alveolar ducts</td>
<td>$8.5 \times 10^5$</td>
<td>0.08</td>
<td>0.1</td>
<td>0.44</td>
<td>14.5</td>
<td>0.01</td>
<td>0</td>
</tr>
<tr>
<td>Atria</td>
<td>$4.2 \times 10^6$</td>
<td>0.06</td>
<td>0.06</td>
<td>0.71</td>
<td>23.4</td>
<td>0.01</td>
<td>0</td>
</tr>
<tr>
<td>Alveolar sacs</td>
<td>$2.1 \times 10^7$</td>
<td>0.03</td>
<td>0.05</td>
<td>0.75</td>
<td>24.8</td>
<td>0.01</td>
<td>0</td>
</tr>
<tr>
<td>Alveoli</td>
<td>$5.3 \times 10^8$</td>
<td>0.015</td>
<td>0.015</td>
<td>4</td>
<td>132</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In the lower tracheobronchial region ($I > 6$), the air stream gradually becomes laminar in flow pattern. The Stokes' number of the remaining airborne particles and the flow rate of the stream will gradually decrease such that deposition by impaction becomes less significant whilst deposition by sedimentation becomes more and more important. However,
deposition at airway bifurcations still occurs preferentially. Deposition in this region (generations $6 < 1 < 16$) which includes the terminal bronchioles is not desirable for most drugs, since drug particles will be removed by mucociliary action. However, this region includes the major sites of action for anti-asthmatic drugs.

In the pulmonary region, gas exchange between the blood-stream and the air takes place. Air flow is very slow, even non-existent, relying primarily on gaseous diffusion. Thus, the Stokes’ number of particles that are still airborne becomes negligibly small, indicating the deposition by impaction is also negligible. Due to the slow air flow, the residence time of particles in this region is greatly increased and hence, deposition occurs mainly by time-dependent mechanisms such as sedimentation and Brownian diffusion. The pulmonary region is considered to be the ideal site of drug deposition especially for drugs administered to elicit systemic effects. This region has been the major target site for the delivery of these drugs.

1.4.2.2 Breathing patterns

The most important features of inhalation are the flow rate, the inhaled volume and the breath-holding time after inhalation (Newman et al., 1982).

1.4.2.2a Flow rate

The inhalation flow rate can change the overall deposition of dry powders by affecting the deaggregation of particles from its formulation, the Stokes’ number of the airborne particles and the turbulence of inhaled airstream and finally, the residence time of airborne particles in each section of the respiratory tract.

As discussed previously, increasing inhalation flow rates usually increases the deaggregation of drug particles, resulting in an improved fraction of small particles. However, in accordance with equation 1-17, an increased flow rate will produce a higher Stokes number for the airborne particles, which will in turn increase deposition by impaction, especially in the upper airways. Furthermore, increasing flow rate will increase
the turbulence of the airstream, mainly in upper airways. This will result in greater contact of particles with the lung walls, thereby increasing the deposition in this region, concomitantly decreasing the deposition in the lower airways. Increasing the flow rate will also reduce the residence time of airborne particles in the respiratory tract and thereby reduce the deposition of particles by sedimentation and diffusion mechanisms in the lower airways. Therefore, slow inhalation is desirable to obtain improved deposition of airborne particles in the lower airways. This has been demonstrated by many studies of deposition from MDIs and nebulisers (Newman et al., 1981 & 1982). Slower inhalation usually results in higher deposition in the lower airways since these devices do not need the inhaled airstream to atomise the drug particles. For example, a slow inhalation of 30 l min⁻¹ was found to be more preferential for the delivery of a bronchodilator both in terms of pharmacological effects and deposition pattern than a fast inhalation of 80 l min⁻¹ (Newman et al., 1981 & 1982). Faster inhalation was less effective since more aerosol impacted in the oropharynx and was unable to penetrate into the lung. By means of mathematical modelling (Martonen and Katz, 1993), the total lung deposition was shown to be inversely related to the inspiratory flow rates due to increased inertial impaction at higher flow rates. In contrast, pulmonary deposition increases with decreasing flow rate due to prolonged residence times. Thus, the tracheobronchial deposition of 6 μm particles was markedly increased from 30% at an inhalation flow rate of 0.5 l s⁻¹ to 50% at 0.04 l s⁻¹ and the deposition in the smaller ciliated airways was also increased (Anderson et al., 1995). This phenomenon was attributed to less deposition in the mouth and throat due to a reduced impaction at slower inhalation flow rates.

The influence of flow rate on deposition is more complicated for dry powders as compared with MDIs and nebulisers. The breath-actuation nature of dry powder inhalers requires that sufficient energy be generated by higher flow rates since too slow a flow rate may not be sufficient to deaggregate cohesive drug particles and detach the adhesive particles from their carriers. Therefore, the total amount of drug particles reaching the lower airways may be much lower at slower inhalation rates than at faster inhalation rates although the probability of the deaggregated individual particles reaching the lower airways may be higher in the former case. Thus, unlike MDIs and nebulisers, DPIs may require a higher inhalation flow rate to effectively deaggregate the drug particles. In a study of fenoterol powder, Padersen
and Steffensen (1986) demonstrated that a significantly increased response was observed in children who inhaled as fast as possible, as compared to children who inhaled only slowly at 16-19 l min\(^{-1}\). Auty et al (1987) found that for sodium cromoglycate (SCG) delivered from a Spinhaler\(^{®}\) a higher plasma concentration of drug was achieved at higher inspiratory flow rates and that a peak inspiratory flow rate around 160 l min\(^{-1}\) probably represented optimal inhalation rate. Pitcairn et al. (1994) used lactose and \(^{99m}\)Tc salbutamol blends to investigate the influence of flow rate on deposition of the drug particles from a multidose Pulvinal\(^{®}\) inhaler. A significantly higher percentage of the nominal dose (p=0.05) was observed to deposit in the lungs at a flow rate of 46.0 l min\(^{-1}\) (14.1 ± 3.2%) as compared to that (11.7 ± 2.3%) attained at slower flow rate of 27.8 l min\(^{-1}\). Newman et al., (1994) investigated the deposition of \(^{99m}\)Tc labelled sodium cromoglycate powder from a Spinhaler at a fast (120 l min\(^{-1}\)) and slow (60 l min\(^{-1}\)) peak inhaled flow rates. Inhalation at 60 l min\(^{-1}\) significantly (p < 0.01) reduced deposition in the lungs compared to inhalation at 120 l min\(^{-1}\). However, Zanen et al (1992) showed that there was no significant difference in bronchodilation of salbutamol after inhalation at 40 or 80 l min\(^{-1}\) through a Cyclohaler\(^{®}\) as particles < 5 \(\mu m\) were reported to be separated completely at low flow rates.

1.4.2.2b Tidal volume and breath-holding

It has long been demonstrated that deep inhalation and breath-holding can improve the deposition of an aerosol in the lung. For example, Riley et al (1976, 1979) found that an isoprenaline MDI was more effective when actuated at a high lung volume, i.e. 80% vital capacity (VC) compared with a low lung volume, 20% VC. A period of breath-holding after inhalation increases the probability of peripheral deposition since it allows time for the particles to settle on the airways by gravitational sedimentation and diffusion. Newman et al (1982) demonstrated that the deposition of \(^{99m}\)Tc teflon particles (aerodynamic diameter 3.2 \(\mu m\)) in patients with obstructive airways disease was greatly influenced by the breath-holding time. At a given vital capacity of 20, 50 or 80%, the increase in breath-holding from 4 to 10 s increased the deposition of teflon particles in the whole lung from 20 to 120%, with the whole lung deposition ranging between 6.5 and 14.3% of the administered dose. However, increasing vital capacity failed to show the predicted commensurate increase in the whole lung deposition. Auty et al (1987) assessed the blood levels of sodium cromoglycate following inhalation from the Spinhaler\(^{®}\) and concluded that an optimised
lunger deposition was obtained by a combination of fast inhalation and breathholding. More recently, Martonen and Katz (1993) employed a mathematical model to predict the effects of tidal volume and breath-holding on the deposition of an inhaled aerosol. According to their calculations, increasing tidal volume should increase the whole lung deposition and pulmonary deposition for all particle sizes. This increased deposition was attributed to the deeper particle penetration into the lung at the increased tidal volumes and the increased particle residence time in peripheral airways, leading to improved deposition due to sedimentation and diffusion.

1.4.2.3 **Formulation factors**

Apart from inhalation manoeuvres, the formulation factors are also important in determining the deposition of inhaled particles in the respiratory tract. Such factors include particle size, size distribution, density, shape and hygroscopicity.

1.4.2.3a **Particle size**

According to equations (1-17) and (1-20), impaction and sedimentation are proportional to the product of the density and diameter\(^2\) (i.e. \(\rho d^2\)) of the particle. Thus, \(\rho d^2\) can be used to predict the deposition site, since for a given inspiratory flow rate, \(V\), the higher the value of \(\rho d^2\) the more likely it is that the particle will deposit in the extrathoracic compartment and the less likely it will be to reach the lower airways (Martonen, 1993). Therefore, the square root of this product (\(\rho^{1/2}d\)) is known as the aerodynamic diameter of the particle, i.e. the diameter of a unit density sphere with the same settling velocity as the particle in question (Agnew, 1984).

The influence of particle size on deposition has been the subject of many reports and numerous mathematical models have been proposed for calculating the extent of retention of particulate material of various sizes in the average adult human lungs (Task Group on Lung Dynamics, 1966). For example, Davies et al (1976) estimated the ideal size for therapeutic aerosols to be between 0.5-7.0 \(\mu\)m. Particles larger than 7.0 \(\mu\)m are believed not to be able to penetrate beyond the trachea, whereas particles smaller than 0.5 \(\mu\)m are most probably exhaled. Curry et al (1975) found a greater response to sodium cromoglycate when
administered as 2.0 μm particles compared with sizes of 6.0 and 11.7 μm. This observation was confirmed by Rees et al (1982), who reported a greater bronchodilator response with terbutaline sulphate of diameter less than 5.0 μm compared with that achieved by particles with a diameter of 5-10 μm or 10-15 μm.

Generally, it is widely accepted (Gonda, 1992) that particles with aerodynamic diameters greater than about 15 μm will entirely deposit in the extrathoracic regions, whereas particles with aerodynamic diameter of 5-10 μm will deposit in the tracheobronchial regions. Particles with diameter of 1 to 3 μm are most likely to deposit in the lower airways, with a maximum alveolar deposition of about 60%. However, even at this optimal alveolar deposition, a significant amount of material will still deposit in extrathoracic (10%) and tracheobronchial regions. During normal breathing, submicron particles are exhaled especially when particles have a diameter of about 0.5 μm. Particles of this size do not have enough mass and momentum to deposit by impaction and sedimentation. At the same time, their sizes are not small sufficiently to exhibit adequate Brownian motion and consequently, a high percentage fail to deposit by diffusion. However, ultrafine particles (< 0.01 μm) may deposit in the lower airways sufficiently rapidly through Brownian motion but the use of such particles is limited since they are difficult to generate in high concentrations and they carry much less mass than larger particles. For an aerosol to be effective, the fraction deposited at the target sites must be equal to or exceed the therapeutic dose. Large particles that are able to penetrate to the deep lung offer the greatest therapeutic advantage. Hence, the optimum size of inhaled drug particles for obtaining therapeutic activities is generally accepted as 1 to 5 μm (Smith et al., 1980) and this size range has been used to guide aerosol generation technology.

1.4.2.3b Particle size distribution

The influence of size distribution on the deposition of inhaled aerosol is related to the particle size of the individual particles. Most aerosols are polydisperse and have a wide range of diameters. The particle size distribution of an aerosol produced by an MDI, DPI and nebuliser usually follows a log normal distribution, that is to say the fraction of particles of a particular size, when plotted against the logarithm of the particle diameter, exhibits a normal, bell-shaped or Gaussian distribution (Raabe, 1978).
The conventional measure used to express the particle size and distribution is by median diameter and geometric standard deviation (GSD). The median diameter of an aerosol is the value of the diameter corresponding to 50% on a plot of diameter vs cumulative fraction such that 50% of all particles are above and below this value. The median diameter can be expressed as a geometric median diameter (GMD), which is characterised by the number of particles, or a mass median diameter (MMD), which is based on the mass or volume of the particles. The size range is expressed in terms of the geometric standard deviation (GSD) which is the ratio of diameters of cumulative fractions between 50% and 84.1% or 50% and 15.9%, the value corresponding to the standard deviation of the median diameter of the curve.

Particles with a narrow distribution have a similar particle size whilst particles with a broad distribution may have the same median diameter but possess a wide range of particle sizes. The log normal distribution of most aerosols indicates that there is a long tail of particles with large aerodynamic diameters. These large particles contain a significant fraction of the administered dose, since it is the amount of drug that dictates the resultant pharmacological effect rather than the total number of drug particles. Therefore, particles of the same MMD but with different polydispersities will exhibit different deposition profiles in the lung and it is always desirable to produce aerosols of the least polydispersity. Several theoretical calculations have shown that the polydispersity of an aerosol should affect the regional deposition of inhaled aerosol. For example, Gonda (1981) calculated that there was a reduction in alveolar deposition from about 60% of the inhaled dose for a monodisperse aerosol with a MMD of 2 μm and a GSD of 1.0 to less than 30 % for a polydisperse aerosol of the same MMD with a GSD of 3.5.

1.4.2.3c Particle shape

Particle shape has an important influence on the rheological properties of the powders. Anisometric particles, which have an elongated or flattened shape, tend to build up open packings of higher porosity. Such particles tend to align along their long axis in the direction of the flow and thus, exhibit less internal friction than more isometric particles (Neumann, 1967). In aerosol science, the use of elongated particles has attracted much
interest. Long objects, such as fibres and needle-like crystals, have aerodynamic diameters almost independent of their length and the diameter is approximately equal to the short dimension of the particle in question (Hinds, 1982). Thus, elongated particles may exhibit a much a smaller aerodynamic diameter than spherical particles of similar mass or volume. Therefore, the controlled growth of crystals in one direction, or other preparations of needle-like particles, can produce particles which have a narrow distribution of aerodynamic diameters as long as their short axes are reasonably uniform, although they may have a wide range of lengths. As the deposition of particles is dependent of their particle size and distribution, elongated particles that have an aerodynamic diameter capable of penetrating into deep lung will have higher mass than spherical particles of similar diameters. Furthermore, the dimension of the shortest axis of an elongated particle can be expected to be reasonably uniform. A narrower size distribution will confer upon the particles a higher selectivity of deposition to the different regions in the respiratory tract. Elongated particles which reach the alveolar region can deposit there by the mechanism of interception whereas spherical particles of the similar aerodynamic diameter (< 2 \( \mu \)m) will have a high probability of being exhaled unless prolonged breath-holding is exercised. Chan and Gonda (1989) were able to prepare elongated crystals of cromoglycic acid by precipitation of the drug with hydrochloric acid from aqueous solutions of cromolyn sodium and subsequent recrystallisation from hot water or mixtures of dimethyl sulphoxide and water. The aerodynamic size distribution, as measured in a cascade impactor, was characterised by a logarithmic normal function with a mass median aerodynamic diameter of 0.7 \( \mu \)m and GSD of 1.9. These particles were thought to be more favourable in terms of deep lung penetration than micronised drug particles. Hickey et al. (1992) obtained elongated disodium cromoglycate particles after the treatment of the aerosol with a hydrophobic molecule, lauric acid. The aerodynamic properties of the elongated particles were investigated using an inertial impactor. The equivalent diameters of the particles (i.e. the diameters of spherical particles that have the same volume as the elongated particles) deposited on each stage of the impactor were markedly higher than the normal cut-off diameters of the stages. As the cut-off diameters were calibrated by spherical particles, these results suggested that elongated particles with larger volume or mass can deposit at the the same stage as spherical particles having a smaller volumes or masses. Each elongated particle can be expected to carry more drug to the stage on which it deposits than an equivalent sphere.
However, this phenomenon needs to be confirmed in vivo and most importantly, in clinical practice.

1.4.2.3d Hygroscopic growth of particles in the airways

Many drugs intended for inhalation are water-soluble, hygroscopic materials. They will absorb the ubiquitous water vapour present within the warm and humid environment of the respiratory tract. Consequently, the sizes and densities of the particles will change after inhalation and this will eventually affect the site of deposition.

The hygroscopic growth of atmospheric pollutants upon entry into the lung has long been a subject of interest following industrialisation and similar effects for therapeutic aerosols have been well documented, especially for aerosols given by MDI and nebulisers (Pritchard, 1987). From a physico-chemical point of view, when the pressure of the water vapour produced by a particle is less than that of the ambient atmosphere, water molecules will condense on the particles until the particle water pressure is the same as the ambient water vapour. For insoluble particles, any adsorbed water molecules will remain on the particle surface to form a thin film that can rapidly reach equilibrium with external pressure of water vapour. Thus, the effect of humidity on the particle size of these materials may be negligible for most practical purposes. However, for a water-soluble particle, a solution will form on its surface and water molecules will penetrate into the particles. The adsorption and absorption of water vapour will result in growth of the particle. The hygroscopic growth of particles can be assessed by growth ratio and rate. The growth ratio is a parameter which takes into account the final diameter of a particle after the growth has reached the equilibrium, i.e. the ratio of particle size after hygroscopic growth to the initial size, whilst growth rate is a dynamic parameter used to assess the rate at which the particle size grows. The growth ratio of a particle is mainly determined by the initial particle diameter and the relative humidity of the environment. For example, the growth ratio of ultrafine particles (< 0.1 µm) increases with initial particle diameter whilst the growth of particles greater than approximately 0.5 µm is essentially a constant, independent of the initial particle size (Xu and Yu, 1985). The growth ratio is drastically increased by a small increase in relative humidity, regardless of the initial particle size (Xu and Yu, 1985). The growth ratio of disodium cromoglycate was calculated as 2.60, assuming a relative humidity of 99.5 % and
growth under equilibrium conditions (Gonda et al., 1981). However, the growth ratio of the same drug was measured experimentally to be 1.31, the measured value being much smaller, as is the case for other drugs, than the calculated value.

The growth rate of a particle is also dependent upon its initial diameter. The smaller the particle, the more quickly it will reach its equilibrium diameter. Morrow (1986) calculated the growth rates of particles with diameters of 0.2, 0.6, 2.0, 6.0 and 10.0 $\mu$m in different generations of airways at inhalation rates of 6 and 60 l min$^{-1}$. Fine particles (< 0.6 $\mu$m) were shown to attain their equilibrium diameters within a residence time of approximately 1 s but larger particles did not reach equilibrium even after 10 s. Since lower inhalation flow rates can increase the residence time of particles in each generation of respiratory tract, the growth of ultrafine particles may reach equilibrium within the 10th generation at 6 l min$^{-1}$ but within the 20th generation at 60 l min$^{-1}$.

The hygroscopic growth of water-soluble particles may be reduced by the coatings of these particles with non-hygroscopic materials such as fatty acids. For example, disodium fluorescein (DF) dry powders have been coated with lauric or capric acid by an adsorption/coacervation technique (Hickey et al., 1988). The \textit{in vitro} hygroscopic growth of the coated and uncoated powders were investigated under controlled temperature (37°C) and relative humidity (20 and 97% RH) (Hickey, 1988; 1990). After exposure to controlled conditions for 40 s, disodium fluorescein particles exhibited a hygroscopic growth ratio of 1.5 at a relative humidity of 97%. This growth was reduced to 1.3 by coating with either 0.15 g of lauric acid or 0.8 g of capric acid per gram of DF, and was eliminated when either 0.2 g of lauric acid or 0.18 g of capric acid per gram of DF were added. The reduced growth ratio was attributed to the retardation of the rate of hygroscopic growth by formation of a hydrophobic coat around the fluorescein particles and an increased capacity to absorb water in order to form droplets whose vapour pressure is the same as that of the surrounding atmospheres. Predictably, the dissolution rate of DF, after coating with the hydrophobic materials was found to decrease with increased surface coverage.

Disodium cromoglycate, a hygroscopic drug, was subject to similar treatment by coating the drug particles with lauric acid (Hickey et al., 1992). In this case, the possible reduction in
the hygroscopic growth of the drug was found to be complicated with a change in the shape of coated particles. Elongated particles were prepared after the coating process and, as discussed above, the geometric change may have brought about some advantages for the controlled lung delivery over more spherical forms even in the absence of any alteration of hygroscopicity.

Coating of hygroscopic drug particles may not only reduce the hygroscopic growth of inhaled particles in the airways but also alter other physico-chemical properties of the drug. For example, the coating of hygroscopic drugs with hydrophobic materials will undoubtedly reduce the absorption of water during preparation and storage. As mentioned previously, water uptake will increase the cohesion and adhesion of powders through capillary forces, decreasing the deaggregation and dispersion of drug particles in the air stream. Through a careful choice of coating materials, powders of desirable physico-chemical properties, which have the optimum interparticulate forces and maximum physico-chemical stabilities, may be prepared to achieve an optimum delivery of drugs to the lung.

1.5 Aim and Scope of the Thesis

It is the aim of this project to investigate the effects of morphological properties of carrier particles and some of the formulation factors of the drug-carrier mixtures on drug delivery from dry powder aerosols, employing a model powder formulation composed of salbutamol sulphate and lactose. Lactose particles will be prepared by crystallisation under different conditions with a view to preparing the carrier particles of different morphological properties such as particle size, particle shape and surface smoothness. The crystal morphology, crystal form and crystallinity will be determined using image analysis, scanning electron microscopy, differential scanning calorimetry and X-ray powder diffraction. Lactose particles will then be blended with salbutamol sulphate using different mixing protocols so as to obtain powder formulations of different, but precisely defined morphology and particle size distributions. The detachment properties and deposition profiles of salbutamol sulphate from different powder formulations will finally be studied using various inertial impaction techniques.
CHAPTER TWO

CRYSTALLISATION OF LACTOSE FROM AQUEOUS SOLUTIONS
2.1 Introduction

Lactose, 4-(β-D-galactosido)-D-glucose, is one of the most widely used excipients in the pharmaceutical industry. Lactose can be obtained in either two basic isomeric forms, α and β-lactose (Figure 2.1), or as an amorphous form. α-Lactose monohydrate is obtained by crystallisation from a supersaturated solution at temperatures below 93.5°C, whereas β-lactose crystals are obtained at temperatures over 93.5°C (Nickerson, 1974). During crystallisation of β-lactose, no water is incorporated in the crystal lattice. Therefore, the crystals of β-lactose exist in a non-hygroscopic, anhydrous form only in contrast with α-lactose, which occurs both as the monohydrate and as anhydrous α-lactose. Dehydration by thermal treatment or desiccation of α-lactose monohydrate can convert it into the anhydrous form. For example, the treatment of α-lactose monohydrate in vacuo at temperatures of 100-130°C can result in the production of a very hygroscopic product known as unstable anhydrous α-lactose (Itoh et al., 1978). However, thermal treatment in a humid atmosphere at temperatures over 110°C, or desiccation with suitable liquids, such as dry methanol, may result in a non-hygroscopic product, called stable anhydrous α-lactose (Itoh et al., 1978).

Amorphous lactose can be prepared after extensive mechanical grinding of crystallised lactose (Morita et al., 1984) or by either spray drying (Fell and Newton, 1971) or freeze-drying of lactose solutions (Morita et al., 1984). The amorphous lactose is softer than crystallised lactose and it improves the compression force/hardness profile of the lactose. The amorphous lactose or crystallised lactose with a portion of the amorphous lactose has been used as a directly compressible excipient in tableting (Lerk, 1993). Different crystal forms of lactose exhibit different physical properties. For example, anhydrous α-lactose was reported to have better binding capacity than α-lactose monohydrate (Lerk et al., 1983). Due to their preferential properties in certain pharmaceutical processes such as tableting, all the crystal forms of lactose mentioned above have been commercially available (Wade and Weller, 1993a).
Chapter two: Crystallisation of lactose from aqueous solutions

α-Lactose monohydrate has been observed in a wide variety of shapes, depending on the conditions of crystallisation. The principal factor determining the crystal habit of lactose is the supersaturation, i.e. the ratio of actual concentration to the solubility at the specific temperature (Herrington, 1934). At high supersaturation, the crystallisation is forced rapidly and only elongated prisms form (A or B in Figure 2.2). As the supersaturation decreases, the dominant crystal shape changes to diamond-shaped plates (C), then to pyramids (D), which result from an increase in the thickness of the diamond. With slow crystallisation, lactose crystals usually exhibit the shape of tomahawks (E), which is the most common shape of lactose crystals.

Figure 2.1 Chemical structures of lactose

α - lactose

β - lactose

Figure 2.2 A diagram showing some crystal habits of α-lactose monohydrate crystals.

These habit modifications arise due both to differences in the growth rates of the individual faces and changes in these relative growth rates with supersaturation (van Kreveld and Michaels, 1965; Michaels and van Kreveld, 1966). It has been shown that lactose crystals
grow only in one direction of the principal axis and therefore such crystals have their nucleus in the apex of the tomahawk (van Kreveld and Michaels, 1965).

The growth rate of lactose crystals is determined by many factors, such as supersaturation, temperature and additives. For example, the growth rate was found to increase with the power of the supersaturation greater than 1 (van Kreveld and Michaels, 1965). Average growth rates of lactose crystals as determined from the population balance approach in a continuous-cooling crystalliser increased with supersaturation (expressed as concentration difference) to the 3.55 power (Griffith et al., 1982). In the crystallisation of α-lactose, the mutarotation of β-lactose to α-lactose in the solution can also be a rate-limiting process. The rate of mutarotation is influenced by both temperature and pH (Nikerson, 1974). Mutarotation is slow at low temperatures but increases as the temperature rises, becoming almost instantaneous at about 75°C. The mutarotation rate is minimum at pH 5.0, increasing with changes in pH on either side of this value. A change in temperature can affect the growth rate by means of changing supersaturation, rate of mutarotation from β-lactose to α-lactose, rate of nucleation and transfer of lactose molecules to the crystal surfaces (Hartel and Shastry, 1991). Although increasing temperature increased the rate of mutarotation, the rate of transfer of molecules from the solution to the crystal surfaces, therefore tending to promote the rate of crystal growth. Such an increase in temperature will decrease the supersaturation which tends to oppose crystal growth and may in some instances cancel out the effects of the opposing former factors. Therefore, the effect of temperature on the growth rate of lactose crystals is dependent upon the supersaturation and the ranges of the temperatures employed. The effects of additives on crystal growth of lactose is even more complicated. Some can cause a marked retardation in the growth rate of lactose crystals, whereas others can accelerate growth rates on specific crystal faces (Michaels and van Kreveld, 1966). Additives may alter the growth rate of lactose crystals by means of either reducing the edge energy at dislocation centres on the crystal face, thereby favouring more rapid step-generation rate by permitting a higher curvature of steps near a dislocation; or retarding step-propagation by adsorption of the additive on the crystal face (Nikerson, 1974). Alternatively, the presence of an impurity changes the kinetics of the crystallisation due to changes in solubility of lactose. The change in solubility results in a change of
supersaturation of the solution, thereby affecting both nucleation and growth rate (Mullin, 1979).

The crystallisation of lactose has received most attention of all sugars especially in the dairy industry and is the subject of some excellent reviews (Hartel and Shastry, 1991; Nickerson, 1974). However, most of the studies have focused on the crystallisation of lactose from commercial cheese whey, since this is the primary source of refined lactose. The principal reasons for the study of lactose crystallisation, in the dairy industry, are either with a view to inhibiting crystal growth of lactose so as to prevent 'sandiness' in some food products such as ice cream (Nickerson, 1962) or to maximising the gross yield of lactose crystals. The rate of lactose crystallisation is expressed as either the rate of reduction in lactose concentration in the solution (Whittier and Gould, 1931) or the increase in the weight of lactose mass (Guu and Zall, 1991). Although different crystal forms of lactose have been commercially available to pharmaceutical industry, few studies have been carried out to investigate the effects of crystallisation conditions on the particle morphology of lactose, such as particle size, particle shape and surface texture. These morphological factors of lactose crystals are of great importance in determining their physical properties and in particular, they play a crucial role in determining the surface interaction of lactose particles with other adhered particles such as drug powders. Therefore, it is the aim of the present work to investigate the effects of some preparative conditions on the morphology of lactose particles with a view to preparing lactose particles of favourable size, shape and surface texture for the delivery of drug to the lower airways. However, the crystallisation kinetics of lactose from different solutions, which is beyond the scope of this work, will not be considered.
2.2 Material and methods

2.2.1 One-step crystallisation from aqueous solutions

A predetermined amount of lactose (Lactochem®, Borculo Whey Ltd., Chester, UK) was dissolved in 100 ml distilled water at 80°C. After filtration through a Whatman filter paper (<0.45 μm), the solution was transferred to a 150 ml glass beaker which had been placed in either an ice bath or a water bath at 40°C. The solution was stirred at 500 rpm (Heidolph Overhead Stirrer, Fisons Laboratory Instruments, UK) with a 4 blade (1×3 cm) stirrer which was situated 2 cm above the bottom of the container. After the crystallisation was allowed to continue for a predetermined period of time, the crystals were filtered and washed sequentially with 60% (v/v) and absolute ethanol, respectively. The crystals were allowed to dry at room temperature overnight before drying in an vacuum oven at 70°C for 3 h. After a small amount of sample (about 0.5 g) was taken from each batch of lactose for the measurement of particle size, shape and surface smoothness, the remaining lactose crystals were poured into a 90 μm sieve which had been placed upon a 63 μm sieve. The particles were then sieved manually and slowly for 1 h so as not to rupture any crystals. The particles were divided into 3 size fractions (< 63, 63-90 and > 90 μm), which were collected and weighed seperately. The lactose crystals thus obtained were transferred to a sealed vial and placed into a desiccator over silica gel until required for further investigation.

2.2.2 Two-stage crystallisation

Lactochem® lactose (200 g) was dissolved in 200 ml distilled water at about 90°C. The solution (about 320 ml) was filtered while still hot through a Whatman filter paper (0.45 μm). It was then transferred to 500 ml glass beaker and stirred at 500 rpm with a 4 blade (1×3 cm) stirrer which was situated 2 cm above the bottom of the container. Lactose was then allowed to crystallise under constant stirring at room temperature at 500 rpm for 2.5 h. The crystals (A) were filtered and the mother liquor was placed back into the beaker and allowed to crystallise further for 16 h to obtain crystals B (Figure 2.3). Both batches of crystals A and B were washed with 60% (v/v) and absolute ethanol, respectively, and were allowed to dry at room temperature overnight. The lactose crystals were poured into a 90
μm sieve which had been placed upon a 63 μm sieve. The particles were then sieved manually and slowly for 1 h so as not to rupture any crystals. The crystals A were classified into batches 13 and 14, which had a particle size range from 63-90 μm and < 63 μm, respectively. The B crystals were classified into batches 15 and 16 with particle sizes of between 63-90 μm and < 63 μm, respectively. The crystals were then dried in a vacuum oven at 70°C for 3 h. The lactose crystals thus obtained were transferred to a sealed vial and placed in a desiccator over silica gel until required for further investigation.

![Lactose solution (50% w/w)](image)

Crystallisation under constant stirring at 500 rpm at room temperature for 2.5 h

Crystals (A) 
Mother liquor

Batch 13 (63-90 μm) 
Batch 14 (< 63 μm)

Crystals (B) 
Mother liquor

Batch 15 (63-90 μm) 
Batch 16 (< 63 μm)

To be recycled

Figure 2.3 The procedures used to prepare lactose particles by two-stage crystallisation

2.2.3 Crystallisation from aqueous solutions with small amounts of organic solvents

Lactochem® lactose (75 g) was dissolved in 100 ml distilled water at about 90°C. The solution was filtered through a Whatman filter paper while hot. After cooling to room temperature without any disturbance, the solution (about 175 ml) was then transferred to a 250 ml beaker and stirred at 500 rpm with a 4-blade stirrer (1x3 cm). A predetermined
amount of an organic solvent (20 ml) was added dropwise to the solution such that the final concentration of the organic solvent in the solution was 10% (v/v). The particle size and morphology were constantly monitored by observation of the crystallisation process using a microscope. After stirring for a fixed period of time, which was dependent upon the crystallisation conditions, the crystals were filtered and washed with 60% and the absolute ethanol. Finally the crystals were allowed to dry at room temperature before being stored in a desiccator over silica gel.

The particle size was measured using an optical microscope (see Section 2.2.4.1) at different periods of crystallisation after nucleation. Only 50 particles were measured for each sample since sizing using this method is a time-consuming process. The crystals were still growing so that measuring more particles would have taken longer time and would have led to less accuracy.

2.2.4 Characterization of lactose crystals

2.2.4.1 Particle size measurement by optical microscopy and image analysis
The particle size of lactose crystals was determined by optical microscopy and image analysis. Slides were prepared by dispersing a very small amount of lactose particles. The fields were selected randomly. The images were projected from the microscope (Nikon Labophot, Tokyo, Japan) onto a a monitor via a video camera and the images were analysed using image analysis software, developed in-house (King’s College London) stored on a computer. At least three hundred particles were measured for each batch of lactose and the surface-volume diameters recorded.

2.2.4.2 Determination of particle shape and surface textures by scanning electron microscopy (SEM)
Particle shape and surface textures were determined by scanning electron microscopy (SEM). Double-sided adhesive tape was placed on an aluminum stub and after stripping off the upper side of the adhesive, a small amount of particles was scattered on the stub and dispersed by tapping lightly on the edge of the stub with a spatula to break agglomerates. The particles were then coated with approximately 15 to 20 nm gold using a sputter coater
(Polaron E5100, Polaron Equipment Ltd., Watford, UK) using an electrical potential of 2.0 kV, 20 mA. Several photomicrographs were produced by scanning fields, selected randomly, at several magnifications with a Philips SEM501B scanning electron microscope (Einhoven, Holland).

### 2.2.4.3 The quantification of rugosity (sphericity) of lactose particles

#### 2.2.4.3a True density of lactose

The true density of lactose was measured using a Beckman Air Comparison Pycnometer (Model 930, Beckman Instrument, Inc., Fullerton, U.S.A). Each sample was measured in triplicate and the mean was taken.

#### 2.2.4.3b Measurement of specific surface area by air permeation

The surface area of the various lactose fractions was determined by an air permeation method employing a Fisher sub-sieve sizer (Gooden and Smith, 1940; Gooden, 1941) which measures the ability of air to flow through a packed powder bed.

A mass of powder equal to its true density was compressed to different porosities in the cell of the Fisher sub-sieve sizer. The flow rate of air through the bed at a fixed pressure differential was transformed by the instrument to an average particle diameter \((d_a)\). The measured diameter increases with the decreasing porosity and finally reaches a plateau. The maximum diameter under low porosity was considered to be the true diameter and employed to calculate the specific surface area \((A_s)\) of the particles according to the following equation:

\[
A_s = \frac{6 \times 10^4}{d_a \rho} \quad (2-1)
\]

where \(\rho\) is the true density of the particles measured by the pycnometer.

Each sample was measured in duplicate and the mean particle size was taken to calculate the specific surface area of the particles.
2.2.4.3c Determination of specific surface area by optical microscopy

The particle size of lactose particles was measured using image analysis optical microscopy as described in Section 2.2.4.1. In order to calculate the surface area of the particles from microscopic data, the following equations were employed:

\[
\begin{align*}
\text{Since } d_s &= \left( \frac{\Sigma n d^2}{\Sigma n} \right)^{1/2} \\
\Sigma n d^2 &= \Sigma n \times d_s^2 \\
\text{Therefore } S &= \Sigma n \times \pi \times d_s^2 = \pi \Sigma n d^2 \\
\text{As } d_v &= \left( \frac{\Sigma n d^3}{\Sigma n} \right)^{1/3} \\
\text{So } \Sigma n d^3 &= d_v^3 \times \Sigma n \\
V &= d_v^3 \times \Sigma n \times \pi / 6 = \pi \Sigma n d^3 / 6 \\
\end{align*}
\]

(2-2)

Where \( d \) is the diameter of individual particles, \( d_s \) is the mean diameter of spherical particles having the same surface area (S) as the particles and \( d_v \) is the mean diameter of spherical particles having the same volume (V) as the particles.

These equations can be applied to obtain the specific surface area (\( A_m \)) of the particles determined by microscopic method:

\[
A_m = \frac{S}{(V \times \rho)} = \frac{\pi \Sigma n d^2}{\rho \times \pi \times \Sigma n d^3 / 6} = \frac{6}{\rho \times \Sigma n d^3 / \Sigma n d^2} = \frac{6 \times 10^3}{\rho d_{sv}} \text{ (m}^2 \text{ kg}^{-1})
\]

(2-3)

\( d_{sv} \) is the surface volume mean diameter of the particles, measured by the microscopic method.

2.2.4.3d Calculation of rugosity of lactose particles

The rugosity (\( R_s \)) of a particle is defined as the ratio between the actual specific surface area, \( A_s \), obtained by the air permeation method (Section 2.2.4.3b) and the specific surface area \( A_m \), obtained by the microscopic method (Section 2.2.4.3c) (Carstensen, 1980).
\[ R_s = \frac{A_n}{A_{\text{area}}} = \frac{d_m}{d_a} \]  

(2.4)

2.2.4.4 Quantification of particle shape

A small amount of lactose particles was scattered on a microscope slide using a small brush ensuring that the particles deposited separately. The slide was then mounted on an optical microscope (Labophot-2, Nikon, Japan) and the images of the particles were transferred to an IBM compatible computer through a Nikon camera. Particle images were analysed automatically using analySIS 2.0 (SIS Image Analysis GmbH, Germany) and a combination of different descriptors was employed to quantify the morphology of lactose crystals (Table 2.1). All the particles that were projected onto the monitor were analysed and more than 100 particles were measured for each batch.

Table 2.1 Some of the morphological descriptors of particles measured by the image analysis (after the User’s Guide to analySIS 2.0)

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>Equations</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elongation ratio</td>
<td>Length / Width</td>
<td>Elongation of the particle</td>
</tr>
<tr>
<td>Shape factor</td>
<td>[ \frac{4\pi \text{area}}{\text{perimeter}^2} ]</td>
<td>A combination of shape and surface smoothness</td>
</tr>
<tr>
<td>Martin's radius mean</td>
<td>[ \frac{\sum r_m}{n} ]</td>
<td>Mean of all distances from a particle's centre of gravity to boundary points ((r_m)).</td>
</tr>
<tr>
<td>Max Martin's radius</td>
<td>(r_{\text{max}})</td>
<td>The maximum distance of a particle's centre of gravity to boundary points.</td>
</tr>
<tr>
<td>Min Martin's radius</td>
<td>(r_{\text{min}})</td>
<td>The minimum distance of a particle's centre of gravity to the boundary points</td>
</tr>
</tbody>
</table>

2.2.4 Data analysis

All data were analysed by two-tailed t-test, multiple regression and ANOVA as appropriate, using Minitab® for Windows Version 10.2 (Minitab Inc., USA).
2.3 Results and discussion

2.3.1 One-step crystallisation of lactose from aqueous solution

Table 2.2 The conditions used to crystallise lactose and the resultant particle size and shape of the lactose crystals prepared.

<table>
<thead>
<tr>
<th>Batch</th>
<th>Lactose (% w/w)</th>
<th>T (°C)</th>
<th>Time (h)</th>
<th>Diameter (d_m) ± SD (μm)</th>
<th>% Particle (&lt; 63)</th>
<th>63-90</th>
<th>&gt; 90</th>
<th>Shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>No 1</td>
<td>33</td>
<td>40</td>
<td>12</td>
<td>83.6 ± 12.8</td>
<td>13.9</td>
<td>45.8</td>
<td>40.3</td>
<td>Tomahawk</td>
</tr>
<tr>
<td>No 2</td>
<td>33</td>
<td>40</td>
<td>24</td>
<td>115.8 ± 14.6</td>
<td>5.6</td>
<td>15.1</td>
<td>79.3</td>
<td>Tomahawk</td>
</tr>
<tr>
<td>No 3</td>
<td>33</td>
<td>0</td>
<td>24</td>
<td>100.3 ± 18.9</td>
<td>15.2</td>
<td>17.2</td>
<td>67.6</td>
<td>Irregular</td>
</tr>
<tr>
<td>No 4</td>
<td>43</td>
<td>0</td>
<td>5</td>
<td>94.4 ± 13.4</td>
<td>19.6</td>
<td>21.8</td>
<td>56.6</td>
<td>Irregular</td>
</tr>
<tr>
<td>No 5</td>
<td>43</td>
<td>0</td>
<td>12</td>
<td>104.5 ± 14.8</td>
<td>14.9</td>
<td>23.2</td>
<td>61.9</td>
<td>Irregular</td>
</tr>
<tr>
<td>No 6</td>
<td>43</td>
<td>40</td>
<td>5</td>
<td>103.8 ± 20.6</td>
<td>14.4</td>
<td>21.6</td>
<td>64.0</td>
<td>Tomahawk</td>
</tr>
<tr>
<td>No 7</td>
<td>33</td>
<td>0</td>
<td>12</td>
<td>63.7 ± 9.4</td>
<td>33.0</td>
<td>40.0</td>
<td>26.8</td>
<td>Irregular</td>
</tr>
<tr>
<td>No 8</td>
<td>43</td>
<td>40</td>
<td>12</td>
<td>100.6 ± 15.3</td>
<td>24.5</td>
<td>17.9</td>
<td>57.6</td>
<td>Pyramid</td>
</tr>
<tr>
<td>No 9</td>
<td>50</td>
<td>40</td>
<td>3</td>
<td>88.8 ± 13.8</td>
<td>27.5</td>
<td>31.9</td>
<td>40.6</td>
<td>Prism</td>
</tr>
<tr>
<td>No 10</td>
<td>60</td>
<td>40</td>
<td>0.3</td>
<td>76.4 ± 15.7</td>
<td>33.8</td>
<td>46.3</td>
<td>19.9</td>
<td>Elongated</td>
</tr>
<tr>
<td>No 11</td>
<td>60</td>
<td>40</td>
<td>1.5</td>
<td>91.8 ± 17.9</td>
<td>26.3</td>
<td>27.6</td>
<td>46.1</td>
<td>Elongated</td>
</tr>
</tbody>
</table>

Lactose particles of different size and shape were prepared under different crystallisation conditions (Table 2.2). All the factors investigated, namely, the initial concentrations of lactose, crystallisation temperature and time period of crystallisation, had an impact on the mean diameter of lactose crystals. With a multiple linear regression by Minitab® for Windows (Version 10.2, Minitab Inc. USA), the following empirical equation can be obtained to assess the effects of different factors on the mean diameter of lactose particles prepared under the specific conditions as employed in the experiment.

Mean diameter (μm)

\[
= 1.50 C_L + 0.13 T_c + 2.46 t_c \\
\text{r} = 0.80
\]  

(2.5)
where \( C_L \) is the initial lactose concentration in % w/w, \( T_c \) is the crystallisation temperature in °C and \( t_c \) is the time period (h) of crystallisation.

According to equation 2-5, the particle size of lactose crystals increases with an increase in the initial concentration of lactose or crystallisation temperature or the time period of crystallisation. For example, the mean particle size was found to increase from 83.8 μm for batch 1 to 100.6 μm for batch 8 when initial lactose concentrations increased from 33% to 43% (w/w) after crystallisation at 40°C for 12 h. This increased mean size of lactose crystals can be attributed to an increased growth rate of lactose crystals at higher initial concentrations.

Crystallisation at higher temperature (40°C) produced larger crystals than at lower temperature (0°C). The effect of temperature on crystal growth is more complicated than that of the concentration. Temperature influences two important aspects of the crystallisation process, namely, supersaturation and the crystallisation rate constant including rate of diffusion, rate of mutarotation and rate of orientation of lactose molecules into the crystal lattice, all of which will increase with temperature. However, supersaturation decreases with temperature. If the supersaturation is not substantially reduced, then increasing the temperature would be expected to accelerate crystal growth by decreasing viscosity and increasing kinetic activity of lactose molecules. For example, increasing the temperature from 30° to 50°C was shown to increase the rate of lactose crystal growth but no further increases in growth were observed between 50 and 70°C (Jelen and Coulter, 1973).

It can be easily expected that the longer the crystals are allowed to grow, the larger the final particles will be. Since lactose particles employed as a carrier for dry powder aerosols are usually required to have a size between 63-90 μm, the time period of crystallisation is one of the major operational parameters to be controlled in order to obtain desirable particle size of lactose crystals. However, lactose crystals prepared under all the conditions investigated exhibited a wide range of particle size distribution with relative standard deviation (SD/Mean) over 15%, resulting in a small portion of particles with a size range between 63-90 μm (Table 2.2). Crystal size distribution was attributed to the inherently different rates of
growth between crystals (Janse and deJong, 1976) although it has also been postulated that crystals may exhibit random fluctuations in growth rates (Randolph and White, 1977). Lactose nuclei generated by a gentle sliding contact exhibited two different populations in terms of growth rate. Some crystals grew rapidly while others barely grew under the same conditions (Shi et al., 1989). Such an observation was further confirmed by using a two component rate distribution model which indicated the existence of two distinct types of nuclei, which can be classified as fast-growing and slow growing crystals (Liang et al., 1991).

Figures 2.4-2.14 shows the scanning electron micrographs of lactose crystals prepared under different conditions. It can be seen that different batches of lactose crystals have different shapes, which appear to be dependent upon the initial concentrations of lactose. At lower initial lactose concentrations (33% and 43%, w/w), the majority of the crystals obtained were either tomahawk- or pyramid-shaped (batches 1, 2, 6 & 8). When the concentration of lactose was increased to 50% (w/w), most of the resultant crystals were prismatic in shape (batch 9). When the concentration was increased to 60%, elongated particles were produced (batches 10 & 11). The change in crystal shape with the initial concentration is in agreement with a previous report (Nickerson, 1974). Different shapes of lactose crystals have been reported to be due to the different relative growth rates on the crystal faces at different supersaturations (Van Kreveld and Michaels, 1965).

Crystals prepared in an ice bath were shown to possess rougher surfaces and more irregular shape than particles prepared at an elevated temperature (40°C). For example, batch 1 lactose crystals (Fig. 2.4) were more regularly shaped with smoother surfaces than batch 3 lactose crystals (Fig. 2.6). It was also observed microscopically that the crystals tended to aggregate when prepared by rapid cooling using an ice bath. There are two opposite mechanisms involving in crystallisation: 1) the transfer of molecules from the solution onto the surface of a crystal, resulting in the growth of the crystal; 2) re-dissolving of the molecules from the crystals into the surrounding solution. If the former exceeds the latter, then the crystal will grow. However, if the latter dominates over the former, the crystal will start to dissolve. During crystallisation under mechanical stirring, crystal defects are
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Figure 2.4 The scanning electron micrograph of lactose batch 1

Figure 2.5 The scanning electron micrograph of lactose batch 2
Figure 2.6 The scanning electron micrograph of lactose batch 3

Figure 2.7 The scanning electron micrograph of lactose batch 4
Figure 2.8 The scanning electron micrograph of lactose batch 5

Figure 2.9 The scanning electron micrograph of lactose batch 6
Figure 2.10 The scanning electron micrograph of lactose batch 7

Figure 2.11 The scanning electron micrograph of lactose batch 8
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Figure 2.12 The scanning electron micrograph of lactose batch 9

Figure 2.13 The scanning electron micrograph of lactose batch 10
Figure 2.14 The scanning electron micrograph of lactose batch 11
unavoidable (Strickland-Constable, 1968). These defects, such as unwanted hills and edges on the crystals, occur owing either to the misarrangement of molecules in the crystal lattice or to the fracture of intact crystals by mechanical stirring. These distortions are more likely to dissolve in the surrounding solutions than the crystal surfaces themselves. An increased in temperature would accelerate the dissolution of these surface distortions, causing the production of smoother surfaces. Therefore, crystallisation at elevated temperatures appeared to favour production of regular crystal shape with improved surface smoothness. However, too high a temperature of crystallisation of lactose may result in a change in the ratio of $\alpha$- to $\beta$-form of the final products which may in turn influence the bulk properties of lactose particles.

Table 2.3 The density, mean diameter, specific surface area (SSA) of lactose particles.

<table>
<thead>
<tr>
<th>Batch No</th>
<th>Density (g cm$^{-3}$)</th>
<th>Diameter (µm)</th>
<th>SSA (cm$^2$ g$^{-1}$)</th>
<th>Rugosity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$d_{sv}$</td>
<td>$d_{air}$</td>
<td>$A_{m}$</td>
</tr>
<tr>
<td>Lactochem</td>
<td>1.53</td>
<td>88.7</td>
<td>47</td>
<td>442.1</td>
</tr>
<tr>
<td>1</td>
<td>1.54</td>
<td>93.6</td>
<td>47</td>
<td>416.3</td>
</tr>
<tr>
<td>2</td>
<td>1.53</td>
<td>95.8</td>
<td>47</td>
<td>409.3</td>
</tr>
<tr>
<td>3</td>
<td>1.55</td>
<td>91.8</td>
<td>34</td>
<td>421.7</td>
</tr>
<tr>
<td>4</td>
<td>1.53</td>
<td>87.6</td>
<td>34</td>
<td>447.7</td>
</tr>
<tr>
<td>5</td>
<td>1.54</td>
<td>88.3</td>
<td>40</td>
<td>441.2</td>
</tr>
<tr>
<td>6</td>
<td>1.54</td>
<td>94.9</td>
<td>42</td>
<td>410.5</td>
</tr>
<tr>
<td>7</td>
<td>1.55</td>
<td>92.1</td>
<td>50</td>
<td>420.3</td>
</tr>
<tr>
<td>8</td>
<td>1.53</td>
<td>91.7</td>
<td>48</td>
<td>427.7</td>
</tr>
<tr>
<td>9</td>
<td>1.52</td>
<td>97.2</td>
<td>65*</td>
<td>406.1</td>
</tr>
<tr>
<td>10</td>
<td>1.52</td>
<td>92.3</td>
<td>40</td>
<td>427.7</td>
</tr>
<tr>
<td>11</td>
<td>1.54</td>
<td>96.6</td>
<td>38</td>
<td>403.3</td>
</tr>
</tbody>
</table>

* estimated value since it exceeds the upper limit of Fisher subsieve sizer.

Table 2.3 shows the true density, specific surface area of different batches of lactose particles. The majority of lactose crystals showed a true density from 1.52 to 1.55 g cm$^{-3}$, similar to the value of 1.54 g cm$^{-3}$ for $\alpha$-lactose monohydrate (Wade and Weller, 1994a).
The rugosity value changes from 1.50 for lactose batch 9 to 2.70 for lactose batch 3. Most of the crystallised lactose particles showed a rugosity value higher than that of Lactochem™ lactose. The rugosity (sphericity), as defined previously (2.2.4.3d), is a term that combines the macroscopic shape and surface texture. Only a sphere with perfectly smooth surface will have a rugosity value of 1. A non-spherical particle will always have a rugosity value greater than 1 even though the particle may have a perfectly smooth surface. The more anisometric the particle is, the higher the rugosity value and vice versa. For example, a more elongated particle will have a higher rugosity value than a less elongated particle even if these two particles have a similar surface texture.

From Table 2.3 and Figures 2.4-2.14, it can be seen that the rugosity value generally correlates well with SEM observation, a higher rugosity value indicating higher surface roughness. Rugosity has been used interchangeably with surface roughness in some previous reports (Kassem, 1990). However, this is true only when the selected particles have similar shapes. For example, according to their SE micrographs, lactose crystals prepared at 40°C (batches 1, 2, 6 & 8) had similar shape to the particles prepared in an ice bath (batches 3, 4 & 5). Hence, the lower rugosity values for the former batches would indicate a smoother surface as compared with the latter batches and this can be justified by visual estimation by the SE micrographs of these particles. The exceptionally low rugosity value measured for lactose batch 7 (1.84), which might not be anticipated on the basis of the SE micrograph of the same batch (Fig. 2.10), might be due to the less elongated shape of these particles as compared with the rest of the batches. Lactose batch 9 had the lowest rugosity value (1.50) and this finding is supported by the SE micrograph of these particles (Fig. 2.12). However, the high rugosity values of batches 10 & 11 (2.31 and 2.54, respectively) may be primarily due to their elongated forms rather than to rougher surfaces. Therefore, great caution should be taken when rugosity is used to compare the surface roughness of crystals with different shapes. In order to represent the macroscopic and microscopic shape of a particle, rugosity should be employed in combination with other shape factors such as those obtained by image analysis.

Table 2.4 shows the results of image analysis of lactose crystals of different batches. It can be seen that lactose batch 3 had the lowest values of shape factor (0.60) and elongation ratio
Lactose batch 9 had the highest value of the shape factor (0.78) whilst lactose batch 11 had the highest value of elongation ratio (2.08). Similar to the rugosity, the shape factor, as defined (Table 2.1), is also a combination of particle shape and surface textures. A spherical particle with a smooth surface will have a shape factor of 1 whilst irregularly shaped particles have shape factor values less than 1. The more irregular the shape and/or rougher the surface, the smaller the shape factor. Elongation ratio is a shape descriptor, the higher the elongation ratio then the more elongated the particle. In general, more elongated particles (higher values of elongation ratio) have smaller values of the shape factor. Martin’s radius is also affected by both the particle shape and surface smoothness. The ratio of the maximum Martin’s radius to the minimum Martin’s radius reflects the change in either surface smoothness or in particle shape. Therefore, a higher ratio would suggest either a rougher surface or a more irregular shape. The combination of shape factor, elongation ratio and Martin’s radius provides a detailed description of the particle shape and surface smoothness. For example, lactose crystals batch 3 have the lowest value of elongation ratio (1.28) and shape factor (0.60) but the highest value (2.59) of ratio of maximum to minimum Martin’s radius. These results suggest that this batch of crystals are the least elongated with the highest surface roughness. Lactose crystals from batch 10 have a low value of shape factor (0.68) but high values of elongation ratio (2.08) and ratio of maximum to minimum Martin’s radius (2.40), indicating they are elongated particles with rough surfaces.
Table 2.4 Some shape factors of lactose crystals (n > 100)

<table>
<thead>
<tr>
<th>Batch No</th>
<th>Shape factor</th>
<th>Elongation ratio</th>
<th>$r_{max}/r_{min}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Lactochem</td>
<td>0.74</td>
<td>0.09</td>
<td>1.68</td>
</tr>
<tr>
<td>1</td>
<td>0.74</td>
<td>0.11</td>
<td>1.39</td>
</tr>
<tr>
<td>2</td>
<td>0.74</td>
<td>0.10</td>
<td>1.39</td>
</tr>
<tr>
<td>3</td>
<td>0.60</td>
<td>0.14</td>
<td>1.28</td>
</tr>
<tr>
<td>4</td>
<td>0.68</td>
<td>0.10</td>
<td>1.29</td>
</tr>
<tr>
<td>5</td>
<td>0.72</td>
<td>0.09</td>
<td>1.30</td>
</tr>
<tr>
<td>6</td>
<td>0.69</td>
<td>0.12</td>
<td>1.64</td>
</tr>
<tr>
<td>7</td>
<td>0.74</td>
<td>0.09</td>
<td>1.34</td>
</tr>
<tr>
<td>8</td>
<td>0.72</td>
<td>0.12</td>
<td>1.37</td>
</tr>
<tr>
<td>9</td>
<td>0.78</td>
<td>0.06</td>
<td>1.63</td>
</tr>
<tr>
<td>10</td>
<td>0.68</td>
<td>0.11</td>
<td>2.08</td>
</tr>
<tr>
<td>11</td>
<td>0.73</td>
<td>0.08</td>
<td>1.71</td>
</tr>
</tbody>
</table>

A close relationship was observed between rugosity and shape factor (Figure 2.15). As might be expected from the equations defining rugosity (section 2.2.4.3d) and shape factor, particles with higher rugosity values have smaller values of shape factor. Higher rugosity represents either a rougher surface or more irregular shape and in either case, the value of shape factor will decrease as a result of an increase in particle perimeter. Such a relationship between the 2-dimensional shape factor and the 3-dimensional rugosity value suggests that different faces of the lactose crystals may have similar surface smoothness.
Both crystallisation temperature and initial concentration of lactose affect the shape of lactose crystals, as expressed by the elongation ratio. Crystallisation from a higher initial concentration of lactose tends to produce more elongated lactose crystals (higher value of elongation ratio) regardless of the temperature of the mother liquor. For example, an initial lactose concentration of $\geq 50\%$ (w/w) produced lactose crystals (batches 9, 10 & 11) with a significantly higher ($p < 0.001$) value ($1.78 \pm 0.48, n = 208$) of elongation ratio than the crystals ($1.39 \pm 0.28, n = 371$) prepared from an initial lactose concentration of $43\%$ w/w (batches 4, 5 & 8), which in turn have a significantly higher ($p < 0.05$) elongation ratio ($1.34 \pm 0.26, n = 277$) than the crystals obtained from an initial lactose concentration of $33\%$ w/w (batches 1-3 & 7). Crystallisation at a higher temperature also appeared to produce more elongated lactose crystals. For example, the combination of all lactose crystals prepared at $40^\circ$C (batches 1, 2, 5, 8-11) have an elongation ratio of $1.60 \pm 0.42 (n = 484)$, which is significantly higher ($p < 0.001$) than the elongation ratio ($1.30 \pm 0.22, n = 373$) of the combination of lactose prepared at $0^\circ$C (batches 2, 3, 4, 5 & 7). According to Table 2-5, crystallisation temperature appears to be dominant ($F = 44.35$) over the initial concentration of lactose ($F = 28.95$) in determining the shape of lactose particles when the initial lactose concentrations were varied between $33-43\%$ w/w at $0^\circ$C and $40^\circ$C. However, at higher
lactose concentrations such as more than 50% (w/w), crystal shape is predominantly
determined by the initial lactose concentrations as can be seen from the more elongated
shape of lactose batches 9, 10 & 11.

Table 2.5 Analysis of variance (ANOVA) of elongation ratio of lactose particles prepared
with an initial lactose concentration between 33 and 43% (w/w) at 0°C and 40°C

<table>
<thead>
<tr>
<th>Source</th>
<th>Degree of Freedom</th>
<th>Sum of Square</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>1</td>
<td>1.8547</td>
<td>1.8547</td>
<td>28.95</td>
<td>0.000</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>2.8417</td>
<td>2.8417</td>
<td>44.35</td>
<td>0.000</td>
</tr>
<tr>
<td>Concentration*Temperature</td>
<td>1</td>
<td>0.9439</td>
<td>0.9439</td>
<td>14.73</td>
<td>0.000</td>
</tr>
<tr>
<td>Error</td>
<td>196</td>
<td>12.5591</td>
<td>0.0641</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>199</td>
<td>18.1996</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Apart from the elongation ratio, crystallisation temperature also affected the shape factor of
the lactose crystals. Crystals produced at 40°C had a shape factor of 0.73 ± 0.11 (n = 766),
which is significantly higher (p < 0.001) than that (0.69 ± 0.12, n = 541) of the crystals
prepared at 0°C. Although initial lactose concentrations of 33% and 43% appeared to
produce lactose of different shape factor (p < 0.01, Table 2.6), the effects of initial lactose
concentration on crystal shape factor might be expected to be more pronounced at higher
lactose concentrations. However, crystallisation temperature played a more important role
(F = 57.43) than initial lactose concentrations (F = 7.19) in determining the shape factor of
lactose crystals when the crystals were prepared using initial lactose concentrations of 33-
43%, w/w, at either 0°C or 40°C (Table 2.6).
Chapter two: *Crystallisation of lactose from aqueous solutions*

Table 2.6 Analysis of variance (ANOVA) of shape factor of lactose particles prepared with an initial lactose concentration between 33 and 43% (w/w) at 0°C and 40°C

<table>
<thead>
<tr>
<th>Source</th>
<th>Degree of Freedom</th>
<th>Sum of Square</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>1</td>
<td>0.1030</td>
<td>0.1030</td>
<td>7.19</td>
<td>0.009</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>0.8222</td>
<td>0.8222</td>
<td>57.43</td>
<td>0.000</td>
</tr>
<tr>
<td>Concentration*Temperature</td>
<td>1</td>
<td>0.2453</td>
<td>0.2456</td>
<td>17.14</td>
<td>0.000</td>
</tr>
<tr>
<td>Error</td>
<td>316</td>
<td>4.5239</td>
<td>0.0143</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>319</td>
<td>5.6944</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Although the absolute difference in the elongation ratio or shape factor of different batches of lactose appeared to be small, differences were found to be significant due to the large number of particles measured. Furthermore, a small difference in the values of these shape factors represent a substantial difference in particle shape as can be seen from their respective SE micrographs.

As found previously, lactose crystals were confirmed to grow faster at 40°C than at 0°C. A higher growth rate has been reported previously to favour the production of more elongated particles (Herrington, 1934), resulting in higher values of elongation ratio of the isolated crystals. Although the crystals prepared at 40°C had higher values of elongation ratio than the crystals prepared at 0°C, the former crystals still had a higher value for the shape factor than the latter. These results suggest that crystals prepared at 40°C have a smoother surface as compared with those prepared at 0°C. Any defects formed on the crystal surface will be more likely to dissolve at 40°C as compared with those formed at 0°C. Furthermore, according to microscopic observation during crystallisation, the crystals prepared at 0°C are more likely to form aggregates than the crystals prepared at 40°C. These aggregates may eventually reduce the uniformity of particle shape and surface smoothness. Therefore, crystallisation at a higher temperature may be more favourable to produce elongated particles with a smoother surface.

Higher initial concentrations of lactose lead to higher rates of crystal growth, resulting in the production of more elongated crystals. However, the effect of initial lactose concentration
on the surface smoothness of lactose is more complicated, depending on many other factors such as the crystallisation temperature, stirring rate, etc. In general, crystallisation from an initial lactose concentration of 43% w/w appears to produce lactose crystals with a smoother surface in comparison with the crystals prepared from an initial lactose concentration of 33% w/w. Crystallisation from an initial lactose concentration of 33% w/w usually took a longer period of time (>12 h) for the crystals to grow to the desirable size (63-90 μm) than crystallisation from an initial lactose concentration of 43% w/w. Since the crystallisation solution was subject to constant mechanical stirring, some crystals were likely to be fractured by the stirrer and this can be seen from their SE micrographs which showed some fractured faces on the crystals. The longer period of time the crystals are subjected to mechanical stirring, the more crystal faces will be fractured. These may be the reasons for crystals prepared from an initial lactose concentration of 33% w/w generally having more surface asperities than crystals prepared from 43% w/w.

2.3.2 Two-stage crystallisation from aqueous solutions

Table 2.7 shows some of the physical properties of lactose obtained at different stages of crystallisation. Since batches 13 and 14 were classified from the same batch of lactose and so were batches 15 and 16, the conditions employed to prepare batch 13 were exactly the same as those of batch 14 but different to those used to prepare batches 15 and 16.

Table 2.7 The density, diameter, specific surface area (SSA) and rugosity values of lactose crystals prepared from different stages of crystallisation

<table>
<thead>
<tr>
<th>Batch No</th>
<th>Density (g cm⁻³)</th>
<th>Diameter (μm)</th>
<th>SSA (cm² g⁻¹)</th>
<th>Rugosity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>dᵥ</td>
<td>d_air</td>
<td>Aᵥ</td>
</tr>
<tr>
<td>13</td>
<td>1.55</td>
<td>104.7</td>
<td>42</td>
<td>369.8</td>
</tr>
<tr>
<td>14</td>
<td>1.53</td>
<td>68.6</td>
<td>28</td>
<td>571.7</td>
</tr>
<tr>
<td>15</td>
<td>1.54</td>
<td>93.0</td>
<td>45</td>
<td>405.9</td>
</tr>
<tr>
<td>16</td>
<td>1.52</td>
<td>65.3</td>
<td>32</td>
<td>604.6</td>
</tr>
</tbody>
</table>

The crystals obtained from the same stage of crystallisation have similar rugosity values regardless of particle size (Table 2.7). For example, lactose batch 13 has a rugosity value of
2.49, which is very close to that of batch 14 (2.45). However, different stages of crystallisation produced lactose particles with different rugosity values. Batches 15 & 16 have a lower rugosity value (2.04 and 2.13) than batches 13 & 14, indicating the crystals prepared at the final stage of crystallisation have a smoother surface and/or a more regular shape than the crystals obtained at the initial stage of crystallisation.

Table 2.8 Some shape factors of lactose batches 13-16 (n > 100)

<table>
<thead>
<tr>
<th>Batch</th>
<th>Shape factor</th>
<th>Elongation ratio</th>
<th>( \frac{r_{\text{max}}}{r_{\text{min}}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
</tr>
<tr>
<td>13</td>
<td>0.65 0.08</td>
<td>1.79 0.31</td>
<td>2.68 0.54</td>
</tr>
<tr>
<td>14</td>
<td>0.65 0.10</td>
<td>1.55 0.30</td>
<td>2.99 1.06</td>
</tr>
<tr>
<td>15</td>
<td>0.69 0.12</td>
<td>1.81 0.45</td>
<td>2.58 1.16</td>
</tr>
<tr>
<td>16</td>
<td>0.72 0.11</td>
<td>1.54 0.33</td>
<td>2.52 1.41</td>
</tr>
</tbody>
</table>

Table 2.8 shows the shape factor, elongation ratio and the ratio of maximum to minimum Martin's radius of the crystals prepared from different stages of crystallisation. Similar to rugosity values, lactose particles from the same stage of crystallisation have similar values of shape factor. There was no significant (p > 0.05) difference between the shape factor values of batches 13 & 14 and no significant difference between the shape factors of batches 15 & 16 (p > 0.05). However, lactose particles prepared in the final stage of crystallisation (batches 15 & 16) have a shape factor value of about 0.70 (0.69-0.72), which is significantly higher (p < 0.001) than that (0.65) of batches 13 & 14. Lactose particles prepared during the first stages of crystallisation (batch 13 or 14) appear to have a similar value of elongation ratio to those prepared at the second stages (batch 15 or 16). However, there is a significant difference (p < 0.001) between the elongation ratio of different size fractions of lactose particles from the same stage of crystallisation. For example, batch 13 has a higher value of elongation ratio than batch 14 whilst batch 15 has higher elongation ratio than batch 16. There was no significant difference (p > 0.05) in the ratio of maximum to minimum Martin's radius between these batches of lactose.

From Table 2.9, it can be seen that the elongation ratio of lactose particles is dependent upon the particle size (p < 0.01) but independent of the crystallisation stage (p = 0.808). The
larger size fraction (63-90 μm) had a higher value of elongation ratio than the smaller size fraction (< 63 μm). It can be therefore concluded from these results that larger lactose particles are more elongated than smaller lactose particles. The more elongated shape of larger particles is due to the different growth rate for the different faces of lactose crystals (Figure 2.16). It was reported that the (010) face grows the slowest whilst the (110) and (010) faces grow the fastest (van Kreveld and Michaels, 1965; Twieg and Nickerson, 1968). Therefore, the crystals will grow along their longitudinal axis, resulting in the larger particles being more elongated than the smaller particles.

<table>
<thead>
<tr>
<th>Source</th>
<th>Level</th>
<th>Degree of Freedom</th>
<th>Sum of Square</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystallisation</td>
<td>1st, 2nd</td>
<td>1</td>
<td>0.0077</td>
<td>0.0077</td>
<td>0.06</td>
<td>0.808</td>
</tr>
<tr>
<td>Particle size</td>
<td>&lt;63, 63-90 μm</td>
<td>1</td>
<td>3.6666</td>
<td>3.6666</td>
<td>28.17</td>
<td>0.000</td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td>197</td>
<td>25.6405</td>
<td>0.1302</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>199</td>
<td>29.3148</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.16 A diagram to show a typical tomahawk crystal of $\alpha$-lactose monohydrate

Statistical analysis of the results (Table 2.10) shows that the shape factor of lactose particles is determined by the stage of crystallisation ($p = 0.01$) but is independent of the particle size fractions ($p = 0.595$). Crystals prepared during the final stages of crystallisation (batches 15 & 16) had a higher value of shape factor than crystals obtained from the conditions prevalent at earlier stages of the crystallisation process (batches 13 & 14). These results suggest that batches 15 & 16 had a more regular shape with a smoother surface than batches 13 & 14. This is quantitatively supported by the visual examination of the SE micrographs of these batches of lactose (Figure 2.17), where batches 13 & 14 show more surface asperities than batches 15 & 16.

Table 2.10 Analysis of variance (ANOVA) of shape factor of lactose crystals with different particle size fractions prepared at different stages of crystallisation.

<table>
<thead>
<tr>
<th>Source</th>
<th>Level</th>
<th>Degree of Freedom</th>
<th>Sum of Square</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystallisation stage</td>
<td>1st, 2nd</td>
<td>1</td>
<td>0.0869</td>
<td>0.0869</td>
<td>6.84</td>
<td>0.010</td>
</tr>
<tr>
<td>Particle size</td>
<td>&lt;63, 63-90 $\mu$m</td>
<td>1</td>
<td>0.0036</td>
<td>0.0036</td>
<td>0.28</td>
<td>0.595</td>
</tr>
<tr>
<td>Error</td>
<td>197</td>
<td>2.5041</td>
<td>0.0127</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>199</td>
<td>2.5946</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Chapter two: Crystallisation of lactose from aqueous solutions

Figure 2.17 The scanning electron micrographs of lactose prepared at different stages of crystallisation. a): batch 13; b): batch 14; c): batch 15 and d): batch 16.
Chapter two: Crystallisation of lactose from aqueous solutions

The more regular shape and smoother surface of the particles prepared during the final stages of crystallisation than those prepared during the initial stages may be due to the fact that the initial crystallisation can 'purify' or 'stabilise' the aqueous solution such that the subsequent crystallisation can be carried out in a more homogeneous solution. Initial crystallisation is accompanied by a rapid drop in temperature and increase in the viscosity. At an initial lactose concentration of 43%, the crystallisation usually starts before the temperature reaches room temperature. Therefore, the crystals grow in an environment of a changing temperature and viscosity, both of which are known to affect the growth of lactose crystals (Hartel and Shastry, 1991). At lower temperatures (<30°C), the rate of mutarotation of lactose in solution was shown to decrease with temperature, leading to a decrease in the crystal growth of lactose (Brinkman, 1976). With a drastic change in temperature and/or viscosity, the initial crystallisation may be carried out in a more 'chaotic' solution than the final stages of crystallisation when the temperature tends towards a constant value. This might be the major reason that lactose crystals prepared at later stages of crystallisation process (batches 15 & 16) have a more regular shape with smoother surface than those prepared during the first stages of crystallisation (batches 13 & 14). Further, even pharmaceutical-grade lactose was reported to contain some impurities that result in most lactose solutions being acidic (pH 5) (Visser, 1980). These impurities were identified as di-lactose phosphate complexes that are incorporated into the crystal lattice (Visser, 1984). Di-lactose phosphate complexes inhibit the growth of lactose crystals by adsorbing onto several of the lactose crystal faces, thereby inhibiting the incorporation of α-lactose molecules into the crystal lattice (Visser et al., 1988). Therefore, it would be reasonable to assume that some of the impurities are incorporated into crystals prepared during the initial stage of crystallisation, leading to a relatively pure solution being generated for subsequent crystallisation. This may further improve shape and surface smoothness of lactose crystals prepared after the initial crystallisation.

2.3.3 Crystallisation from aqueous solutions with small amounts of organic solvents

The mean particle size of lactose varied with the organic solvents added to the mother liquor (Figure 2.18). All the organic solvent except for glycerin and propanol markedly increased the growth rate of lactose crystals from aqueous solutions, in the order of: acetone > ethanol
Chapter two: Crystallisation of lactose from aqueous solutions

> methanol. The use of propanol and glycerine slowed crystal growth. The effects of organic solvents on particle growth rates may be due to three mechanisms. First, since lactose is a hydrophilic compound, its aqueous solubility is reduced in the presence of these water-miscible organic solvents. This will lead to an increased supersaturation and hence an increased rate of lactose crystallisation (Jelen and Coulter, 1973). Although acetone, ethanol and methanol are all water-miscible, they have different hydrophilicities, the order being methanol > ethanol > acetone. Such differences in hydrophilicity may partly account for the different effects of the solvents on crystal growth. Second, the solvents may reduce the surface tension between crystal faces and their surrounding solution and thus facilitate the transfer of lactose molecules to the crystal surfaces and increase the crystal growth. Although alcohol promotes spontaneous nucleation and this may be another factor involved in increasing the crystallisation of lactose such an effect might not be detected by measuring the individual particle size as employed in the current work. The inhibited growth rate of lactose crystals by propanol and glycerine might be due to the increased viscosity of the solution in the presence of these solvents. Increasing viscosity might reduce the molecular mobility and thus, retard the transfer of lactose molecules from the surrounding solution to the crystal surface.

Figure 2.18 The effects of some organic solvents on the growth of lactose crystals (Error bars denote standard deviation, n = 50).
Tables 2.11 & 2.12 show some of the morphological properties of lactose crystals prepared with the aid of different organic solvents. Lactose particles prepared in the presence of different organic solvents appear to have different shape and surface texture. Particles prepared in the presence of ethanol (batch 17) and acetone (batch 19) were shown to have a significantly smaller elongation ratio (p < 0.001) than particles prepared in the presence of glycerine (batch 20) and particles prepared from solutions without any added organic solvents (for example, batches 13-16). Addition of 5% w/w ethanol to the mother liquor was reported to accelerate the growth rate of face (011) by 3 times (van Kreveld and Michaels, 1965), face (110) by 1.4 times and faces (110) and (100) by 1.3 times (van Kreveld, 1969). Therefore, ethanol would appear to accelerate the growth of width and thickness more than the length of the particles. This leads to the production of 'rounder' particles, resulting in the lower elongation ratio of this batch of lactose particles. However, there is no significant difference in the values of shape factors and rugosity (Table 2.12) between batches 17 & 20. Given the higher value of elongation ratio for batch 20, it would be reasonable to assume that lactose batch 20 had a smoother surface than batch 17. This is verified by examination of the SE micrographs (Fig. 2.19) where crystals from lactose batch 20 appear to be more elongated and have smoother surfaces, as compared to particles from batch 17. Furthermore, batch 17 tended to have more aggregates than batch 20. Batch 19 had a significantly (p < 0.001) higher rugosity value but a significantly (p < 0.001) lower shape factor value than either batch 17 or batch 20. This batch of lactose (batch 19) contained particles with a more irregular shape with more surface asperities than batches 17 & 20 (Fig. 2.19).

Table 2.11 Some shape factors of lactose crystals prepared in the presence of different organic solvents (Mean ± SD, n > 150).

<table>
<thead>
<tr>
<th>Batch No</th>
<th>Solvent</th>
<th>Diameter (d_n)</th>
<th>Shape factor</th>
<th>Elongation ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>Ethanol</td>
<td>93.7 ± 15.1</td>
<td>0.76 ± 0.12</td>
<td>1.25 ± 0.19</td>
</tr>
<tr>
<td>19</td>
<td>Acetone</td>
<td>98.7 ± 19.3</td>
<td>0.54 ± 0.09</td>
<td>1.35 ± 0.23</td>
</tr>
<tr>
<td>20</td>
<td>Glycerine</td>
<td>103.7 ± 12.3</td>
<td>0.74 ± 0.12</td>
<td>1.82 ± 0.27</td>
</tr>
</tbody>
</table>
Chapter two: Crystallisation of lactose from aqueous solutions

Figure 2.19 The scanning electron micrographs of lactose prepared in the presence of different organic solvents. a): acetone; b): ethanol and c): glycerine.
Table 2.12 The density, diameter and specific surface area (SSA) of lactose particles prepared in the presence of different organic solvents

<table>
<thead>
<tr>
<th>Batch No</th>
<th>Density (g cm$^{-3}$)</th>
<th>Diameter (μm)</th>
<th>SSA (cm$^2$ g$^{-1}$)</th>
<th>Rugosity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$d_v$</td>
<td>$d_{air}$</td>
<td>$A_v$</td>
<td>$A_{air}$</td>
</tr>
<tr>
<td>17</td>
<td>1.57</td>
<td>93.7</td>
<td>49.58</td>
<td>407.86</td>
</tr>
<tr>
<td>19</td>
<td>1.57</td>
<td>98.7</td>
<td>36.15</td>
<td>387.20</td>
</tr>
<tr>
<td>20</td>
<td>1.55</td>
<td>103.7</td>
<td>54.58</td>
<td>373.29</td>
</tr>
</tbody>
</table>

The more irregular shape and rougher surfaces observed for batches 17 & 19, in comparison with batch 20, may be due to the faster growth rate of lactose crystals, which may result in more crystal imperfections being formed. These imperfections may lead to the production of irregularly shaped lactose with rougher surfaces. The improved crystal morphology observed for batch 20 may have been due to the slower crystal growth rate when glycerine is added. Thus, in order to obtain lactose crystals of regular shape with smooth surfaces, organic solvents which accelerate crystal growth rate should be avoided in the mother liquor. However, addition of a small amount of viscous solvents to the mother liquor may improve the crystal shape and surface texture of lactose crystals.
2.4 Summary

This section of work investigated the effects of some crystallisation conditions such as supersaturation, temperature, some water miscible organic solvents and different stages of crystallisation on the particle shape and surface smoothness of lactose. Lactose particles of different shapes with different surface textures were prepared under different crystallisation conditions. Particle size, shape and surface smoothness were characterized by both image analysis and air permeation methods. The shape factor measured using image analysis correlates well with the rugosity value obtained from the air permeation method. Increasing the crystallisation temperature and/or initial lactose concentrations was shown to produce more elongated lactose crystals with higher surface smoothness. Lactose particles prepared after an initial crystallisation process have a more regular shape with smoother surface than those prepared during the initial crystallisation. The addition of 10% methanol or ethanol or acetone to the mother liquor increased the growth rate of lactose particles whereas addition of propanol or glycerine inhibited the rate of crystal growth. Lactose crystals prepared in the presence of glycerine were more regularly shaped with smoother surface than those prepared in the presence of ethanol or acetone. Having prepared lactose batches with different particle sizes, shape and surface texture, the aim of the next stage of the study was to investigate the effects of these factors on the deposition of micronised salbutamol sulphate. A description of these studies forms the basis of Chapter 4.
CHAPTER THREE

CRYSTALLISATION OF LACTOSE FROM CARBOPOL

934 GELS
Constant stirring is essential for the crystallisation of a substance from solution so as to avoid caking and the formation of other non-dispersible aggregates. However, mechanical stirring is likely to introduce random energy fluctuations in the solution, causing heterogeneous distributions of local concentrations. Such a hypothesis is supported by the phenomenon that a stable supersaturated solution can be induced to nucleate by a mere tap on the side of the vessel. The fluctuations in local concentrations induced by mechanical stirring may result in the heterogeneous growth of crystals since the growth rate is largely dependent upon the supersaturation of its surrounding solution. The heterogeneous growth will thus lead to the production of crystals with different particle sizes and irregular shapes, both of which have been commonly encountered when crystallisation is carried out under agitation. Further, mechanical stirring is known to induce secondary nucleation, which takes place in the presence of existing crystals (Larson, 1981). Thus, if a crystal is growing in a suspension under constant agitation, then, additional nuclei will continually be added to the crystal size distribution. Since the nucleation step also depletes the available supersaturation, in direct competition with the growth of the nuclei, the newly born crystals will grow to a lesser degree than the previously existent crystals. This will further widen the particle size distribution of final crystals. Therefore, mechanical stirring almost always results in the production of crystals with a wide size distribution with a large portion of small crystals and this can be seen from the particle size distribution of lactose prepared under constant stirring (Valle-Vega and Nickerson, 1977). Such a size distribution should be avoided in the preparation of lactose particles intended to be used as the carrier particles for inhalation aerosols since the majority of the particles are required to have a size range of 63-90 μm. As mentioned previously, the crystal shape (habit) of lactose is primarily determined by the supersaturation of the environment from which the crystals have been grown. The difference in supersaturation can be expected to result in the production of lactose crystals of different shape and surface textures. Crystals with such morphological properties would have inconsistent performance when used as carrier particles for any adhered drug particles.

Mechanical stirring (or agitation) also induces collisions between existing crystals, crystals and the wall of the vessel as well as between the crystals and any agitation devices. The
collision energy may cause small pieces of the crystals to be chipped away, which will then be distributed subsequently within the suspension. The chipped parts of crystals may act as stable embryos for new crystals to grow. Any chipped sites on the surface of broken crystals may also act as nuclei for new crystals to grow. Such a phenomenon can be seen in the electron micrograph of batch 3 lactose crystals (Figure 2.6) prepared under conditions involving constant stirring. This batch of lactose had aggregates composed of a large crystal with 1 or more hybrid small crystals, growing from the fractured faces of the mother crystal. Even if no new crystals grow out of any chipped sites of a larger crystal, these fracture planes will undergo irregular growth, increasing the irregularity of both particle shape and surface textures of the affected crystal. Therefore, it may be advantageous to effect crystallisation from an undisturbed system, without any means of agitation, with a view to producing crystals with well defined morphology.

In order for the crystals to grow in an undisturbed system without the formation of any non-dispersible aggregates, one of the most commonly employed methods is to suspend the crystals in a gel (Mullin, 1993). The gel provides a protective barrier for the growing crystals and permits a steady diffusion of crystallising molecules. Without introducing any external turbulence to the solution, the gel can be expected to provide a homogeneous environment in which the crystals can grow and thus, overcome some of the major problems associated with the use of mechanical stirring. Since the crystals are in a stagnant suspension, individual crystals can be grown to a maturity without any fractures. The crystals prepared in this manner always have a more regular shape and a smoother surface than those obtained under mechanical stirring. Therefore, crystallisation from a gel has been widely used to obtain large, single crystals with well-defined morphology (Arora, 1981; Henisch, 1988). Further, secondary nucleation will occur to much lesser extent in a gel than in the case of the solution under agitation (Hartel and Shastry, 1991). The inhibition of such nucleation may result in a narrower size distribution of the final particles. Although the crystallisation from gels has been most widely employed to prepare large, single crystals of inorganic materials (Henisch, 1988), there are few reports dealing with the crystallisation of organic compounds from gels and none was found employing this method to prepare batches of organic crystals. However, monocrystals of α-lactose monohydrate were grown in a 0.7% w/w agar gel (Wong and Aulton, 1987). However, the use of an agar gel may not
prove to be suitable for the preparation of lactose particles intended to be used as a carrier for aerosols due to the following reasons. First, agar is not a pharmaceutical excipient and hence its use to crystallise lactose for use in the respiratory tract may be precluded by Regulatory Authorities. Second, harvesting the bulk of crystals from an agar gel would prove to be problematic due to the relatively high consistency of the gel. Finally, agar is insoluble in most of the common organic solvents and this would make it very difficult to remove any adsorbed agar gel from the crystal surface.

The gel that can be used to prepare lactose crystals intended to be used as a carrier for inhalation aerosol has to meet at least the following criteria. First, the gel must have been employed as a pharmaceutical ingredient for internal usage. The use of the gel has to be proved to be non-toxic, especially to the respiratory tract. Second, the gel must be capable of being efficiently removed from the surface of lactose crystals so as not to affect any physico-chemical properties of lactose and, most importantly, to minimise the possibility of introducing such a compound to the respiratory tract. Third, the consistency or viscosity of the gel can be controlled such that after crystallisation, the bulk of crystals can be harvested more easily without any vigorous treatment that might change the morphology of the crystals.

Carbopols, a group of polyacrylic acid polymers cross-linked with either allylsucrose or allyl ethers of pentaerythritol, might provide an ideal gel that can meet most of the aforementioned criteria. Carbopols have been widely used as suspending agents; emulsifying agents or tablet binders in pharmaceutical industry (Wade and Weller, 1994b). Carbopol gels have also been employed as bioadhesive vehicles for a mucoadhesive drug delivery formulations to prolong drug residence at the application sites (Chu et al., 1991). The viscosity of Carbopol gels was shown to be dependent upon the polymer concentration (Barry and Meyer, 1979a) and therefore, it is possible to obtain a minimal viscosity that can suspend the lactose crystals without substantially inhibiting crystal growth. The consistency of Carbopol gel changes reversibly with the pH value of the solution (Barry and Meyer, 1979b). Carbopols disperse in water to form acidic colloidal solutions of low viscosity which when neutralised, produce highly viscous gels. The viscosity reaches a maximum at pH 6-11 but is considerably reduced if the pH is less than 3 or greater than 12 (Wade and
Weller, 1994b). Therefore, the crystallisation could be carried out in a neutralised Carbopol gel. After which, the gel could be converted to a fluid by acidification such that the crystals may be readily harvested. Carbopol is soluble in either ethanol or glycerine (Wade and Weller, 1994b) whereas lactose is almost insoluble in these solvents. Therefore, any adsorbed Carbopol residue on the lactose crystals may be easily removed by washing the crystals with either ethanol or glycerine without substantially changing the morphology of the crystals.
3.2 Materials and Methods

3.2.1 Crystallisation of lactose from Carbopol gels

A predetermined amount of distilled water was agitated at about 500 rpm with a 4-bladed stirrer (1x3 cm) which was situated 2 cm above the bottom of a 500 ml beaker. The required amount of Carbopol 934 (B. F. Goodrich Chemical Co., Cleveland, Ohio, USA.) with an average molecular weight of approximately 3,000,000, was added into the vortex. When all the Carbopol was dispersed, the liquid was allowed to stand overnight in the dark so as to ensure maximum dissolution of the polymer. A cloudy, colloidal solution of low viscosity was obtained, the pH of which was about 3.2. The required amount of Lactochem® lactose was then dissolved in the Carbopol solution at an elevated temperature (<90°C, depending upon the final lactose concentrations) under constant stirring at 500 rpm to obtain a cloudy solution with a pH value of approximately 2.5. Sodium hydroxide solution (1 M) was then added dropwise to the solution, whilst stirring at about 800 rpm. The viscosity and clarity of the solution increased with pH, until it became a clear homogeneous gel at a pH value of approximately 4.5. After then, the mixer was not sufficiently powerful to disperse the gel and hence, the mixing was continued manually with a spatula. The addition of the neutralising agent (NaOH) was continued so as to obtain a pH value of 7. The gel was then centrifuged at 3000 rpm for about 10 min so as to remove any entrapped air bubbles and insoluble particles. The gel was finally placed in the dark until the majority of the crystals had grown to the size range of 63-90 μm, which was estimated by an optical microscope, the gel was adjusted to pH 3-3.5 with hydrochloric acid (1 M) to obtain a fluid. The crystals were allowed to settle for about 10 min. After decanting the supernatant, the crystals were routinely washed with 60% ethanol twice and absolute ethanol three times. The crystals were finally allowed to dry at room temperature after which, a small amount of sample (about 0.5 g) was taken from each batch of lactose, the remaining lactose crystals were poured into a 90 μm sieve which had been placed upon a 63 μm sieve. The particles were then sieved manually and slowly for 1 h so as to limit the rupture of any crystals. The particles were thus divided into 3 size fractions (<63, 63-90 and >90 μm) which were collected and weighed separately. The classified lactose crystals were dried in a vacuum
oven at 70°C for 3 h before transferring to sealed vials, which were then placed in a desiccator over silica gel.

Crystallisation of the lactose from Carbopol 934 gels were carried out under different conditions by means of altering the crystallisation time and the concentrations of either lactose or Carbopol gels (Table 3.1). Three batches of lactose crystals were prepared under each of the seven conditions listed in Table 3.1 but all of 3 batches were then mixed to prepare final batches of lactose, which were labelled as Car1 to Car 7, respectively. The 63-90 μm fraction of batches Car 1 to Car 7 were labelled as C1 to C7, respectively. Lactose crystals from batch Car 1 were further classified into fractions < 63; 90-125 and > 125 μm, which in turn were labelled as C8; C9 and C10 respectively. Batch C7 was washed directly with 100% ethanol rather than pre-washing with 60% v/v ethanol as described above.

3.2.2 Characterisation of particle morphology of lactose crystals

3.2.2a Particle size measurement by optical microscopy and image analysis
The particle size of lactose crystals prepared from Carbopol gels under different conditions were measured using optical microscopy as described in Section 2.2.4.1

3.2.2b Determination of particle shape and surface textures by scanning electron microscopy (SEM)
The 63-90 μm size fractions of lactose crystals prepared from Carbopol gels under different conditions were analysed using scanning electron microscopy as described in Section 2.2.4.2.

3.2.2c The quantification of rugosity (sphericity) of lactose particles
The true density of the 63-90 μm size fraction of lactose crystals was measured using a Beckman air comparison pycnometer (see Section 2.2.4.3a) and the specific surface area was determined by an air permeation method (see Section 2.2.4.3b). The rugosity value was calculated according to the equation shown in Section 2.2.4.3d.
3.2.2d Particle size and shape factors of lactose crystals
The particle size and shape factors of the 63-90 μm size fraction were measured using an image analysis optical microscopy as described in Section 2.2.4.4.

3.2.3 Characterisation of particle crystallinity

3.2.3a Thermal Gravimetric Analysis
A small amount of lactose crystals (4-5 mg) was placed in an aluminium cup which was then placed in the sample chamber of an STA 625 Differential Scanning Calorimeter (Rheometrics Scientific Ltd., Surrey, UK). The weight loss due to dehydration was measured from ambient temperature to 300°C at a heating rate of 10°C min⁻¹.

3.2.3b Differential Scanning Calorimetry
A small amount of lactose crystals (4.5-6.0 mg) was placed in an aluminium pan which was then transferred into the sample chamber of STA 625 Differential Scanning Calorimeter (Rheometrics Scientific Ltd., Surrey, UK). Thermograms of the samples were measured at a heating rate of 10°C/min from ambient temperature to 280°C under N₂ flowing at 50 ml min⁻¹.

3.2.3c X-Ray Powder Diffraction
The X-ray diffraction pattern of lactose crystals was measured at room temperature with a Philips X'Pert Dual Goniometer (Philips Analytical, Holland). The X-ray source was a copper-Kα operated at a voltage of 40 kV and with a current of 50 mA. Samples were back-filled into 16 mm holders. The traces were recorded over a range of 2-35° 2θ with a step size of 0.04° and a count rate of 1 step s⁻¹.

3.2.4 Characterisation of particle flowability

3.2.4a Angle of repose
Lactose crystals were carefully poured into a copper tube (2.65 cm × 6.90 cm), which had been placed over a flat base with a diameter of 2.53 cm. After the powder heap reached a
height of approximately 4 cm, the addition of powder was stopped and the copper tube was then slowly lifted vertically off the base, on which a cone of powder was formed. The height of the cone was measured using a ruler and, the angle of repose ($\theta_r$) was calculated as:

$$\theta_r = \text{Tangent}^{-1} \left( \frac{h_p}{r_b} \right)$$

(3-1)

where $h_p$ and $r_b$ were the height (cm) of the powder heap and the radius (cm) of the base, respectively.

Each sample was measured at least in triplicate.

3.2.4b The angle of slide

Small amounts of powder can be placed on a flat surface and the surface tilted at an ever-increasing angle until the powder starts to slide. The angle of the plane to the horizontal level at which powder movement occurs, the angle of slide ($\theta_s$), can be employed to express the flowability of the powder bed (Hiestand, 1966). An in-house designed (King’s College London) device (Figure 3.1) was employed to measure $\theta_s$.

Figure 3.1 A schematic diagram showing the device employed for the measurement of the angle of slide.
A small amount (approximately 10 mg) of lactose crystals was placed on a stainless steel plane (6.55 \times 7.00 \text{ cm}), and this was then tilted by screwing a spindle vertically upwards below the plane. When the majority of the powder started to slide, the angle between the tilted plane and the horizontal base, \( \theta \), was directly read from a protractor.

Each sample was measured at least in triplicate.

3.2.5 Rheological characterization of Carbopol 934 gels

3.2.5a Preparation of Carbopol 934 gels
An accurately weighed amount (about 3.0 g) of Carbopol 934 was added slowly into the vortex of 300 ml distilled water agitated at 500 rpm with a 4-blade stirrer (1-3 cm) which was situated 2 cm above the bottom of the container (500 ml beaker). The stirring was continued such that all the polymer was dispersed. The cloudy, acidic (pH 3.2) colloidal solution was then tightly capped and stored in the dark overnight so as to ensure maximum dissolution of the polymer. The solution thus obtained was used as the stock solution for the preparation of a series of Carbopol solutions of different concentrations.

The stock solution was diluted with distilled water to obtain solutions with Carbopol 934 concentrations of 0.2\%, 0.4\%, 0.6\% and 0.8\% w/v, respectively. The polymer solutions were then neutralised by adding 1.0 M sodium hydroxide under vigorous stirring. When the pH value of the polymer solution exceeded 4, the stirring was carried out manually using a spatula. After neutralisation, a clear gel was obtain for all the concentrations. The gels were finally centrifuged at 3,000 rpm for 10 min so as to remove any entrapped air bubbles after which, the gels were tightly capped and stored in the dark until required for further investigation.

3.2.5b Rheological determination of Carbopol 934 gels
The rheology of Carbopol 934 gels was measured using a Carri-Med CSL Rheometer (TA Instruments, Surrey, UK). The operation conditions of the measurement were as follows:
Measurement system type: parallel plate
Plate diameter: 4.0 cm
Measurement system gap: 450 μm
Measurement system inertia: 1.450 μN m s²
Equilibration time: 70 s
Experiment mode: Shear stress sweep
Start temperature: 20°C
End temperature: 20°C
Start stress: 0.50 Pa
End stress: 20.00 Pa
Stress mode: Linear
Ascent time: 60 s
Peak hold time: 10 s
Descent time: 60 s

Six samples of each concentration were tested.

3.2.6 Data analysis

All data were analysed by two-tailed t-test, multiple regression and ANOVA as appropriate, using Minitab® for Windows Version 10.2 (Minitab Inc., USA).
3.3 Results and discussion

3.3.1 Crystallisation of lactose from Carbopol gels

It can be seen from Table 3.1 that lactose crystals with different particle sizes and size distributions were prepared when different concentrations of lactose and Carbopol 934 were employed. Increasing the initial lactose concentrations generally increased the mean particle size of the lactose crystals. For example, the mean particle size of lactose crystals (Car 2) prepared from an initial lactose concentration of 43% w/v was 87.9 μm, which was significantly higher (p < 0.001) than that of the crystals (Car 3) prepared from an initial lactose concentration of 33% w/v. However, increasing the concentrations of Carbopol 934 appeared to reduce the particle size of lactose crystals whilst prolonging the crystallisation generally resulted in the production of lactose crystals of larger particle size. The dependence of the mean diameter of lactose crystals on these preparative conditions can be better seen from the following empirical equation generated by multiple linear regression using Minitab® for Windows Version 10.

Mean particle size (μm)

\[ = 2.12 C_L - 3.9 C_C + 0.2 t_c - 4.4 \]

\[ r^2 = 0.886 \]  

(3-2)

where \( C_L \) and \( C_C \) are the concentrations (% w/v) of lactose and Carbopol 934, respectively; \( t_c \) is the crystallisation time (h).

According to equation 3-2, it can be seen that either increasing the initial lactose concentration or reducing Carbopol concentration or extending the crystallisation time period will increase the mean particle size of the lactose crystals prepared. Increasing the initial lactose concentration was shown previously to increase the particle size of lactose crystals, due to an increase in the growth rate of the crystals at higher supersaturation. However, lactose crystals prepared from Carbopol gels generally have smaller particle sizes than the crystals prepared from aqueous solutions without gels. For example, the mean particle size of lactose batch Car 3 (76.5 μm) was much smaller than that of lactose batch 2 (115.8 μm) or lactose batch 3 (100.3 μm) (see Table 2.1), which were prepared from the
same initial concentrations of lactose (33% w/v) for a similar period of time (24 h) to batch Car 3. The smaller particles prepared in the presence of Carbopol 934 gels may be due to the inhibition of crystal growth by Carbopol gels. Furthermore, in the absence of convection currents in a gel, the only mechanism available for the growth of a crystal is by diffusion (Henisch, 1987) and this determines the rate of transfer of the molecules from solution to the surface of a growing crystal. The lack of convection currents may retard the transfer of lactose molecules from the surrounding solution to crystal surface, which will eventually reduce the growth rate of lactose crystals.

Table 3.1 The crystallisation conditions, mean diameter and particle size distribution of the resultant lactose crystals.

<table>
<thead>
<tr>
<th>Batch No</th>
<th>Lactose (% w/v)</th>
<th>Carbopol (% w/v)</th>
<th>Crystal time (h)</th>
<th>Mean Size (μm)</th>
<th>% Particle (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Car 1</td>
<td>43.0</td>
<td>0.6</td>
<td>72</td>
<td>105.4</td>
<td>5.8 35.4 58.8</td>
</tr>
<tr>
<td>Car 2</td>
<td>43.0</td>
<td>0.3</td>
<td>24</td>
<td>87.9</td>
<td>10.3 56.5 33.2</td>
</tr>
<tr>
<td>Car 3</td>
<td>33.0</td>
<td>0.3</td>
<td>24</td>
<td>76.5</td>
<td>12.2 68.7 19.1</td>
</tr>
<tr>
<td>Car 4</td>
<td>50.0</td>
<td>0.4</td>
<td>48</td>
<td>116.3</td>
<td>8.2 12.6 79.2</td>
</tr>
<tr>
<td>Car 5</td>
<td>50.0</td>
<td>0.6</td>
<td>72</td>
<td>114.2</td>
<td>1.4 22.3 76.3</td>
</tr>
<tr>
<td>Car 6</td>
<td>38</td>
<td>0.4</td>
<td>72</td>
<td>93.3</td>
<td>8.5 53.5 38.0</td>
</tr>
<tr>
<td>Car 7</td>
<td>38</td>
<td>0.4</td>
<td>48</td>
<td>75.4</td>
<td>15.6 73.2 11.2</td>
</tr>
</tbody>
</table>

Lactose particles prepared from Carbopol 934 gels had consistently smaller fractions of particles less than 63 μm in diameter (Table 3.1) than those prepared from aqueous solutions (Table 2.1). The mean % of particles < 63 μm for all 11 batches of lactose prepared under constant stirring (Table 2.1) was 20.8 ± 8.9% w/w of the total particles, which was significantly higher (p < 0.01) than that of all 6 batches of lactose prepared from Carbopol 934 gels (7.73 ± 3.77% w/w total particles). Consequently, the latter batches had higher % particles between 63-90 μm (46.0 ± 8.7% w/w total particles) than the former batches (28.0 ± 11.4% w/w total particles). The difference in the percentages of particles < 63 μm of lactose crystals prepared in the presence and absence of Carbopol 934 gel was more pronounced when the batches of lactose particles with similar mean diameters were
compared. For example, batch Car 3 had a mean diameter of 76.5 μm with 12.2% w/w particles < 63 μm, which was less than half of the 33.6% w/w particles < 63 μm of batch 10 that had a mean diameter of 76.4 μm. The reduced percentage of the smaller particle size fraction of lactose crystals prepared in the presence of Carbopol 934 gels is likely to be due to the gel inhibiting secondary (heterogeneous) nucleation and the lack of any external agitation (Henisch, 1987). The nucleation suppressing property of gels distinguishes crystallisation in gels from ordinary diffusion methods and is responsible for the production of crystals with relatively uniform size distribution. An increase in the percentage of particles between 63-90 μm by crystallisation from Carbopol 934 gels should be beneficial to the preparation of lactose particles which are to be used as a carrier for inhalation aerosols.

Figure 3.2 shows the scanning electron micrographs of lactose crystals prepared under different conditions. It can be seen that most of the lactose crystals prepared from Carbopol 934 gels had a more regular shape with a smoother surface than lactose crystals prepared from aqueous solutions under constant stirring (Figs. 2.4-2.14) All the batches of lactose prepared from the gel had a similar elongated tomahawk shape, regardless of the conditions of crystallisation. As mentioned previously (Section 2.3.1), when crystallisation was carried out in lactose solutions under constant stirring, the crystal shape of lactose changed with crystallisation conditions, especially when the initial lactose concentration was altered. The relatively constant shape of lactose crystals prepared from Carbopol 934 gels containing different lactose concentrations suggests that the effect of supersaturation of lactose on particle shape was suppressed by Carbopol 934 gels such that the shape of lactose crystals prepared from the gels was practically independent of the initial lactose concentration. Lactose molecules can be expected to migrate freely in the crystallisation media under constant stirring and hence, any change in the supersaturation results in a change in the crystallisation pressure in the vicinity of the growing crystals, which will eventually change the crystal shape. However, the framework of the gels may act as a three-dimensional barrier for the free migration of lactose molecules and consequently, lactose concentrations in the immediate vicinity of the growing crystals might be expected to be lower than the apparent concentration of lactose in the bulk of the crystallisation medium. Therefore, any increase in the apparent supersaturation of lactose may not result in a corresponding
Figure 3.2 The scanning electron micrographs of some batches of lactose prepared from Carbopol 934 gels.
increase in the effective concentration driving crystal growth. This would result in a reduction in the sensitivity of crystal habit to lactose concentration.

Of all the batches of lactose prepared from Carbopol 934 gels, batches C2 and C3 appeared to have the most surface asperities (Figure 3.2). These batches of lactose were prepared from Carbopol 934 gels with a concentration of 0.3% w/v. It was observed that Carbopol 934 gel of this concentration failed to suspend the majority of the lactose crystals such that, after crystallisation, most of the crystals sedimented to the bottom of the container. After sedimentation, the crystals tended to form aggregates that had to be dispersed with vigorous shaking. Either crystal sedimentation during crystallisation or the vigorous shaking after crystallisation or their combined effects may be attributable to the surface asperities observed on the surface of the lactose crystals. Therefore, in order to obtain well defined lactose crystals with a smooth surface, the Carbopol 934 gels employed have to have appropriate rheological characteristics which ensure that the majority of the crystals are suspended. However, too high a concentration of the polymer may not be appropriate for at least the following two reasons. First, higher concentrations of Carbopol gels would result in slower crystal growth of lactose, extending the time period for the crystals to grow to the desirable size (63-90 μm). Second, too high a concentration leads to unnecessarily high rigidity of the gel, which will in turn make it difficult to harvest the crystals prepared. A concentration of Carbopol 934 of approximately 0.4% w/w was therefore thought to be suitable for the preparation of lactose crystals.

Lactose batch C7 had some needle crystals adhered to the coarser crystals (Figure 3.2). These needle crystals were introduced during the washing process since this batch of lactose crystals were washed directly with 100% v/v ethanol without prewashing with 60% v/v ethanol as employed for the rest batches of lactose. After separation from the mother liquor, the crystals still had traces of the mother liquor adhered to the crystal surfaces. If the mother liquor is placed in direct contact with 100% ethanol, then, any lactose remaining in solution crystallises so rapidly that only needle crystals are obtained. Therefore, a pre-washing process with lower ethanol concentrations has to be employed so as to remove most of the mother liquor from crystal surfaces without the formation of unwanted crystals. This can be achieved with a lower concentration of ethanol that will not induce lactose to crystallise.
from the mother liquor. However, too low an ethanol concentration should be avoided in the prewashing process with a view to avoiding dissolution and maintaining the integrity of lactose crystals. A concentration of ethanol between 50-60% v/v was considered to be suitable for the prewashing process.

Table 3.2 shows the true density and specific surface area of different batches of lactose particles. It can be seen that all the batches of lactose have a similar density to that of α-lactose monohydrate, 1.54 g cm$^{-3}$ (Wade and Weller, 1994a). The mean diameter of the 63-90 μm size fraction of all seven batches of lactose prepared from Carbopol 934 gels was 108.1 ± 5.8 μm, which was significantly higher (p < 0.001) than the 92.9 ± 3.1 μm of the same size fraction of all eleven batches of lactose prepared under constant stirring, although these samples were prepared by sieving under the same conditions. Such a difference in the particle size of the sieved fractions might be due to the fact that the crystals prepared from Carbopol 934 gels had a more elongated shape than those prepared with constant stirring. Elongated particles with diameters larger than the sieve mesh may also pass through the sieve by aligning their shortest axes (width) along the sieve openings. Therefore, the sieved fractions of more elongated shape should have a larger mean diameter than less elongated particles, if sieved under the same conditions. Although the SE micrographs showed that lactose crystals prepared from Carbopol 934 gels visually had smoother surfaces than those prepared under constant stirring, the rugosity values (2.01 ± 0.23) of all seven batches of lactose prepared from Carbopol 934 gels were only slightly but insignificantly (p > 0.05) lower than those (2.15 ± 0.35) of all eleven batches of lactose prepared under constant stirring (Table 2.2). This is not surprising since, although the crystals from Carbopol 934 gels had smoother surfaces, the decrease in rugosity value due to improved surface smoothness would be diminished by the more elongated shape of these particles.
Table 3.2 The density, mean diameter, specific surface area (SSA) of lactose particles prepared from Carbopol 934 gels

<table>
<thead>
<tr>
<th>Batch No</th>
<th>Density (g cm⁻³)</th>
<th>Diameter (µm)</th>
<th>SSA (cm²/g)</th>
<th>Rugosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>1.54</td>
<td>104.1</td>
<td>61</td>
<td>374.3</td>
</tr>
<tr>
<td>C2</td>
<td>1.56</td>
<td>101.7</td>
<td>48</td>
<td>378.2</td>
</tr>
<tr>
<td>C3</td>
<td>1.55</td>
<td>105.2</td>
<td>46</td>
<td>368.0</td>
</tr>
<tr>
<td>C4</td>
<td>1.54</td>
<td>111.7</td>
<td>57</td>
<td>348.8</td>
</tr>
<tr>
<td>C5</td>
<td>1.53</td>
<td>105.7</td>
<td>61</td>
<td>371.0</td>
</tr>
<tr>
<td>C6</td>
<td>1.54</td>
<td>109.4</td>
<td>55</td>
<td>356.1</td>
</tr>
<tr>
<td>C7</td>
<td>1.55</td>
<td>118.8</td>
<td>52</td>
<td>325.8</td>
</tr>
</tbody>
</table>

The shape factors of lactose crystals prepared from Carbopol 934 gels varied from 0.68 to 0.76 (Table 3.3), which was slightly higher than the shape factors (0.60-0.74) of lactose crystals prepared with constant stirring (Table 2.4) but the difference was not statistically significant (p > 0.05). This was in agreement with the results of rugosity values. However, the standard deviations of the shape factor of all crystals prepared from Carbopol 934 gels ranged from 0.07 to 0.09 (Table 3.3), which was significantly lower (p < 0.05, two-tailed t-test) than that of all eleven batches of lactose crystals prepared under constant stirring (0.06-0.14, Table 2-4), suggesting that the former were more uniformly shaped than the latter. The elongation ratio of all the batches of lactose crystals prepared from the gels was 1.71 ± 0.18, which was significantly higher (p < 0.05) than that of all eleven batches of lactose crystals prepared with constant stirring (1.49 ± 0.25, Table 2.4). A higher elongation ratio for lactose particles, together with the visual observation of their SE micrographs, was indicative of more elongated shape of the lactose crystals prepared from Carbopol gels, as compared with those prepared under constant stirring. The ratio of maximum Martin’s radius to minimum Martin’s radius for lactose crystals from the gels varied from 2.04 to 2.47 (Table 3.3), which taken as a group were slightly but insignificantly (p > 0.05) higher than those of the crystals prepared by stirring (1.89 to 2.59, Table 2.4).
Table 3.3 Some shape factors of lactose crystals (n > 100) prepared from Carbopol 934 gels

<table>
<thead>
<tr>
<th>Batch No</th>
<th>Shape factor Mean</th>
<th>Shape factor SD</th>
<th>Elongation ratio Mean</th>
<th>Elongation ratio SD</th>
<th>r&lt;sub&gt;max&lt;/sub&gt; / r&lt;sub&gt;min&lt;/sub&gt; Mean</th>
<th>r&lt;sub&gt;max&lt;/sub&gt; / r&lt;sub&gt;min&lt;/sub&gt; SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>0.76</td>
<td>0.07</td>
<td>1.58</td>
<td>0.32</td>
<td>2.11</td>
<td>0.48</td>
</tr>
<tr>
<td>C2</td>
<td>0.70</td>
<td>0.09</td>
<td>1.61</td>
<td>0.38</td>
<td>2.37</td>
<td>0.37</td>
</tr>
<tr>
<td>C3</td>
<td>0.68</td>
<td>0.08</td>
<td>1.59</td>
<td>0.35</td>
<td>2.45</td>
<td>0.42</td>
</tr>
<tr>
<td>C4</td>
<td>0.73</td>
<td>0.09</td>
<td>1.85</td>
<td>0.48</td>
<td>2.26</td>
<td>1.04</td>
</tr>
<tr>
<td>C5</td>
<td>0.76</td>
<td>0.07</td>
<td>1.55</td>
<td>0.31</td>
<td>2.04</td>
<td>0.60</td>
</tr>
<tr>
<td>C6</td>
<td>0.71</td>
<td>0.09</td>
<td>2.03</td>
<td>0.40</td>
<td>2.45</td>
<td>0.52</td>
</tr>
<tr>
<td>C7</td>
<td>0.68</td>
<td>0.08</td>
<td>1.78</td>
<td>0.33</td>
<td>2.47</td>
<td>0.46</td>
</tr>
</tbody>
</table>

As mentioned previously, the value of the shape factor is a combination of macroscopic shape and microscopic surface texture. The use of this factor alone will not describe the surface smoothness if the macroscopic shape of the particles concerned differs substantially. For example, lactose crystals of different values of elongation ratio will have different values of shape factor although these particles may have similar tomahawk shape and surface smoothness. Therefore, in order to compare the surface texture of particles having different elongation ratio values more accurately, a new factor has to be introduced, which takes into consideration both the shape factor and elongation ratio. From visual examination of the SE micrographs (Figures 2.4-2.14 and 3.2), all the lactose crystals prepared in this study to date had a shape closer to an elongated cuboid than to a sphere. It would be reasonable to assume that a shape factor calculated from the projected image of an elongated cuboid reflects the shape of lactose crystals more closely than the shape factor based upon the projected image of a sphere. The projected images of an elongated cuboid and a sphere are a rectangle and a circle, respectively. By combining the elongation ratio of the rectangle and the shape factor which was calculated according to a projected image of a spherical particle, it is possible to create a new shape factor that reflects the surface smoothness of the rectangular image of a cuboidal particle only. In order to calculate the new shape factor, the following equations are generated, assuming a rectangular image with length (L) and width (W) that has the same area as that of a circular image with a diameter d.
Since the area of a rectangle \( A_{\text{rec}} = W \times L \)
If Elongation ratio \( E = L / W \), then
\[
A_{\text{rec}} = \frac{L^2}{E};
\]

Perimeter of the rectangle \( P_{\text{rec}} \)
\[
= 2 (W + L)
= 2 (L + L / E)
= 2 L (1 + E) / E. \quad (3-3)
\]

Since the area of a circle \( A_{\text{cir}} = \pi (d/2)^2 \)
if \( A_{\text{cir}} = A_{\text{rec}} \), then
\[
L^2 / E = \pi \times (d/2)^2 \quad \text{or} \quad d = \frac{2L}{\sqrt{\pi E}}
\]

Thus, the perimeter of a circle \( P_{\text{cir}} \) is
\[
P_{\text{cir}} = \pi d = 2L \sqrt{\frac{\pi}{E}} \quad (3-4)
\]

By dividing equation (3-4) by equation (3-3), the following equation is obtained:
\[
P_{\text{cir}} = \frac{\sqrt{\pi E}}{1 + E} P_{\text{rec}} \quad (3-5)
\]

Therefore, the shape factor of a rectangle \( S_{\text{rec}} \) can be calculated as:
\[
S_{\text{rec}} = \frac{4\pi \text{area}}{P_{\text{rec}}^2} = \frac{4\pi \text{area}}{P_{\text{cir}}^2} \times \frac{(1 + E)^2}{\pi E} = S_{\text{cir}} \times \frac{(1 + E)^2}{\pi E} \quad (3-6)
\]

where \( S_{\text{cir}} \) is the shape factor calculated according to a circle and this has been the factor employed in all the previous calculations. \( S_{\text{rec}} \), as defined above, is a factor assuming a shape of rectangle with a known elongation ratio. It is solely dependent upon the particle surface for cuboidal particles and therefore, it is termed 'surface factor'.

If the shape factor \( S_{\text{cir}} \) and elongation ratio \( E \) of a cuboidal particle are known, then the 'surface factor' of that particle can be calculated according to equation (3-6). The value of the 'surface factor' should be between 0 and 1. A cuboidal particle with perfectly smooth
surface should have a value for the 'surface factor' of 1. The rougher the surface, the smaller the 'surface factor' will be.

Table 3.4 shows the calculated values of 'surface factor' of all batches of lactose crystals prepared from either Carbopol 934 gels or from lactose solutions with constant stirring. It can be seen that the 'surface factor' values correlate better with SE micrograph observation (Figures 2.4-2.14 and 3.2) than the shape factor. For example, the lower value of the shape factor (0.68) for batch 10 was indicative of either an elongated shape or rough surface or the combination of both. According to the SE micrograph (Figure 2.13), most of the crystals of this batch of lactose were elongated with a smooth surface. Such an observation was in agreement with a value of 'surface factor' of 0.99. Furthermore, batches 3 and 4 had the lowest values of 'surface factor' (0.78 and 0.88, respectively) of all the batches of lactose crystals, indicating both batches of lactose had the roughest surface. This was justified by their SE micrographs (Figures 2.6 & 2.7)

**Table 3.4 The shape factor ($S_{cir}$), elongation ratio ($E$) and 'surface factor' ($S_{rec}$) of lactose crystals prepared either with agitation or from Carbopol 934 gels**

<table>
<thead>
<tr>
<th>Batch No</th>
<th>$S_{cir}$</th>
<th>$E$</th>
<th>$S_{rec}$</th>
<th>Batch No</th>
<th>$S_{cir}$</th>
<th>$E$</th>
<th>$S_{rec}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.74</td>
<td>1.39</td>
<td>0.97</td>
<td>C1</td>
<td>0.76</td>
<td>1.58</td>
<td>1.02</td>
</tr>
<tr>
<td>2</td>
<td>0.74</td>
<td>1.39</td>
<td>0.97</td>
<td>C2</td>
<td>0.70</td>
<td>1.61</td>
<td>0.94</td>
</tr>
<tr>
<td>3</td>
<td>0.60</td>
<td>1.28</td>
<td>0.78</td>
<td>C3</td>
<td>0.68</td>
<td>1.59</td>
<td>0.91</td>
</tr>
<tr>
<td>4</td>
<td>0.68</td>
<td>1.29</td>
<td>0.88</td>
<td>C4</td>
<td>0.73</td>
<td>1.85</td>
<td>1.02</td>
</tr>
<tr>
<td>5</td>
<td>0.72</td>
<td>1.30</td>
<td>0.93</td>
<td>C5</td>
<td>0.76</td>
<td>1.55</td>
<td>1.01</td>
</tr>
<tr>
<td>6</td>
<td>0.69</td>
<td>1.64</td>
<td>0.93</td>
<td>C6</td>
<td>0.71</td>
<td>2.03</td>
<td>1.02</td>
</tr>
<tr>
<td>7</td>
<td>0.74</td>
<td>1.34</td>
<td>0.96</td>
<td>C7</td>
<td>0.68</td>
<td>1.78</td>
<td>0.94</td>
</tr>
<tr>
<td>8</td>
<td>0.72</td>
<td>1.37</td>
<td>0.94</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.78</td>
<td>1.63</td>
<td>1.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.68</td>
<td>2.08</td>
<td>0.99</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>0.73</td>
<td>1.71</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Lactose particles prepared from Carbopol gels generally have a value of 'surface factor' close to unity, indicating these batches of lactose had such smooth surfaces that the surface asperities, if any, were undetectable by the method employed in the current study. Batches C2 and C3 had lower values of 'surface factor' (0.94 and 0.91, respectively) and this was in agreement with the visually rougher surfaces of these batches of lactose as shown in their SE micrographs (Figure 3.2). Lactose batch C7 also showed a lower value of 'surface factor' (0.94) and this might be due to the adhered small needle crystals on the surface of the coarser lactose crystals. The rest of the batches of lactose crystals all showed a 'surface factor' value of 1, which was higher than most of the lactose crystals prepared under constant agitation. If the concentration of Carbopol gels was sufficient to suspend the majority of the growing lactose crystals (≥ 4% w/v), then the particle shape and surface smoothness of the resultant lactose crystals appeared to be more or less independent of the crystallisation conditions. This is shown by there being no significant difference (p > 0.05) in either the shape factor or elongation ratio of these batches of lactose. Growth of crystals from Carbopol 934 gels may prove to be beneficial in terms of the preparation of lactose particles of regular shape, uniform size distribution and consistent surface textures.

The mean elongation ratio of five batches (C1, C4-7) of lactose crystals prepared from Carbopol 934 gels with concentrations ≥ 0.4% w/v was 1.76 ± 0.20, which was significantly higher (p < 0.05) than the mean value (1.49 ± 0.25) obtained from the eleven batches of lactose prepared under constant stirring. These results suggest that Carbopol 934 gels may slightly alter the crystal habit of lactose crystals, leading to the production of more elongated particles. The former crystals also had a significantly higher (p < 0.05) value (1.00 ± 0.03) of 'surface factor' than 0.94 ± 0.07 of the latter crystals. This further confirmed that lactose crystals prepared from Carbopol 934 gels had a smoother surface than those prepared under constant stirring.

Figure 3.3 shows the SE micrographs of the different size fractions of lactose batch C1. Since these samples were classified from the same batch of lactose, they were prepared under exactly the same conditions. From the SE micrographs, it can be seen that although these crystals generally had a tomahawk shape, there was a slight difference in terms of the shape and surface texture. For example, the fraction of lactose crystals < 63 μm (C8)
Figure 3.3 The scanning electron micrographs of different size fractions of one batch of lactose (Car 1) prepared from Carbopol 934 gels.
Chapter three: *Crystallisation of lactose from Carbopol 934 gels*

contained a combination of prismatic, pyramidal and tomahawk shaped particles. Smaller particles tended to be prismatic, larger particles appeared to be pyramidal whilst the majority of the largest particles were tomahawk-shaped. However, the majority of the fractions of crystals 63-90 µm (C1) and 90-125 µm (C9) were tomahawk-shaped with similar surface textures whereas while the lactose crystals > 125 µm (C10) were also tomahawk-shaped, some aggregates were observed in this size fraction.

From Table 3.5, it can be seen that crystals < 63 µm had the lowest value of elongation ratio, shape factor and ‘surface factor’ but the highest value of rugosity, suggesting that particles of this size fraction had the most irregular and least elongated shape with the most surface asperities. The 63-90 µm crystals exhibited the highest values for the shape factor (0.76) and a value close to unity for the ‘surface factor’, indicating that this size fraction had the most regular shape with the least surface asperities. The 90-125 µm crystals had a lower value of shape factor (0.71) but higher value of elongation ratio (2.02) than the 63-90 µm crystals. This was indicative of a less regular but more elongated shape for the former size fraction than for the latter fraction. The 90-125 µm particles also exhibited a ‘surface factor’ value of 1.02, indicating this size fraction also had smooth particle surface. However, further increasing the particle size above 125 µm appears to reduce the values of all the shape descriptors. Increasing the particle size from < 63 µm through 63-90 µm to 90-125 µm, tended to increase the elongation ratio of the crystals, suggesting larger particles were more elongated than smaller particles. Such a trend was found previously for the crystals prepared under constant stirring (Section 2.3.2). Therefore, similar to growth in aqueous solutions under constant stirring, lactose crystals also grew along their longitudinal axes in Carbopol 934 gels. Further growth of lactose crystals to > 125 µm appears to produce less elongated particles since the crystals > 125 µm had an elongation ratio of 1.83 ± 0.21, which was significantly (p < 0.01) lower than 2.02 ± 0.37 for the crystals 90-125 µm. This phenomenon suggests that although lactose crystals grow more along their length than along their width, the bias toward longitudinal axes might decrease when the crystals exceed a certain limiting diameter.

The shape factor and ‘surface factor’ ($S_{rec}$) of lactose crystals were also shown to be determined by the particle size. The shape factor value first increased from 0.62 ± 0.15 for
crystals < 63 μm to 0.76 ± 0.07 for crystals 63-90 μm (p < 0.01), then decreased to 0.71 ± 0.07 for crystals 90-125 μm (p < 0.05) and further decreased to 0.66 ± 0.06 for crystals > 125 μm (p < 0.01). Crystals within the 63-90 and 90-125 μm size fractions had higher values for the 'surface factors' than either crystals < 63 or > 125 μm. Therefore, crystals of different size fractions had different surface smoothness. Crystals between 63-125 μm had a more regular, elongated shape with smoother surface than smaller (< 63 μm) or larger (> 125 μm) crystals.

Table 3.5 Some physical properties of different size fractions of lactose particles batch C1

<table>
<thead>
<tr>
<th>Size (μm)</th>
<th>Density (g cm⁻³)</th>
<th>Diameter (μm)</th>
<th>Shape factor</th>
<th>Elongation ratio</th>
<th>S_rec</th>
<th>Rugosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;63</td>
<td>1.57</td>
<td>65.6 ± 17.9</td>
<td>0.62 ± 0.15</td>
<td>1.52 ± 0.28</td>
<td>0.82</td>
<td>2.68</td>
</tr>
<tr>
<td>63-90</td>
<td>1.54</td>
<td>104.1 ± 19.1</td>
<td>0.76 ± 0.07</td>
<td>1.58 ± 0.33</td>
<td>1.02</td>
<td>1.70</td>
</tr>
<tr>
<td>90-125</td>
<td>1.53</td>
<td>174.6 ± 19.6</td>
<td>0.71 ± 0.07</td>
<td>2.02 ± 0.37</td>
<td>1.02</td>
<td>ND</td>
</tr>
<tr>
<td>&gt;125</td>
<td>1.54</td>
<td>211.8 ± 26.9</td>
<td>0.66 ± 0.06</td>
<td>1.83 ± 0.21</td>
<td>0.92</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND-not detectable, beyond the limit of the Fisher sub-sieve sizer

3.3.2 Characterisation of crystal forms and crystallinity

3.3.2a Thermal gravimetric analysis (TGA)

Figure 3.4 shows a typical TGA thermogram of lactose crystals. Weight loss between approximately 120° - 190°C was due to the dehydration of crystallisation water instead of the vapourization of free water, which occurred at about 100°C. However, for micronised lactose dehydration was manifest at 95.8°C, suggesting that the majority of the water content in such lactose was surface-adsorbed, free water. The weight loss at 200° - 250°C was due to decomposition of lactose. Lactose is known to melt and decompose at temperatures over 200°C (Nikerson, 1976). From the weight loss of dehydration, it is possible to calculate the water of hydration associated with lactose using the following equation:
Chapter three: Crystallisation of lactose from Carbopol 934 gels

\[
n = \frac{\text{Mole of water}}{\text{Mole of anhydrous lactose}} = \frac{W_{\text{loss}}}{RMM_{\text{water}}} / \frac{(1-W_{\text{loss}})}{RMM_{\text{lactose}}} \tag{3-7}
\]

where \( W_{\text{loss}} \) is the percentage weight loss between 130-170°C, \( RMM_{\text{water}} \) and \( RMM_{\text{lactose}} \) are the relative molecular masses of water (18.0) and anhydrous lactose (342.3), respectively.

From Table 3.6, it can be seen that almost all the batches of lactose crystals were found to have one mole of crystallisation water per mole of anhydrous lactose, suggesting the lactose was mainly monohydrate. Although batch 11 and Lactochem\textsuperscript{TM} lactose were calculated to have a slightly less than unity mole of crystallisation water, this might be due to the underestimation of the weight loss from their TGA thermograms. Although TGA has long been used to measure water content of solids (McCauley and Brittain, 1995), the use of TGA thermograms to measure crystallisation water has two limitations (Khankari et al., 1992). The first limitation is the difficulty in correctly deciding the cut-off point for a specified TGA step. Inaccurate cut-off points may be the cause of an under-estimation in calculating the water of crystallisation associated with batch 11 and Lactochem\textsuperscript{TM} lactose. The second problem is that TGA may not differentiate between various states of binding or water location in the hydrate. However, this problem is not an important concern when measuring the hydration of lactose monohydrate. Interestingly, the micronised lactose showed similar water content to lactose monohydrate. Since the micronised lactose was prepared by micronizing Lactochem\textsuperscript{TM} lactose in an air jet mill, micronised lactose retained the original water content of lactose monohydrate. The majority of the water content of micronised lactose was thought to be free water since the dehydration of the lactose occurred at 95.8°C. Mechanical grinding has been reported to reduce the water content of ground lactose (Morita et al., 1984), however, such a loss was not found to occur in the current study after size-reduction in an air jet mill. Since a large portion of the water content was surface adsorbed water, the resultant capillary forces may be responsible for the highly cohesive and poor flowable properties observed for micronised lactose.
Figure 3.4 The TGA thermograms of some batches of lactose crystals. (A): micronised lactose; (B): batch 14 and (C): lactose C7
Table 3.6 The weight loss (W$_{\text{loss}}$) between 130-170°C and moles of crystallisation water per mole of anhydrous lactose (n) of some batches of lactose crystals calculated from TGA thermograms

<table>
<thead>
<tr>
<th>Batch No</th>
<th>W$_{\text{loss}}$ (% w/w)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>5.02</td>
<td>1.00</td>
</tr>
<tr>
<td>11</td>
<td>4.19</td>
<td>0.83</td>
</tr>
<tr>
<td>13</td>
<td>4.90</td>
<td>0.98</td>
</tr>
<tr>
<td>14</td>
<td>6.05</td>
<td>1.22</td>
</tr>
<tr>
<td>C1</td>
<td>5.41</td>
<td>1.09</td>
</tr>
<tr>
<td>C7</td>
<td>5.52</td>
<td>1.11</td>
</tr>
<tr>
<td>Lactochem$^\text{TM}$</td>
<td>4.42</td>
<td>0.88</td>
</tr>
<tr>
<td>Micronised lactose</td>
<td>5.09</td>
<td>1.02</td>
</tr>
</tbody>
</table>

3.3.2b Differential scanning calorimetry (DSC)

Figures 3.5 and 3.6 show the typical DSC thermograms of one batch of lactose crystals prepared under constant stirring (batch 14) and one batch of crystals prepared from Carbopol 934 gels (batches C1). The DSC-curve of each batch of lactose shows the typical DSC thermogram of α-lactose monohydrate (Lerk et al., 1984a). The endothermic transition starting at about 130°C corresponds to the dehydration of crystallisation water whereas the endothermic peak at about 217°C is the melting endotherm of α-lactose monohydrate. A small exothermic peak was observed at about 177°C, which can be attributed to the crystallisation of amorphous lactose (Lerk et al., 1984b). Different batches of lactose had different intensities of this peak, in an increasing order of micronised lactose > Batch 14 > Batch 13 > Batch 4 > Lactochem$^\text{TM}$ lactose > C1 > C7. Immediately before the exotherm was a small endothermic peak at about 170°C in the DSC thermograms of micronised lactose and lactose batches 4, 13 & 14. The endothermic peak may be due to the rearrangement of molecules in amorphous lactose before transformation to crystalline lactose.

If the enthalpy of dehydration ($\Delta H_d$) that can be measured by the dehydration endothermic peak of the DSC thermogram is the same as the enthalpy of vaporization of water ($\Delta H_v$),
then the number of moles of water per mole of anhydrous lactose can be calculated using the following equation (Khankari et al., 1992):

\[
 n = \frac{\Delta H_d \times RMM_{lactose}}{(\Delta H_v - \Delta H_d) \times RMM_{water}}
\]  

(3-8)

where \(\Delta H_d\) is the enthalpy of dehydration from the dehydration endotherm of DSC thermogram, mJ/mg; \(\Delta H_v\) is the enthalpy of vaporization of water, 2261 mJ/mg (Stark and Wallace, 1976); \(RMM_{lactose}\) and \(RMM_{water}\) are the relative molecular masses of anhydrous lactose (340.3) and water (18.0), respectively.

<table>
<thead>
<tr>
<th>Batch No</th>
<th>Enthalpy of dehydration (mJ/mg)</th>
<th>Moles of water/mole of anhydrous lactose (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>102.6</td>
<td>0.90</td>
</tr>
<tr>
<td>11</td>
<td>97.1</td>
<td>0.85</td>
</tr>
<tr>
<td>13</td>
<td>100.1</td>
<td>0.88</td>
</tr>
<tr>
<td>14</td>
<td>115.3</td>
<td>1.02</td>
</tr>
<tr>
<td>C1</td>
<td>105.9</td>
<td>0.93</td>
</tr>
<tr>
<td>C7</td>
<td>108.7</td>
<td>0.96</td>
</tr>
<tr>
<td>Lactochem(^\text{TM})</td>
<td>99.3</td>
<td>0.87</td>
</tr>
<tr>
<td>Micronised lactose</td>
<td>156.1</td>
<td>1.41</td>
</tr>
</tbody>
</table>

As is shown in Table 3.7, all the batches of lactose crystals had approximately 1 mole of crystallisation water per mole of anhydrous lactose except for the micronised lactose which contained 1.41 moles of water per mole of anhydrous lactose. The mean water content of all the batches investigated measured by DSC was 0.98, which was in excellent agreement with 1.02 measured by TGA. There was no significant difference (paired t-test, \(p = 0.60\)) in the water contents measured by these two methods, indicating either TGA or DSC could be employed to measure the hydration of lactose crystals.
Figure 3.5 The DSC thermograms of micronised lactose and one batch of lactose crystals prepared under constant stirring (Batch 14)
Figure 3.6 The DSC thermograms of some batches of lactose prepared from Carbopol 934 gels
The crystallinity of lactose crystals can be calculated by the heat of crystallisation of amorphous lactose which can be obtained from the DSC thermograms (Saleki-Gerhardt et al., 1994). Given the specific heat of crystallisation of amorphous lactose, $\Delta H_c$ mJ/mg, then the content of amorphous lactose (% amorphous w/w) in the crystalline lactose can be calculated as:

$$\% \text{ amorphous lactose} = \frac{\Delta h_c}{\Delta H_c} \times 100\%$$  \hspace{1cm} (3-9)

where $\Delta h_c$ is the heat of crystallisation of amorphous lactose in mJ/mg lactose crystals and $\Delta H_c$ is taken as 32 mJ/mg for amorphous lactose (Sebhatu et al., 1994). The determined crystallinity of each batch of lactose is shown in Table 3.8.

Table 3.8 Crystallinity of lactose crystals calculated from the heat of crystallisation of amorphous lactose obtained from the DSC thermograms

<table>
<thead>
<tr>
<th>Batch No</th>
<th>Heat of crystallisation</th>
<th>% amorphous lactose</th>
</tr>
</thead>
<tbody>
<tr>
<td>batch 4</td>
<td>2.03 mJ/mg</td>
<td>6.3</td>
</tr>
<tr>
<td>batch 11</td>
<td>1.88 mJ/mg</td>
<td>5.9</td>
</tr>
<tr>
<td>batch 13</td>
<td>2.77 mJ/mg</td>
<td>8.7</td>
</tr>
<tr>
<td>batch 14</td>
<td>9.67 mJ/mg</td>
<td>30.2</td>
</tr>
<tr>
<td>CL</td>
<td>1.35 mJ/mg</td>
<td>4.2</td>
</tr>
<tr>
<td>C7</td>
<td>0.74 mJ/mg</td>
<td>2.3</td>
</tr>
<tr>
<td>Lactochem™</td>
<td>1.82 mJ/mg</td>
<td>5.7</td>
</tr>
<tr>
<td>Micronised lactose</td>
<td>16.58 mJ/mg</td>
<td>51.8</td>
</tr>
</tbody>
</table>

The % amorphous lactose varied from 2.3% for lactose batch C7 to 51.8% for micronised lactose. It is well known that mechanical grinding introduces crystalline disorder on particle surfaces, which results in the particles becoming more amorphous (Ward and Schultz, 1995). Therefore, the high content of amorphous lactose in micronised lactose might be expected since the lactose particles were prepared by extensive energy input, leading to
reduction in the particle size down to about 5 μm. Such a micronisation process would be expected to convert a large portion of crystalline lactose to amorphous lactose. Interestingly, lactose batch 14 possessed a higher amorphicity (30.2%) than the other batches of crystalline lactose although this batch of lactose did not undergo any size-reduction processing. As mentioned previously, batches 13 and 14 were two size fractions from the same batch of lactose crystals (Section 2.3.2). Batch 13 had a mean diameter of 104.7 μm whilst batch 14 had a mean diameter of 68.6 μm (Table 2.7). Therefore, it is reasonable to assume that the higher amorphicity of batch 14 (30.2%) than batch 13 (8.7%) may be due to the smaller particle size for the former particles than in the case of the latter particles. Although smaller particles may have more crystal disorder on their surfaces due to their higher specific surface areas, such a dependence of crystallinity on particle size will be subject to further investigation. Crystals prepared from Carbopol 934 gels (batches C1 and C7) exhibited the highest crystallinity (the lowest amorphicity) of all the lactose crystals investigated. This is not surprising since crystals prepared from gels were allowed to grow in the absence of mechanical agitation which could introduce disorder in the crystal lattice.

3.3.2c X-ray powder diffraction (XRPD)

Figure 3.7 shows the X-ray powder diffraction patterns for different batches of lactose. All batches had similar XRPD patterns to α-lactose monohydrate (Brittain et al., 1991 and Sebhatu et al., 1994). However, different batches showed different peak intensities, which were indicative of different degrees of crystallinity of these lactose crystals.

X-ray powder diffractometry has been widely used to determine the degree of crystallinity of pharmaceuticals (Suryanarayanan, 1995). Some XRPD methods involve the demarcation and measurement of the crystalline intensity and amorphous intensity from the powder patterns (Nakai et al., 1982) whilst others employ an internal standard such as lithium fluoride to measure the crystallinity of drugs (Gerdhardt et al., 1994). Therefore, it is not possible to calculate the absolute degree of crystallinity by the XRPD patterns obtained in current work since neither 100% amorphous lactose nor any internal standard was measured. However, since the degree of crystallinity is a function of either the integrated intensity (area under the curve) or the peak intensity (height), the relative degree of crystallinity of different samples of the same crystal forms may be compared by their peak
intensity at the same diffraction angle. The relative degree of crystallinity (RDC) was defined as the ratio of the peak intensity of a given sample of a single polymorphic form to that of another specimen of the same polymorph which produced the greatest possible response (Ryan, 1986). RDC may be employed to determine the rank order of crystallinity of different batches of lactose crystals. The integrated peak intensities at $2\theta = 12.5^\circ$, $16.5^\circ$, $23.8^\circ$ and $27.5^\circ$, which are characteristic for $\alpha$-lactose monohydrate, were determined by weighing the areas under the curve of the X-ray diffraction profiles. The RDC was calculated by dividing the sum of the four integrated peak intensities of each batch by that of batch C7 since this batch produced the greatest trace of X-ray diffraction. It can be seen from Table 3.9 that the degree of crystallinity decreases in the order of batch C7 > batch C1 > Lactochem™ lactose > batch 11 > batch 14. The results obtained by XRPD were in agreement with the rank order of crystallinity calculated by DSC (Table 3.8).

Table 3.9 Estimates of the integrated peak intensities (cm$^2$) of X-ray diffraction patterns and the relative degree of crystallinity (RDC) of lactose crystals.

<table>
<thead>
<tr>
<th>Angle ($2\theta$)</th>
<th>Lactochem™</th>
<th>Batch 11</th>
<th>Batch 14</th>
<th>C1</th>
<th>C7</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5°</td>
<td>0.72</td>
<td>0.70</td>
<td>0.41</td>
<td>0.58</td>
<td>0.81</td>
</tr>
<tr>
<td>16.5°</td>
<td>0.11</td>
<td>0.88</td>
<td>0.10</td>
<td>0.67</td>
<td>0.68</td>
</tr>
<tr>
<td>23.8°</td>
<td>0.16</td>
<td>0.11</td>
<td>0.16</td>
<td>0.45</td>
<td>0.40</td>
</tr>
<tr>
<td>27.5°</td>
<td>0.04</td>
<td>0.07</td>
<td>0.07</td>
<td>0.19</td>
<td>0.17</td>
</tr>
<tr>
<td>Sum</td>
<td>1.03</td>
<td>0.96</td>
<td>0.74</td>
<td>1.89</td>
<td>2.06</td>
</tr>
<tr>
<td>RDC (%)</td>
<td>50.0</td>
<td>46.6</td>
<td>35.9</td>
<td>91.7</td>
<td>100</td>
</tr>
</tbody>
</table>

The lactose crystals prepared from Carbopol 934 gels had higher degree of crystallinity than lactose particles crystallised under conditions of constant mechanical agitation. Lactochem™ lactose was also found to have a higher degree of crystallinity than the crystals prepared under conditions of constant agitation. This might have been due to the extended period of storage of Lactochem™ lactose, during which some of the amorphous regions of the original particles may have undergone crystallisation. Batch 14 exhibited the lowest degree of crystallinity, confirming the results obtained from DSC. The improved crystallinity of lactose crystals prepared from Carbopol 934 gels was due to the crystals not being subject to any external turbulence, such as that encountered when grown in a stirred
system. The gel framework acts like a three-dimensional crucible in which the crystal nuclei are delicately held in the position of their formation while growth proceeds without the intervention of convective movement of the solute. Furthermore, the crystallisation from the gels occurred at a much slower rate than in the case of crystallisation under mechanical stirring. It is known that the growth rate of a crystal determines the number of defects built into the crystals since the higher the growth rate, the more crystal defects are likely to form in the crystal lattice (Henisch, 1988). All these effects may have contributed to the preparation of lactose crystals with well defined morphology and improved crystallinity by means of crystallisation from Carbopol gels.
Figure 3.7 The X-ray powder diffraction patterns of some batches of lactose crystals.
3.3.3 Flowability of lactose crystals

Table 3.10 shows that different batches of lactose exhibited different degrees of both the angle of repose ($\theta_r$) and the angle of slide ($\theta_s$). Lactose particles from batches 10 & 11 produced significantly ($p < 0.01$) smaller values of $\theta_r$ or $\theta_s$ than the other batches of lactose, indicating that the former had higher flowability than the latter. As mentioned before (Section 2.3.1), the majority of lactose crystals from batches 10 & 11 had an elongated, cuboidal shape. Elongated particles are known to build up open packings of high porosity. In flow, such particles tend to be oriented with their long axes in the direction of the flow and if such an orientation is achieved, these particles show less internal friction than more isometric particles (Neumann, 1967). Batches 14 & 16 produced the largest $\theta_r$ and these particles did not even slide off the plane that had been tilted to an angle of 90° to the horizontal, indicating that these two batches of lactose were highly cohesive and had poor flowability. This is likely to be attributable to the smaller mean diameter (approximately 65 $\mu$m, Section 2.3.2) of batches 14 & 16 in comparison to the other batches of lactose (> 90 $\mu$m) since powders of smaller particle size are known to produce larger $\theta_r$ due to their internal cohesiveness (Neumann, 1967). Lactose particles prepared from Carbopol 934 gels showed more consistent values of $\theta_r$ (40-46°) and $\theta_s$ (40-48°) in comparison to crystals prepared using agitation and this might have been due to more effective control of the particle morphology (Section 3.3.1) of the former particles (Section 2.3.1). Further, the crystals prepared from Carbopol 934 gels appeared to have better flowability than the majority of the batches prepared under constant stirring since they had significantly ($p < 0.01$) smaller values of $\theta_s$ than the latter batches of lactose (batches 1-8). Lactose particles crystallised from Carbopol 934 gels have been shown to have a smoother surface (Section 3.3.1) than lactose prepared under conditions with agitation and smoothing a particle surface is known to reduce the internal friction, which in turn decreases the angle of slide (Hiestand, 1966). Most batches of recrystallised lactose had lower $\theta_r$ and $\theta_s$ than the regular Lactochem® lactose. The angle of slide was shown to be significantly ($p < 0.01$, paired t-test) higher than the angle of repose for all samples taken from the same batch of lactose. The angle of repose differs from the angle of slide in that the former is determined by the least stable particles whilst the latter depends largely on the average conditions for the bulk
of the powder (Hiestand, 1966). Therefore, the angle of slide may correlate more closely with flow properties than the angle of repose.

Table 3.10 The angle of repose ($\theta_r$) and angle of slide ($\theta_s$) of different batches of lactose crystals [Mean (SD), $n \geq 3$]

<table>
<thead>
<tr>
<th>Crystallisation with agitation</th>
<th>Crystallisation in Carbopol 934 gels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch no</td>
<td>$\theta_r$ (°)</td>
</tr>
<tr>
<td>1</td>
<td>43 (1)</td>
</tr>
<tr>
<td>3</td>
<td>41 (1)</td>
</tr>
<tr>
<td>4</td>
<td>43 (1)</td>
</tr>
<tr>
<td>5</td>
<td>46 (2)</td>
</tr>
<tr>
<td>6</td>
<td>53 (1)</td>
</tr>
<tr>
<td>7</td>
<td>38 (0)</td>
</tr>
<tr>
<td>8</td>
<td>56 (2)</td>
</tr>
<tr>
<td>9</td>
<td>37 (1)</td>
</tr>
<tr>
<td>10</td>
<td>34 (1)</td>
</tr>
<tr>
<td>11</td>
<td>32 (1)</td>
</tr>
<tr>
<td>13</td>
<td>58 (1)</td>
</tr>
<tr>
<td>14</td>
<td>60 (0)</td>
</tr>
<tr>
<td>15</td>
<td>57 (2)</td>
</tr>
<tr>
<td>16</td>
<td>59 (1)</td>
</tr>
</tbody>
</table>

3.3.4 Characterisation of Carbopol gels

Figure 3.8 shows that neutralised Carbopol 934 gels at the investigated concentrations were non-Newtonian, pseudoplastic materials possessing a low yield value. Both the apparent viscosity and the yield value increased with concentrations of the gel. There was negligible difference between the up curve (the curve obtained by increasing the shear stress) and the down curve (the curve obtained by decreasing the shear stress). All these results were in agreement with previous reports (Barry and Meyer, 1979a; Berney and Deasy, 1979).
Figure 3.8 Flow curves of neutralised gels containing different amounts of Carbopol 934 obtained using a continuous shear methodology (Error bars denote standard deviation, n = 6). a: 0.2%; b: 0.4%; c: 0.6% and d: 0.8% w/w.

Figure 3.9 shows the change in the apparent viscosity of Carbopol 934 gels with the change in the shear rate of the gels. Increasing the shear rate decreased the apparent viscosity of the gels regardless of the concentration of Carbopol 934 and this behaviour is typical for a non-Newtonian flow. The apparent viscosity also increased rapidly with an increase in the concentration of Carbopol gel (Table 3.11). For example, increasing the concentrations of Carbopol 934 gels from 0.2% to 0.8% w/w increased the coefficient of apparent viscosity, $A$, from 6.19 to 85.76. A constant, $A'$, could be obtained when shear stress was plotted
against the shear rate and this corresponds to the yield value, the minimum shear stress required to exert flow of the gels. According to both Figure 3.8 and Table 3.10, the yield value of the gel also increased as a function of Carbopol concentration.

Table 3.11 The parameters calculated from the flow curves of Carbopol gels (Mean, n = 6)

<table>
<thead>
<tr>
<th>Gel conc. (w/w)</th>
<th>( \eta_{app} = A \times \text{Rate}^B )</th>
<th>Stress = ( A' + B' \times \text{Rate}^C )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2%</td>
<td>6.19, -0.51</td>
<td>0.77, 5.26, 0.53</td>
</tr>
<tr>
<td>0.4%</td>
<td>25.48, -0.52</td>
<td>1.79, 22.05, 0.54</td>
</tr>
<tr>
<td>0.6%</td>
<td>57.07, -0.50</td>
<td>3.49, 52.20, 0.47</td>
</tr>
<tr>
<td>0.8%</td>
<td>85.76, -0.65</td>
<td>5.18, 80.22, 0.41</td>
</tr>
</tbody>
</table>

Apparent viscosity (\( \eta_{app} \)) in Pa.s; rate is shear rate in s\(^{-1}\) and stress is shear stress in Pa.

Figure 3.9 The relationship between the shear rate and apparent viscosity for different concentrations of Carbopol 934 gels (Error bars denote the standard deviation, n = 6).
Chapter three: Crystallisation of lactose from Carbopol 934 gels

The relationship between viscosity and polymer concentrations was determined using the best fit curves according to the least squares criterion. A plot of the log of viscosity against concentration produced the best straight line with $r^2$ over 0.99 when the viscosity was calculated according to the plots of either viscosity against shear rate or the shear stress against the shear rate. The exponential relationship between viscosity and Carbopol concentration is typical of high molecular weight polymer systems (Martin, 1965).

On the basis of the results obtained it was possible to derive an empirical relationship between yield value and Carbopol concentration ($C_c$) as % w/v.

\[
Yield\ value\ (N\ m^2) = 6.91 \times C_c^{1.38} \quad r^2 = 0.997 \quad (3-10)
\]

The ability of Carbopol 934 gels to sustain a relatively high shear stress without exhibiting significant flow has been considered by other workers to be critical in the preparation of suspensions and a highly significant correlation was observed between the sedimentation height of sulphadimidine suspensions and the yield values of the gel (Berney and Deasy, 1979). The yield value for Carbopol gels was found to depend on polymer concentration and to be independent of the stress applied to the systems (Fischer et al., 1961). The yield value can also be expected to be crucial in the crystallisation of lactose particles. From the relationship between the yield value and Carbopol 934 concentrations, it is possible to calculate the minimum theoretical concentration of the gel required to produce a sufficient yield value that can prevent the lactose crystals from sedimentation.

If a spherical particle having a density $\rho_p$ (kg m$^{-3}$) and a diameter, $d$ (m) is suspended in a gel with density, $\rho_g$ (kg m$^{-3}$), then the gravitational force, $F_{grav}$ (N), acting on the particle can be calculated as:

\[
F_{grav} = \frac{\pi}{6} d^3 (\rho_p - \rho_g)g \quad (3-11)
\]

Since the cross-sectional area, $A$ (m$^2$), of the particle is

\[
A = \pi (d/2)^2 = \pi d^2 / 4 \quad (3-12)
\]
Then the pressure, $P_p$ (N m$^{-2}$), of the particle due to the gravitational force acting on the medium can be calculated by dividing equation (3-11) by equation (3-12) and re-arranging to give:

$$P_p = 6.54 d (\rho_p - \rho_g)$$  \hspace{1cm} (3-13)

In order for the particle to be suspended in the gel, the gel should provide a yield value not less than the particle pressure. Therefore, the minimum concentration ($C_{min}$) of the gel required to suspend the particle can be calculated according to equation (3-10) and equation (3-13) as.

$Yield \ value = P_p \ or$

$$6.91 \times C_{\min}^{1.38} = 6.54 d (\rho_p - \rho_g)$$  \hspace{1cm} (3-14)

Equation (3-13) can be re-arranged to obtain:

$$C_{\min} = 0.96 d^{0.72} (\rho_p - \rho_g)^{0.72}$$  \hspace{1cm} (3-15)

Since the concentrations of the Carbopol 934 in the gels were less than 1% w/v, which were much lower than lactose concentrations ($\geq 33$% w/v), the density ($\rho_g$) of the gel can be expected to be primarily determined by lactose. Lactose concentration decreases as crystallisation continues until eventually it reaches the saturated concentration at room temperature (25°C, 21.6% w/w) regardless of the initial concentration. The density of the saturated solution is about 1100 kg m$^{-3}$, which may be employed to represent the density of the gel, $\rho_g$. Therefore, for an $\alpha$-lactose monohydrate crystal ($\rho_p$, 1540 kg m$^{-3}$) with an equivalent diameter of 90 $\mu$m (or 9x10$^{-5}$ m) to be suspended in a Carbopol 934 gel, the minimum concentration of the gel can be calculated using equation (3-15) as 0.10% w/v.

Therefore, for an $\alpha$-lactose monohydrate crystal (90 $\mu$m in diameter) to be suspended in the gel, the concentration of Carbopol 934 in the gel should be $\geq$ 0.10% w/v. However, sedimentation of lactose crystals was found to occur in the gels with Carbopol 934
concentrations less than 0.4% w/w. Such a difference between the theoretically calculated and experimentally observed values may largely be due to these calculations assuming sphericity of the particles, which differs from the real situation in the case of lactose crystals. The pressure acting on the gel for an elongated, tomahawk-shaped lactose crystal changes with the direction of alignment of the particle in the gel, that is to say whether the particle is suspended horizontally (lowest pressure) or vertically (highest pressure) or other directions (intermediate pressures). Since the crystals align randomly in the gel medium, a range of pressures will be exerted. Therefore, the minimum concentration of Carbopol 934 in the gel required to suspend all the maturely grown crystals can be expected to be higher than the theoretical concentration. Nevertheless, the calculation above provides useful theoretical guidance in the choice of a suitable concentrations of the gel, which would be able to suspend any maturely grown lactose crystals without excessively reducing the growth rate of the crystals.
3.4 Summary

This section of work investigated the crystallisation of lactose from Carbopol 934 gels. Neutralised Carbopol 934 gels exhibited pseudoplastic flow with a yield value. Both the viscosity and yield value increased non-linearly with Carbopol 934 concentration. Lactose crystals produced from Carbopol 934 gels exhibited better uniformity both in terms of particle morphology and size distribution than those prepared from aqueous solutions under constant stirring. The growth rate of lactose was slowed by the presence of Carbopol 934 gels and crystals of well-defined, elongated shape with improved surface smoothness were prepared from such gels. When the crystals were suspended in the gel during the crystallisation, then, the particle shape and surface texture of the final products of lactose crystals were practically independent of the concentrations of lactose and Carbopol in the gels. Although Carbopol 934 gels appeared to change slightly the crystal habit of lactose, leading to the production of more elongated crystals, these gels did not change the crystal form of lactose since the lactose crystals prepared from Carbopol 934 gels exhibited a crystal form of α-monohydrate. However, the crystals prepared from Carbopol gels possessed higher crystallinity and better flowability than those prepared under constant stirring and it was decided to investigate the use of lactose prepared from the gels as a carrier for dry powder aerosols.
CHAPTER FOUR

EFFECTS OF THE MORPHOLOGY OF LACTOSE ON THE DELIVERY OF SALBUTAMOL SULPHATE
4.1 Introduction

There may be at least three major processes involved in the introduction of drug particles from a DPI to the respiratory tract. First, the drug-carrier blends have to be dispersed into the air stream from the powder chamber. Second, the drug particles must be detached or dissociated from the air-borne carrier particles if the drag forces of the inhalation air stream exceed the interparticulate forces between the drug and carrier particles. The detached, air-borne drug particles, together with any freely dispersed drug particles, may undergo further deaggregation whilst travelling the respiratory tract to produce a particle size suitable for deep lung penetration. However, the proportion of the drug particles that remain adhered to the carrier particles or any aggregates of the drug particles, are likely to deposit in the upper airways.

Factors that affect any of these processes would be expected to influence the overall deposition of drug particles in the respiratory tract. For example, dispersion and entrainment of a powder in an air stream are known to be determined by the inhalation flow rate (Hindle et al., 1994), particle size (Hickey et al., 1994), particle shape (Rietema, 1991), adhesive forces (Yamamoto, 1990) and design of the inhaler device (Dalby et al., 1996). The detachment and deaggregation of air-borne particles are also dependent upon the inhalation flow rate (Hindle and Byron, 1995), interparticulate forces (Zimon, 1982) and particle properties such as particle size and particle density (Gotoh, 1994a) as well as particle shape (Mullins et al., 1992). Particle deposition in the airways is primarily determined by the particle size (The Task Group On Lung Dynamics, 1966), particle shape (Hickey et al., 1992), breathing patterns (Newman et al., 1982) and airway morphology (Davies, 1961). Although the effects of particle size and size distribution of drug particles on drug deposition in the respiratory tract have been well documented, the effects of such morphological properties of the carrier particles have received considerably less attention. As mentioned above, the carrier particles may play a decisive role in determining the dispersibility of dry powder formulations and the detachment of drug from the carrier particles, both of which are crucial in determining drug delivery from DPIs. For example, increasing the surface smoothness of lactose carrier particles was shown to improve the delivery of salbutamol sulphate from a Rotahaler® (Ganderton, 1992). Improving the surface
smoothness of lactose particles may reduce the adhesion forces to the drug particles and consequently, increase the detachment of drug particles from the carrier particles, leading to more respirable drug particles being generated (Kassem, 1990). Since adhesion is a surface phenomenon, it is also governed by other properties of the interactive particles such as particle shape, particle size and crystal form as well as crystallinity. These factors may also affect the dispersibility and detachment of drug particles and eventually, determine the drug delivery from DPIs.

Four principal methodologies have been available to evaluate the drug deposition from inhalation aerosols: (1) inertial impaction; (2) pulmonary deposition studies using γ-scintigraphy; (3) pharmacokinetic studies and (4) comparative studies of pharmacodynamic and clinical efficacy (Newman, 1992). The inertial impaction studies involve the in vitro characterisation of the aerodynamic particle size of an aerosol, defined as the diameter of a unit density sphere having the same settling velocity, generally in air, as the particle (Agnew, 1984). This diameter is affected by particle shape, density, geometric size, all of which influence the aerodynamic behaviour of the particle. This dynamic parameter can be linked with aerosol deposition and specifically with that in the lung (Hatch and Gross, 1964) and therefore, has been widely used by the pharmaceutical scientists. When an air flow changes direction, for example, to go around a bend as shown in Figure 4.1, the air-borne particles are subject to two forces. The first is derived from their momentum built up whilst travelling in the streamline flow. The second one results from the hydrodynamic forces or friction with the surrounding air stream as it changes direction. The momentum of particles maintains them in their established trajectories whilst the friction with the airflow cause the particles to accelerate in the direction of the new gas flow. Therefore, an air-borne particle will continue to move in the original direction of flow until it loses inertia. It will then 'relax' into the new direction of the flow. Whether or not a particle will deposit on a collection surface placed in the path of its original direction of the air stream is determined by particle momentum and the hydrodynamic forces of the flow acting on the particle.
Chapter four: Effects of the morphology of lactose on the delivery of salbutamol sulphate

The larger the particle diameter and/or higher the flow rate, the more likely a particle will be to deposit on the collection surface. This can be easily understood since increasing either particle size or flow velocity will increase particle momentum. Particles with higher momentum are less likely to relax into the new direction of the flow and hence, they are more likely to impact on the collection surface. On the other hand, smaller particles travelling at a slower flow rate will have a lower momentum. These particles will relax more quickly into the new flow direction than the larger particles and therefore, the former particles have a lower impaction probability than the latter particles. A similar explanation can be given to account for particles with a higher density being more likely to deposit on a collection surface than those with a lower density. The likelihood of particle deposition is decreased by increasing the viscosity of the gas. This is because a fluid with higher viscosity produces stronger hydrodynamic forces than a fluid with a lower viscosity. Therefore, the higher the viscosity of the fluid the more likely it is for suspended particles to flow in the direction of the fluid and this decreases the possibility of particle deposition.

Although a number of devices have been available to measure the aerodynamic diameter of air-borne particles, the most commonly used particle sizers in pharmaceutical sciences are the liquid impingers such as the two-stage and multistage liquid impingers, and the cascade impactors such as the Andersen Cascade Impactor. The twin-stage liquid impinger, also called a twin-impinger, was developed specifically to assess the delivery of drugs from MDIs (Hallworth and Westmoreland, 1987). It was the first device operating on inertial impaction to be adopted by the British Pharmacopoeia (BP) (British Pharmacopoeia
Chapter four: Effects of the morphology of lactose on the delivery of salbutamol sulphate

Commission, 1993). It contains two impaction stages, the upper one (a round bottom flask) which operates at a low velocity and traps the larger particles whilst the lower stage (a conical flask) operates at much higher flow rates and is intended to trap all the smaller particles. The cut-off diameter of the upper stage at 60 l min\(^{-1}\) is 6.4 µm (Hallworth et al., 1978; Miller et al., 1992). One of the major limitations of the device arises from the fact that the twin-impinger is merely a dichotomous sampler; i.e., the total sample is divided into only two size categories. It cannot reveal the particle size distribution, which is critical in determining the \textit{in vivo} deposition of an aerosol (Gonda, 1981). The multistage liquid impinger (MLI), which is based upon a similar principle of particle collection to the twin-impinger, was developed to cope with this problem. MLIs of four stages and five stages have been commercialised and are widely employed in the characterisation of the particle size of aerosols including dry powders. Both pieces of apparatus consist of a glass inlet throat, three or four impaction stages (for 4-stage or 5-stage impinger, respectively) and an integral filter stage. Each stage consists of a single jet and a sintered-glass plate. The cut-off diameters at 60 l min\(^{-1}\) at stages 1, 2, 3, 4 and 5 are approximately 12, 6.8, 3.1 and 1.7 µm, respectively (Hugosson et al., 1993). By constructing a curve of cumulative percentage under size versus particle size, it is possible to estimate the mass median aerodynamic diameter and particle size distribution of the aerosol powders.

The aim of the current studies was to employ these liquid impingers to investigate the deposition profiles of salbutamol sulphate from various batches of lactose crystals prepared in the previous work. This was carried out with the view of determining the effects of particle size, particle shape and surface smoothness of lactose carrier particles on the deposition of the drug.
4.2 Materials and methods

4.2.1 Formulating dry powders composed of lactose and salbutamol sulphate

4.2.1a Preparation of lactose and salbutamol sulphate blends
Salbutamol sulphate and lactose were mixed in a ratio of 1:67.5, w/w in accordance with the ratio employed in the commercial "Ventolin®" formulation. After drying in a vacuum oven at 40°C for 12 h, micronised salbutamol sulphate with mass median diameter 2.0 μm (GlaxoWellcome Group Ltd., Ware, UK) (25 mg), was weighed into a 10 ml stoppered sample vial to which had been added one spatula full of lactose crystals. The vial was stoppered and placed on a Whirlymixer for 5 s. Then, more lactose particles (similar to the amount of the blend) was added to the vial and the blend was mixed on a Whirlymixer for another 5 s. This process was repeated until all the lactose (1.750 g) had been incorporated into the salbutamol sulphate/lactose blend to obtain a ratio of drug to carrier of 1:67.5, w/w. The stoppered vials were then placed in a Turbula mixer (Glen Creston Ltd., Middx, UK) and mixed for 30 min. The samples were then stored in a vacuum desiccator over silica gel until further required.

4.2.1b Measurement of the homogeneity of the mixtures.
Ten samples were taken randomly from each batch. The sample (approximately 33 mg) was weighed accurately and the amount of salbutamol sulphate was measured by HPLC (See Section 4.2.2). The coefficient of variation of the drug content was employed to assess the homogeneity of the mixtures.

4.2.1c Filling of the capsules
Hard gelatin capsules (Size 3, Rotacapsule®, GlaxoWellcome Group Ltd., Ware, UK) were filled with 33.0 ± 1.5 mg of the powder mixture so that each capsule contained 481 ± 22 μg salbutamol sulphate, which was the unit dose contained in a Ventolin Rotacap®. The filling was performed manually.
4.2.2 Determination of salbutamol sulphate by HPLC

4.2.2a HPLC conditions
The basic HPLC method for the determination of salbutamol sulphate was provided by GlaxoWellcome Group Ltd (Ware, UK), employing ethyl paraben (p-hydroxybenzoic acid ethyl ester, Sigma Chemical Co., St. Louis, USA) as an internal standard. The HPLC system consisted of a pump (CM 4000 Multiple Solvent Delivery System, LDC Analytical Inc., Florida, USA), a multiple wavelength UV detector (SpectroMonitor 3100, LDC Analytical Inc., Florida, USA) and a 15 cm x 4.6 mm id column packed with 5 µm C-18 (Hypersil, Phenomenex, Cheshire, England).

The chromatographic conditions were as follows:

Column: Hypersil 5 C18 (15 cm x 4.6 mm id)
Mobile phase: 450 ml of methanol and 550 ml of 0.1% w/w aqueous ammonium acetate (pH 4.5)
Flow rate: 0.8 ml min⁻¹
UV wavelength: 276 nm
Internal standard: Ethyl paraben
Injection volume: 30 µl
Detector sensitivity: 0.02 AUF

4.2.2b Preparation of the internal standard solution
Ethyl paraben was dissolved in the mobile phase to produce a solution with a concentration of 4 µg ml⁻¹.

4.2.2c Preparation of the standard drug solutions
An accurately weighed amount of salbutamol sulphate (20.0 mg) was transferred to a 100 ml volumetric flask, dissolved in the internal standard solution, and made up to volume to obtain a concentration of 0.2 mg ml⁻¹ of salbutamol sulphate (solution A). 10.0 ml of solution A was pipetted into another 100 ml volumetric flask and diluted to volume with the internal standard solution to obtain a solution containing 20 µg ml⁻¹ salbutamol sulphate (solution B).
Aliquots of solution B (0.25, 0.50, 1.00, 2.00, 3.00, 4.00, 5.00, 6.00, 7.00 ml) were pipetted into 10 ml volumetric flasks and made up to volume using the internal standard solution to obtain a series of the standard solutions which contained drug concentrations of 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10, 12 and 14 µg ml⁻¹ respectively. These standard solutions were employed to construct a calibration curve of drug concentration against the peak area ratios of drug to internal standard. The calibration was prepared on a daily basis and a calibration curve with $r^2 > 0.99$ was considered acceptable.

4.2.2d Determination of salbutamol sulphate content in powder mixtures
Approximately 33 mg of the powder mixture was accurately weighed and dissolved in the internal standard solution. After the solution had been sonicated in a water bath for 30 min, it was filtered through a millipore filter (Whatman membrane filters, 0.45 µm, nylon, Whatman Lab. Division, Kent, UK). 30 µl of the filtrate was injected into the HPLC. No interference from the lactose carrier was observed. The concentration of salbutamol sulphate was calculated by interpolation using the previously constructed calibration curve.

4.2.3 Deposition methods

4.2.3a Deposition of salbutamol sulphate in a twin-impinger
HPLC mobile phase containing the internal standard (7 ml; Section 4.2.2b) was introduced into the upper stage and 30 ml of the same solvent into the lower stage of a twin stage liquid impinger. The capsule, to be tested, was placed in a commercially available inhaler (either Rotahaler®, GlaxoWellcome, Ware, UK or Cyclohaler®, Pharbita BV, the Netherlands), which had been fitted into a moulded rubber mouthpiece attached to the throat piece of the impinger. Once the assembly had been checked and found to be airtight and vertical, the vacuum pump was switched on. After the pump had run for 5 s, the dose was released. The pump was allowed to run for another 7 s at 60 ± 1 l min⁻¹ following the release of the dose and it was then switched off. The capsule shells were removed from the inhaler device and the deposition test was repeated until six capsules has been actuated in the same manner. The inhaler body, capsule shells and mouth piece were washed 5 times with the mobile phase containing internal standard (Section 4.2.2b) and the washing solution was made up
to 100 ml with the same solvent. The sample thus obtained was used to measure the amount of drug retained in the inhaler device. The same process was carried out for both the upper and the lower stage of the twin-impinger. All the samples obtained were analysed for the concentration of salbutamol sulphate using the HPLC method as outlined before (Section 4.2.2).

Each experiment was carried out at least in triplicate.

4.2.3b Deposition of salbutamol sulphate in a 4-stage liquid impinger

20 ml of the HPLC mobile phase with internal standard was introduced to each of the upper stages of a 4-stage liquid impinger. A Whatman filter paper (< 0.45 μm) was placed in stage 4 of the impinger. The throat was connected to the neck of the upper stage and wrapped with Sellotape® to ensure the connection was airtight. A Rotahaler® (GlaxoWellcome Group Ltd, Ware, UK) was then fitted into the moulded rubber mouthpiece attached to the throat of the impinger. Finally, a capsule was mounted in the inhaler device. Once the assembly had been checked and found to be airtight and the inhaler device, when inserted, was aligned with the horizontal axis of the throat of the impinger, the vacuum pump was switched on. After the pump had run for 5 s so that the flow rate of the air stream was established, the dose was released. The pump was allowed to run for another 7 s at 60 ± 1 l min⁻¹ following the release of the dose and was then switched off. The capsule shells were then removed from the inhaler device and the deposition test was repeated until four more capsules were actuated in the same manner.

Then, stage 4 of the impinger was dismantled and the filter paper was carefully transferred to a beaker. The filter was washed three times with the mobile phase containing internal standard (Section 4.2.2b) and the washing solution was transferred to a 50 ml volumetric flask and made up to volume with the same solvent. The inhaler body, capsule shells and mouthpiece were washed 5 times with the mobile phase and the washing solution was made up to 100 ml with the same solvent. The throat was washed 3 times with approximately 25 ml mobile phase and transferred to a 100 ml volumetric flask. Then, the inside of the inlet jet tube to stage 1 was rinsed with the solvent and the washing solution was allowed to flow back into the stage. After transferring the solution to the same volumetric flask containing
the washing solution from the throat, stage 1 was washed with the same solvent for 3 times and the final washing solution of this stage and the throat was made up to volume with the solvent. The solution in stage 2 was transferred to a 50 ml volumetric flask and the inside of the stage was washed three times with the mobile phase before making up to volume with the same solvent. Stage 3 was washed using the same procedure as that for stage 2. The concentration of salbutamol sulphate of all the samples obtained were analysed using the HPLC method as outlined above (Section 4.2.2).

Similar deposition tests were carried out at flow rates of 28.3 and 90 l min\(^{-1}\) following the same operational and washing procedures.

Each experiment was carried out at least in triplicate.

4.2.4 Data analysis

4.2.4a Deposition data obtained from a twin-impinger

Table 4-1 summarises the parameters employed to calculate the deposition profiles of salbutamol sulphate in a twin-impinger. The recovered dose (RD) was the sum of the drug collected in the inhaler device, upper and lower stages of the impinger, whilst the emitted dose (ED) was the amount of drug released from the inhaler device, i.e. the sum of drug collected at upper and lower stages of the impinger. However, fine particle dose (FPD) was defined as the amount of drug deposited in the lower stage of the impinger, which has a diameter less than the cut-off diameter of the upper stage of a twin-impinger (6.4 \(\mu\)m at an air flow rate of 60 l min\(^{-1}\)). The fine particle fraction (FPF) was calculated as the ratio of the fine particle dose to either the recovered dose (FPF % RD) or the emitted dose (FPF % ED). The total recovery (% recovery) of the drug was assessed by the ratio of the recovered dose to the theoretical dose, the latter being the dose of salbutamol sulphate in the capsules. For example, the theoretical dose of salbutamol sulphate in one capsule was 481 ± 22 \(\mu\)g, which was equivalent to the filling weight (33.0 ± 1.5 mg) of lactose and salbutamol sulphate blends.
Chapter four: *Effects of the morphology of lactose on the delivery of salbutamol sulphate*

Table 4.1 The parameters employed to quantify the deposition of salbutamol sulphate in a twin stage liquid impinger.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Definition</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovered dose (RD)</td>
<td>$Q_{\text{inhaler}} + Q_{\text{upper}} + Q_{\text{lower}}$</td>
<td>Dose recovered after the study</td>
</tr>
<tr>
<td>Emitted dose (ED)</td>
<td>$Q_{\text{upper}} + Q_{\text{lower}}$</td>
<td>Dose released from the inhaler</td>
</tr>
<tr>
<td>Fine particle dose (FPD)</td>
<td>$Q_{\text{lower}}$</td>
<td>Drug particles $&lt; 6.4 \mu m$</td>
</tr>
<tr>
<td>Fine particle fraction (FPF)</td>
<td>$\frac{Q_{\text{lower}}}{Q_{\text{inhaler}}}$</td>
<td></td>
</tr>
<tr>
<td>FPF (% RD)</td>
<td>$\frac{\text{FPD}}{\text{RD}}$</td>
<td>FPD of recovered dose</td>
</tr>
<tr>
<td>FPF (% ED)</td>
<td>$\frac{\text{FPD}}{\text{ED}}$</td>
<td>FPF of emitted dose</td>
</tr>
<tr>
<td>% Recovery</td>
<td>$\frac{\text{RD}}{\text{Theoretical dose}} \times 100$</td>
<td>% Recovery of the drug</td>
</tr>
<tr>
<td>% Emission</td>
<td>$\frac{\text{ED}}{\text{RD}} \times 100$</td>
<td>% RD emitted from the inhaler</td>
</tr>
</tbody>
</table>

4.2.4b Deposition data from a 4-stage liquid impinger

The 4-stage liquid impinger has been calibrated for use at different inhalation flow rates such as 28.3, 60 and 96 l min$^{-1}$. Each stage of the impinger has a different effective cut-off diameter at different flow rates (Table 4.2) and therefore, the definition of fine particle dose (FPD) or fraction (FPF) will change with the flow rate employed.

<table>
<thead>
<tr>
<th>Stages</th>
<th>28.3 l min$^{-1}$</th>
<th>60.0 l min$^{-1}$</th>
<th>96.0 l min$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18.9</td>
<td>13.0</td>
<td>10.3</td>
</tr>
<tr>
<td>2</td>
<td>9.9</td>
<td>6.8</td>
<td>5.4</td>
</tr>
<tr>
<td>3</td>
<td>4.5</td>
<td>3.1</td>
<td>2.5</td>
</tr>
<tr>
<td>4</td>
<td>2.5</td>
<td>1.7</td>
<td>1.3</td>
</tr>
</tbody>
</table>

The fine particles were defined as the particles $< 4.5$, 6.8 and 5.4 $\mu m$ after aerosolisation at 28.3, 60 and 96 l min$^{-1}$, respectively. Therefore, fine particle dose (FPD) was the amount of
drug collected in stage 4 for inhalation at 28.3 l min\(^{-1}\) but the sum of stages 3 & 4 for aerosolisation at either 60 or 96 l min\(^{-1}\). The fine particle fraction (FPF) was calculated as the ratios of the fine particle dose to either the recovered dose (FPF % RD) or the emitted dose (FPF % ED). Both the recovered dose (RD) and the emitted dose (ED) were as in Section 4.2.4a for deposition within a twin stage impinger. The percentage of recovery and emission as defined in Table 4.1 were also calculated.

4.2.5 Statistical analysis

All data were analysed by two-tailed t-test, multiple regression and ANOVA as appropriate, using Minitab\textsuperscript{®} for Windows Version 10.2 (Minitab Inc., USA).
4.3 Results and discussion

4.3.1 Content uniformity of the powders

The mixtures were found to be homogenous with a coefficient of variation in salbutamol sulphate content of less than 2.2% (n = 10).

4.3.2 The effect of particle shape and surface smoothness of lactose carrier particles on the deposition of salbutamol sulphate in a twin-impinger

4.3.2a Deposition from the Cyclohaler®

The deposition data in Table 4-3 were calculated as one capsule per actuation at 60 l min⁻¹ via a Cyclohaler®. It can be seen that the recovered dose (RD) of salbutamol sulphate varied from 391 µg for the blend containing batch 9 lactose to 508 µg for the blend composed of batch 10 lactose, corresponding to a % recovery of between 81.2-105.5%. The drug recovery was reasonably satisfactory with an average recovery of 94.1% from all of the eight formulations investigated. The emission of drug from the inhaler device ranged from 55.6% for blends containing batch 9 lactose to 70.8% for blends containing batch 10 lactose, with an average drug emission of 66.5%, indicating that a large portion (33.5% RD) of the drug was retained in the inhaler device.

The blends containing batch 9, 10, 11 & Lactochem® lactose produced a similar fine particle dose (FPD) of salbutamol sulphate, which was significantly higher (p < 0.01) than that obtained from the blends which were composed of batch 3, 4 or 7 lactose. The blends containing batch 9 lactose produced the highest FPF in terms of both % RD (25.6%) and % ED (46.2%), which were more than twice the FPF of the formulations containing batch 3 lactose, the FPF of the latter being 12.6 % RD or 19.8% ED. As mentioned previously (Section 2.3.1), these batches of lactose particles had similar particle size but with different surface smoothness and particle shape. The differences in particle shape and surface texture of lactose carrier particles may account for the differences in the deposition of the drug since all the powders are composed of the same batch of salbutamol sulphate. For example, the highest FPF observed for the blends containing batch 9 lactose may be due to that
particular batch having the smoothest surface, the measured ‘surface factor’ having a value of unity (Section 3.3.1). The higher FPF of salbutamol sulphate obtained using the blends containing batch 10 or 11 lactose might be due to the more elongated shape (higher value of elongation ratio) of these batches of lactose than other batches of lactose (Section 2.3.1). The lowest values for FPF of drug, obtained using blends containing batch 3 or 4 lactose may be due to those batches having the roughest surfaces with the least elongated particle shape (Section 2.3.1).

Table 4.3 Deposition of salbutamol sulphate from different batches of lactose in a twin-impinger after aerosolisation at 60 l min⁻¹ via a Cyclohaler® [Mean(SD), n ≥ 3]

<table>
<thead>
<tr>
<th>Batch No</th>
<th>RD (µg)</th>
<th>ED (µg)</th>
<th>FPD (µg)</th>
<th>% RD</th>
<th>% ED</th>
<th>FPF</th>
<th>Recovery %</th>
<th>Emission %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1°Lact®</td>
<td>460(20)</td>
<td>320(37)</td>
<td>101(12)</td>
<td>21.8(1.7)</td>
<td>31.6(3.5)</td>
<td>95.7(4.2)</td>
<td>69.3(6.0)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>432(18)</td>
<td>276(15)</td>
<td>54(10)</td>
<td>12.6(2.4)</td>
<td>19.8(3.9)</td>
<td>89.7(3.8)</td>
<td>63.8(0.9)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>425(24)</td>
<td>294(10)</td>
<td>64(2)</td>
<td>15.1(0.8)</td>
<td>21.8(0.7)</td>
<td>88.3(5.0)</td>
<td>69.1(1.7)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>454(20)</td>
<td>319(14)</td>
<td>91(8)</td>
<td>20.0(1.9)</td>
<td>28.5(1.9)</td>
<td>94.4(4.1)</td>
<td>70.2(1.9)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>398(28)</td>
<td>257(34)</td>
<td>69(18)</td>
<td>17.2(3.3)</td>
<td>26.6(3.6)</td>
<td>82.7(5.9)</td>
<td>64.6(4.0)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>391(48)</td>
<td>217(29)</td>
<td>101(18)</td>
<td>25.6(1.5)</td>
<td>46.2(3.8)</td>
<td>81.2(10.0)</td>
<td>55.6(2.5)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>508(13)</td>
<td>359(5)</td>
<td>113(5)</td>
<td>22.3(1.6)</td>
<td>31.5(1.9)</td>
<td>105.5(2.7)</td>
<td>70.8(0.8)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>450(35)</td>
<td>344(40)</td>
<td>108(7)</td>
<td>21.8(2.5)</td>
<td>31.9(5.4)</td>
<td>103.9(7.3)</td>
<td>68.7(3.7)</td>
<td></td>
</tr>
</tbody>
</table>

°Lact® is Lactochem® lactose

The surface smoothness and particle elongation have been quantified previously using the terms ‘surface factor’ and elongation ratio, respectively (Sections 2.3.1 & 3.3.1). Figures 4.2 and 4.3 show these shape and surface descriptors of lactose carrier particles against the drug FPF of the corresponding blends.
Chapter four: Effects of the morphology of lactose on the delivery of salbutamol sulphate

55.0%
50.00%
45.0%
40.0%
35.0%
30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
0.70 0.75 0.80 0.85 0.90 0.95 1.00 1.05 1.10

'Surface factor' values of lactose carrier particles

Figure 4.2 The relationship between "surface factor" of lactose particles and the FPF of salbutamol sulphate aerosolised at 60 l min⁻¹ via a Cyclohaler® (Error bars denote standard deviation, n ≥ 3).

35.0%
30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
1.1 1.2 1.3 1.4 1.5 1.6 1.7 1.8 1.9 2.0 2.1 2.2

Elongation ratio of lactose carrier particles

Figure 4.3 The relationship between elongation ratio of lactose particles and the FPF of salbutamol sulphate aerosolised at 60 l min⁻¹ via a Cyclohaler® (Error bars denote standard deviation, n ≥ 3).
From Figures 4.2 & 4.3, it can be seen that increasing the surface smoothness of lactose carrier particles, as expressed by the "surface factor", generally resulted in an increase in the FPF of salbutamol sulphate in terms of either % RD or % ED. Interestingly, increasing the elongation ratio of the lactose carrier particles also resulted in an increase in the FPF of salbutamol sulphate (Figure 4.3). These results suggest that apart from surface smoothness, the elongation of carrier particles may also play an important role in determining the FPF of the drug.

4.3.2b Deposition from a Rotahaler®

The % recovery varied from 86.9% for the formulation containing batch 10 lactose to 100.0% for the powder containing batch 3 lactose after aerosolisation at 60 l min⁻¹ via a Rotahaler® (Table 4.4). The average recovery of salbutamol sulphate from all the blends investigated was 94.1%, which was similar to that after actuation via a Cyclohaler® (Section 4.3.2a), suggesting that the overall deposition, washing and analytical procedures were reliable and reproducible.

Table 4.4 Deposition of salbutamol sulphate from different batches of lactose in a twin-impinger after aerosolisation at 60 l min⁻¹ via a Rotahaler® [Mean(SD), n ≥ 3].

<table>
<thead>
<tr>
<th>Batch No</th>
<th>RD (µg)</th>
<th>ED (µg)</th>
<th>FPD (µg)</th>
<th>% RD</th>
<th>% ED</th>
<th>% Recovery</th>
<th>% Emission</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Lact®</td>
<td>565(13)</td>
<td>339(7)</td>
<td>97(7)</td>
<td>20.8(1.0)</td>
<td>28.5(1.7)</td>
<td>96.6(2.6)</td>
<td>72.9(2.5)</td>
</tr>
<tr>
<td>3</td>
<td>482(12)</td>
<td>367(15)</td>
<td>75(2)</td>
<td>15.6(0.4)</td>
<td>20.6(1.1)</td>
<td>100.0(2.5)</td>
<td>76.2(2.9)</td>
</tr>
<tr>
<td>4</td>
<td>430(4)</td>
<td>333(4)</td>
<td>64(9)</td>
<td>15.0(2.2)</td>
<td>19.4(3.0)</td>
<td>89.3(0.9)</td>
<td>77.4(0.4)</td>
</tr>
<tr>
<td>6</td>
<td>475(13)</td>
<td>335(20)</td>
<td>99(6)</td>
<td>20.8(0.8)</td>
<td>29.5(0.8)</td>
<td>98.5(2.7)</td>
<td>70.6(2.3)</td>
</tr>
<tr>
<td>7</td>
<td>436(21)</td>
<td>328(17)</td>
<td>84(3)</td>
<td>19.3(0.3)</td>
<td>25.6(0.9)</td>
<td>90.5(4.3)</td>
<td>75.4(1.7)</td>
</tr>
<tr>
<td>9</td>
<td>457(25)</td>
<td>336(12)</td>
<td>102(5)</td>
<td>22.4(0.9)</td>
<td>30.5(1.1)</td>
<td>95.0(5.1)</td>
<td>73.5(2.2)</td>
</tr>
<tr>
<td>10</td>
<td>419(10)</td>
<td>275(20)</td>
<td>102(2)</td>
<td>24.4(0.8)</td>
<td>34.1(2.5)</td>
<td>86.9(2.2)</td>
<td>65.6(3.3)</td>
</tr>
<tr>
<td>11</td>
<td>462(13)</td>
<td>344(17)</td>
<td>108(6)</td>
<td>23.4(1.5)</td>
<td>31.5(2.3)</td>
<td>95.9(2.8)</td>
<td>74.4(2.4)</td>
</tr>
</tbody>
</table>

*Lact® was Lactochem® lactose

The FPD varied from 64 µg for the blend containing batch 4 lactose to 108 µg for the blend containing batch 11 lactose. The blends composed of batch 9, 10 or 11 lactose generally
produced a higher FPD of salbutamol sulphate than the rest of the blends. Blends containing batch 3 or 4 lactose produced the lowest (p < 0.01) FPD of salbutamol sulphate in comparison to the other batches of lactose. The blends containing batch 3 or 4 lactose also produced the lowest FPF (about 15% RD) whilst the blends composed of batch 9, 10 or 11 lactose produced the highest FPF of the drug (> 22% RD). The formulations containing batch 6, 7 and lactochem™ lactose produced an intermediate FPF of salbutamol sulphate (about 20% RD). The FPF values calculated as % ED followed a similar trend. As mentioned above, the difference in the FPF of the drug may be due to the difference in morphological properties such as surface smoothness and shape of lactose carrier particles. The relationship between FPF of salbutamol sulphate and the morphological properties of lactose carrier particles is shown in Figures 4.4 and 4.5, where values of either “surface factor” or elongation ratio of each batch of lactose are plotted against the FPF of salbutamol sulphate from the corresponding formulations.

Figure 4.4 The relationship between the “surface factor” of lactose and the FPF of salbutamol sulphate after aerosolisation at 60 l min⁻¹ via a Rotahaler® (Error bars denote standard deviation, n ≥ 3).
Figure 4.5 The relationship between the elongation ratio of lactose and the FPF of salbutamol sulphate after aerosolisation at 60 l min⁻¹ via a Rotahaler® (Error bars denote standard deviation, n ≥ 3).

Thus, similar to the results obtained from a Cyclohaler®, the FPF of salbutamol sulphate was dependent upon either the surface smoothness or the elongation of lactose particles. Increasing the surface smoothness of lactose carrier particles, was shown to increase the FPF of salbutamol sulphate. For example, the FPF (% RD) of salbutamol sulphate was increased from about 15% for blends containing lactose particles with a ‘surface factor’ value 0.78 (batch 3 lactose) to more than 20% for the blends containing lactose particles with unity value of ‘surface factor’ (lactose batches 9, 10 & 11). Lactose carrier particles with higher elongation ratio (i.e. more elongated particles) generally produced higher FPF of salbutamol sulphate (Figure 4.5).

No significant difference (paired t-tested, p = 0.6) was observed in the FPF of salbutamol sulphate after delivery from either a Cyclohaler® or a Rotahaler®. Therefore, drug deposition data from each batch of lactose measured by the two inhaler devices were combined and
analysed by linear regression using Minitab® for Windows (Version 10.2) and the following empirical equations were generated.

\[
\begin{align*}
\text{FPF} \, (\% \, RD) &= 6.56 \, E + 24.5 \, S_{rec} - 13.9 \quad r^2 = 0.901 \quad (4-1) \\
\text{FPF} \, (\% \, ED) &= 8.81 \, E + 34.6 \, S_{rec} - 19.0 \quad r^2 = 0.895 \quad (4-2)
\end{align*}
\]

where E and S\textsubscript{rec} were the elongation ratio and "surface factor", respectively.

The FPF (% RD or ED) of salbutamol sulphate increases almost linearly with the values of either the elongation ratio or the 'surface factor', with a linear coefficient, \(r^2\) of approximately 0.90. The coefficients for the 'surface factor' (24.5 or 34.6) were larger than those of the elongation ratio (6.56 or 8.81), suggesting that drug FPF may increase faster with 'surface factor' than with the elongation ratio although the values of 'surface factor' (< 1) were less than the values of elongation ratio (≥ 1). The statistical details of the regression analysis are listed in Table 4.5. Drug FPF in terms of either % RD or % ED increased with the values of either elongation ratio (\(p < 0.05\)) or 'surface factor' (\(p < 0.01\)). The t-ratio values of the FPF generated by changes in the 'surface factor' were higher than those resulting from changes in elongation ratio. This further confirmed that the 'surface factor' might contribute more than elongation ratio to the changes in the FPF.

Table 4.5 Regression analysis and ANOVA of the deposition data obtained with a twin-impinger.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>FPF (% RD)</th>
<th>FPF (% ED)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(SEQ, SS)</td>
<td>(t\text{-ratio})</td>
</tr>
<tr>
<td>Elongation ratio</td>
<td>137.7</td>
<td>4.19</td>
</tr>
<tr>
<td>'Surface factor'</td>
<td>36.6</td>
<td>4.99</td>
</tr>
</tbody>
</table>

The results of the present study indicate that an increase in surface smoothness of lactose carrier particles generally resulted in an increase in the FPF of salbutamol sulphate. Although this was in agreement with the results obtained previously (Kassem, 1990), the effects of the carrier surface smoothness on drug deposition were found to be less pronounced than might have been expected from previous reports (Kassem, 1990). The FPF
of salbutamol sulphate from recrystallised lactose was reported to be almost 6 times as high as that from the Lactochem® lactose due to a very low drug FPF (4%) observed when the latter carrier was employed (Kassem, 1990). However, in the present work, a much higher FPF (about 20%) of salbutamol sulphate was observed from Lactochem® lactose after aerosolisation via both a Rotahaler® and a Cyclohaler.

The elongation ratio of lactose carrier particles was also shown to have similar effects on the FPF of salbutamol sulphate to that of the surface smoothness. Increasing the elongation ratio of lactose particles increased FPF of the salbutamol sulphate. This phenomenon might be due to the improved aerodynamic properties of more elongated particles (Hickey et al., 1992). More elongated lactose particles may travel a longer distance before impacting in comparison to less elongated carrier particles of similar mass. Drug particles adhered to more elongated carrier particles may be subjected to the drag forces of the air stream for a longer period of time. This would result in more drug particles being detached from the carrier particles, leading to a higher FPF of the drug. Such a hypothesis might be confirmed in future by the analysis of the deposition profiles of carrier particles with different elongation ratios.
4.3.3 Effects of particle size, shape and surface smoothness of lactose particles on the deposition of salbutamol sulphate in a 4-stage liquid impinger

4.3.3a Deposition at 28.3 l min\(^{-1}\)

After actuation at 28.3 l min\(^{-1}\) via a Rotahaler\(^{®}\), the recovered dose (RD) of salbutamol sulphate from each capsule varied from 414 µg for blends containing batch 13 lactose to 439 µg for blends composed of batch 14 lactose, corresponding to a % recovery between 86.1% and 90.3% (Table 4.6). The average recovery of all eight formulations was 89.2%. A large portion of drug particles were not emitted from the inhaler since only about half (the average value of % emitted dose being 49.8) of the dose was recovered, indicating that a flow rate of 28.3 l min\(^{-1}\) was not able to efficiently deliver the drug-lactose blends from a Rotahaler\(^{®}\). The emitted dose changed with different lactose carrier particles. For example, the blends containing batch 15, 17 or 20 lactose had much higher emitted dose (about 60% RD) than the blends containing batch 13, 14 or 16 lactose (from 30% to 40% RD). Drug emission tended to be dependent upon the particle size of lactose particles, a larger size fraction (63-90 µm) of the carrier particles producing higher emission of the drug than the smaller size fraction (< 63 µm). For example, batch 13 lactose with a mean diameter of 104.7 µm was fractionated from the same batch of lactose crystals as batch 14, which had a mean diameter of 68.6 µm (Section 2.3.2). Batch 15 (mean diameter 93.0 µm) was fractionated from the same batch of crystals from which batch 16 (mean diameter 65.3 µm) was fractionated (Section 2.3.2). The blends containing batch 13 lactose produced a significantly higher (p < 0.01) emission of the drug than those containing batch 14 lactose. Similarly, the drug from blends of batch 15 lactose was more likely (p < 0.05) to be emitted from the inhaler device than those of batch 16. It was likely that the higher emission observed for blends containing either batch 17 or 20 lactose was principally attributable to the lactose having a mean diameter > 90 µm (Section 2.3.3).

More powders were found to adhere to the inner walls of the inhaler device after the actuation of the blends composed of finer lactose particles than those containing coarser lactose particles, leading to a lower emission of drug from the former than from the latter blends. Once the powders were released from the capsules, either the freely dispersed drug particles or the carrier particles (with adhered drug particles) may be entrained in the air.
stream. The air-borne particles are likely to impact on the inner walls of the inhaler due to the turbulent air flow within the device, which is an essential design feature, introduced to aid particle dispersion and entrainment (Timsina et al., 1994). Under specific aerosolisation conditions, the detachment of any deposited particles from the device walls is akin to the detachment of particles from a stationary surface, which is dependent upon the particle size of the adherent particles. For example, the airflow velocity, required to dislodge 50% of adhered particles from a stationary surface, was shown to be inversely proportional to the particle size, regardless of the surface characteristics (Gotoh et al., 1994a). Therefore, smaller particles are more difficult to dislodge from their adhered sites than larger particles and hence, once impacting on the wall of inhaler device, smaller carrier particles with adhered drug particles are less likely to become air-borne than larger particles. This might explain why the blends of smaller carrier particles produced lower drug emission from the inhaler device than the blends of larger carrier particles. However, neither the blends of the smaller lactose particles nor those of the larger lactose particles produced efficient drug emission at 28.3 l min⁻¹, indicating that such a flow rate is insufficient to extract drugs from the Rotahaler®.

Table 4.6 The deposition profiles of salbutamol sulphate in a 4-stage liquid impinger after aerosolisation at 28.3 l min⁻¹ via a Rotahaler® [Mean(SD), n ≥ 3].

<table>
<thead>
<tr>
<th>Batch No</th>
<th>RD (µg)</th>
<th>ED (µg)</th>
<th>FPD (µg)</th>
<th>% RD</th>
<th>% ED</th>
<th>Recovery</th>
<th>Emission</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>414(2)</td>
<td>169(24)</td>
<td>11(3)</td>
<td>2.6(0.8)</td>
<td>6.5(2.4)</td>
<td>86.1(1.7)</td>
<td>40.8(2.8)</td>
</tr>
<tr>
<td>14</td>
<td>439(20)</td>
<td>130(22)</td>
<td>25(2)</td>
<td>5.7(0.8)</td>
<td>19.3(4.7)</td>
<td>91.3(2.1)</td>
<td>29.6(5.9)</td>
</tr>
<tr>
<td>15</td>
<td>431(8)</td>
<td>287(7)</td>
<td>15(1)</td>
<td>3.5(0.3)</td>
<td>5.2(0.5)</td>
<td>89.6(1.4)</td>
<td>66.6(5.7)</td>
</tr>
<tr>
<td>16</td>
<td>436(10)</td>
<td>166(30)</td>
<td>27(1)</td>
<td>6.2(0.2)</td>
<td>16.2(2.5)</td>
<td>90.6(4.1)</td>
<td>38.1(4.1)</td>
</tr>
<tr>
<td>17</td>
<td>433(15)</td>
<td>274(9)</td>
<td>6(0)</td>
<td>1.3(0.0)</td>
<td>2.1(0.1)</td>
<td>90.0(3.2)</td>
<td>63.3(1.1)</td>
</tr>
<tr>
<td>20</td>
<td>421(3)</td>
<td>255(13)</td>
<td>16(1)</td>
<td>3.9(0.3)</td>
<td>6.4(0.8)</td>
<td>87.5(0.6)</td>
<td>60.5(2.9)</td>
</tr>
</tbody>
</table>

The blends composed of finer lactose carrier particles (batches 14 & 16) produced an FPD almost twice as high as those of the blends containing their corresponding coarser lactose particles (batch 13 & 15) although the former produced lower drug emission than the latter blends. Accordingly, the blends containing finer lactose carrier particles produced
significantly \((p < 0.01)\) higher drug FPF \((\% \text{ RD})\) than the blends of coarser lactose particles. The finer lactose particles produced an FPF \((\% \text{ ED})\) of salbutamol sulphate three times as high as that of the corresponding coarser lactose particles. These results strongly suggest that adhered drug particles were more likely to detach from finer lactose carrier particles than from coarser particles.

The particle size, particle shape and surface smoothness of each batch of lactose were quantified previously (Chapters 2 & 3). It might therefore be possible to establish the relationship between drug FPF and morphology of lactose. Thus, the following empirical equations (4-3 and 4-4) were generated by multiple regression analysis using a Minitab® for Windows (Version 10.2).

\[
\text{FPF \((\% \text{ RD})\) } = 4.92 \times 10^{-4} E - 4.26 S_{\text{rec}} - 0.0968 d + 8.54 \\
\text{FPF \((\% \text{ ED})\) } = 11.7 E + 1.0 S_{\text{rec}} - 0.418 d + 26.1
\]

\(r^{2} = 0.853\) \(r^{2} = 0.846\) \((4-3)\) \((4-4)\)

where \(d\), \(E\) and \(S_{\text{rec}}\) were the mean diameter (\(\mu m\)), elongation ratio and “surface factor” of lactose particles, respectively. The statistical details of the regression analysis are shown in Table 4.7.

<table>
<thead>
<tr>
<th>Predicator</th>
<th>FPF ((% \text{ RD})) SEQ SS</th>
<th>t-ratio</th>
<th>(p)</th>
<th>FPF ((% \text{ ED})) SEQ SS</th>
<th>t-ratio</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter ((\mu m))</td>
<td>24.9</td>
<td>-7.40</td>
<td>0.000</td>
<td>552.7</td>
<td>-7.98</td>
<td>0.000</td>
</tr>
<tr>
<td>Elongation ratio</td>
<td>16.5</td>
<td>5.31</td>
<td>0.000</td>
<td>85.7</td>
<td>3.16</td>
<td>0.007</td>
</tr>
<tr>
<td>‘Surface factor’</td>
<td>6.2</td>
<td>-1.09</td>
<td>0.292</td>
<td>0.0</td>
<td>0.06</td>
<td>0.950</td>
</tr>
</tbody>
</table>

Thus, the FPF \((\% \text{ RD or ED})\) of salbutamol sulphate decreased with the mean diameter of lactose particles \((p < 0.01)\), confirming that smaller carrier particles produced higher FPF of the drug. Increasing the elongation ratio of the carrier particles increased the FPF \((\% \text{ RD or ED})\) of the drug \((p < 0.01)\). This was in agreement with the previous results achieved using a twin impinger operated at a flow rate of 60 l min\(^{-1}\) (Section 4.3.2). However, the surface
smoothness, expressed by the value of "surface factor", did not show a significant effect (p > 0.05) on the FPF (% RD or ED) of salbutamol sulphate. Therefore, at a flow rate as low as 28.3 l min⁻¹, surface smoothness may not be so important as size and elongation of the carrier particles in determining drug deposition.

The blends containing lactose batches 14 & 16 produced aerosolised salbutamol sulphate of similar particle size and size distribution. The particle size distribution of the deposited drug was similar for blends containing lactose 13 & 15, although the plots of cumulative % RD as a function of ECD were less than those obtained for batches 14 & 16 (Fig 4.6). The highest % RD of drug particles < 18.9 μm observed for the blends containing lactose batch 14 or 16 indicates that finer carrier particles produced more efficient aerosolisation of drug than coarser carrier particles employed in the other blends. The lowest % RD of aerosolised drug particles for blends containing batch 17 lactose may be largely due to the rough surface of this batch lactose, which limited the drug detachment from the carrier particles.

Figure 4.6 The particle size distribution of salbutamol sulphate after aerosolisation from different batches of lactose at 28.3 l min⁻¹ via a Rotahaler® (Error bars denote standard deviation, n ≥ 3).
4.3.3b Deposition at 60.0 \( \text{l min}^{-1} \)

As shown in Table 4.8, after aerosolisation at 60 \( \text{l min}^{-1} \) via a Rotahaler®, the average drug recovery of all the deposition tests was 94.7%. All the blends had similar emission of drug particles from the inhaler, with an average emitted dose of 78.9 % RD, indicating that a flow rate of 60 \( \text{l min}^{-1} \) was able to dislodge the aerosol blends from the inhaler devices. Increasing the flow rate of an inhaled air stream has been reported previously to increase the emission of drug from inhaler devices (Hindle et al., 1994). Although smaller lactose carrier particles (batch 14 or 16) still produced slightly lower drug emission than their corresponding larger lactose particles (batch 13 or 15), unlike aerosolisation at 28.3 \( \text{l min}^{-1} \), the dependence of drug emission on carrier particle size became statistically insignificant (\( p > 0.05 \)) at an aerosolisation flow rate of 60 \( \text{l min}^{-1} \).

Table 4.8 The deposition profiles of salbutamol sulphate in a 4-stage liquid impinger after aerosolisation at 60.0 \( \text{l min}^{-1} \) via a Rotahaler® [Mean(SD), \( n \geq 3 \)].

<table>
<thead>
<tr>
<th>Batch No</th>
<th>Recovered dose (( \mu \text{g} ))</th>
<th>Emitted dose (( \mu \text{g} ))</th>
<th>FPD (( \mu \text{g} ))</th>
<th>FPF (% RD)</th>
<th>FPF (% ED)</th>
<th>Recovery (%)</th>
<th>% Emitted</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>442(18)</td>
<td>362(10)</td>
<td>76(6)</td>
<td>14.7(1.3)</td>
<td>18.1(2.6)</td>
<td>94.5(2.9)</td>
<td>81.2(3.9)</td>
</tr>
<tr>
<td>14</td>
<td>448(24)</td>
<td>348(20)</td>
<td>90(3)</td>
<td>17.5(1.3)</td>
<td>23.3(1.2)</td>
<td>94.0(2.9)</td>
<td>74.9(3.5)</td>
</tr>
<tr>
<td>15</td>
<td>455(14)</td>
<td>370(29)</td>
<td>67(4)</td>
<td>17.1(1.0)</td>
<td>20.9(1.2)</td>
<td>91.7(3.6)</td>
<td>81.9(1.9)</td>
</tr>
<tr>
<td>16</td>
<td>453(14)</td>
<td>339(27)</td>
<td>79(8)</td>
<td>20.0(0.5)</td>
<td>25.8(0.7)</td>
<td>92.9(4.9)</td>
<td>77.6(1.6)</td>
</tr>
<tr>
<td>17</td>
<td>474(10)</td>
<td>384(26)</td>
<td>52(4)</td>
<td>10.9(1.0)</td>
<td>13.5(1.8)</td>
<td>98.5(2.0)</td>
<td>80.9(3.8)</td>
</tr>
<tr>
<td>20</td>
<td>464(3)</td>
<td>356(5)</td>
<td>71(2)</td>
<td>15.2(0.3)</td>
<td>19.8(0.4)</td>
<td>96.3(0.6)</td>
<td>76.7(0.7)</td>
</tr>
</tbody>
</table>

Similar to the deposition at 28.3 \( \text{l min}^{-1} \), lactose crystals of smaller size fraction produced higher FPD or FPF of salbutamol sulphate. For example, either the FPD or FPF of salbutamol sulphate from batch 14 lactose was significantly higher (\( p < 0.05 \)) than that of the drug from batch 13 lactose. The FPD or FPF of the drug from batch 16 lactose was significantly higher (\( p < 0.01 \)) than that of batch 15 lactose. These results further confirm that the formulations containing smaller carrier particles produced more efficient delivery of the drug from DPIs than those containing carriers of a larger particle size.
Batches 15 and 16 were prepared from the mother liquor after crystallisation of batches 13 and 14 (Section 2.2.2). The former crystals produced slightly greater but statistically significantly higher (p < 0.05) FPF of salbutamol sulphate than the corresponding particle size fraction of the latter, since lactose crystals batches 15 & 16 had a higher surface smoothness than batches 13 & 14 lactose respectively (Section 2.3.2). These results support the previous findings that increased surface smoothness of lactose carrier particles improved the FPF. Thus, batch 20 lactose included in formulations of salbutamol sulphate resulted in significantly higher (p < 0.01) FPD or FPF of drug in comparison with that deposited using batch 17 lactose. In order to evaluate the relationship between drug FPF and particle morphology of lactose, the following empirical equations (equations 4-5 and 4-6) were generated by multiple regression using Minitab® for Windows (Version 10.2).

\[
\text{FPF (\% RD)} = 11.2 E + 8.9 S_{\text{rec}} - 0.19 d + 6.0 \quad r^2 = 0.901 \quad (4-5)
\]
\[
\text{FPF (\% ED)} = 13.3 E + 11.1 S_{\text{rec}} - 0.259 d + 11.1 \quad r^2 = 0.904 \quad (4-6)
\]

where d, E and \( S_{\text{rec}} \) were defined as before. The statistical details of the regression analysis are shown in Table 4.9.

Table 4.9 Details of regression and ANOVA concerning the relationship between drug FPF and morphology of carrier particles at 60 l/min

<table>
<thead>
<tr>
<th>Predictor</th>
<th>FPF (% RD)</th>
<th>FPF (% ED)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SEQ SS</td>
<td>t-ratio</td>
</tr>
<tr>
<td>Diameter (( \mu )m)</td>
<td>62.8</td>
<td>- 10.02</td>
</tr>
<tr>
<td>Elongation ratio</td>
<td>73.2</td>
<td>8.35</td>
</tr>
<tr>
<td>'surface factor'</td>
<td>2.7</td>
<td>1.58</td>
</tr>
</tbody>
</table>

It can be seen from the regression analysis and ANOVA, that drug FPF (\% RD or ED) was inversely proportional to the mean diameter of lactose carrier particles (p < 0.01). Increasing the elongation ratio of the carrier particles increased the FPF (\% RD or ED) of the drug (p < 0.01). Although an increase in the surface smoothness, expressed by the value of the "surface factor", only resulted in a slight increase in the FPF of the drug (see equations 4-5 & 4-6), the effect of surface smoothness of the carrier particles on the deposition of the drug...
was not statistically significant (p > 0.05, Table 4.9). Thus, similar to the deposition at a flow rate of 28.3 l min⁻¹, particle size and shape (elongation) of the lactose particles plays a more important role in determining the FPF of salbutamol sulphate than surface smoothness.

The inclusion of lactose particles derived from batch 16 in the drug formulation resulted in the most efficient aerosolisation of salbutamol sulphate particles, with the highest % RD of drug particles < 13.0 μm (Figure 4.7). Batches 14 & 15 produced similar pattern of drug aerosolisation. Formulations containing either batch 13 or batch 20 lactose produced a similar particle size distribution of salbutamol sulphate in the impactor, which was lower than the comparable distributions resulting from formulations containing batch 14 or batch 15 lactose (Fig 4.7). Lactose batch 17 resulted in the lowest amount of drug particles being aerosolised. The highest % RD of aerosolised drug particles with a diameter < 13.0 μm, which resulted from blends containing lactose batch 16 may be attributable to the smaller particle size and smooth surface of this batch of carrier particles. Although batch 14 lactose had a smaller mean diameter (68.6 μm) than batch 15 (93.0 μm), the former crystals had a more irregular shape with a rougher surface than the latter crystals (Section 2.3.2). The two opposing factors counteract each other, leading to a more or less similar aerosolisation of salbutamol sulphate from these batches of lactose. Batch 13 lactose had a mean diameter of 104.7 μm and the particles possessed a rough surface. This batch of lactose produced fewer aerosolised drug particles compared with batches 14, 15 or 16. Batch 17 was produced from the similar conditions to batch 20 although the former crystals had a more irregular shape with a rougher surface than the latter (Section 2.3.3). This may be the major factor which resulted in the lowest FPF being produced from formulations containing batch 17 lactose.
**Chapter four: Effects of the morphology of lactose on the delivery of salbutamol sulphate**

**Figure 4.7** The particle size distribution of salbutamol sulphate after aerosolisation using different batches of lactose at 60 l min\(^{-1}\) via a Rotahaler\(^{\circledR}\) (Error bars denote standard deviation, \(n \geq 3\)).

4.3.3c Deposition at 96.0 l min\(^{-1}\)

After aerosolisation at 96.0 l min\(^{-1}\) via a Rotahaler\(^{\circledR}\), the average drug recovery was 95.7% (Table 4.10), which was slightly but insignificantly (\(p > 0.05\)) higher than the average recovery measured at 60.0% l min\(^{-1}\). All the blends showed a drug recovery over 90% nominal dose, which was indicative of an accurate, reproducible experimental procedure encompassing mixing and capsule filling, through deposition, to drug analysis. Similar emission of drug particles from the aerosolisation device was found for all blends, with an average emitted dose of 77.7% RD being obtained. There was no significant difference in the drug emission after aerosolisation at 60 and 96 l min\(^{-1}\), indicating that further increasing the flow rate from 60 l min\(^{-1}\) did not result in a corresponding increase in the emission of the drug from a Rotahaler\(^{\circledR}\).
Table 4.10 The deposition profiles of salbutamol sulphate in a 4-stage liquid impinger after aerosolisation at 96.0 l min⁻¹ via a Rotahaler* [Mean(SD), n ≥ 3].

<table>
<thead>
<tr>
<th>Batch No</th>
<th>RD (µg)</th>
<th>ED (µg)</th>
<th>FPD (µg)</th>
<th>% RD</th>
<th>% ED</th>
<th>Recovery %</th>
<th>Emission %</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>444(5)</td>
<td>337(9)</td>
<td>104(9)</td>
<td>19.2(0.6)</td>
<td>24.6(1.8)</td>
<td>95.7(0.6)</td>
<td>78.3(3.3)</td>
</tr>
<tr>
<td>14</td>
<td>455(17)</td>
<td>353(14)</td>
<td>118(9)</td>
<td>24.3(0.9)</td>
<td>31.5(1.1)</td>
<td>98.6(0.4)</td>
<td>77.1(0.6)</td>
</tr>
<tr>
<td>15</td>
<td>461(3)</td>
<td>361(15)</td>
<td>89(3)</td>
<td>23.4(1.7)</td>
<td>30.9(2.0)</td>
<td>92.2(1.0)</td>
<td>75.9(1.2)</td>
</tr>
<tr>
<td>16</td>
<td>475(2)</td>
<td>366(4)</td>
<td>115(5)</td>
<td>25.8(1.9)</td>
<td>33.3(1.8)</td>
<td>94.4(3.6)</td>
<td>77.7(1.5)</td>
</tr>
<tr>
<td>17</td>
<td>473(13)</td>
<td>389(11)</td>
<td>59(4)</td>
<td>12.4(0.6)</td>
<td>15.0(0.7)</td>
<td>98.2(2.7)</td>
<td>82.2(0.5)</td>
</tr>
<tr>
<td>20</td>
<td>458(16)</td>
<td>343(14)</td>
<td>101(1)</td>
<td>21.9(0.7)</td>
<td>29.3(1.1)</td>
<td>95.1(3.3)</td>
<td>74.8(1.0)</td>
</tr>
</tbody>
</table>

Although the use of batch 14 lactose produced significantly higher (p < 0.01) FPD and FPF of salbutamol sulphate than the use of batch 13 lactose, there was no significant difference (p > 0.05) between the FPF of the drug obtained from formulations employing batch 15 and 16 lactose. Thus, the effect of the particle size of the lactose carrier particles on drug deposition was less marked at an aerosolisation flow rate of 96 l min⁻¹ when compared with that at either 28.3 or 60 l min⁻¹. Lactose particles from batches 15 and 16 had a smoother surface than those derived from batches 13 and 14, respectively, but the former particles resulted in only a slightly but insignificantly (p > 0.05) higher FPF of salbutamol sulphate than the latter particles. Batch 20 lactose produced an FPF of 21.9 % RD or 29.3% ED, which was almost twice as high as the drug FPF (12.4% RD or 15.0% ED) produced by batch 17. In order to evaluate the relationship between the drug FPF and carrier particle morphology, the following empirical equations (equations 4-7 and 4-8) were generated by multiple regression using a Minitab® for Windows Version 10.2.

\[
\text{FPF (\% RD)} = 20.4 E + 13.0 S_{\text{rec}} - 0.278 d + 0.16 \\
\text{FPF (\% ED)} = 29.2 E + 21.4 S_{\text{rec}} - 0.373 d - 7.38
\]

where d, E and S_{rec} were defined as before. Details of the statistical analysis are listed in Table 4.11.
Table 4.11 Details of regression and ANOVA concerning the relationship between drug FPF and lactose morphology at an aerosolisation flow rate of 96.0 l min\(^{-1}\)

<table>
<thead>
<tr>
<th>Predictor</th>
<th>FPF (% RD)</th>
<th>t-ratio</th>
<th>p</th>
<th>FPF (% ED)</th>
<th>SEQ SS</th>
<th>t-ratio</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter ((\mu m))</td>
<td>102.7</td>
<td>-13.53</td>
<td>0.000</td>
<td>160.8</td>
<td>160.8</td>
<td>-13.84</td>
<td>0.000</td>
</tr>
<tr>
<td>Elongation ratio</td>
<td>245.7</td>
<td>14.03</td>
<td>0.000</td>
<td>498.0</td>
<td>498.0</td>
<td>15.28</td>
<td>0.000</td>
</tr>
<tr>
<td>‘surface factor’</td>
<td>5.8</td>
<td>2.13</td>
<td>0.049</td>
<td>15.7</td>
<td>15.7</td>
<td>2.67</td>
<td>0.018</td>
</tr>
</tbody>
</table>

Similar to the results achieved at flow rates of 28.3 and 60 l min\(^{-1}\), the drug FPF (% RD or ED) was found to increase with decreasing particle size of lactose carrier particles (p < 0.01) and in addition, the more elongated the carrier particles the higher the FPF of the drug (p < 0.01) after aerosolisation at a flow rate of 96 l min\(^{-1}\). The surface smoothness of the carrier particles appeared to exert a significant effect on the FPF (p < 0.05) at this flow rate, drug FPF increasing with the surface smoothness of the carrier particles. However, the effect of elongation ratio of lactose carrier particles appeared to be more pronounced than that of the particle size since the former factor produced a higher absolute value of the t-ratio than the latter factor (Table 4.11). These results suggest that whilst the effects of elongation ratio and surface smoothness of lactose particles on drug deposition may become more prominent at 96.0 l min\(^{-1}\), the effect of particle size might diminish at this flow rate.

The differences in the particle size distribution of salbutamol sulphate from different batches of lactose was less pronounced after an aerosolisation flow rate of 96 l min\(^{-1}\) (Figure 4.8) as compared to that observed after aerosolisation flow rate of either 28.3 or 60 l min\(^{-1}\). However, lactose batch 14 still produced a significantly more efficient (p < 0.01) aerosolisation of the drug than batch 13 lactose although the use of batch 16 lactose appeared to result in similar pattern of drug aerosolisation to batch 15. Further, the cumulative % RD of salbutamol sulphate using batch 20 lactose was more than twice that found after use of batch 17 lactose.
Chapter four: Effects of the morphology of lactose on the delivery of salbutamol sulphate

32.0%
28.0%
24.0%
20.0%
16.0%
12.0%
8.0%
4.0%
0.0%

Effective cut-off diameter (ECD, μm)

Figure 4.8 The particle size distribution of salbutamol sulphate after aerosolisation from different batches of lactose at 96 l min⁻¹ via a Rotahaler® (Error bars denote standard deviation, n ≥ 3).

4.3.3d Analysis of the deposition data obtained at different aerosolisation flow rates

The FPF of salbutamol sulphate from different batches of lactose particles obtained at different aerosolisation flow rates were analysed using multiple regression and ANOVA. The following empirical equations (equations 4-9 and 4-10) were generated so as to evaluate the effects of aerosolisation flow rates and particle morphology of lactose carrier particles on the deposition of salbutamol sulphate.

FPF (% RD) = 0.253 V + 12.0 E + 5.6 S_{rec} - 0.188 d - 10.2 \quad r^2=0.905 \quad (4-9)

FPF (% ED) = 0.267 V + 18.0 E + 11.2 S_{rec} - 0.35 d - 6.45 \quad r^2=0.904 \quad (4-10)

where V is the aerosolisation flow rate (l min⁻¹); d, E, S_{rec} are as defined previously. Details of the statistical analysis are listed in Table 4.12.
### Table 4.12 The details of regression and ANOVA of the relationship between drug FPF and aerosolisation flow rates as well as particle morphology of lactose

<table>
<thead>
<tr>
<th>Predictor</th>
<th>FPF (% RD)</th>
<th>FPF (% ED)</th>
<th>SEQ SS</th>
<th>t-ratio</th>
<th>p</th>
<th>SEQ SS</th>
<th>t-ratio</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SEQ SS</td>
<td>t-ratio</td>
<td>p</td>
<td>SEQ SS</td>
<td>t-ratio</td>
<td>p</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flow rate</td>
<td>2654.2</td>
<td>20.05</td>
<td>0.000</td>
<td>2945.2</td>
<td>17.79</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter (µm)</td>
<td>179.4</td>
<td>-6.95</td>
<td>0.000</td>
<td>760.8</td>
<td>-10.93</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elongation ratio</td>
<td>258.7</td>
<td>6.27</td>
<td>0.000</td>
<td>578.6</td>
<td>7.96</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Surface factor’</td>
<td>3.2</td>
<td>0.70</td>
<td>0.489</td>
<td>12.8</td>
<td>1.17</td>
<td>0.247</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

As can be seen from the regression analysis and ANOVA (Table 4.12), aerosolisation flow rate plays the most important role (the highest value of either SEQ SS or t-ratio) in determining the FPF of salbutamol sulphate, an increase in the flow rate resulting in an increase in the FPF of the drug. The aerosolisation flow rate is critical in determining both the dispersion and deaggregation of entrained particle agglomerates. For example, the aerodynamic diameter of aerosolised salbutamol sulphate, was found to decrease with an increase in aerosolisation flow rates until a minimum diameter was reached at a peak flow rate (Hindle and Byron, 1995). The fine particle fraction of salbutamol base has also been shown to increase by over 2-3 times, regardless of the carrier particles, as the aerosolisation flow rates increased from 28.3 to 80 l min⁻¹ (MacRitchie et al., 1995).

Increasing particle size of lactose carrier decreased the FPF of salbutamol sulphate and this might be due to the following reasons. First, large carrier particles have been reported to exert stronger adhesion forces on drug particles than smaller carrier particles (Staniforth et al., 1982). Second, the air-borne smaller carrier particles would have higher mobility than the larger particles. The smaller particles would be expected to rotate more rapidly in the air stream than the larger particles and hence, produce higher centrifugation forces on the adhered drug particles, which in turn would be expected to accelerate the detachment of drug particles from the carrier particles. Finally, the smaller carrier particles would travel a longer distance in the respiratory tract than the larger particles. This would lead to the drug being subject to the drag forces of the air stream for a longer period of time, increasing the possibility of the drug particles being dislodged from the carrier particles. Thus, in terms of drug detachment from air-borne carrier particles, smaller carrier particles may appear to be
more favorable than larger carrier particles. However, smaller particles were shown to have poorer flowability than larger particles (Section 3.3.3), which may result in other problems during powder handling processes. An optimised particle size of the carrier should therefore ensure maximum drug delivery without sacrificing other powder properties such as flowability. This can be achieved by the modification of particle morphology of lactose carrier.

The elongation ratio of lactose carrier particles appears to be of similar importance in affecting the FPF of salbutamol sulphate as particle size. Increasing the elongation ratio of the carrier particles resulted in an increase in the FPF of the drug regardless of the aerosolisation flow rates and inhaler devices. Although increasing the surface smoothness of the carrier particles resulted in a slight increase in the FPF of the drug, this effect was not of statistical significance \((p > 0.05)\). However, the surface smoothness of lactose carrier particles was shown previously to have a significant effect on the deposition of salbutamol sulphate in a twin impinger (Section 4.3.2). The insignificant effects of surface smoothness on drug FPF in this section of experiment might be due to the following reasons. First, the values of ‘shape factor’, an indicator of surface smoothness, of the lactose crystals employed in the current work varied within a narrower range (from 0.868 for batch 14 to 1 for batch 20) than those of the lactose particles in Section 4.3.2, when values ranged from 0.78 to 1. The differences in drug FPF, as a result of minor differences in “surface factor” value, would thus be expected to be less pronounced than that when lactose crystals with more markedly different values of ‘shape factor’ were employed. Second, it must be remembered that the definition of FPF changed with flow rates. FPFs obtained at different flow rates covered different size fractions of the aerosolised drug, the FPF at 28.3, 60 and 90 \(1\ min^{-1}\) being defined as particles < 4.5, 6.8 and 5.4 \(\mu\)m, respectively. Therefore, the combined analysis of the FPF obtained at different flow rates only provides a semi-quantitative insight into the effects of different factors on the deposition of the drug.

4.3.4 Deposition of salbutamol sulphate from lactose crystallised from Carbopol gels

Table 4.13 shows the deposition of salbutamol sulphate from a typical batch of lactose crystals (C1) prepared from Carbopol 934 gels. It can be seen that this batch of lactose
produced significantly higher (p < 0.01) FPF of salbutamol sulphate than all other lactose batches containing a similar size fraction (i.e. 63-90 μm), at all aerosolisation flow rates. For example, the drug FPF from C1 lactose was 5.3% RD at 28.3 l min⁻¹, which was significantly higher (p < 0.05) than the highest FPF (3.9% RD for batch 20 lactose) achievable for lactose crystals of the similar size fraction but prepared with constant stirring (Table 4.6). Similar trends were also observed for aerosolisation at 60 and 96 l min⁻¹. However, there was no significant (p > 0.05) difference in drug FPF from C1 lactose (63-90 μm) and lactose crystals with a size fraction < 63 μm (batches 14 or 16) but prepared with constant stirring. These results suggest that although the use of C1 lactose particles may improve the FPF of salbutamol sulphate, such an effect may be comparable to the effects brought about by reducing the particle size of lactose crystals. Interestingly, C1 lactose generally resulted in a higher (p < 0.05) portion of the emitted dose than all the other lactose crystals. For example, 78.3% RD of salbutamol sulphate was found to be emitted from the inhaler device after aerosolisation of the blends containing C1 lactose at 28.3 l min⁻¹ whilst an average of only 49.8% RD (Section 4.3.3a) was emitted from the device using lactose crystals prepared from stirred aqueous solvent alone. The aerosolisation of salbutamol sulphate from C1 lactose was also more efficient than the majority of the other lactose crystals (Tables 4.8 and 4.10). The mean FPD of salbutamol sulphate from one capsule containing drug blended with C1 lactose was 27.1, 110.9 and 129.4 μg after aerosolisation at 28.3, 60 and 96.0 l min⁻¹, respectively. However, lactose crystals prepared with constant stirring produced an average FPD of salbutamol sulphate 16.4, 72.3 and 97.4 μg for aerosolisation at 28.3, 60 and 96.0 l min⁻¹, respectively. As mentioned before (Section 3.3.1), lactose crystals prepared from Carbopol 934 gels had a more elongated shape with a smoother surface than those prepared with constant stirring. The improved surface smoothness or elongated particle shape may have acted separately or inter-dependently to improve either the drug emission from the inhaler device or the detachment of emitted drug from the carrier particles, resulting in more fine particles of the drug.
Table 4.13 Deposition of salbutamol sulphate from Cl lactose in a 4-stage liquid impinger after aerosolisation at different flow rates via a Rotahaler* [mean(SD), n ≥ 3]

<table>
<thead>
<tr>
<th>Flow rate (l/min)</th>
<th>RD (µg)</th>
<th>ED (µg)</th>
<th>FPD (µg)</th>
<th>FPF (% RD)</th>
<th>Recovery (%)</th>
<th>Emission (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>28.3</td>
<td>508(6)</td>
<td>398(4)</td>
<td>27(1)</td>
<td>5.3(0.2)</td>
<td>103.1(1.2)</td>
<td>78.3(0.3)</td>
</tr>
<tr>
<td>60.0</td>
<td>514(22)</td>
<td>405(21)</td>
<td>111(11)</td>
<td>21.5(1.2)</td>
<td>104.3(4.4)</td>
<td>78.7(0.8)</td>
</tr>
<tr>
<td>96.0</td>
<td>496(8)</td>
<td>399(4)</td>
<td>129(1)</td>
<td>26.1(0.2)</td>
<td>100.6(1.7)</td>
<td>80.5(0.5)</td>
</tr>
</tbody>
</table>

As shown previously, the particle size of the lactose carrier has a profound effect on the FPF of salbutamol sulphate. The effect of particle size of lactose carrier particles on the deposition of salbutamol sulphate was further investigated by the use of different particle size fractions of lactose crystals, namely < 63, 63-90, 90-125 and 125-150 µm fractions which had a mass median diameter (MMD) of 65.6, 118.8, 174.8 and 211.8 µm, respectively (Table 3.5). The deposition of salbutamol sulphate from lactose particles of different size fractions is shown in Table 4.14. The % emission of the drug increased from 72.0% RD for the particles of MMD of 65.6 µm to 91.4% total recovered dose for particles of a MMD of 211.8 µm. Thus, increasing the particle size of the carrier particles increased the entrainment of the particles into the air stream and this was in agreement with some previous reports (Bell et al., 1971; Staniforth, 1995).

Similar to the previous results, both the FPD and FPF of salbutamol sulphate decreased with increasing particle size of lactose. For example, drug FPF decreased from 28.9% RD for blends containing lactose particles with an MMD of 65.6 µm to 8.0% RD for blends containing lactose crystals with an MMD of 211.8 µm. A more pronounced decrease in the FPF of salbutamol sulphate was observed when the FPF was calculated as the % emitted dose. For example, the FPF decreased from 40.2% ED for lactose particles of MMD of 65.6 µm to 8.9% ED for lactose crystals of an MMD of 211.8 µm. All these results suggest that although increasing the particle size of the carrier increased the emission of drug from the inhaler devices, increasing the carrier particle size decreased drug detachment from the carrier particles so remarkably that the overall drug FPD or FPF was greatly reduced with an increase in size of the carrier particles.
Table 4.14 Deposition of salbutamol sulphate from different size fractions of one batch of lactose crystallised from Carbopol 934 gels in a 4-stage liquid impinger after aerosolisation at 60 l min⁻¹ via a Rotahaler [Mean(SD), n ≥ 3]

<table>
<thead>
<tr>
<th>MMD (µm)</th>
<th>Recovered dose (µg)</th>
<th>Emitted dose (µg)</th>
<th>FPD (µg)</th>
<th>% RD</th>
<th>% ED</th>
<th>Emitted</th>
</tr>
</thead>
<tbody>
<tr>
<td>65.6</td>
<td>515(8)</td>
<td>371(39)</td>
<td>149(14)</td>
<td>28.9(2.5)</td>
<td>40.2(2.9)</td>
<td>72.0(1.8)</td>
</tr>
<tr>
<td>118.8</td>
<td>514(22)</td>
<td>405(21)</td>
<td>111(11)</td>
<td>21.5(1.2)</td>
<td>27.3(1.4)</td>
<td>78.7(0.8)</td>
</tr>
<tr>
<td>174.8</td>
<td>440(2)</td>
<td>382(4)</td>
<td>51(5)</td>
<td>11.5(1.1)</td>
<td>13.3(1.1)</td>
<td>86.8(1.3)</td>
</tr>
<tr>
<td>211.8</td>
<td>470(12)</td>
<td>430(11)</td>
<td>38(5)</td>
<td>8.0(1.2)</td>
<td>8.9(1.2)</td>
<td>91.4(0.3)</td>
</tr>
</tbody>
</table>

The particle size distribution of aerosolised salbutamol sulphate from different size fractions of lactose carrier particles is shown in Figure 4.9 and the data confirm that an increase in the size of lactose particles decreases the aerosolisation of the drug, resulting in a lower FPF of salbutamol sulphate.

Figure 4.9 The particle size distribution of aerosolised salbutamol sulphate from fractionated lactose particles after aerosolisation at 60 l min⁻¹ via a Rotahaler (Error bars denote standard deviation, n ≥ 3)
The size of an aerosolised particle is one of the dominant factors in determining its deposition in the respiratory tract (Task Group on Lung Dynamics, 1966). Particles larger than 10 μm in diameter are most likely to deposit in the mouth and throat whereas particles smaller than 5 μm in diameter deposit more frequently in the lower airways. The ideal size for therapeutic aerosols has been proposed to be within the range of 0.5-7.0 μm (Davies et al., 1976). The fine particles measured by the impingers in the current work have a diameter < 6.8 μm and hence, this portion of particles are likely to deposit in the lower airways. The fine particle fraction measured by inertial impaction has been found to correlate with the clinical performance of bronchodilator aerosols (Padfield et al., 1983; Meakin and Stroud, 1983). Therefore, either fine particle dose (FPD) or fine particle fraction (FPF) can be employed to assess the delivery efficiency of DPIs. However, the fine particle dose (FPD) changes with the nominal dose of drug to be actuated whilst the fine particle fraction (FPF) is primarily determined by the specific powder formulations aerosolised under defined conditions. Thus, FPF was the major factor to be considered when comparing the delivery efficiency of different powder formulations. Formulations that produce a higher FPF can be expected to deliver a higher fraction of drug to the lower airways than those which produce a lower FPF.
4.4 Summary

This section of work investigated the effects of particle size, shape and surface smoothness of lactose particles as well as aerosolisation flow rates on the deposition profiles of salbutamol sulphate. Increasing aerosolisation flow rate increased both drug emission and aerosolisation, which eventually increased FPF of salbutamol sulphate. Although increasing the particle size of the lactose was shown to increase drug emission from the inhaler device, larger carrier particles produced lower FPF of the drug. Increasing either the particle elongation or surface smoothness of lactose particles increased the FPF of salbutamol sulphate. Lactose crystals prepared from Carbopol gels produced higher FPF of salbutamol sulphate than lactose particles prepared with constant stirring. However, the blends containing 63-90 μm size fraction of lactose crystals prepared from Carbopol gels produced similar drug FPF to the blends containing the < 63 μm fraction of lactose prepared with constant stirring. Therefore, drug delivery from DPIs can be modified by means of controlling the particle size, shape and surface smoothness of the carrier particles. The effects of other formulation factors such as intermediate-sized carrier particles on drug delivery from DPIs require further investigation and these studies will form the basis of the work described in Chapter 5.
CHAPTER FIVE

EFFECTS OF SURFACE COMPONENTS OF LACTOSE ON

THE DELIVERY OF SALBUTAMOL SULPHATE
5.1 Introduction

Crystallisation of lactose from aqueous Carbopol 934 gels was shown to produce lactose particles with smooth surfaces, uniform shape (pyramid) and narrow size distribution. The use of such particles as a carrier for salbutamol sulphate powder for aerosolisation led to a higher fine particle fraction of the drug being obtained as compared to lactose particles prepared using other crystallisation techniques such as those involving constant stirring (Section 4.3.4). Increased surface smoothness may reduce the mechanical interlocking between the drug and carrier particles. This would increase the detachment of drug particles from carrier particles and hence, increase the respirable fraction of drug. However, when the mechanical interlocking becomes insignificant, further increase in surface smoothness could increase the particle contact area and this would increase adhesion due to Van der waals forces (Zimon, 1982). Thus, it might be easier to detach particles from a rougher surface than from a smoother surface (Gotoh et al., 1994c). An ideal system should ensure minimal mechanical interlocking or entrapment of drug particles into any surface asperity (or cavity) on the carrier particle without substantially increasing particle interactions due to Van der waals forces. This may be achieved by deliberately introducing onto an otherwise perfectly smooth lactose particle surface asperities (or cavities) that have smaller dimensions than any adhered drug particles.

Some areas of carrier surfaces are known to be devoid of binding sites whereas some binding sites are stronger than others (Hersey, 1975). The number of active binding sites is limited and as the concentration of the drug increases, more drug particles will adhere to weaker binding sites or remain free in the mixtures, which eventually reduced the overall interparticulate forces between the drug and carrier particles (Staniforth et al., 1981; Staniforth and Rees, 1983). Thus, increasing the concentration of salbutamol sulphate in the powdered drug-lactose mixture was found to increase the fine particle fraction of drug (Ganderton and Kassem, 1992). The addition of a small amount of a ternary material, such as magnesium stearate was also shown to increase the FPF of salbutamol sulphate by reducing the interparticulate forces between the drug and carrier particles (Ganderton and Kassem, 1992). More recently, L-leucine has been employed to replace magnesium stearate...
to improve the delivery of salbutamol sulphate from lactose carrier particles (Staniforth, 1996). However, inclusion of a ternary component may produce toxicological considerations. A more practical strategy might be to seek to replace such ternary materials with fine particles of carrier.

Reducing the particle size of the lactose carrier was previously shown to improve drug delivery from dry powder aerosols (Section 4.3.3). However, the use of smaller carrier particles results in a poorer flowability of drug-carrier blends than the larger carrier particles, which causes difficulties in powder handling processes such as capsule filling. Therefore, it is not always practical to improve drug delivery from dry powder aerosols simply by means of reducing the carrier particle size. However, a small amount of smaller-sized carrier particles could first be adhered to coarser carrier particles, to saturate any active binding sites or cover some surface cavities, prior to drug particles being mixed with the powder mass. Such a ternary ordered mix would be expected to reduce the interaction between the drug and coarser carrier particles and produce more efficient drug detachment than the commonly employed binary ordered mixture. This can be achieved by blending a small amount (5% w/w of the coarser lactose) of micronised lactose particles (MMD 5 \( \mu \text{m} \)) with coarse lactose particles (63-90 \( \mu \text{m} \)) before mixing with salbutamol sulphate (Zeng et al., 1996). The inclusion of fine particles of lactose with the coarse lactose and salbutamol sulphate was shown to increase significantly the FPF of the drug without substantially reducing the flowability of the final product. However, the use of micronised fine particles may introduce extra amorphous region to the powder formulation since micronised lactose contains a larger portion of amorphous material than crystalline lactose (Section 3.3.2). The use of crystalline carrier particles of intermediate size may solve this problem since fine crystalline lactose may still be able to improve drug delivery through similar mechanisms without introducing extra amorphous regions within powder formulations. A further advantage lies in the possibility of preparing crystals with elongated dimensions, which were shown to produce higher FPF of salbutamol sulphate than more isometric particles (Section 4).

The Andersen Cascade Impactor (ACI) consists of a stack of perforated plates mounted in rings, each plate penetrated by an array of finely drilled holes. The holes in the top plate are
the largest, with a diameter of 1.5 mm, and successive plates carry holes of decreasing diameter, the lowest being 0.25 mm. In each stage, below the holes in the plate, is a circular collection plate coated with a viscous solution, such as silicone oil, that serves to impact the particles that penetrated the previous set of holes (Andersen, 1966). These stages (8 being typical) are preceded by a preseparator that removes large particles (> 9.9 μm), which is in turn connected to an inlet orifice, normally called a throat piece. The aerosol is sprayed into the throat and drawn by the airflow through the holes in successive stages. The diameter and number of holes are chosen so that the airflow velocity increases down the stack, due to the larger holes of the upper plates and smaller holes of the lower plates. As a particle passes down the stack it will ultimately reach a stage at which the jet provides sufficient velocity to allow it to travel to the next, lower plate. The effective cut-off diameters of an ACI at a flow rate of 60 l min\(^{-1}\) are 6.18, 4.00, 3.20, 1.40, 0.76, 0.45 and 0.30 μm for stages 0, 1, 2, 3, 4, 5 and 7, respectively (Internal documents, Glaxo-Wellcome Group Ltd, Ware, 1994). This impactor is superior to both the twin impinger and multistage liquid impinger in that the ACI can produce a more detailed particle size distribution of the aerosolised drug.

The aim of the present work was to employ the ACI to investigate various formulation factors on drug deposition from aerosolised dry powders. These factors included: 1) the inclusion of surface asperities (cavities) on the surface of coarse lactose which had smaller scale than micronised salbutamol sulphate; 2) the addition of a small amount of micronised carrier particles to powder formulations; and 3) addition of a small amount of smaller-sized (5-10 μm) needle crystals of lactose to the powder formulations.
5.2 Material and Methods

5.2.1 Preparation of lactose crystals with or without small scale surface cavities

5.2.1a Crystallisation of lactose from Carbopol gels

Lactose (75 g) was dissolved in 100 ml of 0.4% w/v Carbopol 934 (B.F. Goodrich Chemical Co., Cleveland, Ohio, USA) solutions which had been previously prepared according to the methods outlined in Section 3.2.1. After neutralisation with sodium hydroxide solution (1 M), crystallisation was allowed to continue for approximately 12 h until the majority of the crystals had grown to a size range of 63-90 μm, as monitored by an optical microscope. The crystals were then subjected to similar washing process as described earlier (Section 3.2.1). Four batches of lactose were crystallised from Carbopol 934 gels using identical conditions to those described above and these batches were then mixed to obtain a sample, labelled as lactose 934. The lactose particles were then treated as shown in Figure 5.1.

![Figure 5.1](image-url)  
Figure 5.1 A flow chart to show the classification and treatment of lactose crystals

5.2.1b Treatment of lactose crystals with 95% ethanol

Lactose 934 crystals were then sieved into < 63 and 63-90 μm size fractions (Fig 5.1). Each size fraction was further divided into two equal parts. One part of the crystals was submerged in 95% ethanol for 48 h and then the suspension was filtered to collect the crystals. The other part did not undergo this treatment. Both treated and untreated lactose
crystals were dried in a vacuum oven at 70°C for 3 h before transferring to sealed vials, which were then placed in a desiccator until required.

5.2.2 Preparation of smaller-sized lactose

5.2.2a Micronisation
Lactochem® lactose was subjected to up to 9 passes through a jet mill operated at an air pressure of 15 bars. The milled particles were dried in a vacuum oven at 60°C for 5 h and then placed in a desiccator over silica gel until further required.

5.2.2b Crystallisation of needle lactose crystals
Lactose (Lactochem, 100 g) was dissolved in 100 ml distilled water at 90°C with constant stirring. The solution was filtered through a Whatman filter paper (0.45 μm) whilst hot and allowed to cool to room temperature without agitation. Absolute ethanol was then quickly mixed with the lactose solution to produce an ethanol concentration of 40% (v/v) and the mixture was immediately homogenised at approximately 8,000 rpm for 2 min. The lactose crystallised almost instantly as small needle shaped crystals such that the solution became a cream-like semi-solid due to the massive amount of small crystals produced. The crystals were immediately filtered and washed with 60 % (v/v) ethanol and then absolute ethanol. The crystals were then allowed to dry at room temperature overnight before finally being dried in a vacuum oven at 60°C for 5 h. The crystals were then stored in a desiccator over silica gel at room temperature until required.

5.2.3 Preparation of lactose and salbutamol sulphate formulations

5.2.3a Blends of coarse lactose and salbutamol sulphate
Lactose particles as prepared in Section 5.2.1 were blended with salbutamol sulphate using the methods as reported earlier (Section 4.2.1 a).

5.2.3b Blends of coarse and smaller-sized lactose and salbutamol sulphate
Blends of salbutamol sulphate with smaller-sized lactose and coarse lactose were prepared in the ratio of 1 : 3.3 : 64.2. Thus, coarse lactose (1.925 g) was blended with smaller-sized
lactose (needle crystals or micronised lactose, 0.100 g) in a Whirlimixer for 2 min. The mixture was then blended with salbutamol sulphate (0.030 g) in a Turbula mixer (Glen Creston Ltd, Middx, UK) for 30 min. The samples were then stored in a vacuum desiccator over silica gel until further required.

5.2.3c Filling of capsules
Capsule-filling was carried out using the method as reported in Section 4.2.1c.

5.2.4 Characterisation of carrier particles

5.2.4a Scanning electron microscopy (SEM)
The particle shape and surface texture of the lactose particles, with or without treatment with 95% ethanol, and the micronised lactose as well as the lactose needle crystals were characterised by SEM as outlined earlier (Section 2.2.4.2).

5.2.4b Particle size measurement
Laser light scattering was employed to determine the mass median diameter (MMD), geometric standard deviation (GSD) and cumulative percentage of particles less than 10.8 and 4.84 μm of lactose crystals. Thus, a small amount of lactose powder (about 5 mg) was dispersed in 5 ml butan-1-ol with the aid of sonication for 1 min. The particle size was measured using a Malvern Series 2600 Particle Sizer (Malvern Instruments Ltd., UK) fitted with a 63 mm lens. The particle size analysis was accepted using independent particle modelling when the obscuration was between 10% and 30%.

4.2.4c Measurement of the surface area
The surface area of lactose particles was measured using an air permeation method as described above (Section 2.2.4.3b).

5.2.5 Deposition test

Determination of deposition was carried out using an Andersen Cascade Impactor (ACI) after aerosolisation at 60 l min⁻¹ from a Rotahaler®.
The cascade impactor consists of eight stages, 0 to 7, and a preseparator. The impactor plates were coated with silicone oil by immersing in a 1% v/v solution of Dow Corning 200/1000 cS silicone oil in hexane and allowed to dry prior to each analysis. A filter paper (Whatman, cut off < 0.45 μm) was placed in stage 8 of the impactor and 10 ml of the mobile phase with the internal standard was introduced into the preseparator. The impactor was then assembled and a Rotahaler® (GlaxoWellcome Group Ltd., Ware, UK) was then fitted into a moulded rubber mouthpiece attached to the throat of the impinger. A capsule was finally inserted into the inhaler device. After the impactor was found to be airtight and the inhaler device lined up along the horizontal axis of the throat of the impactor, the pump which was connected to the outlet of the apparatus was switched on and allowed to run for 5 s prior to the release of the dose. The pump was then allowed to run for another 7 s at 60 ± 1 l min⁻¹. After the broken capsule shell was removed from the inhaler device, the deposition test was repeated until five capsules had been actuated in the same manner.

After all the capsules had been used, the impactor was dismantled and the filter paper of stage 8 was carefully transferred to a beaker. It was then washed with the HPLC mobile phase containing internal standard (Section 4.2.2b) three times. After transferring to a 50 ml volumetric flask, the washing solution was made up to volume with the same solvent. The inhaler body, capsule shells and mouth piece were washed 5 times with the mobile phase and the washing solution was made up to 100 ml with the same solvent. Both the throat and preseparator were washed 3 times with the mobile phase and the separate washing solutions were made up to 100 ml with the same solvent. The impaction plates of the rest stages of the impactor were also washed individually with the same solvent but each washing solution was made up to 50 ml. The concentration of salbutamol sulphate in each of the samples was analysed using the HPLC method as outlined above (Section 4.2.2a).

Each formulation was tested five times.
5.3 Results and discussion

5.3.1 Particle shape and surface texture of lactose

5.3.1a Particle shape by scanning electron microscopy
Treatment of lactose particles with 95% ethanol was shown to introduce surface asperities or cavities to the crystals without substantially changing the particle shape of lactose (Figures 5.2 & 5.3). Before this solvent treatment, both size fractions (< 63 and 63-90 µm) of lactose crystals were tomahawk-shaped with a smooth surface (Figures 5.2a & 5.3a). The coarser lactose (63-90 µm) appeared to have more small particles adhered to its surface than the finer lactose (< 63 µm). Treatment of lactose particles with 95% ethanol introduced small shallow asperities (cavities) onto the particle surfaces, the majority of which were < approximately 5 µm in diameter, such that the crystal surface had an appearance similar to that of a sponge (Figures 5.2b & 5.3b). Although no substantial change in particle shape was observed, the lactose crystals after treatment with ethanol tended to be pyramidal. This subtle change in particle shape indicates that the crystals may have undergone some recrystallisation process during the treatment with ethanol. On contact with ethanol, some of the lactose molecules on crystal surface may dissolve in the solvent until a saturated solution is formed. Such an ethanolic solution will only contain a very low concentration of lactose since lactose is practically insoluble in ethanol. The dissolved molecules can then crystallise, inducing further dissolution of lactose into the solvent. Thus, dissolution and crystallisation may eventually reach a dynamic equilibrium. Since dissolution of lactose is unlikely to distribute uniformly on the particle surface, some parts of the surface may have disappeared whilst the other parts may have been affected very little. Regions with higher energy such as edges are more likely to dissolve than those with lower energy and thus, some faces will diminish whilst other faces can become enlarged, leading to a slight change in the particle shape after solvent treatment.

5.3.1b Specific surface area of lactose by air permeation method
The specific surface area (SSA) of lactose crystals of lactose was found to be markedly increased after treatment with 95% ethanol. For example, SSA was increased from 857 cm² g⁻¹ for the 63-90 µm size fraction before treatment to 1365 cm² g⁻¹ for the same size fraction.
Figure 5.2 The scanning electron micrographs of lactose crystals (< 63 μm) before (a) and after treatment with 95% ethanol.
Figure 5.3 The scanning electron micrographs of lactose crystals (63-90 μm) before (a) and after treatment with 95% ethanol.
after treatment whilst the SSA increased from 1257 cm$^2$ g$^{-1}$ for < 63 μm before treatment to 2089 cm$^2$ g$^{-1}$ after treatment. The increase in surface area after treatment with ethanol was due to the introduction of many small asperities (cavities) on the particle surface.

### 5.3.2 Particle size characterisation by laser light scattering

Solvent treatment was also shown to change the particle size distribution of lactose particles (Table 5.1). For example, the mass median diameter (MMD) of the lactose of 63-90 μm size fraction was increased from 73.5 μm to 82.4 μm by the treatment of lactose with 95% ethanol but the same treatment did not produce a significant (p > 0.05) effect on the MMD of the lactose of < 63 μm size fraction. However, solvent treatment reduced the fraction of lactose particles less than 10.8 μm regardless of the size fractions of lactose particles being treated. For example, before treatment lactose particles of 63-90 μm fraction had 8.4% v/v particles < 10.8 μm but this fraction was reduced to 5.8% after solvent treatment, which also reduced the portion of particles < 10.8 μm when particles < 63 μm were used. Smaller particles are more likely to dissolve in the solvent than the larger particles since the smaller particles have a higher specific surface area. The dissolution of these small particles may result in re-crystallisation of lactose onto the larger particles, leading to an increase in MMD and a reduction in the fraction of fine particles. Interestingly, lactose particles of 63-90 μm size fraction contained a significantly (p < 0.05) higher fraction of particles < 10.8 μm (8.4%) than lactose of < 63 μm (6.2%). This can also be seen quantitatively from the SE micrographs (Figures 5.2 & 5.3) where more fine particles are shown to adhere to the crystals of 63-90 μm than to < 63 μm crystals. Since these two size fractions of lactose were classified by manual sieving from the same batch of lactose, the size fraction < 63 μm would be expected to have more fine particles (for example, those < 10.8 μm) than the size fraction of 63-90 μm. The opposite observation may imply that fine particles (< 10.8 μm) may be more likely to adhere to the larger particles (63-90 μm) than to the smaller particles (< 63 μm). This phenomenon may indirectly support the previous findings (Section 4.3.3) that the drug particles are less likely to detach from the larger carrier particles than from the smaller carrier particles.
After mixing with salbutamol sulphate (MMD, 2.0 µm), the blends generally exhibited an approximately 1.5% w/w higher fraction of particles < 4.8 µm than the corresponding lactose particles without drug and this was due to the addition of about 1.5% micronised drug particles to the blends. However, mixing salbutamol sulphate with untreated lactose of 63-90 µm failed to produce a significant (p > 0.05) increase in the fraction of particles < 4.8 µm.

Table 5.1, The mass median diameter (MMD), geometric standard deviation (GSD) and fractions of particles < 10.8 and 4.8 µm of lactose particles [Mean(SD), n ≥ 3].

<table>
<thead>
<tr>
<th>Size fraction</th>
<th>Treatment</th>
<th>Samples</th>
<th>MMD (µm)</th>
<th>GSD</th>
<th>% &lt; 10.8 µm</th>
<th>% &lt; 4.8 µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>63-90 µm</td>
<td>untreated</td>
<td>lactose</td>
<td>73.5(2.6)</td>
<td>1.4(0.1)</td>
<td>8.4(1.3)</td>
<td>7.5(1.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>blend</td>
<td>77.0(2.1)</td>
<td>1.3(0.0)</td>
<td>8.1(0.2)</td>
<td>7.6(0.2)</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>lactose</td>
<td>82.4(0.4)</td>
<td>1.3(0.0)</td>
<td>5.8(0.2)</td>
<td>5.4(0.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>blend</td>
<td>82.0(0.4)</td>
<td>1.3(0.0)</td>
<td>7.2(0.3)</td>
<td>7.0(0.3)</td>
</tr>
<tr>
<td>&lt; 63 µm</td>
<td>untreated</td>
<td>lactose</td>
<td>59.3(1.8)</td>
<td>1.6(0.0)</td>
<td>6.2(0.4)</td>
<td>5.9(0.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>blend</td>
<td>59.4(0.5)</td>
<td>1.6(0.0)</td>
<td>7.7(0.1)</td>
<td>7.4(0.1)</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>lactose</td>
<td>60.5(1.4)</td>
<td>1.6(0.0)</td>
<td>4.7(1.1)</td>
<td>3.9(0.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>blend</td>
<td>57.8(0.9)</td>
<td>1.6(0.0)</td>
<td>6.5(0.1)</td>
<td>5.9(0.1)</td>
</tr>
</tbody>
</table>

5.3.3 The effect of solvent-treatment of lactose particles on the deposition of salbutamol sulphate

The deposition data in Table 5.2 were calculated as a nominal dose of one capsule (containing 481 ± 22 µg salbutamol sulphate). It can be seen that a similar recovered dose (RD) of salbutamol sulphate was obtained for all four formulations, ranging from 449 µg for blends containing untreated lactose of < 63 µm size fraction to 469 µg for blends composed of untreated lactose of 63-90 µm size fraction, corresponding to a recovery of between 93.2% and 97.4% nominal dose. Such a high and consistent recovery of salbutamol sulphate
indicates a high reliability, accuracy and reproducibility of the overall procedures including mixing, capsule filling, deposition, washing and analysis.

Table 5.2 Deposition profiles of salbutamol sulphate from untreated and solvent-treated lactose of different size fractions in an ACI after aerosolisation at 60 l min\(^{-1}\) via a Rotahaler\(^\circledR\) [Mean (SD), n = 5].

<table>
<thead>
<tr>
<th>Size fraction</th>
<th>Treatment</th>
<th>RD (µg)</th>
<th>FPD (µg)</th>
<th>Inhaler % RD</th>
<th>Throat % RD</th>
<th>Presep. * % RD</th>
<th>FPF % RD</th>
</tr>
</thead>
<tbody>
<tr>
<td>63-90 µm</td>
<td>untreated</td>
<td>469(13)</td>
<td>80(4)</td>
<td>16.0(1.5)</td>
<td>4.9(0.8)</td>
<td>61.9(0.9)</td>
<td>17.1(0.5)</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>455(15)</td>
<td>66(4)</td>
<td>18.7(2.8)</td>
<td>6.5(1.9)</td>
<td>60.2(2.0)</td>
<td>14.6(0.5)</td>
</tr>
<tr>
<td>&lt; 63 µm</td>
<td>untreated</td>
<td>449(9)</td>
<td>88(2)</td>
<td>16.2(1.1)</td>
<td>6.8(2.9)</td>
<td>56.6(3.3)</td>
<td>19.6(0.3)</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>464(30)</td>
<td>69(5)</td>
<td>16.3(0.9)</td>
<td>10.6(3.0)</td>
<td>57.3(4.5)</td>
<td>14.9(1.1)</td>
</tr>
</tbody>
</table>

* preseparator

These formulations showed a similar drug emission since there was no significant (p > 0.05) difference in the percentage of drug retained in the inhaler device. They also produced similar drug deposition in the preseparator of the ACI. However, the amount of drug collected in the throat piece of the ACI was significantly higher (p < 0.05) for blends containing solvent-treated lactose as compared with those composed of untreated lactose of the corresponding size fraction. The powder blends composed of the solvent-treated lactose appeared to be more poorly fluidised than those of the untreated-lactose. After aerosolisation, the former blends were more likely to stick to the walls of the throat piece, leading to a higher portion of drug particles being retained in that part of the device compared to blends employing untreated lactose. As a result of less drug particles being retained in the throat piece, the blends containing untreated lactose produced significantly higher (p < 0.01) FPD of salbutamol sulphate than those containing the solvent-treated lactose of the corresponding particle size fractions. For example, drug FPD decreased from 80 and 88 µg to 66 and 69 µg after the treatment of lactose carrier particles of 63-90 µm and < 63 µm size fractions, respectively (Table 5.2). Therefore, treatment of lactose with 95% ethanol was found to reduce markedly the FPF of salbutamol sulphate regardless of the carrier size fractions. For example, the mean FPF was significantly (p < 0.01) reduced from 17.1% RD for blends containing untreated lactose of 63-90 µm size fraction to 14.6% RD.
for blends composed of the treated lactose. The blends containing untreated lactose of < 63 μm size fraction produced a mean drug FPF of 19.6% RD, which was reduced to 14.9% RD for treated lactose of the same size fraction. The untreated lactose particles of < 63 μm fraction produced a significantly higher (p < 0.001) FPF of salbutamol sulphate than the untreated lactose particles of 63-90 μm size fraction. This was in agreement with the previous findings that smaller carrier particles produced a higher drug FPF (Section 4.3.3). However, after its treatment of the carrier particle with 95% ethanol, there was no significant difference (p = 0.56) between drug FPF from blends with carrier particles in the size range < 63 μm and 63-90 μm, suggesting that the effects of particle size on drug deposition might have been eliminated by solvent treatment of the carrier particles.

The relative importance of particle size and solvent treatment of the carrier particles on drug deposition can be analysed using ANOVA (Table 5.3). It can be seen that both particle size and the solvent treatment of lactose had a significant (p < 0.001) effect on the FPF of salbutamol sulphate. However, the F-value resultant from the solvent treatment was much higher than that of particle size of lactose, suggesting that the solvent treatment of lactose contributed to the change of drug FPF more than the particle size of lactose carrier. The effects of the solvent treatment and particle size of lactose on drug FPF were also significantly (p < 0.05) dependent upon each other.

<table>
<thead>
<tr>
<th>Source</th>
<th>Degree of freedom</th>
<th>Sum of square</th>
<th>Mean square</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size fraction</td>
<td>1</td>
<td>10.368</td>
<td>10.368</td>
<td>22.28</td>
<td>0.000</td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>64.800</td>
<td>64.800</td>
<td>139.28</td>
<td>0.000</td>
</tr>
<tr>
<td>Size*Treatment</td>
<td>1</td>
<td>6.050</td>
<td>6.050</td>
<td>13.00</td>
<td>0.002</td>
</tr>
<tr>
<td>Error</td>
<td>16</td>
<td>7.444</td>
<td>0.465</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>88.662</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The untreated lactose particles < 63 μm consistently produced the highest cumulative % of drug particles < 4.00 μm, whilst the treated lactose (< 63 or 63-90 μm) produced the lowest
cumulative % of drug particles < 4.00 μm (Fig. 5.4). Before treatment with solvent smaller carrier particles resulted in more efficient aerosolisation of salbutamol sulphate than the larger carrier particles whereas after treatment with 95% ethanol, there was no significant (p > 0.05) difference in the aerosolisation of salbutamol sulphate between the two size fractions of lactose particles.

Figure 5.4 Particle size distribution of salbutamol sulphate after aerosolisation from different lactose particles (Error bars denote standard deviation, n = 5)

Thus, the introduction of small scale asperities onto the surface of lactose particles by treatment with 95% ethanol reduced the aerosolisation of the adhered salbutamol sulphate and this resulted in decreased FPF of the drug. The effect of such solvent-treatment predominated over the influence of particle size of the lactose. Solvent treatment of lactose particles was shown to change substantially the appearance of the particles. Lactose particles appeared to be shiny and polished before treatment but became duller after solvent treatment. Poorer fluidisation of the solvent-treated lactose may arise from increased
frictional forces between the particles due to their rougher surface compared with untreated particles. Poorer fluidisation may be the major cause of the reduced FPF of salbutamol sulphate rather than less efficient drug detachment when the solvent-treated lactose was employed as the carrier. This can be seen from the data which show higher retention of drug in the inhaler device and throat piece from the solvent-treated lactose but similar drug deposition from both lactose crystals in the preseparator after aerosolisation (Table 5.2). These results suggest that although the introduction of small scale surface asperities or cavities onto carrier particles failed to show significant effects on drug detachment, it was likely to be due to the reduced fluidisation of the blends containing the treated lactose that led to reduced aerosolisation efficiency of the drug particles. These results were not supportive of the original hypothesis that introduction of small surface asperities on the surface of the carrier particles would increase deposition of the drug by reducing interparticulate forces between the drug and carrier particles. In contrast, they further confirm the importance of surface smoothness of drug deposition, i.e. the smoother the surface of carrier particles, the higher the FPF of drug particles.

5.3.4 Effects of smaller-sized lactose on deposition of salbutamol sulphate

5.3.4a Effects of micronised lactose
Table 5.4 shows the particle size distribution of different formulations composed of the micronised lactose, coarse lactose (< 63 or 63-90 μm) and salbutamol sulphate. It can be seen that the formulations containing treated lactose 63-90 μm as the coarse lactose had the largest MMD. The MMD of all three formulations containing 5% w/w of micronised lactose was of the same order of magnitude as the MMD of the coarse lactose reported above (Table 5.1). However, all the formulations were found to contain similar concentrations of particles that were < 10.8 or 4.8 μm, and these were approximately 5% higher than the blends without added micronised lactose (Table 5.1).
Chapter five: Effects of surface components of lactose on the delivery of salbutamol sulphate

Table 5.4 Particle size distribution of different formulations with 5% w/w micronised lactose [Mean(SD), n ≥ 3].

<table>
<thead>
<tr>
<th>Lactose</th>
<th>MMD (μm)</th>
<th>GSD</th>
<th>&lt; 10.8 μm</th>
<th>&lt; 4.8 μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated lactose (63-90 μm)</td>
<td>66.4(1.7)</td>
<td>1.5(0.0)</td>
<td>16.9(1.6)</td>
<td>12.7(1.0)</td>
</tr>
<tr>
<td>Treated lactose (63-90 μm)</td>
<td>73.8(2.1)</td>
<td>1.4(0.0)</td>
<td>18.4(2.4)</td>
<td>12.6(1.3)</td>
</tr>
<tr>
<td>Untreated lactose (&lt;63 μm)</td>
<td>52.9(1.3)</td>
<td>1.7(0.0)</td>
<td>17.4(0.9)</td>
<td>12.9(0.2)</td>
</tr>
</tbody>
</table>

The deposition data in Table 5.5 are shown from a dose of one capsule. Similar deposition profiles of salbutamol sulphate were observed from all three formulations, composed of different batches of coarse lactose but with the same added micronised lactose. The recovered dose of salbutamol sulphate ranged from 454 μg for blends containing untreated lactose of < 63 μm to 501 μg for blends composed of untreated lactose 63-90 μm, corresponding to a drug recovery of between 94.3% and 104.1%. No significant difference (p > 0.05) was observed in the percentage of drug retained in the inhaler device, throat piece and preseparator after aerosolisation of the three formulations. However, more drug particles tended to remain in the inhaler device and throat piece but less drug particles were collected in the preseparator when the blends containing untreated lactose of < 63 μm were actuated in comparison to blends composed of lactose 63-90 μm. Thus, all three formulations produced similar FPD and FPF of salbutamol sulphate although they were composed of different grades of coarse lactose particles as the major carrier particles.

As mentioned earlier, untreated lactose of < 63 μm produced the highest FPF of salbutamol sulphate whilst the solvent-treated lactose 63-90 μm resulted in the lowest drug FPF. The untreated lactose of 63-90 μm produced an intermediate FPF. The addition of 5% w/w micronised lactose (MMD 4.9 μm) to the coarser lactose before mixing with the drug tended to eliminate the effects of particle size and surface texture of the coarse lactose particles on drug deposition such that salbutamol sulphate exhibited similar deposition profiles after aerosolisation from these formulations containing different grades of coarse lactose albeit with the same amount of added micronised lactose.
Introducing micronised lactose to the powder formulations was shown to change the deposition profiles of salbutamol sulphate from all three formulations. After actuation of the blends with micronised lactose, more drug particles were retained in the inhaler device than in the case of the blends without micronised lactose. For example, approximately 17% RD of drug was retained in the inhaler device following the actuation of the blends without micronised lactose (Table 5.4) whereas approximately 30% RD drug particles were collected in the device after actuation of the formulations with micronised lactose (Table 5.5). Similarly, more drug particles were found to deposit in the throat piece from the formulations with micronised lactose (8.3-11% RD) than from those without micronised lactose (4.9-6.8% RD). However, drug deposition in the preseparator was markedly reduced from 56.6-61.0% RD for blends without micronised lactose to 36.1-41.5% RD for the formulations with micronised lactose. All these results suggest that the introduction of a small amount of micronised lactose to the powder blends improve drug detachment from the carrier particles and consequently, improve the FPF of salbutamol sulphate. However, micronised lactose was shown to have a more pronounced effect on drug deposition from the coarser lactose 63-90 μm than from the finer lactose < 63 μm. For example, micronised lactose increased the FPF of salbutamol sulphate from 14.6% and 17.5% RD to 20.8% and 21.6% RD for the solvent-treated and untreated lactose 63-90 μm, respectively, whereas addition of micronised lactose to the untreated lactose < 63 μm resulted in a slight but statistically insignificant (p > 0.05) increase in FPFs of the drug (from 19.6% to 20.6% RD).

<table>
<thead>
<tr>
<th>Lactose</th>
<th>RD (μg)</th>
<th>FPD (μg)</th>
<th>Inhaler % RD</th>
<th>Throat % RD</th>
<th>Presep. % RD</th>
<th>FPF % RD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated 63-90 μm</td>
<td>501(7)</td>
<td>108(3)</td>
<td>27.7(2.2)</td>
<td>8.3(1.2)</td>
<td>41.5(0.8)</td>
<td>21.6(0.7)</td>
</tr>
<tr>
<td>Treated 63-90 μm</td>
<td>460(3)</td>
<td>96(6)</td>
<td>29.4(0.8)</td>
<td>10.3(1.7)</td>
<td>38.5(1.7)</td>
<td>20.8(1.2)</td>
</tr>
<tr>
<td>Untreated &lt; 63 μm</td>
<td>454(29)</td>
<td>93(11)</td>
<td>31.5(2.7)</td>
<td>11.0(2.5)</td>
<td>36.1(2.6)</td>
<td>20.6(2.1)</td>
</tr>
</tbody>
</table>

As shown in Figure 5.5, the formulations containing micronised lactose produced a consistently higher percentage of fine particles (< 4.00 μm) of salbutamol sulphate than the blends without micronised lactose. Although it was shown previously that the solvent-
treated lactose produced a lower FPF of drug particles than the untreated lactose, the incorporation of micronised lactose in such formulations resulted in a similar pattern of particle size distribution of the aerosolised drug particles (Fig 5.5). Thus, addition of micronised lactose to the powder blends appeared to reduce the effects of surface roughness of the coarser lactose on drug deposition and the effects of improving drug deposition by incorporating micronised lactose into the formulation may be more pronounced for coarse particles of rougher surface than those of smoother surface.

Figure 5.5 Particle size distribution of salbutamol sulphate after aerosolisation from lactose particles 63-90 μm without or with 5% w/w micronised lactose fine particles (Error bars denote standard deviation, n = 5).

Adding micronised lactose particles may reduce the drug-carrier interactions by the fine lactose occupying possible drug binding sites on the larger lactose particles. The filling of the surface crevices or cavities of lactose or the formation of multiple layers of fine lactose may also occur in the presence of a greater proportion of fine particles, thereby hindering
direct contact of drug and carrier and promoting drug particle detachment from the carrier surface during aerosolisation.

5.3.4b Effects of needle shaped crystals of lactose

The needle shaped crystals of lactose had a slightly larger particle size (MMD 5.9 μm) and wider size distribution (GSD 3.6) than micronised lactose (MMD ± GSD, 5.2 ± 2.1 μm). However, light and SE microscopy (Figure 5.6) suggested that the micronised lactose particles were more irregularly shaped than the crystalline fine lactose, most of which were elongated or needle-like in shape. The micronised lactose particles were obtained after 9 consecutive passages of Lactochem™ lactose through an air-jet microniser. The needle shaped lactose crystals were obtained by a one-step crystallisation from the same starting material, which was much less laborious and time-consuming than micronisation.

Blends containing the smaller-sized lactose particles (either micronised or needle shaped lactose crystals) had a slightly smaller MMD than powders without added lactose particles (Table 5.6). The formulation containing needle shaped lactose crystals had a similar particle size and size distribution to that of the formulation containing micronised lactose. Rotacap® blends had the smallest MMD with the widest particle size distribution (the highest value of GSD).

Table 5.6 Particle size distribution of the blends composed of coarse lactose 63-90 μm and salbutamol sulphate with 5% w/w either micronised or crystallised lactose fine particles

<table>
<thead>
<tr>
<th>Samples</th>
<th>MMD (μm)</th>
<th>GSD</th>
<th>% &lt; 10.8 μm</th>
<th>% &lt; 4.8 μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotacaps®</td>
<td>50.2(0.9)</td>
<td>1.9(0.0)</td>
<td>16.1(0.2)</td>
<td>8.5(0.2)</td>
</tr>
<tr>
<td>Coarse lactose + drug</td>
<td>77.0(1.9)</td>
<td>1.3(0.0)</td>
<td>8.1(0.6)</td>
<td>7.4(0.3)</td>
</tr>
<tr>
<td>Coarse &amp; micronised lactose + drug</td>
<td>65.1(1.7)</td>
<td>1.5(0.0)</td>
<td>17.9(0.9)</td>
<td>12.9(0.2)</td>
</tr>
<tr>
<td>Coarse &amp; needle lactose + drug</td>
<td>67.3(1.2)</td>
<td>1.6(0.0)</td>
<td>15.4(0.7)</td>
<td>9.2(0.5)</td>
</tr>
</tbody>
</table>
Figure 5.3 The scanning electron micrographs of micronised lactose (a) and needle shaped crystals of lactose (b).
The deposition data of salbutamol sulphate were calculated as a function of the nominal dose from one capsule (Table 5.7). The recovered dose varied from 447 μg for the formulations containing small needle shaped crystals of lactose to 501 μg for the blends composed of micronised lactose, corresponding to an average recovery of approximately 99% of the nominal dose.

Table 5.7 Deposition of salbutamol sulphate from different formulations in an ACI after aerosolisation at 60 l min⁻¹ via a Rotahaler® [Mean(SD), n ≥ 4].

<table>
<thead>
<tr>
<th>Formulations</th>
<th>RD (μg)</th>
<th>FPD (μg)</th>
<th>Inhaler % RD</th>
<th>Throat % RD</th>
<th>Presep. % RD</th>
<th>FPF % RD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotacaps*</td>
<td>497(8)</td>
<td>83(2)</td>
<td>28.6(1.9)</td>
<td>13.1(1.8)</td>
<td>40.5(1.5)</td>
<td>16.6(0.3)</td>
</tr>
<tr>
<td>Without fine</td>
<td>469(13)</td>
<td>80(4)</td>
<td>16.0(1.5)</td>
<td>4.9(0.8)</td>
<td>61.9(0.9)</td>
<td>17.1(0.5)</td>
</tr>
<tr>
<td>With micronised</td>
<td>501(7)</td>
<td>108(3)</td>
<td>27.7(2.2)</td>
<td>8.3(1.2)</td>
<td>41.5(0.8)</td>
<td>21.6(0.7)</td>
</tr>
<tr>
<td>With needle</td>
<td>447(6)</td>
<td>121(3)</td>
<td>25.9(0.2)</td>
<td>7.9(0.6)</td>
<td>37.6(0.9)</td>
<td>27.0(0.5)</td>
</tr>
</tbody>
</table>

As mentioned earlier, the introduction of smaller-sized carrier particles should improve drug detachment and dispersion by means of reducing the interaction of drug particles with coarse carrier particles. This can be further seen from the different distribution patterns of drug particles within the inhaler device, throat piece and preseparator of the impactor. The blends without added intermediate lactose had the highest (p < 0.001) drug deposition in the preseparator (61.9% RD) but the lowest drug retention in the inhaler device (16.0% RD) (p < 0.001) and throat piece (4.9% RD) (p < 0.05). The blends without added smaller-sized lactose had the larger MMD (Table 5.6) in comparison with other formulations and it is this that is likely to have contributed to more drug particles being emitted from the inhaler device. The direct adherence of drug to coarse carrier particles in the blends is likely to produce stronger adhesion forces of drug to coarse carrier particles. The stronger forces result in poorer drug detachment from the carrier and this leads to higher drug deposition in the preseparator compared to the formulations containing smaller-sized lactose. Inclusion of smaller-sized carrier particles with the drug and coarse carrier particles limits the direct interaction of the latter two components and consequently, improves detachment of the drug from the carrier, resulting in higher retention of drug in the inhaler device and throat piece.
but lower drug deposition in the preseparator. The formulations with needle shaped lactose crystals produced a significantly lower (p < 0.001) drug deposition in the preseparator (37.6% RD) than the formulations containing micronised lactose (41.5% RD) although there was no significant difference (p > 0.05) in drug retention in inhaler device (26-28% RD, p = 0.15) and throat piece (7.9-8.3% RD, p = 0.47) between these two formulations. These results suggest that although both formulations may provide similar drug emission from the inhaler device, the formulations with lactose needle crystals may produce more efficient drug detachment from the lactose carrier than the formulations containing micronised lactose. The Rotacap® formulation produced the highest drug deposition in the throat piece (13.1% RD). After actuation of these blends, it was apparent that more powder (either fine lactose, drug aggregates or aggregates of drug and lactose) was retained on the walls of the throat piece than the other formulations. This might be due to the smaller particle size of the Rotacap® blends in comparison to the experimental formulations.

The formulation containing needle shaped lactose crystals produced the highest FPD (p < 0.001), followed by the formulation containing micronised lactose whereas the formulation without added fine particles and the Rotacap® formulation produced the lowest FPD of salbutamol sulphate. Therefore, the formulation containing lactose needle crystals produced the highest FPF of salbutamol sulphate whilst the Rotacap® and blends without added fine lactose produced the lowest FPF of the drug. The formulations containing micronised lactose gave an intermediate FPF of the drug. For example, the fraction of fine drug particles < 6.18 μm from the formulation containing needle shaped lactose (27.0% RD) was about 25% higher than that of the formulation containing micronised lactose (21.6% RD), which was in turn more than 25% higher than that of the blends without added intermediate lactose (17.1% RD). Although the Rotacap® formulation had the smallest MMD, it produced the lowest FPFs of salbutamol sulphate.

As shown in Figure 5.7, the formulation containing 5% w/w needle shaped crystals of lactose produced a similar percentage of aerosolised drug particles < 2.30 μm to the formulations containing 5% w/w micronised lactose but the former formulations released a significantly (p < 0.01) higher percentage of aerosolised drug particles between 3.20 and 6.18 μm than the latter formulations. Both formulations were composed of the same batch
Chapter five: Effects of surface components of lactose on the delivery of salbutamol sulphate

of coarse lactose and salbutamol sulphate, blended with the smaller-sized lactose in the same ratio using the same mixing process. Further, they also showed similar MMD and fractions of particles < 4.8 µm (Table 5.6). Their major differences lies in the crystallinity and particle shape of the added smaller-sized lactose particles. As discussed previously, micronised lactose is rich in amorphous regions and amorphous material is physically softer than the crystalline form. If micronised drug particles are bound to these amorphous regions, then deformation on the binding sites would be more likely to occur than the binding sites of the crystalline lactose with increased hardness. The drug particles and coarser lactose carrier would thus be expected to bind to the micronised lactose more strongly than to the crystalline lactose. Further, the intermediate crystalline lactose had a more elongated shape than the micronised lactose. The needle crystals are more likely to disperse in an airflow and remain airborne than more isometric (spherical) micronised particles. As a result, more drug particles may be detached from the needle crystals than from the micronised lactose under the same airflow conditions. Even for drug particles which are not detached, the needle shaped lactose crystals may act as carrier to aid deep lung penetration of the drug. This may explain why the major difference in the amount of fine particles of drug from these two formulations was for particles sized between 3.20 and 6.18 µm. Finally, the effects of crystallinity on drug deposition may be pronounced in the longer term. In the case of present work, the deposition test was carried out within 2 weeks of the powders being stored in a desiccator over silica gel and thus, crystallisation of amorphous lactose on micronised particles would be expected to be negligible.

The formulation containing needle shaped lactose crystals also produced a much higher FPF of salbutamol sulphate than that obtained from Ventolin Rotacap® (Figure 5.7). The FPF of salbutamol sulphate from the formulation containing needle shaped crystals was 1.64, 1.60, 1.54, 1.64, 2.77, 5.50 and 5.67 times those obtained from Ventolin Rotacap® calculated as particles less than 6.18, 4.00, 3.20, 2.30, 1.40, 0.76 and 0.45 µm, respectively. Thus, the improvement in drug delivery by the use of needle shaped lactose crystals appeared more pronounced in terms of the smallest drug particles liberated, such as those less than 2.30 µm. The improvement in the delivery of drug particles less than 2.30 µm may be of clinical importance since particles of this size range are more likely to reach the lower airways than larger particles.
Chapter five: Effects of surface components of lactose on the delivery of salbutamol sulphate

The powder contained in Ventolin Rotacap® had a smaller particle size than the formulations containing needle shaped lactose crystals but nevertheless, there were similar amounts of fine particles of lactose in both formulations (Table 5.6). As mentioned earlier (Section 4.3.3), a decrease in carrier particle size increased FPF of aerosolised drug. Thus, the smaller carrier particles employed in the powders of Ventolin Rotacap® might have been expected to produce a higher FPF of drug than the formulations employing lactose needle crystals. Therefore, the improved drug delivery obtained using the blends with incorporated needle shaped lactose crystals provides further evidence to support the hypothesis that the use of such crystals of intermediate size increases the delivery efficiency of drugs from dry powder aerosols. Both the Rotacap® and needle shaped lactose formulation had similar dose uniformity (relative standard deviation < 2%). However, the experimental formulation
produced a significantly (p < 0.05) higher percentage of emitted dose relative to total dose (74.1 ± 0.2%) than Ventolin Rotacaps (71.4 ± 1.9%). On this basis, such a formulation would be superior to blends of Ventolin Rotacaps® in terms of delivery efficiency of drugs to the lower airways.
5.4 Summary

This section of work describes the effects of introducing surface asperities of smaller scale than drug particles onto the carrier surface and also determined the effect of adding smaller-sized lactose particles on the deposition of salbutamol sulphate. The treatment of lactose with 95% ethanol was shown to introduce surface asperities or cavities, the majority of which had a smaller scale than the drug particles without substantially changing the particle shape. The solvent-treated lactose produced an FPF of salbutamol sulphate which was significantly lower than from untreated lactose of a similar size fraction. The reduction in drug FPF caused by the solvent treatment counteracted the increase in drug FPF induced by decreasing particle size such that the use of treated lactose of two different size fractions (< 63 and 63-90 μm) produced similar deposition profiles of salbutamol sulphate. The reduction in drug FPF might be largely due to the reduced fluidisation in the air stream rather than a decreased drug detachment from the treated lactose carrier. The effects of such surface asperities or cavities of lactose were offset by introducing a small amount of smaller-sized lactose (5-10 μm) to the powder formulations. The smaller-sized lactose increased the FPF of salbutamol sulphate to such a level that all lactose batches, regardless of particle size distribution or whether solvent treated, produced a similar FPF of salbutamol sulphate. The use of lactose needle crystals was found to be superior to micronised lactose in terms of the delivery efficiency of the drug. The formulations, composed of coarse lactose (63-90 μm), needle shaped lactose crystals (5.9 μm) and salbutamol sulphate (1.9 μm) in a ratio of 64.125 : 3.375 : 1 (w/w), produced an FPF of salbutamol sulphate 1.64, 1.60, 1.54, 1.64, 2.77, 5.50 and 5.67 times those of Ventolin Rotacaps® calculated as drug particles less than 6.18, 4.00, 3.20, 2.30, 1.40, 0.76 and 0.45 μm, respectively. Therefore, such a formulation would provide an important means of improve the delivery of drug to the lower airways.
CHAPTER SIX

GENERAL DISCUSSION
All dry powder inhalers (DPIs) have four basic features: a) a dose-metering mechanism; b) an aerosolisation mechanism; c) a deaggregation mechanism and d) an adapter to direct the aerosol into a patient’s mouth. The major components of most DPIs are micronised drug powders adhered to coarse lactose particles, a drug reservoir or premetered individual dose, the body of the device, and a cover to prevent ingress of dust or moisture. In order for the drug to gain access to the lower airways, it is generally accepted that a prime requirement is that the drug particles have an aerodynamic diameter between 1-5 μm (Newman and Clarke, 1983; Gonda, 1990). An important consequence of the requirement of fine particles for inhalation arises from the fact that powder flow properties are dependent on the particle size distribution, fine particles generally flowing less well than coarse ones. The final formulation must flow sufficiently well either to be dispensed from the bulk reservoir to give an adequately reproducible dose, or be capable of being handled well on automatic filling machines to produce the unit dose forms for use in a device. Small particles are also notoriously difficult to disperse (Hickey et al., 1994) due to the highly cohesive nature of fine particles. The fine drug particles must therefore be formulated to have appropriate properties such as reasonable flowability and high dispersibility. Two major approaches have been employed to improve the flow and dispersion of drug particles. The first one involves the controlled aggregation of the undiluted drug to form loosely adherent floccules (Bell et al., 1971); such an approach takes advantage of the inherent cohesiveness of the drug particles. The alternative approach is to use formulations comprising fine drug particles blended with coarser carrier particles (Ganderton, 1992).

Drug particles for inhalation are always prepared by mechanical micronisation (Hickey, 1993), a process which imparts a large quantity of energy to the micronised particles (Otsuka and Kaneniwa, 1990; Florence and Salole, 1976). As a consequence, such micronised particles often have a high associated surface energy (Buckton et al., 1988) and a large portion of amorphous drug is usually present (Saleki-Gerhardt et al., 1994). Different micronisation conditions may induce different surface energies and surface disorders of micronised materials (Buckton et al., 1988). These amorphous regions are thermodynamically unstable and will convert to more stable crystal forms under proper environmental conditions such as high temperatures and/or relative humidities. For
example, after micronisation, salbutamol sulphate showed an extra, small exothermic peak at 85°C, which disappeared after storing the micronised sample at 40°C/75% relative humidity (RH) for 24 h (Ward and Schultz, 1994). These results suggest that during the micronisation process, crystal disorder was introduced into the powdered salbutamol sulphate and that these amorphous regions reconverted to the crystalline state after treatment at relatively high temperature and humidity. The transformation was shown to result in a distinctive change in particle morphology and to increase interparticulate bridging. The mass median diameter (MMD) of micronised particles, measured by laser light diffraction, was increased from 1.5 μm to 3.9 μm after 24 h storage at 40°C/75% RH. These results are indicative of the importance of crystallinity disorder, however small it may seem, in determining the bulk properties of the micronised powders. If small amounts of amorphous regions exist on a particle surface, then any absorbed water vapour will be likely to concentrate in such regions (Ahlneck and Zografi, 1990). Thus, a seemingly insignificant amount of water relative to the powder mass, could result in a high concentration of water in those specific areas. The effects of water may thus be amplified since even small amounts of water can greatly change the molecular mobility in disordered regions and result in a significant change in physical and even chemical (Konno, 1990) properties of the bulk powders. Surface disorders of particles induced by micronisation may bear even more significance in dry powder aerosols than in other delivery systems since the in vitro and in vivo behaviour of dry powders is largely dependent upon their physical properties. Therefore, the surface energy and amorphism of micronised drug should also be controlled in line with the particle size and morphology of the final products. Since the formation of crystal disorder is unavoidable during mechanical micronisation, it may be necessary to carry out some treatment of the particles after micronisation under controlled conditions such as storing the micronised samples at elevated temperatures and humidities to deliberately convert any unstable amorphous regions. Such a transformation would greatly decrease the sensitivity of powders to surrounding environments and thus enhance uniformity and reproducibility of in vitro and in vivo performances of dry powders.

Spray drying has also been employed to prepare drug particles for inhalation (Chawla et al., 1994; Golbach et al., 1993). This process involves the formation of droplets with subsequent rapid evaporation of solvent. Thus, spray-dried particles are often spherical in nature and are
mostly amorphous in form (Chawla et al., 1994). A lower FPF was obtained using spray-dried salbutamol sulphate than when micronised drug was employed although the former particles had smaller geometric size than the latter particles (Chawla et al., 1994; Venthoye et al., 1995). After storage at a relative humidity of 60%, spray dried amorphous disodium cromoglycate was shown to transform to crystalline drug and the transformation reduced the drug FPF (< 7.1 μm) from about 30% to less than 10% nominal dose (Vidgren et al., 1988). As a result of its higher amorphous content, spray dried particles are often softer than micronised particles and this will lead to higher interparticulate forces due to the deformability of the soft particles. This might account for the lower dispersibility of spray dried particles in comparison to micronised particles. Furthermore, the amorphous nature of spray dried particles may render the particles more sensitive to environmental humidities than micronised particles. Any water uptake and the subsequent crystalline transformation may increase interparticulate forces and change some particle characteristics such as particle size and shape. Therefore, spray drying may not provide an ideal means of preparing drug particles for inhalation.

Supercritical fluid (SCF) recrystallisation to obtain fine particles suitable for deep lung penetration has attracted some attention in recent years (Philips and Stella, 1993). After recrystallisation from a SCF, drug particles were shown to retain their crystalline structure (Larson and King, 1986). One of the major problems associated with the use of SCF to produce inspirable particles is the likelihood of the fine particles forming large aggregates, due to high surface energy intrinsic to ultrafine particles (Philips and Stella, 1993). This may lead to an increase not only in the effective particle size of the bulk powder but also the interparticulate forces between the drug and carrier particles. Rapid recrystallisation may also produce amorphous drug and metastable crystal forms. Another problem may arise from the low solubility of many drug entities in supercritical CO₂ although the use of gas anti-solvent recrystallisation, which requires the drug be dissolved in an organic solvent and then precipitate from the solvent by an SCF (Sacchetti and van Oort, 1996) may partly solve this problem.

Crystallisation of drug particles from ordinary solvents under carefully controlled conditions may provide a powerful alternative to mechanical micronisation, spray drying and
supercritical crystallisation. In order to obtain crystals of suitable size and shape for inhalation, the basic procedures may involve dissolving the drug in a solvent and then adding the solution to a second solvent. The drug should not be soluble in the second solvent, which should be miscible with the first solvent. Therefore, after mixing with the second solvent, the crystallisation of drug particles is so rapid that only small crystal nuclei of the drug are prepared. For example, in the present work, salbutamol sulphate was crystallised by adding its aqueous solution to absolute ethanol to obtain elongated crystals (needle-like) of salbutamol sulphate with MMD of 5.49 μm (Figure 6.1). After blending with Lactochem® lactose, the recrystallised salbutamol sulphate gave an FPF (< 6.4 μm) of 22.8% recovered dose, which was more than double the FPF (10.8% recovered dose) of micronised salbutamol sulphate with an MMD of 4.79 μm. The possibility of preparing elongated fine particles of drug by recrystallisation may offer another opportunity of optimising drug delivery from dry powder aerosols since elongated objects such as fibres and needle-like crystals have aerodynamic diameters largely dependent upon their shorter axes but practically independent of their lengths (Hinds, 1982). These particles may be expected to have smaller aerodynamic diameters as compared with more spherically shaped particles of the similar geometric size. Since the aerodynamic diameters of such crystals depend largely on their shorter axes, which are much smaller than the mean diameters of spherical particles of the similar size, it can be expected that such axes are more uniformly distributed, leading to more uniform aerodynamic particle size distribution than more isometric particles. Once inhaled, the elongated particles may possess a high selectivity of deposition in the respiratory tract. In a similar manner to fibre pollutants, elongated particles may deposit in the airways through interception (Timbrell, 1965). Moreover, recrystallised drug particles may have higher crystallinity, lower surface energy and electrostatic charge than micronised particles. Also, the shape of crystallised particles may be more easily controlled than micronised particles. Overall, in this work, the elongated crystallised particles exhibited much higher FPF than micronised powder. If this phenomenon can be further substantiated, it will undoubtedly provide another means of improving drug delivery from dry powder aerosols.
Figure 6.1 The scanning electron micrographs of micronised (a) and recrystallised salbutamol sulphate (b).
Chapter six: General discussion

The material used as a carrier for inhalation aerosols should be readily available in an acceptable pharmaceutical grade, be chemically and physically stable and not interact with the drug substance. Most importantly, the carrier must not have any side effects and once delivered to the respiratory tract, it should be easily metabolised or rapidly cleared from the airways. The carrier must not have any unpleasant taste and odour and it should be inexpensive, easy to prepare and present in a crystalline form with low hygroscopicity. Ideally, the carrier particles should ensure maximal dose uniformity and delivery efficiency of drug particles. Much work needs to be done to find a carrier that can meet all the above requirements. However, lactose has been most commonly used to aid the drug flow and dispersion (Byron and Jashnani, 1990) because it fulfils most of the aforementioned criteria. It is possible to obtain lactose in a wide spectrum of particle size distributions but a typical particle size of lactose used as carrier for inhalation aerosols is between 63-90 μm (Bell et al., 1971). In order for the drug particles to reach the lower airways, they must therefore dissociate or detach from the carrier particles (Ganderton, 1992). Thus, the drug particles are dispersed and can traverse the upper respiratory tract whilst the excipient particles do not pass beyond the mouth-piece of the device or the mouth and throat of the patient. The drug particles failing to detach from the carrier particles on inhalation may be one of the major reasons of the inefficient drug delivery encountered for most dry powder inhalers.

Drug detachment is determined by interparticulate forces between the drug and carrier particles. Reducing the interparticulate forces may provide one of the major strategies to improve delivery efficiency of the drug. Interparticulate forces are mainly composed of van der Waals, electrostatic and capillary forces (Rietema, 1991). Although van der Waals forces are dominant over other forces under normal conditions for most pharmaceutical ordered mixes (Staniforth, 1985), the contribution of electrostatic forces to the overall interparticulate forces increase with decreasing particle size (Bailey, 1984). Capillary forces only begin to be manifest at relative humidities (RH) between 50 and 60% and at RH between 65 and 100% they may be the main factor in adhesive interaction (Zimon, 1982). Since in a typical aerosol powder formulation, the drug particles (2-5 μm) are adhered to coarse carrier particles (63-90 μm), the entrapment or incorporation of drug particles within any existent surface cavities of the carrier particles may also markedly increase the overall adhesion forces of the drug to the carrier particles.
Many factors are known to affect the interparticulate forces such as crystal habit, surface textures and particle size, etc. For example, increasing the surface smoothness of carrier particles may decrease the proportion of drug particles entrapped in any surface cavities of the carrier particles and consequently reduce the overall interparticulate forces between the drug and carrier particles. Also, increasing the surface smoothness of lactose particles was reported to increase the respirable fraction of salbutamol sulphate (Kassem, 1990). The respirable fraction of the drug was shown to increase from 4.1% for regular crystalline (Lactochem®) lactose with a rugosity value of 2.6 to 23.0% for recrystallised lactose with a rugosity value of 1.2 after aerosolisation at 60 l min⁻¹ via a Rotahaler®. The improved respirable fraction was attributed to the increased surface smoothness of the recrystallised carrier particles. However, other factors such as particle shape and processing history may also have differed between the commercially available Lactochem® lactose and the batch of recrystallised lactose. For example, Lactochem® lactose as supplied had previously undergone a milling process whilst the recrystallised lactose did not undergo any such comminution treatment (Kassem, 1990). Further, the recrystallised lactose and the regular crystalline lactose may have had different particle shapes. All these factors were not taken into account in these earlier studies and the data were not subjected to sound statistical analysis (Kassem, 1990; Ganderton, 1992). Therefore, the direct comparison of drug deposition from formulation containing Lactochem® lactose with that from formulations employing recrystallised lactose might not reveal the true effects of surface smoothness of the carrier particles on the deposition of the drug. In order to investigate the effects of a specific morphological factor of the carrier particles on drug deposition, the contribution of all the other factors of the carrier particles to the adhesion forces of drug particles, have to be minimised or kept at similar level. This was achieved in the current work by comparing drug deposition from different batches of lactose particles that had undergone similar preparative procedures.

It is shown in the present work that lactose particles of different particle shape, surface textures and particle size distribution can be prepared by varying the conditions of crystallisation. The particle shape (elongation ratio), particle size, surface smoothness of lactose particles and inhalation flow rate all have an impact upon the FPF of salbutamol
sulphate. Increasing the inhalation flow rate or increasing particle elongation ratio and/or surface smoothness and/or decreasing the particle size of lactose particles may act independently or interdependently to increase the FPF of the salbutamol sulphate. However, there have been no strict criteria to control the particle size and morphological properties of the carrier particles employed as the carrier for inhalation aerosols. Variation in such factors affecting carrier particles may be one of the major causes of the batch-to-batch variation in drug delivery encountered for most dry powder aerosol formulations. Therefore, the morphology and particle size of the carrier particles should be carefully controlled so as to improve the delivery efficiency and reduce the batch-to-batch variations of dry powder aerosols.

Different conditions of crystallisation also resulted in the production of lactose particles of different amorphous content. Such amorphous regions on the surface of carrier particles may lead to similar problems associated with those on the surface of drug particles. Therefore, the crystallinity of the carrier particles should also be controlled. The degree of crystallinity of pharmaceutical solids has been measured using XRPD (Blask and Lovering, 1977); infrared spectroscopy (Blask and Lovering, 1977); NMR spectrometry (Murari, 1989); DSC (Saleki-Gerdardt et al., 1994); density (Duncan-Hewitt and Grant, 1986) and dissolution rate (Hendriksen, 1990). All these techniques are known to measure the average degree of order throughout the bulk powder and thus have limited capability when measuring small amounts of disorder. For example, XRPD, DSC and density measurement revealed good linearity with the percentage disorder and these techniques had an acceptable detectability down to about 10% (Saleki-Gerhardt et al., 1994). These techniques may not be sensitive enough to detect the small, but critical amounts of surface disorder that may be induced during processing. Isothermal heat-conducting microcalorimetry provides an alternative to DSC but with much higher sensitivity for measurement of crystal disorder with a resolution of at least 1% (Sebhatu et al., 1994; Briggner et al., 1994). Unlike DSC which detects and measures the heat of crystallisation of an amorphous material from a scan of increasing temperature, isothermal microcalorimetry measures the heat flow from ongoing processes that proceeds at a specified (slightly elevated) relative humidity in a sample vessel kept at a specific (room) temperature. The humidity can be maintained by sealing the sample in an ampoule with a small tube of a saturated salt solution. The response
recorded power (the rate of change of heat) as the function of time, the area under the curve being the total heat released from the sample. Given the specific heat capacity or by preparing a calibration curve, it is possible to calculate the amount of the amorphous material in the sample. Isothermal moisture uptake profiles may also be able to probe the surface amorphism of a powder. Since, as mentioned above, moisture will preferentially be adsorbed by the amorphous regions in powder samples. Thus, both the extent and rate of moisture uptake are likely to be of value in probing order/disorder features of the samples and eventually discriminate between physically (particle size, etc.) and chemically (structure) equivalent samples (York, 1994). Future work would involve the use of these techniques to characterise crystallinity of either carrier or drug particles prepared under different conditions.

Crystallisation from Carbopol 934 gels led to the preparation of more regularly shaped lactose with smoother surface and higher crystallinity in comparison to crystallisation under constant stirring. The blends containing these batches of lactose produced a higher FPF of salbutamol sulphate than the highest achievable FPF of the blends containing lactose of the same size fraction (63-90 μm) but prepared under constant stirring. Furthermore, lactose particles prepared from Carbopol 934 gels had a higher crystallinity than those prepared under conditions with constant stirring. However, despite the elongated shape and perfectly smooth surface, lactose crystals prepared from Carbopol gels still failed to produce satisfactory drug FPF, the values being only comparable to those that resulted from blends incorporating lactose prepared under constant stirring but with a size < 63 μm. Therefore, it may not be sufficient to improve drug FPF solely by means of engineering carrier particles. However, such control of crystallisation conditions may be employed in combination with other approaches such as the manipulation of components of the powder formulations, with a view to further improving the FPF of drug from dry powder formulation.

A possible strategy proposed to improve drug detachment from carrier particles has been the incorporation of fine particles of a third component such as l-leucine into the powder formulations (Staniforth, 1996) with a view to the fine particles occupying the sites of strong adhesion. However, the use of third components in powder formulations for the lung will require stringent toxicological testing. An ideal strategy would involve the precise
engineering of the coarse carrier particles together with the introduction of fine particles of the carrier into the powder formulation. Inclusion of intermediate-sized carrier particles between the drug and coarse carrier particles would be expected to prevent the direct interaction of the two components and consequently, improve detachment of the drug from the carrier. The effects of addition of the intermediate-sized carrier particles to dry powder formulations on drug delivery were more pronounced for needle crystals than for micronised particles. Such a strategy to maximise the FPF of drug from dry powder formulations may also be applicable to other drugs in other powder formulations employing other sugars as carriers. Such an approach may be commercially exploitable more rapidly than those employing a third component in the powder formulations. Future work would investigate the possibility of improving delivery of other drug particles employing similar strategies. The effects of addition of dissimilar intermediate-sized carrier particles such as glucose on drug delivery from formulations containing lactose as the principal carrier should also be studied.

Apart from the physical properties of the interactive particles, chemical structure may also affect the interparticulate forces. Generally, materials having similar polarity or hydrophilicity adhere to each other more strongly than materials of different polarities (Podczeck et al., 1996). Carriers of different chemical entities may exhibit different interparticulate forces with a specific drug particle. Thus, carrier particles of different materials may produce different deposition profiles of the same drug particles. For example, the FPF of salbutamol base was shown to be the highest after aerosolisation from sorbitol, followed by crystalline lactose and maltose with the least FPF resultant from dextrose and spray dried lactose (MacRitchie et al., 1995). Although this work did not specify whether the difference in drug delivery was due to different chemical structures of the different carriers or due to different particle size and morphology of the carrier particles, different sugars have different polarities and hydrophilicities and therefore, would be expected to exert different adhesion forces to the drug particles, leading to different drug delivery from these carriers. Further, different sugars should have different characteristics of crystallisation, controlled crystallisation of these sugars may produce a variety of crystal habit and crystal form, which may provide a wealth of opportunities of optimising drug delivery from dry powder aerosols. Obviously, the various permutations of employing two
different carrier types with different size distributions, both prepared under conditions of controlled crystallisation, offer extensive scope for future investigation.

The interaction between the drug and carrier particles may also be affected by the mixing procedures that are used to produce the ordered mixture. Under extensive blending, particles may attempt to achieve the orientation with the least potential energy, which involves the shortest separation and largest contact area, especially for elongated and plate-like particles and this may result in higher interparticulate forces being generated (Ganderton and Kassem, 1992). Under extensive blending, particle triboelectrification may also be increased, which may further increase the interparticulate forces. For example, the adhesive tendency in a model drug-carrier system was found to increase with blending time (Kulvanich and Stewart, 1988). Different mixing orders produced different FPFs of the salbutamol sulphate from the same formulation composed of coarse, fine lactose with salbutamol sulphate (Tee, 1996). Powders prepared by adding drug particles to a mixture of coarse and fine carrier particles produced a mean respirable fraction of 14.7%, which was significantly higher (ANOVA, p < 0.05) than the 9.1%, which resulted from powders prepared by adding fine lactose to the mixture of drug and coarse lactose particles. This difference in respirable fraction may be best explained by the fact that in the former case, the fine lactose particles were used to saturate active binding sites on the coarse lactose particles whereas in the latter case, the drug particles were firstly exposed to the active binding sites. Thus, adhesion between drug and carrier particles can be expected to be stronger in the latter case than in the former case. Therefore, the mixing procedures may also need to be clearly defined so as to ensure the uniformity of the mixing without substantially increasing the interparticulate forces between the drug and carrier particles.

Uniformity of mixing is a prerequisite for the uniformity of subsequent dosing. Ideally, drug particles should be evenly distributed among the carrier particles after mixing. This requires that the adhesive forces between drug and carrier particles are stronger than the cohesive forces between the fine drug particles. Thus, during powder mixing, higher adhesion forces are favoured so as to obtain a desirable uniformity of the ordered mix since increased adhesion forces would lead to more even distribution of drug particles amongst the carrier particles (Staniforth, 1995). The ordered mixes should maintain their uniformity during
powder handling and storage. The stability of ordered mixes toward mechanical vibration was shown to improve by either increasing the van der Waals (Staniforth and Rees, 1983), electrostatic (Staniforth and Rees, 1982) and capillary forces (Staniforth, 1985). However, increasing the interparticulate forces between the drug and carrier particles will decrease the detachment and dispersibility of drug particles. These contradictory factors have to be carefully balanced so as to ensure dose uniformity and delivery efficiency of drugs from dry powder aerosols.

In conclusion, the current work has investigated the effects of the morphological properties of carrier particles on the drug delivery from dry powder aerosols. It was shown that drug delivery from a model DPI, Rotahaler®, depends upon many factors, which includes particle size, particle shape, surface smoothness and powder formulations as well as the inhalation flow rate. A more elongated shape, smoother surface, or smaller mass median diameter of the carrier particles or the introduction of intermediate-sized carrier particles to the powder formulations increased the FPFs of drug particles. Aside from the design of inhaler device, the careful control of such properties of the powder formulation by means of particle engineering may provide a powerful tool for the optimisation of drug delivery from dry powder aerosols.


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