Preparation and Isolation of Drug Particles with Improved Therapeutic Response

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DECLARATION

I hereby declare that this dissertation is my own work and contains nothing which is the outcome of work done in collaboration with others, except where specific reference is made in the text and Acknowledgements. The contents of this dissertation have not been submitted in whole or in part for consideration for any other degree or qualification in this, or any other university.

Teresa Tierney

Oct 2016
Faith is a force that is greater,
Than knowledge or power or skill,
And many defeats turn to triumph
If you trust in God's wisdom and will.

Helen Steiner Rice
ACKNOWLEDGEMENTS

This thesis could not have happened without the help of many people.

Firstly, my supervisors, Dr. Sarah Hudson and Prof Ake Rasmuson, thank you for the years of discussion, guidance and help. Sarah, your constant encouragement, enthusiasm and level-headedness, particularly in the face of adversity, motivated me to persevere with my work, and to tackle challenges head on and with composure. Ake, your challenging insights, useful suggestions and constructive criticism helped me to break through my self-defined limits and raise the standard of my work. In times of difficulty, I will always remember your advice to ‘sit in the sun, with a beer in your hand, look up at the sky and open your mind’.

I would like to thank my friends and colleagues in the SSPC, the MSSI, the CES department and the University of Limerick as a whole, who made it a joy to come to work every day, but an even greater joy to leave work and participate in our social outings and events. Because of this PhD, I have met many great people and made many great friends. I will dig out the hundreds of photos in years to come.

A sincere thanks to my parents for encouraging me to begin this PhD journey four years ago; for your unfaltering confidence in my abilities, and your never ending support along the way.

Finally, I must send my most profound thanks to Rónán, for being there by my side every step of the way. For the support you have given me during the last four years, I will be eternally grateful.
ABSTRACT

Crystals of a particular size and habit can be engineered by manipulating crystallisation process conditions. In the pharmaceutical industry, size control of the final drug product is vital for preparing a medicine with a specific therapeutic outcome. This work focuses on controlling the crystal size and shape of drugs with limited aqueous solubility, so that upon oral administration, they will dissolve at a desirable rate in the aqueous environment of the gastrointestinal tract, thus allowing efficient delivery of their therapeutic response.

Crystals of salicylic acid and fenofibrate were prepared at nano and/or micron size ranges by antisolvent-based precipitations from supersaturated solutions. The outcome in terms of particle size and shape depended on the mass available, the degree of supersaturation and the choice of stabilising additives employed during the preparation process. Micro-particles of salicylic acid were prepared at supersaturations < 8 with sizes ranging from 20-150 µm for the pure system and as low as 6 µm for an additive-stabilised system. Selection of a supersaturation of > 3000 facilitated generation of nanosized particles of fenofibrate. In their suspended state, the nanoparticles dissolved instantaneously upon addition to the dissolution medium. However, as a result of their small size the particles were physically unstable and grew into the micron size range within 2 min, leaving a limited time window for their isolation from suspension into the solid state. Solubilised additives (eg. PVA) were capable of stabilising the nanoparticles in suspension and extending the time period over which the particles remained at the nano size range. However, isolation and drying of these small, fragile and highly energetic systems by freeze-drying presented further difficulties, with drying-induced aggregation and fusion causing the nanoparticles to forego their nanoparticle-induced dissolution enhancement.

An alternative, all in one, stabilisation and isolation strategy was developed to overcome the primary challenges associated with nanoparticle formulations. Surface-functionalised insoluble, micro-scale carrier particles were designed to attract drug nanoparticles from suspension onto their surface. Carrier-attached nanoparticles were stable from growth and aggregation both in suspension and during drying. A simple filtration step was sufficient to separate the nanoparticle-carrier composites from suspension, to produce a solid-state material which maintained its nanoparticle-induced fast dissolution rate. The process was successfully demonstrated with two BCS class II drugs, fenofibrate and mefenamic acid, with potential applicability to a far wider range of drug substances.
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PATENT APPLICATION


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II. 46th annual conference of the “British Association for Crystal Growth” London, England June 2015

III. 66th Irish Universities Chemistry Research Colloquium (winning presentation) Galway, Ireland June 2014

### Poster presentations

I. National University of Ireland Galway and University of Limerick Annual Research Day  
   Galway, Ireland  
   April 2015

II. The Institute of Chemistry of Ireland Congress 2014 (winning poster)  
    Limerick, Ireland  
    Sept 2014

III. 45th annual conference of the “British Association for Crystal Growth”  
     Leeds, England  
     July 2014

IV. 44th annual conference of the “British Association for Crystal Growth”  
    Manchester, England  
    June 2013
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<td>-</td>
</tr>
<tr>
<td>$A$</td>
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<td>cm$^3$ s$^{-1}$</td>
</tr>
<tr>
<td>AFM</td>
<td>Atomic Force Microscopy</td>
<td>-</td>
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<tr>
<td>$A_s$</td>
<td>Surface area</td>
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</tr>
<tr>
<td>AS/S</td>
<td>Antisolvnet/solvent volume ratio</td>
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<tr>
<td>$c$</td>
<td>Concentration</td>
<td>mg/mL</td>
</tr>
<tr>
<td>$c_{eq}$</td>
<td>Saturation concentration (solubility)</td>
<td>mg/mL</td>
</tr>
<tr>
<td>$c_r$</td>
<td>Size-dependent saturation solubility</td>
<td>mg/mL</td>
</tr>
<tr>
<td>$c_{\infty}$</td>
<td>Saturation solubility of infinitely large particle</td>
<td>mg/mL</td>
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<td>Carboxy Methyl Cellulose</td>
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<tr>
<td>CNT</td>
<td>Classical Nucleation Theory</td>
<td>-</td>
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<td>$D$</td>
<td>Diffusion coefficient</td>
<td>m$^2$ s$^{-1}$</td>
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<td>DLS</td>
<td>Dynamic Light Scattering</td>
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<td>DLVO</td>
<td>Derjaguin, Landau, Venvey and Overbeek</td>
<td>-</td>
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<tr>
<td>DSC</td>
<td>Differential Scanning Calorimetry</td>
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<td>FDA</td>
<td>Food and Drug Administration</td>
<td>-</td>
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<tr>
<td>FF</td>
<td>Fenofibrate</td>
<td>-</td>
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<td>Growth rate</td>
<td>m s$^{-1}$</td>
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<tr>
<td>GRAS</td>
<td>Generally Recognised As Safe</td>
<td>-</td>
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<tr>
<td>$h$</td>
<td>Diffusion layer thickness</td>
<td>m</td>
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<td>HPMC</td>
<td>Hydroxy Propyl Methyl Cellulose</td>
<td>-</td>
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<td>J or N</td>
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<td>Pa</td>
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<tr>
<td>PVA</td>
<td>Poly Vinyl Alcohol</td>
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<tr>
<td>$r$</td>
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<tr>
<td>$r_c$</td>
<td>Critical radius</td>
<td>m</td>
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<td>RESS</td>
<td>Rapid expansion of a supercritical fluid</td>
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<tr>
<td>$S$</td>
<td>Supersaturation ratio</td>
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<td>SAS</td>
<td>Supercritical antisolvent recrystallisation</td>
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<td>SDS</td>
<td>Sodium Dodecyl Sulphate</td>
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<td>SEM</td>
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<td>Scanning electron microscopy</td>
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<td>$T$</td>
<td>Temperature</td>
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<td>$V_R$</td>
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<td>XRD</td>
<td>X-ray diffraction</td>
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<tr>
<td>$\gamma$</td>
<td>Interfacial energy</td>
<td>N m⁻¹</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Activity coefficient</td>
<td>kg mol⁻¹</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Chemical potential</td>
<td>J mol⁻¹</td>
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1.1 Challenges in pharmaceutical development

In the past 20 years, automation of drug discovery methods has led to a significant increase in the number of therapeutic compounds coming through from drug discovery into the development stages. Of every 10,000 compounds which are discovered, only one will make it through the rigorous development life cycle to gain FDA approval for commercialisation as a new medicine [1], Figure 1.1. Potential drug candidates may be discontinued from further development for any number of reasons (eg. issues relating to clinical efficacy, pharmacokinetics, human safety or manufacturability).

Figure 1.1  Drug discovery and development timeline (modified from data in [1])
A study on the fate of compounds which entered the development cycle between the years of 1964 and 1985 was published in 1988 and identified that a poor pharmacokinetic profile (including poor bioavailability) was the single biggest factor in development failure, contributing to almost 40% [2, 3], Figure 1.2. Pharmacokinetics describes the effect the body has on the drug as it moves through the body including its absorption, distribution, metabolism and elimination. A drug must be solubilised before it can be absorbed by the body and exert its therapeutic effect [4]. Therefore, the rate at which a drug is released from a solid dosage form into the solubilised state (through disintegration, de-aggregation and dissolution) also has an influence on its pharmacokinetic profile. Solubilisation is governed by thermodynamic (equilibrium solubility) and kinetic (dissolution rate and kinetic/apparent solubility) factors. Solubility is an inherent property of a solid which is governed by its crystal and molecular properties, while dissolution describes the rate at which molecules leave the particle (through the diffusion layer) and enter the bulk solution [5]. Both factors influence the absorption and thus the pharmacokinetics of orally administered drugs.

**Figure 1.2** Reasons for withdrawal of drugs from development based on a study of 319 drugs in the development phase between the years 1964 and 1985 (taken from data published by Prentis et al. 1988 [3])
Bioavailability, an important component of pharmacokinetics, is a term used to describe the rate and extent at which the active drug becomes available at its site of action (chemically unchanged) when introduced to the body [6]. Certain medications (eg. pain relievers or antihistamines) require immediate therapeutic response, and so their bioavailability is crucial for drug performance. Drugs that are administered intravenously have potentially high bioavailability, while drugs administered through other routes (eg. oral, pulmonary, topical administration) are likely to have reduced bioavailability [7, 8].

For an orally administered drug to be 100% bioavailable, it must be completely released from the dosage form (eg. tablet, capsule); be fully dissolved and stable in the gastrointestinal fluids; and be able to permeate through the lipophilic gastrointestinal barrier and the liver to enter systemic circulation and reach its site of action unchanged. Dissolution in the aqueous environment of the gastrointestinal tract is favoured by hydrophilic drugs, while permeation across biological membranes is favoured by lipophilic (hydrophobic) drugs. Due to dissolution rate or permeation rate limitations, achieving an appropriate bioavailability can often prove challenging and can become the reason for the developmental failure of many potential drug candidates.

The biopharmaceutical classification system (BCS), introduced in 1995, broadly categorises drug substances into one of four classes according to their water-solubility and membrane-permeability, so that the rate-limiting step in intestinal absorption can be predicted [9], Figure 1.3. In short, the BCS classification allows anticipation of potential issues in pharmaceutical development so that probable failures can be detected. BCS Class I drugs are the most, and BCS Class IV drugs the least, preferred. The permeability issues associated with Class III drugs are difficult to overcome since permeability is an inherent biological property. However Class II drugs, with dissolution-limited bioavailability, can be manipulated using formulation and processing technologies. Of an evaluation of 135 orally administered drugs on a list of national essential medicines, 20.7% of the drugs fell into the BCS class II category [10].
Figure 1.3  The Biopharmaceutical Classification System as defined by Amidon et al. [11]

The current drug discovery methodology has a tendency to produce candidate drugs of increasing molecular size and hydrophobicity, which has led to a sharp decrease in solubility and an increase in the number of drugs falling into the undesirable BSC Class II, and IV categories [5]. Until the 1980s an aqueous solubility of < 20 mg/L was almost unheard of [12]. Today, about one third of new compounds lie in this range, and solubility’s of < 1 mg/L are quite common [12]. The hydrophobic/lipophilic nature of a compound can be assessed by determining its partition coefficient (Log P) between immiscible phases in a biphasic system (eg. octanol-water). The Log P scale allows molecules to be ranked in terms of their hydrophobicity, whereby a high log P indicates a highly hydrophobic compound and a Log P of 0 implies equal distribution to both phases [13]. The Log P scale ranges from approx. -3 (highly hydrophilic – water loving) to 8 (highly hydrophobic – water repelling) [14]. Table 1.1 shows a list of BCS Class II drugs with their corresponding Log P values.
Table 1.1  A list containing some BCS Class II drugs and their approximate Log P values as taken from the DrugBank [15].

<table>
<thead>
<tr>
<th>Active ingredient</th>
<th>Log P</th>
<th>Active ingredient</th>
<th>Log P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amiodarone</td>
<td>5.95</td>
<td>Indinavir</td>
<td>2.81</td>
</tr>
<tr>
<td>Amprenavir</td>
<td>2.43</td>
<td>Indomethacin</td>
<td>3.53</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>5.39</td>
<td>Itraconazole</td>
<td>5.48</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>2.67</td>
<td>Ketoconazole</td>
<td>4.19</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>4.19</td>
<td>Lovastatin</td>
<td>3.90</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>3.90</td>
<td>Magestrol acetate</td>
<td>3.48</td>
</tr>
<tr>
<td>Clofazimine</td>
<td>7.30</td>
<td>Mefenamic acid</td>
<td>5.40</td>
</tr>
<tr>
<td>Curcumin</td>
<td>3.00</td>
<td>Naproxen</td>
<td>2.99</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>6.92</td>
<td>Nifedipine</td>
<td>2.97</td>
</tr>
<tr>
<td>Danazol</td>
<td>3.46</td>
<td>Ritonavir</td>
<td>5.22</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>4.26</td>
<td>Saquinavir</td>
<td>2.58</td>
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<td>Ibuprofen</td>
<td>3.84</td>
<td>Warfarin</td>
<td>2.74</td>
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</table>

1.2 Solving the problems of limited bioavailability

Compounds with limited bioavailability may be physically/chemically modified or reformulated to improve their therapeutic response. In contrast to developing completely new drugs, introducing upgraded or advanced formulations greatly reduces the risk, time and capital invested in drug development. Several approaches can be employed to increase the bioavailability of drugs intended for immediate-release oral formulation, with particular focus on BCS Class II drugs. Common formulation strategies for poorly soluble drugs include pH adjustment, salt formation, co-solvency, micellular solubilisation, amorphous formulations, solid dispersions, cocrystals, inclusion complexes, lipid-based formulations and particle size reduction (all of which have been covered in detail in several recent reviews [5, 16, 17], but some of which will be discussed briefly here). The appropriate choice of formulation depends on numerous factors including required dose,
shelf-life, manufacturability, and properties of the API (e.g. molecular weight, lipophilicity, ionisation ability and solubility). The formulation choices available differ in complexity and efficiency but are largely focused around improvements in the solubility and/or the dissolution rate [5, 18].

While the solubility ($c_{eq}$) is dependent on the molecular structure (e.g. activity coefficient) and crystal lattice properties (e.g. melting point), the dissolution rate is dependent on the solubility, the surface area and the diffusion layer surrounding the dissolving particle. The Noyes-Whitney equation (Eq. 1.1), shows the mathematical relationship between the rate of dissolution of a drug particle ($dc/dt$), its surface area ($A_s$) and its solubility ($c_{eq}$) in the dissolution medium, all of which are modifiable physical properties [19]. By increasing the surface area (e.g. by size reduction or shape modification) or by increasing the solubility (e.g. by changing the solid-state form or by introducing solubilising excipients), the dissolution rate can be enhanced.

$$\frac{dc}{dt} = A_s \frac{D}{h} (c_{eq} - c)$$  

Eq. 1.1

where $D$ is diffusion coefficient, $h$ is diffusion layer thickness, $c_{eq}$ is the saturation solubility at the particle surface and $c$ is the concentration in the bulk solution [19].

Solid materials can exist in different forms (e.g. polymorphic crystalline forms, co-crystals or amorphous forms), each with different stabilities and melting points which influence their aqueous solubility. Polymorphism describes a condition where a chemical compound exists in more than one crystalline form; one of which will always be the most thermodynamically stable (and least soluble) under a given set of conditions [20]. Stable crystalline forms generally possess a high melting point and a reduced ability to get into the aqueous phase. Therefore, reducing the stability (and melting point) can lead to more favourable solubilisation. Co-crystals refer to materials where molecules of two or more substances crystallise in the same crystal lattice, which often induces decreased melting points (e.g. eutectic mixtures) [21, 22]. Similarly, non-crystalline amorphous materials which lack long range molecular order have lower melting points [23-25]. Therefore, metastable (less stable) crystalline polymorphs, co-crystals or amorphous forms (e.g. solid dispersions) are generally associated with reduced stability, lower melting points and, as a
result, increased solubility. Despite this potential strategy for formulating poorly soluble drugs, their reduced stability increases their tendency to revert to a stable crystalline form through crystallisation or polymorphic transformation [20, 23-25]. Therefore, the solubility enhancement achieved for metastable, co-crystal or amorphous forms may be short lived and is often termed ‘kinetic’ or ‘apparent’ solubility. However, strategies are continuously developing for stabilising these solid forms, eg. by selection of suitable stabilising excipients [26], by generating an amorphous solid dispersion in which the drug is molecularly dispersed within a polymer matrix to form a single phase amorphous mixture [27-30], or by precipitating a drug in a confined space (eg. in mesoporous material) so that its conversion to crystalline form is spatially prevented [31, 32].

Salt formation provides a further formulation strategy for solubility enhancement. Salt formation requires an ionisable compound so that a stable ionic bond can form. Intramolecular ionic interactions increase the crystal lattice energy and thus the melting point of salts which can hinder their solubilisation process. On the other hand, ion-dipole interactions between the dissociated ion and water can accelerate hydration and solubilisation. Therefore, the solubility of a salt depends both on its crystal structure and on the solvent properties [5]. Ionisable (ie. acidic or basic) drugs exhibit a pH dependent solubility whereby acidic drugs are more soluble at pH>pKa (ionisation constant) and basic drugs are more soluble at pH<pKa [17]. Upon salt formation of weakly acidic or basic drugs (which account for nearly 70% of all drug substances [17]), counter ions can be selected to produce favourable pH conditions upon dissolution in water (eg. by selecting a counterion with a pKa higher than the pKa of an acidic drug or lower than the pKa of a basic drug [33]. For maximum benefit in terms of drug delivery, a drug should be ionised at low pH to promote solubilisation in the acidic environment of the stomach, before converting to its unionised form toward neutral pH to promote absorption across the lipophilic intestinal lining (at pH 6 - 7.4). Counter ions must be selected that are pharmaceutically approved, and that are not negatively influenced by other ions in gastric fluids (ie. common ion effect). Due care must be given to selecting a salt form which avoids any unwanted effects which could lead to clinical failure. The salt form is considered a new chemical entity which must obtain new approval from regulatory authorities [5]. A review of the effects of salt formation on drug solubility is given by Serajuddin [34].
An additional route to solubility improvement is through inclusion complexes (eg. cyclodextrin inclusion complexes). Cyclodextrins are excipients which have the ability to interact/complex with poorly water-soluble drugs through their lipophilic central cavity, while their hydrophilic outer surface give them the ability to increase the apparent solubility of the active pharmaceutical ingredient (API) [35-38]. Janssen Pharmaceuticals were the first company to get US FDA approval for a cyclodextrin-based drug formulation in 1999 (itraconazole-cyclodextrin), but several other drugs have since been reformulated in a similar way [17].

An additional strategy for enhancing the bioavailability of hydrophobic drugs is through particle size reduction, which is the strategy of focus in this thesis. Particle size (and surface area) can influence both the dissolution rate (Noyes-Whitney Eq. 1.1) and the solubility (Ostwald-Freundlich Eq. 1.2 [39, 40]) of drug substances. Solubility of a solute in a solvent is influenced by the intermolecular interactions which occur between the solute and the solvent. Decreasing the solute particle size increases the surface area to volume ratio, and therefore increases the solute-solvent interaction capability. However, the effect of size on solubility only becomes important at very small particle sizes (typically ≤100 nm) [41].

\[
\ln \frac{c_r}{c_\infty} = \frac{2\gamma v}{r k T}
\]

Eq. 1.2

where \(c_r\) is the size dependent saturation solubility, \(c_\infty\) is the solubility of a particle with infinitely large size, \(\gamma\) is the interfacial energy, \(v\) is the molar volume of the solute, \(r\) is the particle radius, \(k\) is the Boltzmann constant and \(T\) is the temperature.

A much greater impact of particle size reduction is on dissolution rate. According to Eq. 1.1, the dissolution rate of the API is proportional to both its surface area and its saturation solubility. Figure 1.4 shows how the surface-area to volume ratio is influenced both by the size and shape of a particle, particularly so at the small micron or nano scales. Reducing the particle size is one of the simplest approaches to increasing the dissolution rate of poorly-soluble drugs. In the field of pharmaceutics and medicine, the term nanoparticle is used for particles in the submicron size range (typically 200-700 nm), while the term microparticle is used for particles ranging from 1 to 1000 µm [42]. A
pioneering example of the influence of particle size on bioavailability was for the BCS Class II drug griseofulvin, where size reduction from 10 µm to 2.7 µm approximately doubled its bioavailability [8]. Other examples of drugs where size reduction to micron scale resulted in enhanced bioavailability include progesterone, spironolactone and diosmin [43]. However, for drugs with very poor solubility (high log P), nanonisation may be needed to acquire an effective increment in bioavailability [42, 44-47]. Nano-sized particles provide sufficient increase in the surface-area to volume ratio to induce notably different behaviour compared to traditional microparticles.

**Figure 1.4** The influence of (a) size and (b) shape on surface area to volume ratio
1.3 Strategies for preparing drug micro/nanosuspensions

Nano or micron-size particles can be prepared by top-down (i.e. disintegration/diminution) or bottom-up (i.e. crystallisation/precipitation) techniques, Figure 1.5. Top-down processes involve mechanically reducing the size of large particles into smaller fragments using various wet milling techniques such as media milling (NanoCrystal® technology [42]), and high pressure homogenisation (Dissocubes®, Nanopure® technologies [42]). Bottom-up processes begin at a molecular level and involve growing the particle to the desired size in a controlled manner via molecular association (hydrosols [42]). The two approaches present different limitations in terms of usability, and the conditions encountered for each approach can greatly affect the particle characteristics.

![Figure 1.5 Bottom-up versus top-down approaches to preparing nanocrystals [48]](image)

1.3.1 Top-down technology. All media milling techniques require high energy input and are highly inefficient in terms of energy usage. Energy dissipated in the form of heat can lead to thermal degradation of temperature-sensitive materials, and unavoidable abrasion of the milling media can introduce product contamination which may not be well tolerated. Milling, since it is a disintegration technique, allows limited control over the particle morphology and the surface properties. Increased surface energetics can induce disordering or localised amorphisation, resulting in crystal defects which can affect both the physical and chemical stability of processed materials. Finally, milling is a time consuming process. Size reduction to the nanoscale may not always be achievable within
a reasonable time, particularly for hard materials [45, 49-52]. Despite these limitations, milling has broad commercial and industrial application and has been the industrial method of choice for preparing nanoparticles since the first nano drug formulation, Rapamune (Sirolimus) was launched in 2000 [42]. It is generally a straight-forward process which can be applied to the majority of drug particles, regardless of their solubility in aqueous or non-aqueous solvents [53]. Milling has become a well-established, and a technologically-developed unit operation in pharmaceutical processing. It has been used to prepare a range of micro/nanoformulations at industrial scale [53]. Examples of commercially available oral drug formulations which utilise nanoparticles to reach their delivery goals include Rapamune (sirolimus, milled), Emend (aprepitant, milled), TriCor (fenofibrate, milled), Megace ES (magestrol acetate, milled) and Triglide (fenofibrate, homogenised).

1.3.2 Bottom-up technology. Crystallisation/precipitation allows an alternative, controlled approach to produce micro or nano sized particles, starting at the molecular level and gradually building particles to the desired size by molecular addition. Such an approach introduces flexibility in controlling the particle size and morphology. During solution crystallisation, the drug is dissolved in a solvent and later crystallised/precipitated by changing the environment (eg. by changing the solvent composition or temperature). In order for this approach to be viable, the drug must be soluble in at least one solvent; a criterion which may only be satisfied for some compounds if harsh or undesirable solvents are employed. If the solvent is difficult to remove, any residue may compromise the physical and chemical stability of the formulation [49]. However, precipitation through reverse antisolvent addition, first patented in 1995 [54], provides a cheap and technologically easy route to prepare micro- or nano-size drug particles in suspension. It has an operational merit that temperature operation is unnecessary, and that quantity of production is secured. Liquid antisolvent precipitation is a rapid, straight-forward, one-step technique which can be performed at ambient conditions, using low cost equipment, with low energy consumption and good scale-up prospects [46, 55, 56]. Not all precipitation processes are conducted in liquid phase. Precipitation using supercritical fluids has grown in popularity in recent years, Figure 1.6. Most supercritical fluid technologies are based on supercritical antisolvent recrystallisation (SAS) and rapid expansion of supercritical solutions (RESS). In the SAS method, similar to the liquid antisolvent precipitation method, a supercritical fluid such as supercritical CO2 acts as an
antisolvent to precipitate the drug from a solution. In the RESS process, the supercritical fluid is the solvent, after which the pressure is reduced to precipitate the drug [57, 58].

**Figure 1.6** Phase diagram of carbon dioxide as a function of temperature and pressure [59].

The primary challenge of both liquid and supercritical precipitation approaches to size control is in preserving the desired character of the growing crystals, particularly at ultrafine size scales. Despite vast academic interest, precipitation techniques are not currently used to fabricate drug nanoparticles at industrial scale [60].

### 1.4 Thermodynamics and kinetics of solid formation

Solid formation occurs by crystallisation or precipitation; terminology often used interchangeably, distinguished primarily by the driving force [61]. Crystallisation is a critical unit operation in the pharmaceutical industry and is a key component of almost all pharmaceutical manufacturing processes. It is used as a separation process for intermediates and as the final step in the manufacture of active pharmaceutical ingredients (APIs) [62]. Control over crystallisation is one of the most important techniques in modern materials science due to its potential to produce well-defined particles with unique structures in the nanometre and micrometre size ranges. Materials in the solid-state can be crystalline, amorphous or a combination of both. Crystalline materials are those in which the molecules are arranged in defined and repetitive three dimensional patterns, to produce a crystal lattice with long-range order. Amorphous materials on the other hand (eg. glass), lack long range order.
Chapter 1                                                                                                            Introduction

Crystallisation describes the process by which a solute is transferred from the liquid to the solid phase, and may occur from a melt or from a solution in which the solute is dissolved in a solvent. Crystallisation from solution, which dominates crystallisation processes in the pharmaceutical industry, will be the focus of this section.

Crystallisation is best described as a two-step process consisting of nucleation and growth [63]. Before a crystal can develop, a crystallisation centre (ie. a nucleus) must exist in solution which is made up of a number of minute solid bodies (eg. molecules). Nucleation is the initial creation of the new phase and is accompanied by an increase in the free energy of the system, which makes the process energetically unfavourable from a thermodynamic view point. Nucleation will only occur if a driving force exists (ie. supersaturation) which exceeds the thermodynamic force working to prevent formation of a crystal nucleus. Crystal growth, the second step in the crystallisation process, takes over when it becomes more energetically favourable for the new phase to grow than to shrink. Although described sequentially, nucleation and growth can occur simultaneously [64].

1.4.1 Nucleation. Nucleation refers to the process by which a solute crosses a phase boundary. Initial nucleation theories which were outlined by Gibbs (19th century) and further by Volmer and Weber (20th century) described condensation of a vapour into a liquid droplet. This work set the basis for development of liquid-solid nucleation theories (such as that outlined by Vekilov [65]). Although a number of nucleation theories exist, the process is most widely described by the classical nucleation theory (CNT) [64]. According to the CNT, nucleation occurs when molecules, through their random motion, colloid and aggregate to form a molecular cluster of a critical size. This standard theory for nucleation assumes a spherical nucleus with a well-defined surface, whose free energy is estimated from its interfacial energy ($\gamma$).

Nucleation can be described as primary if it is not influenced by other crystals, or secondary if it is influenced by the presence of already existing crystals. Primary nucleation can be further classified as homogeneous or heterogeneous, depending on the presence of foreign substances in the system [64]. The first stage in crystal nucleation from solution is in achieving non-equilibrium conditions of supersaturation, which drives the nucleation process. A solution is supersaturated when its solute concentration exceeds the saturation concentration (ie. solubility, $c_{eq}$). Supersaturation is a direct measure of the difference in chemical potential ($\mu$) between molecules in solution $\mu_s$ and in the bulk of a
new crystal phase $\mu_c$, Eq. 1.3. The chemical potential difference ($\Delta \mu$, ie. the fundamental driving force for homogeneous nucleation) describes the free energy response of molecules transferring from the liquid to the solid phase [66].

$$\Delta \mu = \mu_s - \mu_c \quad \text{Eq. 1.3}$$

Conditions of supersaturation ($S$) are achievable due to the fact that phase transitions occur at finite rates. Special approaches are required to generate a supersaturated solution (eg. by reducing the solubility or by increasing the solute concentration). The solubility curve forms a boundary between supersaturation and undersaturation as shown in Figure 1.7. Any process which places the solution concentration above the saturation concentration generates supersaturation. The most common methods of generating supersaturation are by reducing the temperature (since solubility is generally lower at lower temperature), by increasing the concentration (eg. by evaporating some solvent) or by changing the solvent composition (eg. by adding a miscible solvent in which the solute is less soluble, ie. an antisolvent) [67], all of which are depicted in Figure 1.7.

Figure 1.7  Typical solubility curve of a solute as a function of temperature or solvent/antisolvent ratio.
The chemical potential difference ($\Delta \mu$) is related to supersaturation ($S$) as outlined in Eq. 1.4, which can be further expressed in terms of the solution activities (effective concentration under non-ideal conditions). Since solution activities are difficult to measure directly, real supersaturation is often approximated by solution concentration ratios, Eq. 1.5 [68]. However, the concentration-based supersaturation estimation is only accurate if the ratio of the activity coefficients is close to unity, which is often assumed for simplicity. A supersaturation value of $>1$ indicates a thermodynamically unstable solution, from which precipitation will eventually occur [69].

\[
\Delta \mu = kT \ln S = RT \ln \left( \frac{a}{a_{eq}} \right) = RT \ln \left( \frac{\gamma c}{\gamma_{eq} c_{eq}} \right)
\]  

Eq. 1.4

Where $k$ is the Boltzmann Constant, $T$ is the temperature, $a$ and $a_{eq}$ are the activities of the solute, $\gamma$ and $\gamma_{eq}$ are the activity coefficients, and $c$ and $c_{eq}$ are the solute concentrations at supersaturation and equilibrium respectively.

\[
S = \frac{c}{c_{eq}} \quad (\text{when } \frac{\gamma}{\gamma_{eq}} = 1)
\]  

Eq. 1.5

As mentioned previously, CNT outlines that homogeneous nucleation will only occur if a crystallisation centre (molecular cluster) of a critical size is created. CNT assumes that clusters grow by step-by-step molecular addition and that the cluster has the same structure as the crystals which form. This assumption contrasts with the two-step aggregation/reconstruction nucleation theory which was proposed more recently, but is still not well understood [65]. According to CNT, clusters are created by collision and coagulation of molecules in solution which are under constant Brownian motion. Clusters continuously form and redissolve before nucleation eventually occurs. The total free energy changes associated with a nucleation process may be described in terms of the Gibb’s free energy change, $\Delta G$. Gibbs defined the free energy change necessary for the formation of a stable nucleus ($\Delta G$) to be the sum of the free energy change associated with phase transformation ($\Delta G_{V, \text{volume free energy}}$) and the consequential free energy change associated with the formation of a new surface ($\Delta G_{S, \text{surface free energy}}$) [64, 70].

\[
\Delta G = \Delta G_{V} + \Delta G_{S}
\]  

Eq. 1.6
For a cluster to become a stable crystal nucleus, the favourable reduction in free energy which is associated with the new solid phase ($-\Delta G_v$) must dominate the unfavourable increase in free energy induced by the introduction of a new solid-liquid interface ($+\Delta G_s$), so that the total free energy change is negative ($-\Delta G$). The system approaches this condition when the cluster surpasses a certain critical size ($r_c$), Figure 1.8. Above $r_c$, a stable nucleus exists. Every additional molecule which joins the nucleus will reduce its free energy and thus increase its stability further. The stable nucleus may consist of about 10 to several thousand individual molecules, depending on the species nucleating and the degree of supersaturation. Assuming a spherical cluster, the volume ($\Delta G_v$) and the surface ($\Delta G_s$) terms depend on the cluster size ($r$) by differing degrees, whereby $\Delta G_v \propto r^3$ and $\Delta G_s \propto r^2$ [64, 70], Eqs 1.7 and 1.8.

$$\Delta G_S = 4\pi r^2 \gamma \quad \text{Eq. 1.7}$$

$$\Delta G_V = \frac{4}{3} \pi r^3 \Delta G_v \quad \text{Eq. 1.8}$$

Where $\Delta G_v$ is the free energy change of the transformation per unit volume and $\gamma$ is the interfacial energy.

The volume free energy term varies more rapidly with changes in $r$ than does the surface free energy term. At small $r$, the $r^2$ surface term dominates and the free energy change is positive (unfavourable) while at large $r$, the $r^3$ volume term dominates and the free energy change is negative (favourable). Due to the opposite signs and the different dependencies of $\Delta G_v$ and $\Delta G_s$ on $r$, the total free energy of formation of a new solid phase ($\Delta G$) passes through a maximum value corresponding to the critical nucleus size ($r_c$) which creates a barrier for nucleation, Figure 1.8 [64].
The critical free energy, $\Delta G_{\text{crit}}$, can be interpreted as an ‘activation energy’ that must be overcome for nucleation to occur, and is a function of the supersaturation ($S$, Eq. 1.11). At $\Delta G_{\text{crit}}$, $\Delta G$ is momentarily unaffected by $r$, so therefore:

$$\frac{d\Delta G}{dr} = 0$$

Eq. 1.9

By combining Eq. 1.9 with Eqs. 1.6, 1.7 and 1.8, and further with the Ostwald-Freundlich equation which correlates the solubility of a particle to its size, an expression for both the $r_c$ and the $\Delta G_{\text{crit}}$ can be derived [64], Eq. 1.10 and 1.11. As shown in Eq. 1.11, $\Delta G_{\text{crit}}$ decreases with increasing $S$, making nucleation more favourable at higher supersaturation.

$$r_c = -\frac{2\gamma}{\Delta G_v}$$

Eq. 1.10

$$\Delta G = \frac{16\pi\gamma^3v^2}{3k^2T^2(lnS)^2}$$

Eq. 1.11

Where $\gamma$ is the interfacial energy, $v$ is the molecular volume (molar volume / Avogadro number), $k$ is the Boltzmann constant, and $T$ is the temperature.
In kinetic terms, the rate of nucleation ($J$), which is the number of nuclei formed per unit volume per unit time, can be described by an Arrhenius type equation, with $kT\ln S$ as the driving force and $\Delta G_{\text{crit}}$ as the energy barrier, Eq. 1.12. Therefore, the nucleation rate is also a function of supersaturation.

$$J = A \exp \left(-\frac{16\pi \gamma v^2}{3kT^3(lnS)^2}\right)$$  \hspace{1cm} \text{Eq. 1.12}

Where $A$, the pre-exponential factor, accounts for the net rate by which molecules move from solution into the cluster and where the exponential factor incorporates the free energy barrier for formation of a stable nucleus.

Eq. 1.12 indicates how strongly the nucleation rate depends on temperature, supersaturation and interfacial energy, each with 2nd or 3rd powers in the argument of an exponential. The reciprocal of the nucleation rate, ie. the induction time, is a measure of the time until nucleation at any given supersaturation. At low supersaturation, the energy barrier for the formation of a new surface is high and so the induction time is long. Induction time approaches infinity as supersaturation approaches 1 (unity). The energy barrier for nucleation and thus, the induction time can be reduced in conditions of low supersaturation by addition of a surface to the supersaturated solution (ie. secondary nucleation - seeding).

1.4.2 Growth. Nucleation is followed by crystal growth. Growth of a crystal occurs when the number of molecules joining the surface of the nuclei exceeds the amount leaving (ie. by simultaneous dissolution). Crystal growth of nucleated crystals is thermodynamically favourable in conditions of supersaturation since after reaching the critical nucleus size, the attachment of every additional molecule decreases the free energy of the system [64], Figure 1.8. Surface molecules are energetically less stable than interior molecules due to the surface energy which they introduce by uncoordinated surface molecules, Figure 1.9. Therefore larger crystals, due to their smaller equivalent surface area and corresponding smaller surface energy, are energetically favoured over smaller crystals.
Several mechanisms have been proposed to describe the growth of a crystal (eg. non-uniform lateral step growth, or uniform normal growth of entire surface [71-73]). The crystal surface is usually envisaged as periodic arrangements of flat regions (terraces), raised partial layers (steps) and incomplete steps (kinks), Figure 1.10. Molecular attachment is more favourable at kinks than terraces due to additional bonding capabilities with neighbouring molecules. The rate at which molecules are added to a crystal, for a given solute concentration, is a function of kink density [74]. The equilibrium shape of a crystal is determined by the specific surface energies of each crystal face. The crystal faces with the lowest surface energies define the equilibrium shape.

Increasing the supersaturation will increase not only the growth rate but also the number of imperfections that are introduced to a growing crystal [47]. No crystalline material is perfect; dislocations and imperfections come about due to incorrect attachment of a molecule to a surface. Simultaneous growth and dissolution can help to correct any crystalline errors and imperfections. Although growth dominates dissolution in conditions of supersaturation, its domination increases with increasing supersaturation, and so the
error correction mechanism becomes inefficient [76]. Therefore, the density of crystal
defects increases with supersaturation.

Following crystal growth, subsequent particle enlargement processes can occur through
Ostwald ripening and agglomeration, both of which are thermodynamically-driven
spontaneous processes which occur because larger particles (with reduced surface area to
volume ratio) are more energetically favoured to smaller particles. Wilhelm Ostwald put
forward a crystal growth theory which has since been denoted Ostwald ripening [77, 78].
This theory suggests that in a system containing crystals of various sizes, the larger
crystals will grow at the expense of smaller crystals. Thus, the energetically unfavourable
smaller crystals will dissolve and their molecules will detach and re-deposit onto larger
crystals by diffusion through the dispersing phase [66, 67, 79]. For a similar objective,
small energetically unstable particles can agglomerate (ie. stick together) to generate a
large secondary particle, with reduced surface energy. Polycrystal and mesocrystal are
terms used to describe agglomerated primary crystals. A polycrystalline material is one in
which the primary crystals within the agglomerate are randomly orientated with no
preferred direction. A mesocrystalline material is one in which the primary crystals are
partially orientated in a preferred direction with respect to other crystals within the
agglomerate [80]. The agglomeration process is governed both by the rate of collision
of particles and the sum of attractive (Van der Waals) and repulsive (electrostatic) forces
between colliding particles. The smaller the particle size, the bigger the agglomeration
effect becomes.

Both nucleation and growth rates increase with increasing supersaturation (with differing
dependencies) and continue simultaneously until supersaturation is consumed. Their
relative kinetics controls the final particle size distribution. The rates of both nucleation
(expressed here as N) and growth (G), which were outlined (in simplified from) by Nyvlt
in 1968 [81] (Eqs. 1.13 and 1.14), are expressed as power functions which highlight their
dependence on S, given that \( n \approx 5-15 \) and \( g \approx 1-2 \) [82, 83]. The stronger dependence of
the nucleation rate on S results in the formation of a larger number of nuclei at increasing
S. As a result of a high nucleation rate, the amount of solute available per nucleus for
further growth is reduced, resulting in smaller crystals at higher S [84, 85]. Therefore, by
increasing the nucleation rate to a level at which the extent of growth becomes negligible,
fine particles in the micro or nano size ranges can be produced [47, 86]. However, agglomeration of these small particles must be controlled.

\[ N = k_n S^n \]  \hspace{1cm} \text{Eq. 1.13} \\
\[ G = k_g S^g \]  \hspace{1cm} \text{Eq. 1.14}

Where \( S \) is the supersaturation ratio, \( N \) is the nucleation rate, \( k_n \) is the nucleation rate constant, \( n \) is the kinetic “order” of nucleation, \( G \) is the growth rate, \( k_g \) is the growth rate constant and \( g \) is the kinetic “order” of growth.

Therefore, for particle size control, high \( S \) is chosen if small particles are required and low \( S \) is chosen if large particles are required [87]. Rapid and effective mixing is necessary to avoid accidental zones of local supersaturation. A uniform supersaturation is necessary to ensure a narrow particle size distribution [88, 89]. However, additives are sometimes required for additional manipulation of nucleation and growth rates and their effect on the crystallisation outcome (eg. size) [90].

1.5 Stabilisation of particles in suspension

Section 1.4 highlighted the influence of supersaturation on the size of precipitated particles. By controlling the relative rates of nucleation and growth, and by employing rapid mixing, a narrow particle size distribution can be achieved in the small micron or even nano size ranges. However, the smaller the particle size, the higher the surface energy, and the lower the stability. Particle stability is determined by the interactions which can occur between the particles during collision. Fine particles, particularly nanoparticles, have a natural tendency to grow or agglomerate to reduce the free energy of the system, often making size-control difficult [91-93]. To preserve a small particle size, the growth of nucleating crystals must be immediately arrested [56].

Stability issues associated with nanosuspensions have been widely investigated and can be categorized as physical (eg. size, crystalline form) and chemical (eg. hydrolysis or degradation) stability [91]. Physical stability issues can include crystal growth,
agglomeration, sedimentation and change of the crystalline state, but this review focuses only on issues relating to stability of the particle size. Addition of stabilising additives is a common strategy to preserve the small size of precipitated particles. In order to obtain a small particle size with a narrow size distribution, additives should ideally be capable of accelerating nucleation and subsequently decelerating growth and agglomeration. Additives which are surface active have the ability to reduce the interfacial energy for nucleation (Eq. 1.11 and 1.12) by absorbing at the nucleus-solution interface, thus enhancing the nucleation rate and reducing the high surface free energy of nanoparticles which form [85, 94]. Following nucleation, the adsorbed additives should impart steric or electrostatic stability to minimise inter-particle contact as outlined in Figure 1.11. However, since the total surface area of nanoparticles is typically orders of magnitude larger compared to a coarse suspension (Figure 1.4a), large quantities of additives may be necessary to ensure adequate stabilisation [92].

The main functions of a stabiliser are (i) to wet the drug particles thoroughly, and (ii) to prevent Ostwald ripening and agglomeration of nanoparticles in order to yield a physically stable formulation [93]. Stabilising additives attach to the particle surface by adsorption at a specific adsorption site. Additive-particle attachment can occur through hydrophobic/hydrophilic, hydrogen bonding or electrostatic/ionic interactions [95]. Upon attachment, the additives block the adsorption site from approaching molecules and thus disrupt the natural growth process. Once bound, the additive properties (eg. size and charge) can impart an additional mechanism of stabilisation to minimise growth and agglomeration.
Polymeric (eg. non-ionic) and surfactant (eg. ionic) based stabilisers are generally employed to stabilise nanosuspensions through steric and electrostatic protective barriers [47, 96]. The list of stabilisers which are typically selected for nanosuspension production is rather limited compared with the number of potential stabilisers that could be applied for this purpose [97], potentially due to safety and regulation considerations. The most common stabilisers utilised in nanoparticle formulations include polymers; povidones, cellulose derivatives and poloxamers, and surfactants; sodium dodecyl sulphate and Tween-80, Table 1.2. Of this list, the stabilisers which appear most often in academic and industrial applications are in bold. All additives in Table 1.2 have GRAS designation by the FDA (ie. generally recognised as safe).
Table 1.2  List of stabilisers which commonly appear in nanoparticle formulations

<table>
<thead>
<tr>
<th>Common name</th>
<th>Name</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVA</td>
<td>Polyvinyl alcohol</td>
<td>Non-ionic polymer</td>
</tr>
<tr>
<td>PVP K12</td>
<td>Polyvinyl pyrrolidone (povidone)</td>
<td>Non-ionic polymer</td>
</tr>
<tr>
<td>PVP K15</td>
<td></td>
<td></td>
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<tr>
<td>PVP K17</td>
<td></td>
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<tr>
<td>PVP K30</td>
<td></td>
<td></td>
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<tr>
<td>PVP K25</td>
<td></td>
<td></td>
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<tr>
<td>PVP VA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEG 400</td>
<td>Polyethylene glycol</td>
<td>Non-ionic polymer</td>
</tr>
<tr>
<td>PEG 300</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluplus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPMC</td>
<td>Cellulose ethers (eg. hydroxyl propyl methyl cellulose, hydroxyl propyl cellulose, methyl cellulose, ethyl cellulose, hydroxy ethyl cellulose, carboxymethyl cellulose)</td>
<td>Non-ionic polymers</td>
</tr>
<tr>
<td>HPC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC</td>
<td></td>
<td></td>
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<tr>
<td>HEC</td>
<td></td>
<td></td>
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<tr>
<td>Na-CMC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pluronic F127</td>
<td>Poloxamer/pluronic PEG-PPG-PEG</td>
<td>Non-ionic block copolymer</td>
</tr>
<tr>
<td>Pluronic F108</td>
<td>(also poloxamer 407)</td>
<td></td>
</tr>
<tr>
<td>Pluronic F68</td>
<td>(also poloxamer 188 and Kolliphor 188)</td>
<td></td>
</tr>
<tr>
<td>Soluplus</td>
<td>PEG-PVCap-PVAc</td>
<td>Non-ionic block copolymer</td>
</tr>
<tr>
<td>Polyphosphoesters</td>
<td>Polyphosphates</td>
<td>Anionic polymers</td>
</tr>
<tr>
<td>Polyphosphonates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
<td>Anionic polymers</td>
</tr>
<tr>
<td>HSA</td>
<td>Human serum albumin</td>
<td>Anionic polymers</td>
</tr>
<tr>
<td>Chitosan derivatives</td>
<td>Polysaccharide</td>
<td>Cationic polymer</td>
</tr>
<tr>
<td>Dextran</td>
<td></td>
<td>Nonionic polymer</td>
</tr>
<tr>
<td>Sodium alginate</td>
<td></td>
<td>Anionic polymer</td>
</tr>
<tr>
<td>Tween-80</td>
<td>Tween/Span/polysorbate</td>
<td>Non-ionic surfactant</td>
</tr>
<tr>
<td>Tween-20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Span-85</td>
<td></td>
<td></td>
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<tr>
<td>Span-80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPGS 1000</td>
<td>D-α-tocopherol polyethylene glycol succinate</td>
<td>Non-ionic surfactant</td>
</tr>
<tr>
<td>VES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phospholipon 90</td>
<td>Phospholipids</td>
<td>Zwitterionic surfactant</td>
</tr>
<tr>
<td>Lipoid S75</td>
<td></td>
<td></td>
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<tr>
<td>Lecithin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDS / SLS</td>
<td>Sodium dodecyl Sulphate</td>
<td>Anionic surfactant</td>
</tr>
<tr>
<td>DOSS</td>
<td>Docusate sodium</td>
<td>Anionic surfactant</td>
</tr>
<tr>
<td>NaDC</td>
<td>Sodium deoxycholate</td>
<td>Anionic surfactant</td>
</tr>
</tbody>
</table>
Polymers (e.g. non-ionic and amphiphilic) generally impart steric stabilisation, by adsorbing to the particle surface through an anchor segment, and extending their long polymeric strands into the bulk medium. The polymeric layer produces a separation barrier which sterically hinders interaction of two adjacent particles, Figure 1.11. The coverage and thickness of the polymeric layer is influential on the stabilisation efficiency, and thus polymer concentration, molecular size and conformation are important [40]. Alternatively, electrostatic stabilisation can be achieved through adsorption of ionically charged species to provide an energetic barrier to inter-particle contact.

The concept of an energetic barrier can be explained by the classical DLVO theory (Derjaguin, Landau, Venney and Overbeek), which describes how charged solid particles in liquid medium interact through (a) attractive Van der Waals forces and (b) repulsive electrostatic forces between the electrical double layers surrounding particles in solution [98, 99]. According to the DLVO theory, the summation of these forces allows prediction of the interaction between charged particles immersed in a liquid, Figure 1.12. Solid particles may become charged for example by ionisation of certain functional groups of the particles (e.g. $-\text{COOH}$ or $\text{NH}_2$ groups) or through selective adsorption of a particular ionic species (e.g. an ionic surfactant) [96]. Particles which are charged in suspension have a surface potential which indicates their stability. The surface potential can be approximated by measuring the zeta potential (electrical potential at shear plane) and used as an indicator of the stability of particles in suspension. Zeta potential defines the degree of repulsion between adjacent, similarly-charged solid dispersed particles. As a rule of thumb, a zeta potential of greater than $\pm 30$ mV indicates adequate system stability for purely electrostatically-stabilised particles [40, 100]. Employment of high molecular weight stabilisers (e.g. polymers) invalidates the rule (by shifting the shear plane).
Figure 1.12  Illustration of the classical DLVO theory showing the interactive potential energy as a function of interparticle distance. Attractive forces ($V_A$) dominate at very small and large distances. Repulsive forces ($V_R$) dominate at intermediate distances.

Charged surfactants like SDS are more affected by physiological conditions than steric stabilisers. Electrolytes, which are present in the gastrointestinal tract, reduce the zeta potential and physical stability of a previously stabilised system. Steric stabilisers in comparison to electrostatic stabilisers are less impaired in their effect by electrolytes. On the other hand, steric stabilisers are more sensitive to temperature fluctuations than are electrostatic stabilisers [47]. Combinations of steric and electric interactions are often complementary for stabilising nanoparticles in suspension.

Douroumis and Fahr (2007) characterised the influence of different polymeric additives on the stabilisation of carbamazepine nanosuspensions through a screening process and found the best growth inhibition in the presence of HPMC or MC and PVP [101]. Dong et al. (2009) again employed a trial and error stabiliser screening approach to identify HPMC as the most effective stabiliser for spironolactone nanoparticles [56]. Pandya et al. (2011) used a $2^3$ factorial design to study the effect of independent variables (eg. concentration of each additive) on the dependent variables (eg. particle size) of the drug simvastatin. A pre-screening study identified PVPK-30 and SDS (combination of steric and electrostatic) as the optimum stabiliser system for preparing particles with a size of 300 nm [102]. Similarly, Meng et al. (2009) showed that a combination of cellulose ether
and SDS reduced the average particle size of fenofibrate and griseofulvin particles more effectively than either cellulose ether or SDS alone [103].

The current strategy for stabiliser selection is generally a trial and error screening approach with no prior knowledge of their ability to interact with the drug [104]. Some studies have focused on developing relationships between stabiliser efficacy and molecular properties to generate a general guide to stabiliser selection. Choi et al. (2005) and Lee et al. (2008) attempted to select efficient stabilisers by matching surface energies of the polymer and the drug but concluded that specific interactions between the stabiliser and drug was more influential than comparing surface energies [105, 106]. Van Eerdenbrugh et al. (2009) performed a screening study with a variety of stabilisers and drug compounds and concluded that the hydrophobicity of the drug surface played a decisive role in the production of stable nanoparticles [107]. Verma et al. (2009) investigated local interactions between non-ionic polymeric stabilisers and a surface using atomic force microscopy to visualise the stabiliser arrangement/conformation on the drug surface (ibuprofen). The stabilisation efficiency of the stabilisers correlated with the surface coverage and adhesion strength to the drug surface, as seen by AFM [104]. Khan et al. (2013) looked at specific interactions between the drug and stabiliser and found that the choice of stabiliser was both critical and specific for each drug molecule. They concluded that there is probably no generic set of stabilisers that yield stable, low particle-size nanocrystals [108]. Tuomela et al. (2016) alluded to the fact that a stabiliser should be selected not only on the basis of its ability to impart physical stability, but also on the basis of how the stabiliser affects the downstream processing and bioavailability in the final formulation [109], potentially to bridge the gap between primary and secondary manufacturing. Additional excipients are generally required during secondary manufacturing (eg. fillers, binders, lubricants, disintegrants). With some consideration, additives with dual functionality may be employed. However, to date it seems that no study has given adequate assistance in the systematic selection of appropriate stabilisers for nanosuspensions, and the trial and error approach continues.
1.6 Antisolvent precipitation for generation of nanoparticles

As outlined in section 1.3.2, antisolvent precipitation is a promising approach for the industrial preparation of nanoparticles, because of its simplicity, scalability and convenience of processing [110]. The process can be performed either in the absence or in the presence of a stabilising agent, as required. Briefly, the process requires two miscible solvents; one in which the drug has good solubility and one in which the drug has poor solubility (antisolvent). The drug is dissolved in the solvent, and is quickly added to an excess amount of antisolvent (usually water) under rapid agitation. Diffusion of the antisolvent into the solvent phase induces conditions of supersaturation from which the drug precipitates [46, 108, 111-113]. A high degree of supersaturation is achievable as soon as cosolvent mixing takes place, but the supersaturation level depends on both the concentration in the cosolvent mixture (c) and the solubility in the cosolvent mixture (c_{eq}), Eq. 1.5. The lower the solubility in the cosolvent mixture, the higher the supersaturation, thus making the approach well suited to preparing small particles of hydrophobic drugs with limited water-solubility.

Although this approach to fine particle production (to the nanoscale) has been used in many academic studies, industrial applications are so far limited. The conversion of the attractive process into practice is fraught with difficulties, since the model concepts on particle formation in the individual case can only supply approximate information on the optimal composition of the active compound/polymer/solvent/stabilizer system [67]. The process requires that; (1) nucleation takes places at the highest possible level of supersaturation in order to maximise the nucleation rate, (2) that the supersaturation level is rapidly reduced after nucleation so as to limit particle growth and generate a narrow size distribution (possibly under the influence of growth inhibitors), and (3) that agglomeration of primary particles is prevented (eg. with viscosity-increasing additives, or by steric/electrostatic barriers) [67]. Antisolvent precipitation facilitates rapid generation of supersaturation by quick addition of the drug solution to the antisolvent (red line, Figure 1.13). The solute concentration momentarily exceeds the critical nucleation concentration, before quickly falling back down to the saturation concentration following an instantaneous burst of nucleation and subsequent growth. However, increasing the
speed of the process limits the time spent in the nucleation and growth regions after precipitation, thus enabling preparation of smaller particles, Figure 1.13.

![Diagram of supersaturation generation, nucleation, growth, and agglomeration](image)

**Figure 1.13** Schematic illustration of the processes of (red) fast and (blue) slow supersaturation generation, followed by nucleation, growth and agglomeration to return the system to equilibrium concentration (modification of LaMer representation [114]).

Upon identification of a drug with dissolution-limited bioavailability, particle size reduction by antisolvent precipitation should be considered as an option for improving the bioavailability. Drugs with dissolution-limited bioavailability are those which fall into the BCS Class II category. In such cases, the surface area increase which accompanies size reduction will improve its dissolution rate. The poorer the aqueous solubility of the drug substance (greater hydrophobicity), the higher the supersaturation will be, leading to a smaller particle size. Partition coefficient (Log P) values can be used to identify drug compounds on the basis of their hydrophobicity (see Table 1.1, Section 1.1). Control over the process parameters employed during antisolvent precipitation facilitates control over the precipitation product, and therefore drug particles of a desired size can be prepared.
1.6.1 *Selection of Antisolvent Process Criteria.* Primary considerations for batch precipitations are choice of solvent, antisolvent, drug concentration in the solvent, method and speed of addition of the solvent phase to the antisolvent phase, antisolvent:solvent ratio, temperature, agitation rate, use of stabilisers (in solvent or antisolvent phase, present during precipitation or added later), aging time allowed and isolation/drying method (which will be discussed in section 1.7).

In terms of solvent and antisolvent selection, the primary criteria are that the two solvents are miscible. Water is generally selected as the antisolvent. Solvent choice is limited to toxicological safety and the requirement of complete miscibility with water. It is beneficial if the solvent can solubilise the drug in large amount so that high yields can be achieved [67]. Methanol was the solvent of choice for Wang et al. to prepare ultrafine beclomethasone dipropionate because of the higher degree of supersaturation that could be achieved compared to other miscible solvents [110]. Chung et al. investigated solvent choice in terms of drug solubility and solvent polarity (dielectric constant) and its effect on the nanocrystal growth. On increasing the dielectric constant from 4 (DEA) to 8 (THF) to 21 (acetone) to 47 (DMSO), the size of perylene nanocrystals reduced linearly from 215 nm to 95 nm. However, the inverse of this trend was observed for anthracene nanocrystals [115].

In terms of drug concentration, higher concentrations of drug solution should produce a higher degree of supersaturation upon mixing with the antisolvent, which is desirable in terms of nanoparticle preparation. This trend was observed in several studies on the effect of drug concentration on size [56, 110, 111, 116]. However, agglomeration of the precipitated fine particles must be carefully controlled. Other studies have shown an increase in particle size with increase in drug concentration due to the effects of agglomeration [86, 117]. Furthermore, mass available for crystallisation also directly influences the particles size and should be considered in addition to supersaturation.

The rate at which the drug solution is added to the solvent and the mixing efficiency are closely connected in terms of their effect on particle size and size distribution. Addition of the drug solution to the antisolvent generates a momentary localised increase in supersaturation. Whether the rate of particle formation is slower or faster than the time to
complete homogeneous mixing, which is defined by the rate of diffusion of the molecules, is a critical consideration of the particle formation process [67]. The faster the drug solution is added to the solvent, the more efficient the mixing must be to distribute the supersaturation. The degree of supersaturation prior to precipitation varies locally as a function of the mixing process until the solution is fully mixed. Since nucleation rate is highly dependent on supersaturation (Eq. 1.12), efficient mixing is required to obtain a uniform supersaturation and a narrow size distribution [85]. Wang et al. (2007) showed a decrease in the particle size of beclomethasone dipropionate from > 4 to 1.3 µm as the agitation rate was increased from 500 to 2000 rpm [110]. A similar trend was observed by Hatkar et al. [116]. Ultrasonication is sometimes incorporated into the antisolvent precipitation process to improve the mixing conditions. Ultrasonic waves generate cycles of compression and rarefaction (decompression) in the liquid medium, leading to cavitation (bubbles and voids). Collapse of the bubbles sends intense shock waves through the medium, resulting in homogeneous micromixing of the solution and antisolvent and consequential precipitation of finer particles [84, 86]. Furthermore, the use of sophisticated mixing devices in combination with the antisolvent precipitation process has been explored to further intensify the mixing, particularly when high production rates are required (eg. confined impinging jets [118, 119], T-mixers [120, 121], and static mixing devices [122]). Matteucci et al. (2006) investigated the effect of drug addition rate (5 to 130 mL/min), method of addition (eg. pump, dropwise or pouring) and mixing energy on the resulting particle size. They found that the particle size was large and the population density was small following slow addition (5 mL/min), whereas the particle size was small and the population density was large following fast addition (130 mL/min). Slow, dropwise addition gave particles which formed from the first drops sufficient time to grow during the remaining addition period [85]. A similar trend was observed by Dalvi et al. [86].

Increasing the antisolvent to solvent ratio (AS/S) at constant concentration in the supersaturated solution increases the degree of supersaturation, which again should lead to smaller particles in situations where agglomeration and Ostwald ripening are controlled; a trend observed by Hatkar et al. 2012 [116]. Both Dalvi and Wang indicated that increasing the AS/S ratio above 20/1 did not give any further benefit in terms of particle size reduction since the nucleation rate was then approaching its maximum value [86, 110]. Changing the temperature of the antisolvent changes the saturation
concentration of the solute (as per Figure 1.7) and therefore, also changes the supersaturation (as per Eq. 1.5). Reducing the temperature should increase the supersaturation and decrease the particle size, as has been observed in several other studies [85, 86, 110]. However, again, the effect of changing the antisolvent to solvent ratio on the solubility and thus on the mass available for crystallisation should also be considered.

The use of stabilisers during antisolvent precipitation is highly influential on the resulting particle size, as discussed in Section 1.5. Stabilisers can inhibit or enhance the nucleation, growth and agglomeration of precipitated nanoparticles. The selected stabiliser must have good affinity for the drug particles and possess a fast diffusion rate and effective adsorption onto the surface of the drug particles [56]. However, in addition to the choice of stabiliser system, consideration must be given to where the stabiliser is introduced (eg. in the solvent or antisolvent phase) and when it is introduced (eg. before or after nucleation). The majority of antisolvent precipitation studies have the stabiliser dissolved in the aqueous phase before precipitation. However, few investigations have focused on its best placement position. Matteucci 2006 found that under identical experimental conditions, changing the stabiliser location from the aqueous phase to the organic phase gave a more desirable (smaller) average size [85]. Placement of the stabiliser in the organic phase may make it more available to the precipitating drug particles upon mixing since diffusion of the stabiliser molecules across the aqueous/organic interface; or throughout the entire volume of the relatively larger aqueous phase is not necessary [85]. Liu et al. distributed the stabilisers in both the aqueous (SDS) and organic (VES) phases perhaps due to solubility restrictions [123]. Bodnár et al. (2016) explored the timing of stabiliser addition and found beneficial results in terms of particle size and stability when the surfactant was present during precipitation (to enhance nucleation) and the polymeric stabiliser was added subsequently (to prevent particle growth) [124]. Certain polymers can function as nucleation inhibitors and can thus restrict the nucleation rate, and stabilise supersaturation conditions [125-127].

The stability of nanoparticle systems achieved by choice of optimum stabilisers is, in general, only temporary. Isolation of the nanoparticles from suspension is required for longer term stability. In temporarily stabilised systems, the aging time of particles in suspension (time period between addition of the drug solution to the time of particle
isolation from suspension) should be as short as is feasible. Zhang et al. showed that HPMC only stabilised nanoparticles of atorvastatin calcium at < 450 nm for 10 min after precipitation. By 1 hr, particles had grown to 1200 nm [111]. Hatkar et al. took the first particle size measurement of salicylic acid crystals at 1 hr and beyond this time (up to 12 hrs) the particle size was constant, indicating that crystal growth was complete by 1 hr [116]. Khan et al. showed that if the optimum stabiliser system is found for a specific compound, the effects of aging time are reduced. By using various combinations of HPMC, Pluronic F127, PVP K30 and sodium deoxycholate, they were able to stabilise nanoparticles of ibuprofen, glyburide and artemisinin from significant growth over 30 days. However, for all other stabiliser combinations tested, the aging time had a significant influence on the particle size [108]. In most cases, the limited system stability introduces the need for almost immediate drying [128-130].

### 1.7 Isolation of suspended particles into solid state

For long term stability and for convenience during drug administration, most pharmaceutical suspensions are converted into dry powder form for use in solid dosage formulations such as tablets and capsules. Residual moisture must be low enough to prevent product deterioration during storage. All isolation and drying processes of pharmaceutical suspensions involve separation of the solid from the liquid phase and subsequent drying by evaporation or sublimation. Common pharmaceutical separation/isolation and drying devices include filters, centrifuges, spray-dryers, freeze-dryers and fluidised-bed dryers [131, 132]. Selection of the most suitable approach requires consideration of the heat sensitivity of the material and its physical characteristics (eg. size), the nature of the liquid to be removed, and the scale of operation [132].

Filtration facilitates separation of an insoluble solid from a fluid by means of a porous medium that retains the solid but not the fluid, through a straining mechanism (eg. membrane filters). The rate of filtration must be fast enough to ensure the manufacturing process can be carried out economically. Filtration rate is affected by the area available for filtration, the filter pore size, the pressure difference (increased by drawing vacuum), the fluid viscosity, the thickness of the filter medium (eg. build-up of filter cake) and the
particle size relative to the size of the filter pores. Cross-flow filtration, where the feed moves parallel to the filter medium, (as opposed to dead-end filtration) is employed when the cake layer thickness needs to be controlled. Rotary vacuum filtration is commonly used when large scale filtration under continuous operation is required [131]. Centrifugation employs centrifugal force to drive filtration or sedimentation. Centrifugal filters are common for industrial scale pharmaceutical separations.

Spray-dryers function by generating a large surface area in the liquid for efficient heat and mass transfer, by atomisation of the liquid into small droplets which are then sprayed into a stream of hot air to dry. Due to the nature of the drying, spray-dried products are generally spherical and hollow, sometimes with a small hole (from release of internal vapour). Spray-drying affords very rapid evaporation so that very high temperatures can be avoided. Spray drying is most convenient for drying reasonably large quantities of material. Alternatively, freeze-drying is used for temperature-sensitive materials. The suspension is frozen and pressure is reduced so that the water is removed by sublimation. Freeze-drying is beneficial in cases of poor suspension stability, since molecular diffusion and thus growth cannot occur in the frozen state. Fluidised bed dryers have been used to dry wet particles due to the good contact which can be obtained between the warm air and the particles which are under vigorous motion. However, care must be taken to avoid loss of fines to the air stream, by implementing a suitable filter [132].

While all the above mentioned isolation and drying techniques are commonplace within the pharmaceutical industry, drying problems can arise when the particle size is reduced to the nanoscale. Filtration of nanosuspensions presents a challenge as the cake will be compressible, leading to longer filtration time. Furthermore, the requirement of a filter with a small pore size will impede the flow rate, often making filtration unfeasible, particularly for larger volumes. If filtration is possible, then the dry powder will be highly subject to agglomeration. For this reason filtration is rarely the chosen method of isolating nanoparticles. For similar restrictions related to the particle size, centrifugation and fluidised bed drying are often avoided when dealing with nanosized particles. The centrifugal force which can be achieved during centrifugation at large scale is often insufficient to sediment ultrafine particles. Spray-drying and freeze-drying are currently the most common industrial techniques for the isolation and drying of nanoparticles [5, 133]. However, isolation and drying are complex processes and the stresses which they
induce can destabilise fragile nanoparticle systems, even those which are sufficiently stable in the aqueous phase. Additional excipients (lyoprotectants and cryoprotectants) are often required to minimise aggregation and promote redispersibility after drying, so that the original particle size and surface area is preserved. Typical drying protectants include sugars (eg. sucrose, saccharose, lactose), sugar alcohols (eg. mannitol, sorbitol) and water-soluble polymers (eg. PVP, PVA, PEG) [134]. However, selection of an effective drying protectant and its concentration is not always straightforward. The influence of lyo/cryoprotectants is largely determined on a trial and error basis, and studies have indicated that concentrations as high as 40% are required to acquire redispersible nanoparticles after freeze-drying [135].

Van Eerdenbrugh et al. investigated the effect of spray-drying and freeze-drying on the nano size of nine model drug compounds, and reported nanoparticle agglomeration for all dried powders. Furthermore, they detected that compounds with higher Log P values and a more hydrophobic surface produced agglomerates which were harder to redisperse [130]. In another study, Van Eerdenbrugh compared the use of sucrose and microcrystalline cellulose as matrix formers during the freeze-drying of itraconazole nanosuspensions. In the absence of a drying protectant, freeze-drying compromised the fast dissolution behaviour of the drug. Both drying protectants improved the dissolution rate of the dried material but concentrations up to 200% relative to the weight of the drug were required in certain cases [136]. An additional study reported on the use of alternative matrix formers (eg. microcrystalline cellulose, CaHPO₄, SiO₂ and hydrophobically-modified polyfructose) during spray drying of drug nanosuspensions which are known to forego their fast dissolution upon drying in the absence of matrix formers. Although results were slightly varied for each drug-protectant combination, the best results (in terms of dissolution upon redispersion) were achieved in the presence of SiO₂ and polyfructose protectants [134]. These studies highlight that the isolation of nanoparticles into the solid state while preserving the fast dissolution that stems from the nanoparticle size is no easy task and may be the limiting factor in preparing fast dissolving nanoformulations of BCS class II drugs.
1.8 Characterisation of nanoparticles

The essential characterisation parameters for nanocrystal suspensions are listed below [137].

1.8.1 Size and size distribution. The particle size and distribution are vitally important since they govern other characteristics such as surface area, and therefore dissolution rate and physical stability. The particle size of nano/microsuspensions is typically measured by laser light scattering techniques such as Laser Diffraction (LD) in the reliable size range of 100 nm to 1000 µm or Dynamic Light Scattering (DLS) in the reliable size range of 20 nm to 2 µm. Laser diffraction measures the angle of diffraction of light scattered by particles as they pass through a laser beam and reports a volume-weighted size distribution on the assumption of spherical particles. Dynamic Light Scattering (also Photon Correlation Spectroscopy) measures the rate of fluctuation of light scattered by particles which are moving under Brownian motion, on the basis that large particles move more slowly than small particles. DLS reports the intensity distribution (Z-average) and is therefore not directly comparable to laser diffraction. The reported particle size is only valid in relation to the measurement technique used. Electron microscopy can give visual information about the particle size and distribution by examination of each particle, which makes it possible to distinguish between individual particles and agglomerates/aggregates.

1.8.2 Shape (habit). Particle shape is a complex geometric characteristic which again governs other particle characteristics such as surface area and dissolution rate. The sphere is the shape with the smallest surface area to volume ratio. This value will increase as the shape deviates from perfect roundness (eg. for needles or plates). Transmission and scanning electron microscopes (TEM and SEM) facilitate easy determination of the particles shape. The shape of drug crystals depends on their crystalline structure as well as their conditions of crystallisation, and can be clearly visualised by electron microscopy.

1.8.3 Surface properties and zeta potential. The particles’ zeta potential, which is an indication of the particles surface charge and the degree of electrostatic repulsion, is one of the fundamental parameters known to affect particle stability. The tendency for
agglomeration can be predicted by measurement of the zeta potential. According to the literature, a zeta potential of \( > \pm 30 \text{ mV} \) is desired to obtain a physically stable nanocrystal suspension [40].

1.8.4 Crystalline state. Evaluation of the crystalline state is necessary in terms of nanoparticle solubility and stability. The high supersaturation conditions employed during antisolvent precipitation may encourage the formation of amorphous or metastable crystalline forms, which have reduced stability and may subsequently convert to the more stable crystalline form over the long term. X-ray diffraction (XRD) and differential scanning calorimetry (DSC) can be used to evaluate the crystalline structure of drug nanocrystals.

1.8.5 Saturation solubility and dissolution rate. Measurement of the saturation solubility facilitates selection of drug compounds that may be suitable candidates for particle size manipulation for improved bioavailability. The saturation solubility should be low so that size reduction can have a remarkable improvement on their dissolution behaviour. Dissolution rate is generally recognised as the key attribute of nanoparticle formulations and its measurement facilitates comparison to other formulations (eg. micron formulations) as well as giving an indication of in vivo performance. Dissolution testing should be conducted in accordance with the methods described in the United States Pharmacopoeia (USP) in sink conditions (x3 times undersaturated).

1.9 Conclusions and resulting project motivation

A thorough review of the literature indicated that there is a route to nanoparticle generation, through bottom-up precipitation approaches, which has not yet been exploited at industrial scale. Given the number and range of hydrophobic BCS class II drugs coming through the development pipeline from discovery, and given the number of these drugs which are shelved on the basis of their poor bioavailability, there is an opening for the development of more advanced methods for formulating these nanoparticles eg. through precipitation from the molecular scale. Preparation of drug nanocrystals allows a means of substantially improving the dissolution behaviour of hydrophobic drugs and could potentially save many drugs with high therapeutic potential from developmental discontinuation.
Furthermore, precipitation in comparison to disintegration (top-down approach) may alleviate some of the disadvantages associated with current size reduction techniques, which can exclude certain drug substances, such as those which are thermolabile or those of hard materials which are incapable of being reduced to the nanoscale through mechanical means. However, nanoparticles cannot generally be prepared using conventional crystallisation approaches (eg. through temperature control). High supersaturation conditions are required to prepare the smallest particles, but small particles, due to their enhanced surface area and resulting surface energy are highly unstable. Stabilisation, isolation and characterisation therefore become more difficult as a result of the reduced length scale.

The aim of this project is to prepare micron and nano sized particles through precipitation from a supersaturated solution, to examine the effect of stabilisers on the growth rates of particles and on their resulting particle sizes and shapes, to stabilise nanoparticles of BCS class II drugs with significantly enhanced dissolution rates, and to isolate these nanoparticles from suspension into the solid state while preserving the fast dissolution which stems from their nanoparticle size. Significant research will be conducted in the area of nanoparticle isolation since this is an area which has remained largely neglected in the literature in comparison to nanoparticle formation.

### 1.10 References


Chapter 1  
Introduction


Chapter 2

Precipitating Micron-Sized Salicylic Acid Crystals

2.1 Abstract

Crystals of a particular size and habit can be engineered by manipulating crystallisation conditions. During an antisolvent precipitation process, conditions such as temperature, drug concentration and volume of drug solution added to the antisolvent were varied, and the effects of these variations on the size of salicylic acid crystals were analysed. Changes in the process conditions induced simultaneous changes in both the supersaturation level and the mass available for crystallisation which in turn provided competing influences on the particle size. In a supersaturation dominated system (i.e. $\Delta S > \Delta m$), particle size decreases with increasing supersaturation. In a mass dominated system (i.e. $\Delta m > \Delta S$), particle size increases with increasing mass. Therefore, by selecting process changes (e.g. $\Delta T$, $\Delta c$, $\Delta V$) and determining their effect on $\Delta S$ and $\Delta m$, the final particle size can be controlled and/or predicted.

In the pure system, the length of precipitated crystals ranged from 20 and 150 $\mu$m, and the crystal habit ranged from solid square prisms to hollow/indented square prisms to rods by varying the process conditions. The proposed mechanism of hollow crystal formation is through an internal dissolution and concomitant external recrystallisation process, attributed to the packing orientation of salicylic acid molecules in the crystal lattice, centred by an internal channel of carboxylic acid dimers. The indentations were more pronounced at higher supersaturation; a condition which increases the density of crystal defects and thus accelerates dissolution. Indentations were suppressed in the presence of
certain additives, indicating additive adsorption and consequential protection from dissolution. Of the range of additives tested, methyl celluloses had the most significant effect on the size and habit of precipitated crystals, compared to when no additives were present. Both HPMC and CMC, when present during precipitation and growth, converted the habit of salicylic acid crystals from square prisms to fine needles. We propose that their mechanism of action is through face-selective hydrogen bonding with surface-exposed fragments of salicylic acid molecules which have hydrogen bonding capabilities, ie. through their hydroxyl and carbonyl groups.

2.2 Introduction

Crystallisation is one of the most important techniques in modern material science due to its potential to produce well-defined particles with unique structures in the micrometre and nanometre ranges [1]. The vast majority of pharmaceuticals are generated in crystalline form [2]. Crystal uniformity in terms of size and habit/morphology is often a critical requirement of pharmaceutical crystallisation processes, influencing not only the final product performance (eg. bioavailability, dissolution rate, solubility and stability) but also downstream processing operations and product properties (eg. filtration, washing, compressibility, flowability and mixing behaviour) [3, 4]. During crystallisation, the crystal size and habit of the product can be tailored by controlling the crystallisation process conditions or by introducing additives which selectively disrupt the crystallisation progression.

Crystallisation conditions can be tuned to prepare crystals of a desired size and shape. If particles of a small size are required (eg. in the nano or small micron size ranges), the nucleation rate must first be maximised, followed by suppression of potential growth and ripening processes. Additives (typically surfactants or polymers) may be required to arrest these processes and maintain the small size of the particles which nucleate. Additives with an affinity for the target compound may bind to the molecules in solution to alter the nucleation rate or to the growing crystal surfaces to alter the growth rate [5]. Exposed functionalities at the surface of a crystal often differ from face to face, leading to different interaction capabilities with an additive or with the crystallisation environment (eg. solvent, supersaturation etc.). Selective inhibition of growth in one direction can change
the crystal shape remarkably [3]. The equilibrium shape is that which minimises the total surface free energy of the crystal and thus, low energy, slow-growing faces are preferentially expressed [6, 7].

In terms of particle size control, Dalvi et al. (2009) and Zhang et al. (2009) showed that the particle size of griseofulvin [8] and atorvastatin calcium [9] decreased with increasing drug concentration. Agglomeration of the small particles occurred at higher concentrations. Wang et al. (2007) and Dong et al. (2009) saw only a decreasing trend for the particle size of beclomethasone dipropionate [10] and spironolactone [11] with increasing concentration, within the concentration ranges tested. In terms of particle shape control, Munk et al. (2012) used a tailor made polymeric additive (poly(N-isopropyl acrylamide) to alter nitrofurantoin crystals from needle to dendritic shape [12]. Kumar et al. (2008) and Zimmermann et al. (2009) used polymers (PVP or HPMC) or a surfactant (SDS) to modify the crystal shape of mebendazole [13] and siramesine hydrochloride [14] from needles to plates. Furthermore, rapid particle formation which results from high supersaturation can reduce the quality of the crystalline product and can introduce crystal defects as studied by Takiyama 2012 [3]. Therefore, S is vitally important in designing crystals of a desired specification [15].

This work investigates the influence of antisolvent process conditions (and consequentially supersaturation and available mass) and additives on the size and shape of salicylic acid crystals following crystallisation in water-methanol mixtures. Salicylic acid (log P 2.3 [16]) a compound with pain relieving capabilities, was chosen as a model compound due to its absence of polymorphism, thus making it easier to attribute changes in its crystal habit to the crystallisation conditions. However, its slight solubility in water induces a limitation to the maximum supersaturation level which can be achieved during the antisolvent precipitation process using a water/methanol system. The molecular structure of salicylic acid consists of a hydrophobic aromatic ring functionalised with a hydrophilic carboxylic acid and an alcohol group on one side, giving the molecule amphiphilic nature [17], Figure 2.2a. Its hydrophilic side has both hydrogen bond donating and accepting capabilities, which may partake in inter- and intra- molecular hydrogen bonding [4]. However, the dominating feature of its crystal structure is an intermolecular centrosymmetric carboxylic acid dimer [18], Figure 2.2b. Salicylic acid
generally crystallises with a rod-like morphology, indicating anisotropic growth of its crystal faces [4].

![Molecular structure of salicylic acid and (b) the carboxylic acid dimer which is favoured in the crystal structure.](image)

**Figure 2.2** (a) Molecular structure of salicylic acid and (b) the carboxylic acid dimer which is favoured in the crystal structure.

During an antisolvent precipitation process, the effects of temperature, drug concentration, and drug solution injection volume on the resulting particle size were monitored. A diverse range of additives were tested including HPMC (non-ionic polymer – high MW), CMC (anionic polymer – high MW), a range of pluronic (non-ionic surfactant block copolymers – medium MW) and SDS (anionic surfactant - low MW). All are well-known excipients with excellent safety profiles and broad FDA clearance as pharmaceutical additives [19, 20]. Their structures are shown in Figure 2.3.

![Molecular structures of the tested stabilisers](image)

**Figure 2.3** Molecular structures of the tested stabilisers
2.3 Experimental Details

2.3.1 Materials. Salicylic acid (SA, ≥ 99.0% purity), methanol (99.7% purity), hydroxypropyl methyl cellulose (HPMC, ~26 kDa), carboxy methyl cellulose sodium salt (Na-CMC, ~250 kDa), sodium dodecyl sulphate (SDS) and Pluronic F127 (P127, ~12.6 kDa) were purchased from Sigma Aldrich. Pluronic 25R8 (P25, ~3.6 kDa) and Pluronic F108 (P108, ~14.6 kDa) were purchased from BASF corporation. Distilled water was used for sample preparations.

2.3.2 Solubility experiments. The solubility of SA in methanol, water and in water/methanol mixes (at 2.5% v/v and 10% v/v methanol) was measured gravimetrically at 5 °C and 25°C, following a 24 hr equilibration period in saturated conditions.

2.3.3 Particle preparation. 0.25 or 1 mL of an organic solution of salicylic acid in methanol (34.5, 116 or 358 mg/mL solution) at 25°C was quickly introduced by Eppendorf pipette to water (totalling 10 mL, in a 20 mL glass vial) at 25 or 5°C under rapid agitation (800 rpm, using magnetic stir bars, 20 x 2.5 mm) with or without additives (0.05, 0.005, or 0.0025% w/v) to precipitate the drug. Methanol and water were selected as a suitable solvent/antisolvent combination due to their miscibility and due to a large difference in their respective ability to dissolve salicylic acid [18]. Following visual crystallisation, precipitated particles were primarily isolated from suspension by vacuum filtration using a 0.45 µm PVDF membrane filter, and air dried before analysis. However, particles prepared via Prep E and Prep F (Table 2.1) were isolated by centrifugation (6 min at 4000 rpm) in 20 mL glass vials using a Hettich Universal 32R centrifuge. All preparation conditions used are outlined in Table 2.1.
Table 2.1 Preparation conditions employed during antisolvent precipitation

<table>
<thead>
<tr>
<th>Name</th>
<th>Drug conc. (mg/mL)</th>
<th>Inj. Vol (mL)</th>
<th>Temp. (°C)</th>
<th>Additive</th>
<th>Add. Conc (%w/v)</th>
<th>SS ratio</th>
<th>Mass added (mg/10 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prep A</td>
<td>116</td>
<td>0.25</td>
<td>25</td>
<td>N/A</td>
<td>N/A</td>
<td>2.6</td>
<td>29</td>
</tr>
<tr>
<td>Prep B</td>
<td>116</td>
<td>0.25</td>
<td>5</td>
<td>N/A</td>
<td>N/A</td>
<td>5.2</td>
<td>29</td>
</tr>
<tr>
<td>Prep C</td>
<td>358</td>
<td>0.25</td>
<td>25</td>
<td>N/A</td>
<td>N/A</td>
<td>8.0</td>
<td>89.5</td>
</tr>
<tr>
<td>Prep D</td>
<td>116</td>
<td>1.00</td>
<td>25</td>
<td>N/A</td>
<td>N/A</td>
<td>6.0</td>
<td>116</td>
</tr>
<tr>
<td>Prep E</td>
<td>116</td>
<td>0.25</td>
<td>25</td>
<td>HPMC</td>
<td>0.0025</td>
<td>2.6</td>
<td>29</td>
</tr>
<tr>
<td>Prep F</td>
<td>116</td>
<td>0.25</td>
<td>25</td>
<td>CMC</td>
<td>0.0025</td>
<td>2.6</td>
<td>29</td>
</tr>
<tr>
<td>Prep G</td>
<td>116</td>
<td>0.25</td>
<td>25</td>
<td>Pluronic HPMC SDS</td>
<td>0.05</td>
<td>2.6</td>
<td>29</td>
</tr>
<tr>
<td>Prep H</td>
<td>358</td>
<td>0.25</td>
<td>25</td>
<td>Pluronic HPMC SDS</td>
<td>0.05</td>
<td>8</td>
<td>89.5</td>
</tr>
</tbody>
</table>

2.3.4 *Induction Time Experiments.* Induction times were determined based on the first visual detection of the crystallisation event as seen by a hazy suspension.

2.3.5 *Scanning Electron Microscopy.* Sizes (in terms of length of individual crystals) and habits were analysed using a Joel CarryScope JCM-5700 scanning electron microscope (SEM). Dried particles were spread on carbon tape on an aluminium stub and coated with an ~8 nm gold deposit using an EMITECH K55. Particles were imaged at a voltage of 8 kV, and a working distance in the range 23-49 mm. The image analysis software, Image J, was used to analyse particles sizes from SEM images. A minimum of 100 particles were analysed for each sample and a mean value is reported. Variation around the mean is also reported.

2.3.6 *Modelling the Crystal Morphology.* Mercury CSD 3.7 © was employed to visualise the crystal structure and calculate the BFDH morphology of salicylic acid crystals.
2.4 Results

2.4.1 Characterisation of salicylic acid ‘as received’. SA ‘as received’ had an irregular, rod-shaped crystal habit with an average particle size of 333 µm and a wide particle size distribution (±178 µm), Figure 2.4. Solubility data ($c_{eq}$) for SA in methanol, water, and water/methanol mixes at 25°C and 5°C is shown in Table 2.2. The solubility of SA was substantially higher in methanol (solvent) than in water (antisolvent). The solubility data was used to calculate the supersaturation level for each precipitation experiment using Eq. 1.5 and the mass available for crystallisation when the solubility was taken into account, Table 2.3.

![SEM image and particle size distribution of SA ‘as received’](image)

**Figure 2.4**  SEM image and particle size distribution of SA ‘as received’.

**Table 2.2**, Solubility data for salicylic acid in methanol (M), water (W) and methanol/water (M/W) mixtures at 5°C and 25 °C

<table>
<thead>
<tr>
<th>Solvent system</th>
<th>Temp (°C)</th>
<th>Solubility (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% Methanol (M)</td>
<td>25</td>
<td>358.0 ± 7.3</td>
</tr>
<tr>
<td>10% Methanol</td>
<td>25</td>
<td>1.94 ± 0.23</td>
</tr>
<tr>
<td>(1 mL injection)</td>
<td>5</td>
<td>0.55 ± 0.07</td>
</tr>
<tr>
<td>2.5% Methanol</td>
<td>25</td>
<td>1.12 ± 0.11</td>
</tr>
<tr>
<td>(0.25 mL injection)</td>
<td>5</td>
<td>0.56 ± 0.04</td>
</tr>
<tr>
<td>100% Water (W)</td>
<td>25</td>
<td>1.24 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.51 ± 0.05</td>
</tr>
</tbody>
</table>
Table 2.3  Supersaturation and mass available for crystallisation for each set of process conditions

<table>
<thead>
<tr>
<th>Expt.</th>
<th>S ratio</th>
<th>Mass available (mg/10 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prep A</td>
<td>2.6</td>
<td>17.8</td>
</tr>
<tr>
<td>Prep B</td>
<td>5.2</td>
<td>23.4</td>
</tr>
<tr>
<td>Prep C</td>
<td>8.0</td>
<td>78.3</td>
</tr>
<tr>
<td>Prep D</td>
<td>6.0</td>
<td>96.6</td>
</tr>
</tbody>
</table>

2.4.2  Precipitation of SA from pure water. Precipitation of SA in the antisolvent (water) by the standard process conditions outlined in Table 2.1 (Prep A) generated particles with an average size of 20.7 ± 7.0 µm, Figure 2.5a. Particles developed as well-faceted rectangular prisms with a square base (7.4 ± 2.2 µm). Precipitated particles were uniform in both size and shape throughout the entire sample, and were stable in size and shape when held in suspension for at least 3 hours (aging), Figure 2.5. However, indentations were observed at the prism base of some particles throughout the sample, leading to hollow or partially hollow structures, Figure 2.6a. While the external crystal surfaces were generally well-faceted with sharp edges and defined facet angles, the internal cavity (when present) was rounded and irregular. Although the indentation was not clearly evident in every crystal, instances were seen consistently throughout the sample. The indentations were much deeper, more defined and prevalent when the particles were precipitated using a higher drug concentration, Figure 2.6b.

Figure 2.5  SEM images of precipitated salicylic acid crystals prepared via Prep A at aging times of (a) 2.5 min, (b) 6 min, and (c) 3 hrs, n=100
2.4.3 Effect of antisolvent process conditions on size. The effect of temperature, drug concentration, and drug solution injection volume (into a total volume of 10 mL) on the particle size was investigated. Decreasing the precipitation temperature from 25 °C to 5°C (Prep A→Prep B) had no significant influence on particle size, Figure 2.7a, b. Increasing the concentration of the SA solution by a factor of ~3 from 116 mg/mL to 358 mg/mL (Prep A→Prep C) resulted in a modest increase in particle size (eg. length increased from 20.7 to 30.5µm), Figure 2.7a, c. Increasing the volume of SA solution which was added to the antisolvent (totalling 10 mL) from 0.25 mL to 1 mL (Prep A→Prep D) resulted in a significant increase in particle size, with the average length increasing from 20.7 µm to 149.8 µm, Figure 2.7a, d.

The effects of process changes on the supersaturation ratio and on mass available for crystallisation are outlined in Table 2.4, whereby an increase in supersaturation is expected to decrease the particle size and an increase in mass available is expected to increase the particle size. The factors by which the supersaturation and mass are changed (ie. ΔS and Δm) for given changes in process conditions are shown in Table 2.4.
**Figure 2.7** SEM images of SA crystals precipitated under process conditions which deviate from the standard precipitation conditions outlined in Prep A (ie. addition of 0.25 mL of a drug solution (116 mg/mL) into water at 25 °C).

**Table 2.4** Influence of the process parameter changes on supersaturation, mass and size. Bold arrows show the effects which contribute to the observed size result.

<table>
<thead>
<tr>
<th>Process Parameters Changed</th>
<th>Temp</th>
<th>AS/S ratio</th>
<th>S</th>
<th>Mass available</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prep A→B 25→5°C</td>
<td>↓</td>
<td>=</td>
<td>↑(x2)</td>
<td>↑(x1.3)</td>
<td>No significant change in size</td>
</tr>
<tr>
<td>Prep A→C 116→358 mg/mL</td>
<td>=</td>
<td>=</td>
<td>↑(x3.1)</td>
<td>↑(x4.4)</td>
<td>Slight increase in size</td>
</tr>
<tr>
<td>Prep A→D 0.25→1 mL</td>
<td>=</td>
<td>↓</td>
<td>↑(x2.3)</td>
<td>↑(x5.4)</td>
<td>Large increase in size</td>
</tr>
</tbody>
</table>
2.4.4 Effect of additives on size and habit. Both the type and concentration of additive affected the resulting size and habit of the precipitated particles. The particle size decreased with increasing concentration of HPMC and CMC, but this trend was more pronounced for HPMC. Increasing the concentration of HPMC in the antisolvent from 0.0025% w/v to 0.05% w/v reduced the particle length from 32 µm to 6.4 µm, Figure 2.8 a-c, but agglomeration of the small particles occurred. Increasing the concentration of CMC in the same concentration range, did not significantly reduce the particle length but reduced the width, transforming the crystal habit from rods to fine needles, Figure 2.8 d-f.

![Figure 2.8](image)

Figure 2.8 SEM images of SA crystals precipitated in the presence of (a-c) HPMC [Prep E] and (d-f) CMC [Prep F] following a 0.25 mL injection of drug solution (116 mg/mL) into the antisolvent at 25°C containing (a,d) 0.0025%, (b,e) 0.005% and (c,f) 0.05% of each additive

The habit of SA crystals was manipulated from square prisms with no additives (sometimes indented), to needles with HPMC and CMC (polymers), to rods with the pluronics (amphiphilic block copolymers) to blocks with SDS (surfactant) (all at 0.05% w/v), Figure 2.8f, Figure 2.9, (Prep G). The basic habit and size obtained from precipitation in the different Pluronics were equivalent, and hence only images from the Pluronic 25R8 system are presented in Figure 2.9. Although the shape of the crystals
precipitated in the presence of SDS were similar to those of pure crystals (square prisms), no hollow crystals or crystal indentations were observed, Figure 2.9d.

**Figure 2.9** SEM images of SA crystals precipitated on addition of 0.25 mL of a drug solution (116 mg/mL) into an antisolvent consisting of (a) water, or aqueous solutions containing 0.05% (b) pluronic 25R8, (c) HPMC or (d) SDS,

2.4.5 Influence of additives on nucleation and growth. The influence of additives on the nucleation and growth kinetics was investigated by monitoring induction times. Induction time describes the time delay between generation of supersaturation and the first detection of crystals and incorporates the time required for formation of a nucleus and for its growth to a detectable size. As a control, induction times were monitored for the pure system as a function of supersaturation, and were found to decrease with increasing supersaturation, until they became too short to measure at supersaturations ≥ 6, Figure 2.10. However, for a fixed supersaturation, induction times were clearly influenced by the presence of additives during precipitation. At a supersaturation of 2.6 (Prep A), the induction time in the pure system was 2 min. Addition of HPMC and CMC to the antisolvent extended the induction time, while addition of pluronics and SDS shortened
the induction time, Figure 2.11. Of all the additives tested, HPMC had the most pronounced effect on induction time. At a supersaturation of 8 (Prep H), again, an extension in induction time was observed for HPMC, Figure 2.11. However, in this case, the effects of pluronics and SDS could not be seen since the induction time was too short to measure.

**Figure 2.10** Influence of supersaturation on induction time in a pure, additive-free system

**Figure 2.11** Influence of additives on induction time at fixed supersaturation (2.6 and 8).
2.5 Discussion

Uniformly shaped particles with a size in the small micron range were prepared during an antisolvent precipitation process by adding a portion of a salicylic acid solution to an aqueous antisolvent, Figure 2.5. The crystallisation outcome in terms of particle size and shape was, at times, influenced by changes in the process conditions under which the particles were precipitated and/or by introducing additives to control nucleation and growth kinetics. The process conditions used during antisolvent precipitation influence additional factors such as supersaturation and mass available for precipitation; both of which contribute to changes in the resulting size. As discussed in Chapter 1 (Section 1.4), at equal mass, larger supersaturation produces smaller particles. However, in cases where the available mass is not constant, its direct effect on size must also be considered. For a cubic crystal, the particle size in terms of its length ($L$) is influenced both by supersaturation ($S$) and mass ($m$) according to Eq. 2.1 [21] and rearranging to Eq. 2.2; whereby it is understood that the number of particles ($N_p$) is a function of $S$. The combined effects of supersaturation and mass changes may explain the particle sizes which were observed in Figure 2.7.

$$N_p = \frac{m}{\rho L^3} \quad \text{where } N_p = f(S) \quad \text{Eq. 2.1}$$

$$L = \sqrt[3]{\frac{m}{\rho N_p}} \quad \text{Eq. 2.2}$$

The influence of changes in the process conditions (ie. temperature, concentration and drug solution addition volume) on both the supersaturation and mass available are shown in Table 2.4. By reducing the precipitation temperature (Prep A$\rightarrow$B), the supersaturation ratio increased by a factor of 2 thus promoting smaller particles, while simultaneously, the mass available for crystallisation increased by a factor 1.3 (due to reduced solubility) thus promoting larger particles, Eq. 2.1. Therefore, for experiment set A$\rightarrow$B, supersaturation is (by a marginal amount) the dominating influence on size and should lead to a marginally smaller particle length at the reduced temperature (ie. 14.5 $\mu$m). However, due to the uncertainty in particle size measurements from SEM images (ie. 20.7 $\pm$ 7.0 $\mu$m), this effect was masked, Figure 2.7a,b.
By increasing the concentration of SA (Prep A→C), the supersaturation ratio increased by a factor 3.1 thus promoting smaller particles, while simultaneously, the mass available for crystallisation increased by a factor 4.4 thus promoting larger particles. Therefore, for experiment set A→C, mass is the dominating influence on size and is responsible for the slight increase in particle size at the higher drug concentration, Figure 2.7a,c.

For experiment set A→D, whereby the drug solution addition volume was increased, the influence of process conditions on supersaturation and mass available were more complex. Increasing the volume, decreased the supersaturation and mass available for crystallisation due to increased solubility in the solvent/antisolvent mixture but increased the supersaturation and mass available due to increased mass added. However, ultimately, by increasing the drug solution injection volume (Prep A→D), the supersaturation increased by a factor 2.3 thus promoting smaller particles, while simultaneously, the mass available for crystallisation increased by a factor 5.4, thus promoting larger particles. Therefore, for experiment set A→D, mass is the dominating influence on size and is responsible for the large increase in particle size at the higher volume of drug solution, Figure 2.7a,d.

Therefore, for the purpose of controlling the particle size of a pure, additive free system, process parameters should be altered to provide a dominating supersaturation factor if small particles are required or to provide a dominating mass factor if larger particles are required. Typically, very high supersaturations (>3000) are employed during antisolvent precipitation for the production of nanosized particles.

Induction time experiments were used to monitor the influence of supersaturation and additives on the crystallisation outcome. Since induction time combines the time required for nucleus formation and for its growth to a detectable size, distinction between nucleation and growth based on induction times alone is difficult. In the pure system, the induction time decreased with increasing supersaturation (Figure 2.10), indicating faster nucleation, but also faster growth to a detectable size. As outlined in Section 1.4, both the nucleation and growth rates increase with supersaturation but with nucleation having a stronger dependence on supersaturation. Therefore, the faster induction time at higher supersaturation is primarily attributed to faster nucleation. The use of HPMC as an additive during precipitation (at fixed S) induced two effects; an increase in induction time (either due to reduced nucleation and/or growth rates) and a decrease in particle size.
However, since a reduced nucleation rate should lead to a larger particle size, the appearance of smaller particles at longer induction time in the presence of HPMC (Figure 2.9c) compared to the control (Figure 2.9a) must be attributed primarily to a decrease in the growth rate. HPMC can slow growth by adsorbing onto the crystal surfaces and preventing the incorporation of drug molecules into the crystal lattice. HPMC has an abundance of hydrogen bond accepting and donating groups throughout its bulky polymeric structure which may hydrogen bond with the carboxyl/hydroxyl groups from SA molecules at the crystal surface, and impart additional steric stabilisation. However, it is possible that the drug-polymer interactions exist in both the solid and solution phases and therefore, may interfere with both nucleation and growth processes.

The pluronics and SDS on the other hand, decreased the induction time for the same fixed supersaturation (S: 2.6, Figure 2.11a). Both pluronics and SDS have amphiphilic structures, giving them surfactant properties. Pluronics have a central hydrophobic chain centred between two hydrophilic end groups, Figure 2.3. The central hydrophobic block can anchor to the hydrophobic portion of SA molecules in solution leaving the hydrophilic blocks free to extend into the bulk solution [22]. Similarly SDS molecules, with their hydrophilic heads and hydrophobic tails, can self-organise around clusters of SA molecules, leaving only the hydrophilic portion exposed to the surrounding water. Through their surfactant properties, both pluronics and SDS have the ability to reduce the solute-solvent interfacial tension, thus reducing the energy barrier for nucleation and consequently reducing the induction time. Figure 2.12 shows that after crystal formation, it is predominantly hydrophilic groups (hydroxyl and carbonyl group oxygens) which protrude from the dominating {110} surfaces of the SA crystals. Therefore, it is likely that these additives will interact with the SA in the solution phase, while the hydrophobic portion is still exposed.
Figure 2.12  Simulation of the crystal morphology of salicylic acid using the BFDH method and with a view along the \{110\} faces, highlighting the exposed hydrophilic groups (phenol and carbonyl group oxygen) on these surfaces.

Supersaturation level and the use of additives were found to influence not only the particle size but also the particle shape. In the pure system, some SA crystals developed a hollow or partially indented centre in their base, Figure 2.6. The indentation was more defined, deep and prevalent for particles which were precipitated in conditions of higher supersaturation (S: 8) than those at lower supersaturation (S: 2.6), Figure 2.6. At both supersaturations, the internal walls of the cavities were irregular with a roughened surface and no obvious faceting. At the higher supersaturation, where the indentations were deeper and more defined, the outer surfaces were also smoother and more faceted, Figure 2.6b. Based on these observations and based on the knowledge that crystals dissolve with a roughening surface and grow with a smoothening surface [23, 24], an internal dissolution and a concurrent external recrystallisation process is hypothesised. Based on this hypothesis and on SEM observations, it appears that the indentations start at the surface and deepen along the inside of the long axis, gradually transforming the solid prism into a completely hollow tubular structure (Figure 2.6). Nordstrom and Rasmuson, during a solubility investigation for SA, reported a hollow tube habit for salicylic acid when crystallised from water through evaporative crystallisation [18]. However, no explanation for this crystal habit was attempted. Here, we rationalise that the hollowing effect is caused by the unique packing geometry of the SA molecules in the crystal lattice.
Examination of the predicted crystal morphology (BFDH method) of the salicylic acid crystal in Figure 2.13 shows that the \{001\} faces contain the hydrophilic carboxylic acid groups of two adjacent SA molecules at their centre, forming stacked centrosymmetric carboxylic acid dimers, thus giving the crystal a central hydrophilic channel, Figure 2.13b. Tiwari et al (2006), following an investigation of the structure of salicylic acid-water complexes, demonstrated that hydrogen bonding between the carboxylic hydrogen of SA and the oxygen of water is the strongest of all hydrogen bonds possible in the system [17]. Therefore, in an aqueous system, it is probable that water molecules will attack this central channel through intermolecular hydrogen bonding between a water molecule and the surface-exposed carboxylic acid portion of a SA molecule, leading to site-specific dissolution and hollowing of the crystal from the inside out, starting at the centre of the \{001\} faces. Dissolved material may then recrystallise on the outer walls of the crystals to produce highly defined facets. The deeper indentations at higher supersaturation may be explained by an increase in supersaturation-induced crystal defects, which aid in the dissolution process and may also account for the “arms” which developed on the edges of some tubes, Figure 2.6b. Some examples of hollow crystals formed via dissolution-recrystallisation [25-27], melt/sublimation-recrystallisation [28, 29] or variations of these mechanisms [30] have been reported in the literature.

**Figure 2.13** (a) Simulation of the crystal morphology of salicylic acid using the BFDH method and (b) a view into the simulated crystal through the centre of the 001 face, highlighting its central hydrophilic channel.

Additives were also found to influence the crystal shape. Face-selective interactions between the additives and the exposed functional groups on the SA crystal can account for the change in crystal shape. The predicted crystal structure of salicylic acid in Figure
2.12 shows the exposed functional groups on the dominant \{110\} (long axis) and the \{001\} faces. The \{110\} faces expose a high surface density of hydroxyl groups and oxygen atoms from adjacent carbonyl groups, giving those faces strong affinity for hydrogen bonding with HPMC and CMC molecules at the crystal-solution interface, so that upon attachment, they slow the growth of those faces. Crystal morphology is determined by the slowest growing face and therefore, attachment of HPMC and CMC polymers to the \{110\} faces, explains the needle-like crystal habit observed in Figure 2.8. No indentations or hollowing was observed at the \{001\} faces in the presence of any of the additives. Due to the increase in aspect ratio of the particles in the presence of polymeric additives (ie. from rods to needles) it is likely that the \{001\} faces, which are less likely to interact with the stabilisers, are faster growing and are thus ‘grown out’. However, in the presence of SDS, no significant change in the aspect ratio occurred compared to the pure SA crystals, Figure 2.9a,d. In this case, SDS-attachment at the \{001\} face may account for the absence of indentation, by providing a dissolution-preventative protective layer at that face.

### 2.6 Conclusions

In this work, the size and habit of salicylic acid crystals were tailored by varying precipitation conditions, and by introducing additives during an antisolvent precipitation process. Changes in process conditions induced changes in both the supersaturation ($\Delta S$) and the mass available for crystallisation ($\Delta m$); both of which are factors that influence the particle size. The relative influences of $\Delta m$ and $\Delta S$ determined the particle size trend which was observed with changes in process conditions.

Changes in the induction time in the presence of additives served as an indicator of drug-additive interactions. Precipitation in the presence of HPMC extended the induction time and reduced the particle size further than all other systems analysed.

Supersaturation and the use of additives influenced not only the size but also the particle shape. Hollowing of the salicylic acid crystals occurred under certain conditions of supersaturation during precipitation in a pure system. Hollow crystals were accounted for by the packing geometry of salicylic acid molecules in the crystal structure to create a hydrophilic channel down the centre of their long axis, thus encouraging a dissolution-
recrystallisation process. Additives altered the crystal habit of salicylic acid through face-selective interactions with exposed functional groups on the dominating crystal faces, resulting in anisotropic growth and alteration of the crystal shape from square prisms to rods to needles. These results show that control over the precipitation conditions and the additives used during precipitation can allow crystals of a particular size and shape to be engineered.

2.7 References


Chapter 3
Precipitation, Stabilisation and Isolation of Fenofibrate Nanoparticles

Content from this chapter appeared in:

3.1 Abstract
Nano to small-micron size particles of the BCS Class II, hydrophobic drug fenofibrate were prepared in order to enhance its dissolution behaviour, Figure 3.1. Nanoparticles (200-300 nm) were generated in suspension from a supersaturated solution during an antisolvent precipitation process, which matched the size and dissolution behaviour of a commercial wet-milled formulation of the drug. Although their preparation was straightforward, retaining their small size and high surface area in suspension and during isolation was a challenge. Solubilised additives were employed to temporarily stabilise the nanosuspension, and extend the time window for nanoparticle isolation and drying. However, isolation/drying of these small, fragile and highly energetic systems into a redispersible powder proved difficult (eg. by filtration, centrifugation, spray-drying, and freeze-drying). Additives which were effective at stabilising the nanoparticles in aqueous suspension were not always effective at stabilising the nanoparticles during the stress-
inducing drying stage, causing them to aggregate and forego their nanoparticle-induced dissolution enhancement. Additional excipients (e.g., soluble cryoprotectants and insoluble carriers) were required to improve the redispersibility of the dried nanoparticles.

**Figure 3.1** Reducing the particle size increases the exposed surface area to favor faster dissolution

### 3.2 Introduction

As discussed in Chapter 1, a surprisingly large proportion of new drug candidates emerging from drug discovery programmes have poor water solubility and therefore poor bioavailability, often leading to abandoned development efforts [1, 2]. As per the Noyes Whitney equation (Eq. 1.1) BCS Class II drugs, whose bioavailability are dissolution-limited, can be formulated with reduced particle size in order to improve their dissolution behaviour. Size reduction to the small micron or nano scale offers the opportunity to address many of the deficiencies associated with hydrophobic drug formulations and could potentially save many prospective drug candidates from abandonment after discovery.

Chapter 2 discussed the role of additives and supersaturation on the crystallisation outcome in terms of particle size at the micron scale. However, often micronisation is insufficient to reach the desired bioavailability. In such cases, nanonisation may be required. The high supersaturation conditions achievable during antisolvent precipitation (often >3000) facilitate preparation of drug nanoparticles. Antisolvent precipitation has been used to prepare nanosuspensions of the drug substances resveratrol [3], itraconazole
[4], simvastatin [5], carbamazepine [6], and mefenamic acid [7] among many other examples. The process is primarily challenged by the physical stability of the suspended nanoparticles in terms of their particle size, and so, additives are generally required to stabilise their size in suspension. Khan et al. (2013) used combinations of Pluronic F127, PVP-K30, HPMC and sodium deoxycholate to stabilise suspended particles of artemisinin (400 nm), ibuprofen (92 nm) and glyburide (298 nm) following antisolvent precipitation [8]. Hu et al. (2011) used a similar approach to prepare and temporarily stabilise a suspension of fenofibrate (300-400 nm) for 6 min using HPMC, SDS and lactose [9]. Stable nanosuspensions allow greater flexibility in the subsequent downstream processing (ie. in the drying timeline and technique). Additional excipients or matrix formers are often required to promote re-dispersibility and minimise aggregation and loss of surface area during the drying stage, as discussed in Section 1.7. Nanoparticle isolation is a critical and challenging step in the overall production of drug nanoparticles, but is an area which has not been given sufficient attention in the literature.

The work in this chapter focuses on the entire nanoparticle preparation process for a model compound fenofibrate in terms of nanoparticle formation, stabilisation and isolation into solid state. Fenofibrate is a cholesterol-controlling, hydrophobic, BCS Class II drug (log P 5.24) with extremely limited aqueous solubility (<0.5 mg/L at 25°C) [10]. It is a pharmacologically inactive pro-drug (MP: 80-81°C), which undergoes rapid hydrolysis at the ester bond to form the active metabolite fenofibric acid (MP: 179-183°C) [11]. Its molecular structure is shown in Figure 3.2. Several alternative strategies for formulating fenofibrate with enhanced bioavailability have recently been reported in the literature including an amorphous form stabilised in a mesoporous silica carrier [12], entrapment of amorphous fenofibrate into solid lipid nanoparticles [13], nanoparticle dispersion within a polymeric matrix layer surrounding internal lactose particles by fluidised bed processing [14], or nanoparticle loading into polymer strip films [15], each with their own advantages and disadvantages.

Figure 3.2  Molecular structure of fenofibrate.
In this work, nanosuspensions of fenofibrate were generated from supersaturated solutions using a simple antisolvent precipitation process. The impact of antisolvent process conditions on the particle formation/size were examined, including the solvent-antisolvent volume ratio, drug concentration, stirring rate, stabiliser type, stabiliser concentration and aging time in suspension. A discussion is herein provided as to why certain additives have superior or inferior suspension stabilisation capabilities based on the molecular chemistry of both the drug and the additive molecules. The optimum stabiliser system determined in this work improved the stabilisation period of a fenofibrate nanosuspension beyond that which was achieved in a previous report, prepared under comparable conditions [9]. Furthermore, the impact of drying on the redispersibility of the nanoparticles was examined, with a focus on the freeze-drying technique. Additional excipients were included to protect the nanoparticles from aggregation during the drying stage so as to promote their redispersion upon resuspension. Redispersibility was monitored in terms of dissolution characteristics of the dried product, since enhanced dissolution can be considered as the main advantage of a nanosized system.

3.3 Experimental Details

3.3.1 Materials. Fenofibrate (FF) (99.7% purity) was generously gifted from Abbvie Laboratories. Ethanol (99.8% purity) was purchased from Merck Millipore. Stabilisers; polyvinyl alcohols (PVA, MW: 9-10 kDa, 13-23 kDa, 85-124 kDa, 146-186 kDa), hydroxylpropyl methyl cellulose (HPMC, ~ 26 kDa), sodium dodecyl sulphate (SDS), α-lactose monohydrate (lactose), polyvinyl pyrrolidone K30 (PVP-K30, 40 kDa), Pluronic F127 (~12.5 kDa) and Kolliphor 188 (7.9 kDa) were purchased from Sigma Aldrich and polyethylene glycol (PEG, 3.4 kDa) was purchased from Polysciences Inc.. Drying aids; α-lactose monohydrate, D(+)-sucrose, D-mannitol, montmorillonite K-10, calcium phosphate dibasic dehydrate, kaolin and silicon dioxide were purchased from Sigma Aldrich and microcrystalline cellulose (36 kDa) was gifted from FMC BioPolymer. Hydrochloric acid and Tween-80 were purchased from Sigma Aldrich for use in dissolution testing. TriCor tablets (commercial milled nanoformulation containing 22.3% FF, 22.3% sucrose and 20.3% lactose monohydrate) were purchased from Abbott. Distilled water was used for sample preparations.
3.3.2 Antisolvent Process Parameters. The antisolvent process conditions were optimised by monitoring the effects of drug concentration (10, 30, 50 mg/mL), antisolvent to solvent (AS/S) volume ratio (5, 10, 20, 40 [constant FF mass added to AS/S mixture: 1.82 mg/mL]), agitation rate (200, 500, 800 rpm) and aging time (up to 31 min) on the resulting size and morphology after precipitation. The aging time refers to the time over which the precipitated particles are held in suspension before analysis or isolation. The AS/S experimental set was conducted at 30°C. All other experiments were conducted at 25°C. The solubility of FF in pure ethanol and in a 9.1 vol % aqueous solution of ethanol at 25°C, were determined by UV-visible spectrophotometry (Cary 300 Bio UV-vis at 289 nm) for calculation of the supersaturation level present during precipitation under optimum conditions.

3.3.3 Suspension Preparation and Stabilisation. An organic solution of FF in ethanol (1 mL, 50 mg/mL) was quickly introduced by eppendorf pipette to water (10 mL) with or without additives at the compositions outlined in Table 3.1. Solutions/suspensions were maintained at a constant temperature of 25°C under rapid agitation (800 rpm) throughout the precipitation process. On contact with the antisolvent, the FF immediately precipitated giving a fine milky suspension. The pure system refers to an un-stabilised system in which no additives were present in the antisolvent during precipitation.
### Table 3.1 Additive systems used during antisolvent precipitation

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Additive system</th>
<th>Additive conc. in AS (%w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PVP-K30</td>
<td>0.1 %</td>
</tr>
<tr>
<td></td>
<td>Pluronic F127</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PVA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HPMC</td>
<td></td>
</tr>
<tr>
<td>Additive screening</td>
<td>SDS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kolliphor 188</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PEG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Combinations of above additives</td>
<td>0.1 % each</td>
</tr>
<tr>
<td></td>
<td>PVA (9-10 kDa)</td>
<td>0.1 %</td>
</tr>
<tr>
<td></td>
<td>PVA (13-23 kDa)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PVA (85-124 kDa)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PVA (146-186 kDa)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PVA: Concentration</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PVA (9-10 kDa)</td>
<td>0.01 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.05 %</td>
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<tr>
<td></td>
<td></td>
<td>0.1 %</td>
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<tr>
<td></td>
<td></td>
<td>0.2 %</td>
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<tr>
<td></td>
<td></td>
<td>0.35 %</td>
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<tr>
<td></td>
<td></td>
<td>0.5 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.75 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0 %</td>
</tr>
<tr>
<td>Prep A</td>
<td>Pure system</td>
<td>N/A</td>
</tr>
<tr>
<td>Prep B</td>
<td>HPMC</td>
<td>0.05 %</td>
</tr>
<tr>
<td>Prep C</td>
<td>PVA (9-10 kDa)</td>
<td>0.2 %</td>
</tr>
<tr>
<td>Prep B</td>
<td>SDS</td>
<td>0.05 %</td>
</tr>
<tr>
<td>Prep B</td>
<td>Lactose</td>
<td>1 %</td>
</tr>
</tbody>
</table>

#### 3.3.4 Isolation and Drying

Following precipitation and aging in suspension (fixed time), particles were isolated into the solid phase by evaporation (oven-drying, 55°C), vacuum filtration (0.22 μm nylon membrane filter), centrifugation (4 min at 4000 or 13,400 rpm [1/4 scale]), spray-drying (B-290 Büchi Mini Spray Dryer) or freeze-drying (Dura-Dry Microprocessor Control freeze-dryer, < 27 Pa).

The evaporative method involved quick oven-drying of a drop of suspension at 55°C. The filtration method was only used to isolate particles from suspension in which the full size distribution lay above 220 nm. Centrifugation at 4000 rpm was used to sediment microsuspensions (Hettich Universal 32R centrifuge) in 20 ml glass vials, while centrifugation at 13,400 rpm was used to sediment nanosuspensions (MiniSpin Eppendorf...
microcentrifuge) in 2 mL Eppendorf tubes. The supernatant was decanted and sedimented pellets were frozen in liquid nitrogen and freeze-dried at <27 Pa overnight. Spray-drying was conducted in a semi-continuous fashion whereby FF was precipitated repeatedly (8 times) under batch conditions at 50 mL antisolvent scale, and the suspension (totalling 2 g FF) was immediately and continuously fed to the spray-dryer by peristaltic pump and dried in <6 min. An inlet temperature of 150°C, a feeding rate of 9.1 mL/min, an aspirator value of 100% and an air flow of 30 mm were used. Freeze-drying at <27 Pa for 48 hrs was conducted following flash freezing of the suspension in liquid nitrogen. In some cases, drying aids (listed in Section 3.3.1) were dissolved or suspended in the antisolvent phase before or 0.5 min after precipitation at solution concentrations of 0.5%, 1%, 5% and/or 10% w/v.

3.3.5 Particle Size Analysis. Particle size measurements were performed by laser diffraction on a Malvern Mastersizer 2000 (Mie theory), with water as the dispersion medium. Precipitated suspensions were diluted by a factor of 5 with water (0.91 mg/mL FF) prior to their introduction into the sample chamber. An obscuration of 7-10%, a stir rate of 2600 rpm, a one minute pre-measurement delay and 5% sonication power (wrt Malvern Mastersizer 2000 sonication scale) were used during all size measurements. Runs were conducted at room temperature and four measurements were recorded for each run. When no stabilising additives were present during precipitation, HPMC and SDS were added to the water dispersant (0.038 mg/mL each) to minimise variation between four consecutive measurements. Size distributions for FF were calculated using a refractive index of 1.55 and an absorption index of 0.01. Freeze-dried particles were redispersed in water at 0.91 mg/mL, and inverted 50 times, before measuring the size. The D[4,3] volume-weighted mean diameter was reported for each size distribution. Sizing experiments were carried out at least in duplicate.

The size of FF particles in TriCor tablets were measured using dynamic light scattering on a Malvern Zetasizer. Tablets were ground with a pestle and mortar for 2 min and the powder was suspended in water (1 mg/mL wrt FF content). The large insoluble excipients were removed by vacuum filtration with a hydrophilic 1.2 μm Millipore membrane filter (RAFT04700). The size of the sub-1.2 μm particles was measured (Appendix 3.1) and these particles were captured by further filtering the suspension through a 0.22 μm PVDF
membrane filter. FTIR (Perkin Elmer Spectrum 100 FT-IR Spectrometer) confirmed that the submicron particles were FF.

3.3.6 Scanning Electron Microscopy. Sizes and morphologies of particles in dried samples were analysed using a Hitachi SU-70 high-resolution scanning electron microscope (SEM). Oven-dried particles were prepared by drying a drop of suspension directly on carbon tape on an aluminium SEM stub in an oven at 55°C (drying took approx. 12 min). For all other drying techniques, a small amount of powder sample was placed on the carbon tape. All samples were coated with an ~8 nm gold deposit using an EMITECH K55 and the particles were imaged in field-free mode at a voltage of 10 kV and a working distance of 10 mm. The image analysis software, ImageJ, was used to analyse particle sizes (maximum dimension) from SEM images. All SEM images show particles which were fully representative of the entire sample analysed in each case.

3.3.7 X-ray Diffraction. Powder X-ray diffraction (XRD) was used to identify the crystalline form and to monitor the degree of crystallinity of the ‘as received’, and precipitated and dried samples. Diffraction patterns were recorded using a PANalytical Empyrean diffractometer in transmission mode using Ni filtered Cu Kα radiation (λ = 1.54 Å) at 40 kV and 40 mA. Powder samples were prepared by trapping a small amount of powder between two strips of amorphous scotch tape and scanning the samples in the angular range of 5° (2θ) to 35° (2θ).

3.3.8 Dissolution Studies. A 0.1M HCl solution containing 0.4% w/v Tween-80 was used as the dissolution medium (DM).

Solubility: Prior to dissolution rate testing, the solubility of FF (‘as received’, TriCor and precipitated samples) in the DM was measured in order to determine sink conditions for dissolution testing. The solubility was determined by UV-visible spectroscopy (Cary 300 Bio, λ - 288 nm) by increasing the FF amount in the DM until the saturation limit was reached, as seen by a plateau effect in the calibration curve (Appendix 3.2).

Dissolution rate testing: Dissolution tests were carried out in sink conditions at approx. 1/5 of the solubility concentration. Tests were carried out at 42°C and were conducted by adding a sample (solid or suspension) containing 25 mg FF to 900 mL of the DM. TriCor tablets were ground by pestle and mortar for 2 min, prior to dissolution testing. After sample addition, 4 mL aliquots were taken at regular intervals from the bulk solution in
preheated (45°C) plastic syringes and filtered through preheated (45°C) PTFE 0.2 μm syringe filters. The dissolved FF concentration was measured by UV-visible spectroscopy (Cary 300 Bio, λ - 289 nm). Dissolution rates are expressed as the % dissolved/second over the first 30 seconds of dissolution. Dissolution tests were carried out at least in duplicate. It was noted that the presence of ethanol (0.055 mL/100 mL DM) in the suspended samples had no significant impact on the dissolution rate or overall solubility.

3.4 Results

3.4.1 Effect of Antisolvent Process Parameters. The solubility of FF in pure ethanol (solvent) and in a 9.1 vol % aqueous solution of ethanol (antisolvent) at 25°C, were found to be 63 mg/mL and 1.17 µg/mL respectively (AS/S volume ratio: 10), making them a suitable combination for antisolvent precipitation. The highest drug concentration tested during antisolvent precipitation (Prep A, 50 mg/mL) produced the smallest particles (200-300 nm, aging time 0.5 min), with a monomodal distribution, Figure 3.3. Lower drug concentrations produced bimodal distributions consisting of both submicron and micron range particles. The antisolvent to solvent (AS/S) volume ratio and the agitation rate had no obvious impact on the particle size over the ranges tested (Appendix 3.3).

![Particle size distributions of FF precipitated from varying drug concentrations at an aging time of 0.5 min.](image)

**Figure 3.3** Particle size distributions of FF precipitated from varying drug concentrations at an aging time of 0.5 min.

Based on the size results obtained for the pure sample (no additives, Prep A), a drug concentration of 50 mg/mL, an AS/S ratio of 10:1 and an agitation rate of 800 rpm were
chosen as the standard process conditions for additive-based precipitation studies. Under such precipitation conditions, a supersaturation level of 3888 was present (Appendix 3.4).

3.4.2 Preparation and Stabilisation of FF Nanosuspensions. Additives were introduced to the antisolvent phase to stabilise the size of the nanoparticles which precipitated. All additive systems enhanced the stability of the suspended nanoparticles compared to the pure system, Figure 3.4. Results pinpointed PVA as the most effective stabiliser both by preserving the particles in the nano size range for the longest time (11 min) and by providing long term stabilisation for the 4-5 µm particles which developed by 21 min. Kolliphor 188 also stabilised particles in the submicron range for at least 11 min, but its longer term stabilisation ability was poor, with the particles reaching 20 µm by 2 hrs (data not shown). No further improvements in stabilisation were observed when PVA was used in mixtures with other additives, Figure 3.4. Additional analysis indicated that PVA with a molecular weight of 9-10 kDa at a concentration of 0.2% w/v provided the optimum stabilisation conditions for FF (Figure 3.5). Increasing the PVA concentration above 0.2% did not improve the stabilisation. Furthermore, the antisolvent process was scalable by a factor 5 without compromising the size of the PVA-precipitated nanoparticles (Appendix 3.5).

Figure 3.4 Size of precipitated FF as a function of additive system and aging time.
**Figure 3.5** Analysis of the effect of PVA concentration and molecular weight on FF nanoparticle stability

Figure 3.6 shows the time-resolved particle size distributions for three antisolvent systems: pure system (no additives, Prep A), a solution of HPMC/SDS/lactose (as a comparison to results by Hu et al. [9], Prep B) and a solution of PVA (Prep C). The additive-induced nanoparticle size stabilisation was only temporary. The time-resolved charts in Figure 3.6 show the time at which the particles grow out of the nano size range.

**Figure 3.6** Evolution of the particle size distributions over time for FF precipitated from (a) a pure system (no additives), (b) solution of HPMC/SDS/lactose and (c) solution of PVA
The mean particle size was determined for each distribution. Figure 3.7b shows that nanoparticles were preserved for 2 min for the pure system, 6 min when precipitated in HPMC/SDS/lactose and 11 min when precipitated in PVA. In each system, the initial period of slow growth was followed by a period of accelerated growth, before the size plateaued and no further growth occurred (during a monitored 24 hr period), Figure 3.7a.

Figure 3.7  Particle size evolution of precipitated FF with aging time in suspension

FF nanoparticles in suspension which were precipitated in the pure system (~600 nm, 3.18%/s), or in a solution of HPMC/SDS/lactose (~200 nm, 2.28%/s) or PVA (~300 nm, 2.55%/s) dissolved at a rate equivalent to that of a commercial nanoformulation of the drug (TriCor, ~260 nm, 2.45%/s). However, in accordance with the Noyes Whitney equation, the dissolution rate decreased as the size increased into the micron range, Figure 3.8. PVA-precipitated particles which reached a stable size of ~4.5 µm, dissolved at a rate of 0.55%/s (at 31 min aging time), while pure precipitated particles which reached a stable size of ~30 µm, dissolved at a rate of 0.23%/s (at 31 min aging time).
Figure 3.8  Dissolution profiles of FF suspensions precipitated in pure and additive systems at short (0.5 min) and long (31 min) aging times. Dissolution profiles of commercial and ‘as received’ FF powder samples are shown for comparison.

3.4.3  Isolation and Drying of Nano and Microsuspensions. The influence of isolation and drying on FF nano and microsuspensions was investigated (focusing on the PVA-stabilised system), with the objective being to preserve an unmodified particle size, and maintain enhanced dissolution rates upon redispersion.

Evaporation. Dried particles with sizes equivalent to those of the particles in suspended state were obtained across the nano and micron size range when a drop of suspension containing PVA-precipitated particles (at various aging times) was quickly dried by evaporation in an oven at 55°C. Figure 3.9a depicts segregated FF nanoparticles (~380 nm) at the shortest aging time (as determined by drying time: ~12 min). Following a rapid growth stage, particles reached a stable size of 4.7 µm (Figure 3.9c), which was again representative of the original pre-dried particle size in suspension (Figure 3.7a). Particles matured from spherical to faceted parallelepiped crystals over time. While samples dried in this way were representative of the particles in suspension, only small quantities could be isolated.
Figure 3.9  SEM images of oven-dried, PVA-precipitated FF particles at aging times of (a) ~12 min, (b) ~12.7 min, and (c) ~13.3 min. The particle size and morphology observed in image (c) remained unchanged with additional aging time.

Filtration. Filtration (0.2 µm) of a PVA-precipitated nanosuspension (~300 nm) was inefficient due to loss of the finer particle fraction (31.5%) through the filter, until eventual filter blockage occurred. However, particles in the micron range (4-5 µm, 31 min aging) were easily filtered, and maintained an unmodified particle size (Figure 3.10). As with fully-grown, oven-dried particles, fully-grown filtered particles were parallelopiped with well-faceted, sharp edges, and were representative of the particle size in suspension, Figure 3.10. However, filtration (at least under the conditions tested) was unsuccessful for smaller particles.

Figure 3.10  SEM image of PVA-precipitated fenofibrate crystals, aged in suspension for 31 min before filtering and vacuum drying.
Centrifugation. A low speed 4,000 rpm benchtop centrifuge successfully sedimented PVA-precipitated microparticles (31 min aging, 4-5 µm) but could not sediment freshly-precipitated nanoparticles (0.5 min, 300 nm). A higher speed 13,400 rpm eppendorf microcentrifuge was successful for sedimenting the nanoparticles into a solid pellet. However, only a small quantity of material could be isolated in this manner (2 mL/Eppendorf). Figure 3.11a shows significant aggregation of primary nanosized particles (~530 nm) in the dried sample. The parallelepipeded microparticles (at 31 min aging time) which were isolated by centrifugation were comparable in size and habit to those isolated by evaporation (Figure 3.9c) and filtration (Figure 3.10).

Figure 3.11  SEM images of centrifuged, decanted and vacuum-dried PVA-precipitated FF (a) nanoparticles (0.5 min aging) at 13,400 rpm and (b) microparticles (31 min aging) at 4,000 rpm.

Spray-drying. Spray-drying produced a combination of micron-sized spheres and irregular aggregates containing visible nanosized particles, Figure 3.12. XRD analysis showed characteristic peaks for fenofibrate, indicating that the crystalline form was present, Figure 3.13. The curved baseline is attributed to the amorphous scotch tape used to hold the sample in place when XRD is run in transmission geometry. However, it cannot be verified if any of this curvature is caused by partial amorphous content. Due to the aggregation of nanoparticles, the dissolution rate of the spray-dried material was significantly poorer than that of the freshly precipitated, dispersed nanoparticles in suspension, Figure 3.14. Following fast burst dissolution of 40% of the FF content, the remainder took approx. 7 hrs to dissolve completely.
Figure 3.12  SEM images of spray-dried PVA-precipitated FF at <6 min aging time

Figure 3.13  X-ray diffraction patterns of fenofibrate (a) as received and (b) after precipitating in PVA solution and spray-drying
Figure 3.14 Dissolution profiles of suspended and spray-dried FF nanoparticles after precipitating in a PVA solution. The dissolution profile of TriCor, the commercial nanoformulation of FF, is shown for reference.

Freeze-drying. The influence of freeze-drying on FF particles which were precipitated from all three antisolvent systems (pure water, HPMC/SDS/lactose and PVA) was first investigated, followed by a more detailed analysis of the PVA-based system.

Time-resolved SEM imaging captured the particle development over time for each antisolvent system. Precipitation from pure water produced chunky, faceted FF crystals which grew to a mean size of 4.1 µm within 15 min (Figure 3.15a). Precipitation in HPMC/SDS/lactose showed a non-uniform crystal habit over time. Some sub-micron spheres (0.9 µm) were observed when aged for 2 min, but they gradually evolved into flat elongated 4 µm plates with fused tips to form aggregated structures of approx. 10 µm in length (Figure 3.15b). Over time, PVA-precipitated FF particles developed from smooth-surfaced but rounded, immature and irregular particles at 0.5 min, to roughly-surfaced particles at 11 and 21 min, to smooth-surfaced parallelepiped-shaped particles with defined edges at 31 min (Figure 3.15c, this morphological development is discussed further in Chapter 5).
Figure 3.15  Time-resolved SEM images of freeze-dried FF precipitated in (a) pure water, (b) HPMC/SDS/lactose solution, and (c) PVA solution.

XRD analysis of the freeze-dried formulations at the shortest aging times (< 2 min) clearly showed characteristic peaks for fenofibrate, indicating that the crystalline form was present, Figure 3.16. Again, it cannot be said with certainty that none of the baseline curvature is attributed to partial amorphous content.
Figure 3.16  X-ray diffraction patterns of fenofibrate (a) as received, and after freeze-drying following precipitation in (b) pure water, (c) a solution of HPMC/SDS/lactose and (d) a solution of PVA (< 2 min aging in suspension)

Dissolution testing of freeze-dried samples (Figure 3.17) showed that dissolution of the pure material aged for 2 min was higher than that of a sample which was aged for 30 min. The dissolution rate of HPMC/SDS/lactose-precipitated particles, aged for 2 and 30 min were similar. The dissolution rate of PVA-precipitated particles aged for < 2 min was surprisingly lower than that of particles which were aged for 31 min. The 31 min PVA-precipitated sample showed the best dissolution rate of all the dried samples (0.45% /s). Additional tests showed that the additives did not alter the solubility of the FF in the dissolution medium at the concentrations present in the dried samples (Appendix 3.2).
Figure 3.17  Dissolution profiles of freeze-dried FF, precipitated in pure water, HPMC/SDS/lactose solution, and PVA solution at short (< 2 min) and long (> 30 min) aging time. The dissolution profiles of TriCor and the 'as received' material are shown for reference.

Due to the opposing influence of aging time on dissolution rate for freeze-dried (Figure 3.17) vs suspended (Figure 3.8) PVA-precipitated particles, additional analysis of this system was conducted. Dissolution rates were categorised into fast, medium and slow dissolution regions (Figure 3.18). The commercial nanoformulation (TriCor) and the freshly precipitated nanoparticles in suspension (≤ 11 min aging time) lay in the fast dissolution region. Suspended particles which had grown into the micron size range (≥ 21 min) and the majority of the dried particles lay in the medium dissolution region. However, only the 0.5 min dried sample lay in the slow dissolution region (Figure 3.18). After drying, the 4 min sample had the highest dissolution rate (0.65%/s). The difference in dissolution rates between suspended and dried particles decreased as aging time (and thus particle size) increased. Particle sizing of dried and redispersed material showed that micron range particles (4.5 µm) could maintain their original size during drying while nanoparticles could not. Particles with a pre-dried size of 300 nm grew/agglomerated to 16 µm during drying (Figure 3.19), explaining their poor dissolution.
Figure 3.18  Dissolution profiles comparing the effects of aging time on dissolution rate for suspended (blue/green) and freeze-dried (red/pink) FF precipitated from a PVA solution. The dissolution profiles of TriCor and the ‘as received’ material are shown for reference.

Figure 3.19  Time-resolved particle sizing of PVA-precipitated FF (♦) in suspension (before drying), and (■) after freeze-drying and re-suspending.
3.4.4 Soluble and Insoluble Cryoprotectants. Due to the instability of the nanoparticles during drying, additional drying aids (cryoprotectants) were incorporated into the system to protect the nanoparticles from drying-induced irreversible aggregation. Both soluble and insoluble cryoprotectants were tested.

Use of water-soluble cryoprotectants (e.g. lactose, sucrose and mannitol) in the PVA-based antisolvent phase during antisolvent precipitation improved the dissolution rates of the freeze-dried nanoparticles compared to a PVA-precipitated control sample (at 0.5 min aging), Figure 3.20. Preliminary tests indicated that dissolution rates of samples prepared with water-soluble cryoprotectants were highest when the cryoprotectant was present in the antisolvent during precipitation, at a solution concentration of 5% w/v, and when the aging time was short (e.g. 0.5 min). Under these conditions, initial dissolution rates were highest in samples containing lactose (2.41% dissolved/s) and sucrose (2.40% dissolved/s) and those rates were comparable to that of the commercial reference nanomaterial (TriCor, 2.45% dissolved/s). However, after an initial burst release to 72%, the dissolution rate slowed considerably until complete dissolution was eventually reached (< 7 hrs, exact time unknown).

Figure 3.20 Dissolution profiles of freeze-dried FF, precipitated in a PVA-based antisolvent containing additional lactose, sucrose and mannitol (5% w/v, 8.8 wt % FF) and aged for 0.5 min before freezing. The dissolution profiles of TriCor and the ‘as received’ material are shown for reference.
Incorporation of water-insoluble excipients/carriers as cryoprotectants (e.g., montmorillonite, microcrystalline cellulose, calcium phosphate, kaolin, silicon dioxide) into the PVA-based antisolvent phase during antisolvent precipitation had varying impact on the dissolution rates of the freeze-dried material. Preliminary tests indicated best dissolution results in conditions where the cryoprotectant was present in the antisolvent during precipitation, and when the aging time was increased from 0.5 to 4 min. Dissolution rates were higher than that of a PVA-precipitated control sample (at 4 min aging, 0.65% dissolved/s) when MMT (1.91% dissolved/s) and MCC (0.76% dissolved/s) were present. All other excipients reduced the dissolution rate compared to the control sample, Figure 3.21.

These results highlight the importance of the drying step in the generation of nanoparticle formulations.

**Figure 3.21** Dissolution profiles of freeze-dried FF, precipitated in a PVA-based antisolvent containing additional insoluble excipients (5% w/v) and aged for 4 min before freezing. The dissolution profiles of TriCor and the ‘as received’ material are shown for reference.
3.5 Discussion

Precipitation is a solid-forming process which is triggered by conditions of high supersaturation. During precipitation, rapid nucleation is followed by particle growth. The supersaturation level controls the nucleation rate which in turn controls the particle size. High supersaturation which is induced by poor solubility, high drug concentration and high AS/S ratio encourages nucleation to dominate over growth, resulting in the formation of smaller particles, as reported previously for many compounds [16-18]. The inverse trend between concentration and size validates that it is not the growth rate, but the nucleation rate, and ultimately the number of particles than governs the particle size. Variations in the concentration had a bigger influence on the particle size than variations in the AS/S volume ratio. This result can be explained by differences in the local supersaturation at the point of injection of the drug solution. The drug mass varies with variations in the concentration (at a fixed AS/S ratio). Higher concentration, and thus higher drug mass, induces a steep local supersaturation gradient at the drug solution/antisolvent interface before uniformity is achieved by mixing. In our system, the effects of local supersaturation gradients were smaller for changes in the AS/S volume ratio, since the added drug mass was constant, and solubility changes were minimal.

Using optimised antisolvent process parameters (50 mg/mL FF solution, 1 mL addition into 10 mL antisolvent, 800 rpm), nanosized particles were successfully produced by antisolvent precipitation. While nanoparticle preparation was relatively straightforward, stabilisation of their small size was a greater challenge. Additives were required to stabilise the nanoparticles in suspension and during their isolation into the solid state. The growth rate of precipitated particles depended on the additive system used. The interaction strength between the drug particles and the stabilising additives governed the stabilisation efficiency. Of the systems tested, PVA provided the most successful stabilisation for FF particles in suspension. PVA is an FDA-approved GRAS additive (generally recognized as safe), which has been reported to stabilise particles by coating them with a thick and stable protective layer [19]. This polymer has an abundance of hydrogen bond donors while FF has four hydrogen bond acceptors and no donors. This combination gives PVA high potential to hydrogen bond with FF surfaces. While many of the other tested additives have functional groups capable of hydrogen bonding (eg. the
pluronic, PEG, HPMC, PVP-K30), the presence of hydrogen bond accepting groups in these molecules limits their ability to interact with FF. Molecules with both accepting and donating capabilities can form intramolecular and interchain hydrogen bonds, thus decreasing their drug-polymer interaction potential. The initial stabilisation induced by Kolliphor 188 was attributed to its lower molecular weight compared to the other polymers tested, which lessens its steric effects on interacting with the FF molecule. However, the presence of both hydrogen bond accepting and donating groups in this molecule dampens its longer term stabilisation abilities. The stabilisation efficiency of PVA for the precipitated particles was further improved by selecting the concentration and molecular weight which induced the slowest growth rate. It is likely that these optimum conditions indicate the point at which the surface coverage and coating thickness of the PVA were respectively optimised. PVA enhanced the stability of FF nanoparticles compared to that reported previously for this compound under comparable preparation conditions [9]. In addition, it allowed replacement of an electrostatic/steric (SDS/HPMC) stabilisation combination with a single steric stabiliser. Electrolytes which are present in the gastrointestinal tract can impair the efficiency of electrostatic stabilisers by reducing zeta potential and physical stability. Steric stabilisers are less impaired in efficiency in electrolyte containing environments, making them a more attractive stabiliser choice. Furthermore, unlike other reports on FF nanoparticles [9, 20-22], a commercial nanoformulation (TriCor, milled) was used as the reference gold standard in terms of desired particle size (260 nm) and dissolution rate (2.45%/s). This size and dissolution rate was matched by PVA-precipitated particles in suspension (~300 nm, 2.55%/s).

However, even at optimised process conditions with the optimal stabiliser, the stability of the PVA-precipitated nanosuspension was only temporary. Suspended particles gradually evolved from nano to small micron size in a manner corresponding to Ostwald ripening, until the nanosized fraction was fully depleted and a stable size of 4-5 µm was reached (Figure 3.6c). A corresponding reduction in dissolution rate ensued. For long term stabilisation and for convenience during oral administration, nanoparticles are generally isolated into the solid state for incorporation into solid dosage forms such as tablets. However, due to the limited stability of the nanoparticles in suspension (11 min), a rapid isolation process was required. Evaporation, filtration, centrifugation, spray-drying, and freeze-drying approaches were explored for bringing the nanoparticles into the solid-state.
PVA-precipitated particles across the nano and micron size ranges were effectively dried by evaporation in an oven at 55°C, to produce dried material with suspension-equivalent particle sizes (as determined visually by SEM analysis). The evaporation temperature was limited by the melting point of FF (81-83°C). Due to the aqueous nature of the antisolvent and the high heat of vaporisation of water, evaporation was slow and was thus only capable of drying small amounts of suspension (1 drop) within the period of stabilisation. The evaporation technique was therefore limited in its scalability. Particle isolation by filtration, which is an industrially friendly separation/drying technique, was unsuccessful for isolating nanoparticles using a 0.22 µm pore filter at 10 mL antisolvent scale. During vacuum filtration, the smaller particle fraction initially passed through the filter before nanoparticles concentrated in the filter pores, caked and ultimately blocked the filter. On the other hand, centrifugation could successfully separate (most) nanoparticles from suspension by sedimentation into a solid pellet, but only under high centrifugal forces (13,400 rpm) at small 2 mL scale. Despite their limitations for nanoparticle isolation, both filtration and centrifugation were successful, industrially-feasible and scalable techniques for isolating particles in the small-micron range (4-5 µm) with suspension-equivalent particle sizes (as determined visually by SEM analysis, Figures 3.10, 3.11b). Spray-drying and freeze-drying are the most commonly used processes for solidification of pharmaceutical nanosuspensions [19] but are known to often induce aggregation of nanoparticles as solvent is removed. Dissolved stabilisers that are incorporated into the system to stabilise the nanosuspension may precipitate upon concentration, rendering them unavailable to protect the nanoparticles from aggregation. Strong aggregation of nanoparticles will reverse the advantage of rapid dissolution which is central to nanosuspension formulations.

For all three antisolvent systems (pure, HPMC/SDS/lactose and PVA), the dissolution rates of the freeze-dried particles (Figure 3.17) were poorer than those of their suspended equivalents (Figure 3.8). The dissolution rate of the pure dried sample (no additives, Figure 3.17) decreased with increasing particle size (aging time, as observed by SEM, Figure 3.15a). The dissolution rate of the HPMC/SDS/lactose-prepared dried sample (Figure 3.17) remained constant with aging time due to the comparable surface area to volume ratios of 0.9 µm spheres (2 min) and 4 µm plates (30 min), Figure 3.15b, thus highlighting the importance of particle shape in addition to particle size. The poorer dissolution rate of PVA-precipitated FF (Prep C) at 0.5 min compared to 31 min can only
be explained by aggregation and fusion of insufficiently stabilised submicron particles (at 0.5 min) during the destabilising drying process, a trend which is discussed in greater detail in Chapter 5.

SEM imaging of spray-dried and freeze-dried PVA-precipitated FF (Figure 3.12 and 3.15c) indicated visually that the precipitated nanoparticles were not preserved as individual entities during drying. The addition of soluble (lactose, sucrose and mannitol) or insoluble (montmorillonite and microcrystalline cellulose) drying aids in addition to PVA improved the dissolution rate of the freeze-dried material by aiding in nanoparticle segregation during drying. The fastest dissolution for dried FF samples was obtained in the presence of lactose (soluble), sucrose (soluble) and montmorillonite (insoluble) at solution/suspension concentrations of 5% w/v. In the case of the soluble sugars, dissolution testing showed an initial burst release to >70% dissolved within 0.5 min, followed by a slower gradual increase in the dissolved content towards 100%. This two phase dissolution trend indicated preservation of a large portion of the nanoparticles (which dissolved quickly) followed by slower dissolution of the smaller fraction of nanoparticles which aggregated during drying. Soluble sugars (cryoprotectants) function by immobilising nanoparticles within their glassy matrix to protect the nanoparticles both from the mechanical stresses of ice crystal formation and from forced aggregation and potential fusion of nanoparticles upon concentration of the liquid phase during freezing. Certain cryoconcentrated solutions such as those containing lactose and sucrose reach a maximum concentration (~80%) before they vitrify into a glassy matrix within which the nanoparticles are dispersed [19]. Cryoconcentrated mannitol, on the other hand, is known to precipitate into its own phase (crystalline or amorphous) during the freezing step [19, 23], which may limit its ability to protect the nanoparticles from aggregation. This could explain the poorer results afforded by mannitol compared to sucrose and lactose. In the case of the soluble drying aids, shorter aging time resulted in faster dissolution. However, in the case of the insoluble drying aids (carrier particles), an extended aging time of 4 min was required to allow sufficient contact time for attachment of the PVA-stabilised nanoparticles to the surface of the carrier particles. In contrast to soluble drying aids, the goal of the insoluble carrier particles is to adsorb a segregated dispersion of nanoparticles on their surface, so that the nanoparticles become desensitised to freezing and drying stresses. While montmorillonite and microcrystalline cellulose improved the dissolution rates of the dried material compared to a control sample, other carriers such as calcium
phosphate, kaolin and silicon dioxide all contributed to reduced rates of dissolution. This may be attributed to poor or incomplete adsorption of the nanoparticles to the carriers, and subsequent destabilisation of the nanoparticles in the liquid pockets between adjacent carrier particles. Despite the improvement in dissolution rates in the presence of certain drying aids, in no case was the dissolution profile of the commercial nanoformulation (TriCor) fully replicated for the freeze-dried samples. Chapter 4 will discuss an alternative approach for isolating FF nanoparticles while maintaining TriCor-equivalent dissolution rates.

3.6 Conclusions

Nanosized FF particles (200-300 nm) were successfully precipitated from a supersaturated solution with an equivalent size and dissolution rate to that of a milled commercial nanoformulation. The stability of the particles in the nanosize range was short-lived and additives were required to slow their growth rate. PVA showed the most effective stabilisation for FF due to the characteristic hydrogen bonding capabilities of both PVA and FF, compared to the other additives tested. The stabilisation of the suspended nanoparticles by PVA was only temporary and so immediate isolation from suspension was necessary. All drying techniques tested facilitated successful drying of particles which lay in the small micron size range. However, drying of high-energy nanoparticles with preserved particle sizes and dissolution rates was more problematic. Only evaporative drying at small volume enabled size preservation of both nano and microparticles during the drying step. The use of additional drying protectants aided in the redispersibility of freeze-dried particles (as indicated from dissolution rates), but even under optimised conditions, the fast dissolution rates of the suspended nanoparticles or the commercial nanoformulation (TriCor) were not quite replicated. However, this work demonstrated the important influence of additives and isolation method on the final size and shape of the crystals, both of which are determining factors on the resulting dissolution rate.
3.7 References


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Chapter 4

Carrier Particle Design for the Stabilisation and Isolation of Drug Nanoparticles

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4.1 Abstract

Nanoparticles of poorly water-soluble drugs were prepared in suspension via antisolvent precipitation in order to improve their dissolution behaviour. Insoluble, surface-functionalised, micron-range, clay carrier particles were employed for the dual purpose of stabilising the nanoparticles in suspended state, and facilitating their unhindered isolation to solid state; often challenging steps in nanoparticle production. The carrier particles attracted the precipitated nanoparticles to their surface at drug loadings up to 9% w/w. Carrier-bound nanoparticles were stable in suspension for 24 hours and were easily filtered to produce solid-state nanoparticle composites, with fast dissolution rates stemming from their high surface area. The process was validated with two poorly water-soluble BCS Class II drugs; fenofibrate and mefenamic acid.
4.2 Introduction

The isolation of drug nanoparticles from suspension into the dried state is a research area that has remained quite unexplored to date. Many academic studies have focused on controlled crystallisation/precipitation approaches to prepare nanoparticles in suspension, as discussed in Chapters 1 and 3. However, drug nanoparticles in suspended state have a predisposition to physical and chemical instability and have a tendency to grow or aggregate to reduce their free energy. Solubilised additives are generally required to stabilise the nanoparticle size in suspension, requiring rigorous stabiliser screening approaches; but isolation from suspension into the solid-state is normally necessary for longer term stabilisation.

Conventional nanoparticle isolation techniques (eg. freeze-drying [1-3], spray-drying [2, 4-6] and centrifugation [7]) are quite complex processes which can induce nanoparticle aggregation and consequential reduction in dissolution rate compared to the suspended nanoparticles (as was observed in Chapter 3). Furthermore, certain drying techniques are only suitable for isolating small amounts of material, eg. ultracentrifugation [7], rotary evaporation [8] and oven-drying [9], which limits their potential for scaling up.
In recent years, some novel strategies have been proposed for isolating nanoparticles from suspension. Nekkanti et al. reported a method of converting a drug nanosuspension into solid form by layering the suspension onto water-soluble carriers in a fluid bed processor. The granules are then dried as they move upward in the airflow of the fluid bed [10]. Basa et al. used this method to coat lactose monohydrate with a layer of ketoconazole nanoparticles [11, 12]. Matteucci et. al described a method of salt flocculation to induce reversible clustering of nanoparticles in suspension, thus allowing a more efficient filtration. This method is applicable to polymer-stabilised nanosuspensions, whereby the addition of a salt weakens the hydrogen bonding strength between the drug and the polymer, causing a malfunction in the stabilisation, and subsequent flocculation. Open flocs were produced which were highly redispersible [13]. Dong et al. (2014) used montmorillonite clay as a matrix former, to prevent agglomeration of fenofibrate nanoparticles during spray drying. Agglomeration occurred when a sugar-based matrix former was used, causing clogging of the spray-drying apparatus. Replacement with the clay-based system prevented agglomeration and allowed smooth operation of the spray dryer without clogging [4]. Palo et al. investigated the use of flexographic printing onto edible substrates to convert nanosuspensions of indomethacin and itraconazole into solid dosage forms [14]. In 2014, Khan et al. showed that insoluble carrier particles composed of dibasic calcium phosphate could be used to recover nanocrystals of ibuprofen and glibenclamide from suspension by filtration [15]. Filtration is a desirable particle separation technique at industrial scale, but can become problematic when dealing with small micron or nanosized particles. Adsorption of nanoparticles to relatively large carrier materials can facilitate their filtration. The concept of using carrier particles has had widespread application in the preparation of dry-powder inhaler formulations [16-18] and in ordered mixing [19] where sugars have regularly been employed as carrier particles. However, the report by Khan et al. presented the first and, to our knowledge, only application for carrier mediated-filtration of drug nanocrystals from suspension. However, the process was notably limited by a low maximum drug loading of 0.35%, restricting its application to high potency drugs [15]. In addition, prior knowledge of drug-specific, soluble stabilisers was required, which was the focus of an earlier study by the group [7].

This work presents a novel one-step method for preparing fast-dissolving, stable nanoparticles of BCS Class II drugs (fenofibrate and mfenamic acid) in the solid state.
Fenofibrate (log P 5.24 [20]) and mefenamic acid (log P 4.83 [21]) are highly hydrophobic, poorly water-soluble BCS class II drugs, with dissolution-limited bioavailability. Their molecular structures are shown in Figure 4.2. Nanosizing has been shown to enhance the dissolution behaviour of both drugs [4, 22-26]. Previously, our group prepared and stabilised nanosuspensions of fenofibrate [22], and mefenamic acid [26] by antisolvent precipitation in the presence of dissolved polymer and surfactant-based additives. However, in both cases, the dissolved stabilisers only provided short-term stabilisation in suspension and were incapable of stabilising the nanoparticles during isolation and drying, causing them to forego their nanoparticle-induced dissolution enhancement. In the present work, the precipitated nanoparticles were captured from suspension and stabilised on the surface of a functionalised clay carrier excipient. Carrier-bound nanoparticles were isolated from suspension by filtration, and their fast nanoparticle-induced dissolution rates were preserved during the isolation and drying processes. The previously used optimum additives for each drug system were omitted in the presence of carrier particles, as their function was made redundant. Nanoparticle/carrier composites were prepared with a drug loading of up to 9%, for further use in the final drug formulation.

The carrier (montmorillonite, MMT) is an insoluble, negatively-charged aluminosilicate clay with high ion-exchange capacity. Its surface functionalisation agent (protamine, PA) is a positively-charged cationic polymer which can easily adsorb to the clay following an ion-exchange process [27, 28] and attract negatively-charged drug nanoparticles to its surface. Both MMT and PA have FDA approval as inactive ingredients [29].

Figure 4.2 Molecular structures of (a) fenofibrate and (b) mefenamic acid
4.3 Experimental Details

4.3.1 Materials. Fenofibrate (as received, 99.7% purity) and fenofibrate choline salt (crude) were generously gifted from Abbvie Laboratories. Converted fenofibrate (FF) was prepared from the salt form as outlined in Section 4.3.2. Ethanol (99.8%) was purchased from Merck Millipore. Mefenamic acid (MEF, Form I, >98%), N, N-dimethylacetamide (DMA, >99.9%), montmorillonite K10 (MMT, 220-270 m²/g), protamine sulphate salt from salmon (PA, amorphous, approx. 5.1 kDa), hydrochloric acid, Tween-80, isopropanol (≥ 99.9%), thionyl chloride (≥ 99.9%) and potassium carbonate (≥ 99%) were purchased from Sigma Aldrich. Potassium dihydrogen phosphate and disodium hydrogen phosphate were purchased from VWR International. TriCor tablets (commercial nano formulation of FF) were purchased from Abbott. Ponstan capsules (commercial micron formulation of MEF) were purchased from Chemidex Pharma Ltd. Distilled water was used for sample preparations.

4.3.2 Conversion of Fenofibrate Choline Salt to Fenofibrate. Fenofibrate choline salt (1 eq.), a choline salt of fenofibric acid, was dissolved in water (2 volumes). HCl (1.2 eq.) was added to protonate the salt, causing the insoluble fenofibric acid to precipitate. Fenofibric acid was isolated by filtration, washed 3 times with cold water (1 volume for each wash) and recrystallised from a minimum amount of hot isopropanol. The recrystallised product was filtered, washed with 1 volume cold water and oven-dried at 60°C overnight. Fenofibric acid was converted to fenofibrate by esterification according to a patented procedure [30] with a yield of 94.8%. Converted fenofibrate was characterised by ¹H NMR (Joel EX 270 NMR (270MHz) with DMSO-d6 solvent), FTIR (Perkin Elmer 100 FT-IR at 4 cm⁻¹), XRD (PANalytical Empyrean diffractometer in transmission mode, using Ni filtered CuKα radiation (λ= 1.54 Å) at 40 kV and 40 mA in the range 5–40 °2θ) and SEM (high resolution Hitachi SU-70) and compared to that of the as received fenofibrate (see Appendix 4.1- 4.4). Only SEM images depicted any difference between the two materials, with slightly larger, smoother particles present in the converted material (unimportant since FF was subsequently dissolved). The melting point range of both materials was 81-83°C (Stuart SMP20 MP apparatus). Converted FF was used for all further experiments.
4.3.3 Functionalisation of the Montmorillonite Surface. Protamine sulphate salt was dissolved in 10 mL water at concentrations ranging from 0.05 - 10 mg/mL. Montmorillonite clay (0.4 g – 1 g) was added to the protamine solution, and agitated at 25°C for > 2 hrs. The surface coverage of protamine on montmorillonite was altered by increasing the ratio of protamine to MMT (2 - 1000 mg PA/g MMT) until the saturation limit of the MMT surface was reached. PA-MMT samples were equilibrated for > 2 hrs, before vacuum-filtering using Whatman filter paper 50 (2.7 µm pore, 35 mm cross-section). The concentration of protamine remaining in the filtrate was measured by UV-visible spectroscopy (Shimadzu UV-1280) at a wavelength of 200 nm and served as an indication of the loading of protamine to the MMT.

4.3.4 Zeta Potential Determination. Zeta potential measurements were conducted on a Malvern Zetasizer Nano ZSP system. Zeta potential was determined from the electrophoretic mobility using the Smoluchowski approximation. The precipitated samples (without dilution) were filled into a folded capillary cell and equilibrated at 25°C for 120 s before measurement. Three measurements were taken per run and each sample was run twice. The average value and the variation between measurements were reported.

4.3.5 Synthesis of Nanoparticles and their Loading onto Carrier Particles. Suspended nanoparticles of both fenofibrate (FF) and mefenamic acid (MEF) were generated by antisolvent precipitation. An organic solution of FF in ethanol (1 mL, 50 mg/mL) was quickly introduced by Eppendorf pipet to 10 mL antisolvent containing (a) water, (b) an MMT suspension in water (100, 80, 50, 30 mg/mL, equilibrated for >2 hrs) or (c) a protamine-modified MMT suspension in water (50 mg/mL, 4.6 – 189.9 mg PA/g MMT, equilibrated for >2 hrs). Solutions/suspensions were maintained at 25°C under rapid agitation (800 rpm) throughout the precipitation process. For standard experiments, particles were aged for 1 min before drying. Exceptions were made for stability testing. Aging time refers to the time period from precipitation to isolation during which the particles are held in suspension. Two additional experiments were conducted at (i) 100 mL scale and (ii) where a PA-MMT suspension (50 mg/mL, 4.6 mg PA/g MMT, equilibrated for >2 hrs) was added to the water-precipitated fenofibrate suspension at 20 s after precipitation, and aged for an additional 1 min before drying. FF nanoparticles from preparation (a) were isolated by freeze-drying on a Dura-Dry Microprocessor Control
freeze-dryer at <20 Pa for 48 hrs after flash-freezing in liquid nitrogen. Particles from all other preparations were isolated by vacuum-filtration (Mini diaphragm vacuum pump, VP 86) using Whatman filter paper 50 (2.7 µm pore, 35 mm cross section), and washed with 2 mL water. Filter cakes were dried under vacuum (<20 Pa) for 24 hrs.

Suspended nanoparticles of MEF were prepared and isolated according to the procedure outlined by Bodnar et al. [26] with slight modification. An organic solution of MEF in DMA (0.5 mL, 40 mg/mL, 25 °C) was quickly introduced by Eppendorf pipet to a 9.5 mL aqueous solution of docusate sodium salt (0.53 mg/mL, 5 °C) antisolvent under rapid agitation (1200 rpm). After 1 min aging, free nanoparticles were isolated from suspension by direct filtration with a nylon membrane (0.2 µm). To prepare nanoparticle-carrier composites, MEF was precipitated under the same conditions but with the replacement of the docusate sodium stabiliser in the antisolvent for protamine-modified MMT (42 mg/mL MMT in water, 4.6 mg PA/g MMT). After 1 min aging, particles were vacuum filtered in the same way as the fenofibrate-carrier composites.

Nanoparticle attachment to the carrier particles was indirectly monitored by measuring the drug content remaining in the filtrate after filtration (2.7 µm filter pore) using a Shimadzu UV-1280 UV-visible spectrophotometer (λ = 289 nm). Unattached nanoparticles could pass through the filter to produce a milky filtrate, while carrier-attached nanoparticles could not, resulting in a clear filtrate. An aliquot of the filtrate was diluted by a factor of 10 in methanol and equilibrated for 24 hrs to dissolve any drug particles present before measuring the dissolved concentration.

Centrifugation was further used to distinguish whether the nanoparticles adsorbed to the PA-modified MMT or if it simply served as a filtration aid to remove the nanoparticles from suspension. Samples containing free FF nanoparticles, a PA-MMT control, and a FF-PA-MMT composite were aged for 0.5 min before centrifuging at a speed of 5000 rpm for 2 min. The supernatant was decanted into a separate vial and the FF content in the supernatant was measured by UV-visible spectroscopy after dissolving a portion in methanol and leaving to equilibrate for 24 hrs. On knowing the FF content in the supernatant, a mass balance was used to estimate the % of FF which sedimented. This was used as an indicator of the % of FF which adsorbed to the PA-MMT carrier from suspension.
4.3.6 Dissolution Testing. The dissolution medium for FF samples consisted of a 0.1 M HCl solution containing 0.4% w/v Tween-80 at 42°C. The dissolution medium for MEF samples consisted of a 0.05 M pH 7.4 phosphate buffer with 0.05% w/v Tween-80 at 37°C. Dissolution tests were carried out in sink conditions by adding a sample (powder or suspension) containing 12.5 mg API to 450 mL dissolution medium under agitation of 400 rpm. TriCor tablets (commercial FF) were ground to a powder by pestle and mortar for 2 min prior to dissolution testing. The powder contained in Ponstan capsules (commercial MEF) was extracted from the capsules for use in dissolution testing. After sample addition, 4 mL aliquots were taken at regular intervals from the bulk solution in preheated (45°C) plastic syringes and filtered through preheated (45°C) PTFE 0.2 µm syringe filters. The dissolved drug concentration was measured by UV-visible spectroscopy (Shimadzu UV-1280) at a wavelength of 289 nm. Dissolution tests were carried out at least in duplicate.

4.3.7 Particle Size Analysis. Particle size measurements were performed by laser diffraction using a Malvern Mastersizer 3000, with water as the dispersion medium. In cases where the particle size was not stable (Prep (a) in section 4.3.5), HPMC and SDS were added to the water dispersant at a concentration of 0.038 mg/mL each to minimise variation between consecutive measurements. Precipitated drug suspensions were diluted by a factor of 5 with water prior to their introduction to the measurement vessel. An MMT control was treated by the conditions used during antisolvent precipitation before measuring its particles size. An obscuration rate of 7-10%, a stir rate of 2300 rpm, a 1 min pre-measurement delay (including 20 sec with 5% sonication power) were the conditions used during all size measurements. A refractive index of 1.55 and an absorption index of 0.01 were used for FF measurements. A refractive index of 1.55 and an absorption index of 0.1 were used for MMT measurements. Four measurements were taken per run and each sample was run twice. The D_{50} diameter was reported for each size distribution and averaged across all measurements. Measurement variations were also reported.

4.3.8 X-ray Powder Diffraction. X-ray diffraction patterns of the powders were recorded using a PANalytical Empyrean diffractometer in transmission mode, using Ni filtered CuKα radiation (λ=1.54 Å) at 40 kV and 40 mA. The XRD data was recorded in the range of 20.5–23.0 °2θ for FF samples and the range 14.5-16.5 °2θ for MEF samples.
4.4 Results

4.4.1 Solid-State Drug-Carrier Composites. Drug nanoparticles of FF and MEF which were precipitated from a supersaturation solution, captured by a functionalised carrier (PA-MMT), filtered and vacuum dried maintained a dissolution rate which was equivalent to that of the suspended (and stabilised) nanoparticles (300-400 nm) reported previously by our group [22, 26], Figure 4.3. The solid-state particles prepared in this way preserved their fast, nanoparticle-induced dissolution rates during isolation and drying, unlike when freeze-drying or direct filtration (0.2 µm) were used, Figure 4.3. The PA-MMT carrier alone was able to stabilise the nanoparticle size and surface area (as indicated by dissolution rates) of both FF (9.1% loading) and MEF (4.8% loading) during drying, without the need for the drug-specific dissolved stabilisers which were previously reported for each system [22, 26]. The fast dissolution rates achieved by the dried drug-carrier composites were comparable to or faster than that of their respective dry-powder commercial drug formulations (TriCor [nano] and Ponstan [micron]), which were prepared by milling, Figure 4.3.

Figure 4.3 Dissolution profiles of (a) fenofibrate and (b) mefenamic acid comparing commercial formulations, and precipitated material in suspension and after drying (1 min aging, in the absence and presence of carrier particles at 9.1% FF loading on FF-PA-MMT and 4.8% MEF loading on MEF-PA-MMT).
Under standard conditions, the functionalised carrier was present during precipitation of the drug, precipitation was conducted at a 10 mL antisolvent scale, and the aging time in suspension was short (1 min). However, additional tests with the FF system confirmed that it was non-influential (in terms of preserving a fast dissolution rate) whether the carrier was present during precipitation or added 20 s later, if the process was scaled up to 100 mL antisolvent scale or if the aging time in suspension was increased to 24 hours, Figure 4.4. In comparison to the limited stability of a pure FF nanosuspension, which was demonstrated by its significant size evolution and corresponding dissolution rate reduction over 30 min aging (Appendix 4.6), the result in Figure 4.4 shows the high stabilisation ability of the functionalised carrier for the FF nanoparticles in suspended state. Furthermore, nanoparticles in the nanoparticle-carrier composites were stable in the dried state, over a monitored period of 10 weeks (Appendix 4.7).

Figure 4.4 Dissolution profiles of dried FF composites highlighting influence of variation from standard conditions (ie. carrier present during precipitation, 1 min aging, 10 mL scale)

Particles in the dried drug-carrier composites were designated as ‘nano’ particles on the combined basis of their known nanosize in suspension (without carrier particles) and their preserved fast dissolution rates when attached to the carrier particles. Although highly unstable, free nanoparticles of FF ($D_{50}$: 0.75±0.09 µm, Figure 4.5) and MEF ($D_{50}$: 0.84±0.05 µm [26]) existed in suspension at short aging times following their precipitation from water alone.

Figure 4.5 Particle size distribution of FF precipitated in pure water (no carrier, 1 min aging)
Similarly, nanoparticles in the dried nanoparticle-carrier composites were designated as ‘crystalline’ on the combined basis of their known crystallinity when no carrier was present (Figure 4.6), their similar dissolution rates regardless of whether the carrier was present during or added shortly after precipitation (Figure 4.4), and based on the diffraction peaks (albeit low intensity) obtained from nanoparticles in the nanoparticle-carrier composites as shown in Figure 4.6.

![Figure 4.6](image)

**Figure 4.6** X-ray diffraction patterns for (a) fenofibrate and (b) mefenamic acid, comparing commercial formulations and precipitated material after drying (1 min aging time)

### 4.4.2 Nanoparticle Adsorption to the Carrier

Attachment of the suspended drug nanoparticles to the PA-MMT carrier particles (average size: 28.0 ± 1.3 µm, Appendix 4.8) was confirmed by the inability of the carrier-bound FF nanoparticles (0.75 µm) to pass through a 2.7 µm filter, as indicated by a clear FF-free filtrate (containing < 0.5% FF). The filtration process was fast, taking < 0.5 min for the mother liquor (11 mL) to pass through the filter. In comparison, control tests showed that >80% of free nanoparticles, precipitated from water or from a PA solution passed through the 2.7 µm filter when no carrier was present (milky filtrate). A schematic of the method used to screen for nanoparticle attachment to the clay is shown in Figure 4.7.
Figure 4.7  A filtration test used to indicate nanoparticle adsorption to the carrier. (a) No carrier present, (b) no attachment to the carrier and (c) attachment of nanoparticles to the carrier.

Centrifugation further probed whether the PA-MMT carrier adsorbed the FF nanocrystals to its surface or just served as a filtration aid to separate the nanocrystals from suspension during filtration. Centrifugation allowed the sedimentation ability of the free nanocrystals versus the carrier-attached nanocrystals to be assessed. A control sample of PA-MMT, treated by antisolvent conditions, sedimented during centrifugation. A second control of free FF nanoparticles in suspension only partially sedimented (30%) under the same centrifugation conditions, leaving a dense white haze in the supernatant. However, in the presence of the PA-MMT carrier, 98% sedimentation of the FF nanocrystals occurred, giving additional indication of their adsorption to the carrier.
4.4.3 Role of the PA Modifier. Free FF nanocrystals (-25.3±0.9 mV, Figure 4.8), free MEF nanocrystals (-35.3±1.3 mV [26]) and unmodified MMT carrier particles (-26.9±1.2 mV, Figure 4.8) all possess a negative electrical charge in aqueous media. The surface charge of the MMT was modified from negative to positive by the adsorption of protamine, a cationic protein. Figure 4.8 shows that the zeta potential of MMT in water changed from -26.9 to -6.1 to +14.8 mV as the surface coating of adsorbed PA on MMT increased from 0 to 4.6 to its saturation value of 200 mg PA/g MMT.

Figure 4.8  Zetapotential distributions unmodified MMT (-26.9 mV), sparsely PA-modified MMT (-6.1 mV, 4.6 mg PA/g MMT) and PA-saturated MMT (+14.8 mV, 200 mg PA/g MMT)

The adsorption isotherm for PA onto MMT followed a non-linear Langmuir-type increase in adsorbed protamine which tended towards its maximum value of 200 mg/g MMT, Figure 4.9a. The zeta potential of the carrier was dependent on the coating density of protamine on the MMT (mg PA/g MMT), and was easily tuned to between -26.9 mV and +14.8 mV by varying the PA coverage, Figure 4.9b. A sparse coating of PA increased the zeta potential significantly.
Both the nanoparticle loading and the PA loading on the carrier had a bearing on dissolution rates of the dried material, Figure 4.10. For the FF system, the maximum drug loading at which nanoparticle-induced fast dissolution rates were preserved in the dried form was 4.8% w/w when no PA-functionalisation was present. However, by introducing sparse PA-functionalisation and by controlling its coverage on the MMT (4.6 mg/g MMT), the maximum drug loading at which the fast dissolution rate was preserved was increased to 9.1% w/w. Further increase in the PA-coverage on MMT towards its saturation point (200 mg/g MMT) had a negative influence on the dissolution rate, Figure 4.10b. Therefore, a sparse PA coverage was required for optimum dissolution at high drug loading. For the MEF system, 4.8% w/w was found to be the maximum loading on sparsely PA-functionalised MMT (4.6 mg/g MMT) at which the dissolution rate of the dried material was comparable to the nanoparticles in suspension. The initial dissolution rate was marginally slower at the higher loading of 9.1% w/w, but was still complete in < 4 min (Appendix 4.5).
For both the FF and MEF systems, the sparse PA coating on the MMT increased the speed of filtration; an important consideration in terms of scalability. Filtration of FF-PA-MMT composites (9.1% loading) was 10 times faster than filtration of FF-MMT composites at 10 mL antisolvent scale (0.5 min vs 5 min), while filtration of MEF-PA-MMT composites (4.8% loading) was 6 times faster than MEF-MMT composites (1.2 min vs 7.5 min).

Figure 4.10  Dissolution profiles of FF from dried (a) FF-MMT composites at various drug loadings and (b) FF-PA-MMT composites at various PA coverages

4.5 Discussion

In this work, BCS Class II drugs were formulated into fast-dissolving, solid-state nanoparticle composites using a simple one-step approach. Nanoparticles of fenofibrate and mefenamic acid were precipitated in suspension in the presence of an insoluble excipient (a functionalised clay carrier) which enabled stabilisation, fast filtration, drying, and fast dissolution of the surface-adsorbed nanoparticles. Adsorption of nanoparticles to the carrier was demonstrated both by filtration and centrifugation techniques and the nanoparticles were found to maintain their crystalline form when bound to the carrier.
Free nanoparticles of FF and MEF in suspension were previously found to be highly unstable, particularly so in the absence of dissolved stabilisers [22, 26]. This work demonstrated prolonged stabilisation of the nanoparticles in suspension (during 24 hrs, Figure 4.4) once bound to the functionalised carrier, without the need for the previously reported dissolved stabiliser systems which were identified on the basis of a laborious trial and error stabiliser-screening approach [22, 26]. The prolonged stabilisation in the suspended state introduced flexibility to the isolation timeline. The carrier-bound nanoparticles were stable during their isolation (by filtration) and drying stages, as demonstrated by the preservation of fast nanoparticle-induced dissolution rates of the dried material. This result is an improvement to nanoparticles isolated by freeze-drying or by direct filtration with a nano-pore filter; two isolation approaches which contributed to reduced dissolution rates, Figure 4.3. The nanoparticles in the dried nanoparticle-carrier composites yielded dissolution behaviour equivalent to that of the suspended nanoparticles, and equivalent to or better than those of the commercial formulations (prepared by milling). Furthermore, the use of relatively large carrier particles (~28 µm) facilitated an exchange of more complex, but frequently used nanoparticle isolation methods (eg. spray-drying or freeze-drying) for a simple filtration method. An additional advantage of the carrier approach is the incorporation of the carrier as an excipient at the particle formation step (at drug concentration up to 9%) which bridges the gap between primary and secondary manufacturing, while simultaneously eliminating API-excipient segregation issues.

The surface of montmorillonite clay contains segregated regions of hydrophobicity and charge-induced hydrophilicity. Siloxane (≡Si-O-Si≡) units cover much of its exposed surface, giving the material a hydrophobic nature due to strong bonding interactions between silicon and oxygen atoms [31]. However, isomorphous substitution of selected surface atoms (eg. Si $^{4+}$ by Al $^{3+}$) provides diffuse regions/patches of permanent (hydrophilic) negative surface charge, Figure 4.11 [27, 32-34]. Negative charges are balanced by adsorption of loosely-bound exchangeable inorganic counter-ions (eg. Na$^+$, K$^+$), giving the material high ion exchange capacity. In the presence of water, the counter-ions migrate from the surface and gain a hydration layer which introduces a regional hydrophilic environment between patches of hydrophobic surface [33], Figure 4.11.
Figure 4.11 Graphic description of the MMT surface in the unhydrated (upper) and hydrated (lower) state.

The surface properties of the clay greatly affect the affinity and thus the binding potential and binding mechanism of drug nanoparticles [34, 35]. In the liquid state, protamine (a cationic polymer) can easily adsorb to the clay following an ion-exchange process [27, 28, 35], providing it with a positively-charged coating. Protamine is rich in arginine which has a basic side chain (pKa of 12.5), giving the material a positive charge in acidic, neutral and even moderately basic environments [36]. The charge reversal of MMT from -26.9 mV to +14.8 mV upon saturation with protamine confirmed successful deposition of the cationic coating onto the clay, Figure 4.8.

Although the negatively-charged drug particles were capable of attaching to the unfunctionalised clay (eg. through hydrophobic interactions between methyl/benzene groups and the siloxane surface or through hydrogen bonding with the dangling hydroxyl end groups on MMT [37, 38]), the maximum drug loading at which fast dissolution rates were preserved after drying was low. Due to charge repulsions, the negatively-charged nanoparticles would strictly avoid the negatively-charged patches on the clay surface, thus limiting the available favourable adsorption sites, Figure 4.12c. At higher drug loadings, nanoparticles may aggregate at the hydrophobic site, resulting in a loss of nanoparticle-induced high surface area and causing a consequential reduction in dissolution rate, Figure 4.12d. Surface-modification of the negatively-charged clay with the positively-charged PA was necessary to introduce favourable charged adsorption sites to the clay surface so that the drug could attach both through electrostatic and
hydrophobic interaction. It is hypothesised that the increase in the availability of favourable adsorption sites on the MMT surface allowed a homogenous, segregated dispersion of negatively-charged nanoparticles to adsorb to the functionalised carrier, based on their fast dissolution rates after drying, Figure 4.12a. However, the surface coverage of PA on the MMT was also influential in preserving this fast dissolution rate. Overcompensation of the surface with positive charge could induce nanoparticle aggregation surrounding positively charged patches, which is likely the explanation behind the reduced dissolution rates at high PA coating, Figure 4.12b. Adsorption of dispersed, fast-dissolving nanoparticles (at >99.5% FF recovery from suspension) was achieved by significantly reducing the surface coverage of protamine molecules (Figure 4.10b).

Figure 4.12 Mechanistic description of the potential interactions between the negatively charged drug nanoparticles and (A,B) the PA-functionalised and (C,D) the unfunctionalised MMT carrier.

MMT has an expanding 3-layer structure with an interlayer spacing of approx. 1-2 nm [37]. Some previous reports have ascribed the adsorption abilities of clays such as montmorillonite for applications in capturing molecules from solution to its large internal surface (eg. in contamination control [35, 39] and drug delivery [28, 37, 40]). In our work however, nanoparticles were first precipitated from solution and their relatively large size permitted adsorption only to the external surfaces of the PA-MMT carrier. Whether the carrier was present during the drug precipitation step or added somewhat later was
irrelevant (Figure 4.4), as long as nanoparticles were nucleated from solution and the carrier particles were added before too much growth/aggregation occurred.

The carrier-mediated approach to nanoparticle stabilisation and isolation outlined in this work was validated by two drugs, but has generic potential in the preparation and isolation of all negatively-charged drug nanoparticles. Furthermore, the same rational may be applied to other combinations of carriers and polymers for tailored application to any drug substance. The antisolvent precipitation technique for nanoparticle preparation, coupled with carrier-mediated isolation by filtration therefore provides an effective means of formulating particles with enhanced dissolution behaviour, and could have significant application in formulating BCS Class II drugs.

### 4.6 Conclusion

This work reports a method by which nanoparticles can be isolated into the dried, solid powder form while retaining dissolution rates which are comparable to or surpass those of the nanoparticles in suspension and currently marketed commercial formulations. Negatively charged nanoparticles generated in suspension from a supersaturated solution during an antisolvent precipitation method were recovered from suspension using a microparticle carrier system, functionalised with a cationic polymer. The drug nanoparticles were stable on the nanoparticle/microparticle composite (at up to 9% drug loading) in suspension for 24 hours without the requirement for the more traditional nanosuspension stabilisers. The composites were quickly filterable to produce a dried sample with a maintained high drug dissolution rate that stemmed from the nanoparticle size. A more efficient isolation was achieved using a polymer-functionalised carrier, as compared to when an un-functionalised carrier or no carrier was present during filtration. This nanoparticle isolation system was validated by application to particles of two negatively charged drugs, fenofibrate and mefenamic acid. While success is anticipated for all such drugs, the process has potential to be tailored to an even wider range of drug molecules with modification to the nature of the carrier and its functionalising agent.
4.7 References


Chapter 5

A Morphological Investigation of Freeze-Dried Fenofibrate

Content from this chapter appeared in:

5.1 Abstract

Chapter 3 described the process for preparing fenofibrate nanoparticles in suspension by antisolvent precipitation and showed the growth of the particles in suspension by Ostwald ripening. This chapter highlights the effect of freeze-drying on the growth pathway. Freeze-drying stresses were found to destabilise the fragile nanoparticle system. The growth pathways of particles during freeze-drying and during rapid oven-drying were compared. Time-resolved morphological analysis of the oven-dried particles showed gradual enlargement of the particles through a process concurrent with Ostwald ripening/molecular addition. Freeze-dried particles, on the other hand, appeared to follow an orientated attachment or aggregation based growth mechanism, inducing speculation of the development of ordered nanoparticle assemblies (ie. mesocrystals). Despite the morphological differences between oven-dried and freeze-dried particles, an investigation of the electron diffraction patterns and the cross-sectional inner-particle surfaces indicated that the growth mechanisms were the same; growth by molecular addition. The observed differences in the time-resolved particle morphologies were found to be an artefact of the freeze-drying process.
Chapter 5                                                   Morphological Investigation of Freeze-Dried FF

Figure 5.1    Schematic showing two proposed particle growth pathways for PVA-precipitated, freeze-dried fenofibrate

5.2 Introduction

Conventional (classical) crystal growth theories generally postulate that crystals grow by the gradual addition of individual ions, atoms or molecules to an interfacial region, resulting in crystal enlargement (ie. Ostwald ripening) [1]. However, in recent years, an increasing number of reports on crystal growth through orientated attachment and assembly have appeared (ie. non-classical growth) [2, 3]. In such cases, crystals are grown through a non-equilibrium process, with nanoparticles, rather than individual molecules, serving as the building blocks for growth. Both growth mechanisms have been reported for particles prepared through antisolvent precipitation. Some reports suggest that once precipitated, nanoparticles can self-assemble into an ordered superstructure or ‘mesocrystal’. The term ‘mesocrystal’ describes a crystallographically aligned intermediate between a polycrystalline aggregate and a single crystal. Adjacent nanoparticles can subsequently fuse to form single crystals, but the extent of fusion depends on the extent of alignment [4].
It is stated that a temporary stabilisation is crucial for nanoparticle orientation to a mesocrystal, so that rotation and ultimate crystallographic alignment can be achieved. If the stabilisation is too strong, the nanoparticles repel each other, while if the stabilisation is too weak, the nanoparticles stick to each other and can no longer change their mutual orientation [2]. Nanoparticle stabilisers such as polymers or surfactants can drive the self-assembly process through hydrophobic interactions between the stabiliser chains and molecules, while simultaneously preventing immediate single crystal formation or uncontrolled aggregation of the assembled nanoparticles [2, 5, 6]. It is well known that two identical crystal faces can orient each other when brought into close proximity to each other [4, 7, 8]. The short range repulsive force generated by the stabilisation layer allows rotation of the nanoparticles in the aggregate until they reach a position of crystallographic alignment. Nanoparticles, with identical or complementary crystal faces in register with each other, can then fuse to transform the mesocrystal into a single crystal. The attached stabiliser may remain at the sites between primary crystallites, resulting in significant stabiliser content and consequential variation in crystal properties [6].

However, the high order that exists between the crystalline subunits has made distinction between mesocrystals and single crystals quite difficult [6, 9]. While some defend the existence of mesocrystals, others argue an alternative spherulitic growth mechanism to describe polycrystalline solids of various shapes. Spherulites form by growth front nucleation when a new crystal grain nucleates at the surface at a different lattice orientation than the parent crystal [10]. Mis-orientated nucleates are then said to grow through the classical route of molecular addition to form a polycrystalline solid.

The term ‘mesocrystal’ was first applied in 2005 to inorganic calcium carbonate polymorphs that were precipitated in the presence of polymer additives [11-13]. However, mechanisms of formation of the three CaCO$_3$ polymorphs (vaterite, aragonite and calcite) have been described by conflicting classical (molecular addition) and non-classical (self-assembly) growth theories. Xu et al. reported a method of synthesising calcite mesocrystals in the presence of an anionic polymer [14]. Zhou and colleagues argued that the porous structure and slight misorientation within aragonite crystals were evidence for oriented attachment of separate, individual nanoparticles into mesocrystals [15]. However, both Sand and Beck interpreted the underlying particle enlargement mechanism...
of calcium carbonate crystals as spherulitic growth through classical ion-by-ion attachment rather than self-assembly [5, 10]. Kim et al. demonstrated that the data commonly used to assign mesocrystal structure (ie. SEM and XRD) are frequently misinterpreted, using calcite crystals as a test case [13].

While mesocrystal assembly has been reported for a number of inorganic systems, it is more uncommon for organic compounds. In 2008, Medina et al. reported organic mesocrystals of DL-alanine from supersaturated solutions in water-alcohol mixtures. The existence of mesocrystals was depicted from differences in X-ray diffraction patterns and differences in their thermal behaviour, as analysed by DSC [16]. In 2014, Thorat et al. reported superstructure formation of curcumin nanoparticles, precipitated from an ethanol solution in the presence of ultrasound and additives. SAED patterns from TEM analysis were used primarily to rationalise the presence of superstructures [3]. Choi et al. used polymer-directed antisolvent crystallisation to generate mesocrystals of a model drug atorvastatin calcium and investigated the usefulness of mesocrystals (and their polymer content) for enhancing the performance of drug substances in terms of release, stability and processability [6]. They suggested that the polymer layers on the surfaces of the primary crystallites within the composite (as detected by melting point reduction) could function as a protective diffusion barrier, to achieve improved stability and processability. In 2015, Cho et al. formed hybrid mesocrystals of ibuprofen and calcium carbonate (aragonite) with environment-responsive release behaviour through a ball milling process. The stability of aragonite was found to be the controlling factor for the release kinetics of ibuprofen [17].

Despite the increased attention to orientated aggregation and non-classical growth theories over the past 10 years, mesocrystal formation is still experimentally difficult to prove and critical analysis of experimental indicators is required [13]. However, investigation into mesocrystal existence is important from a fundamental scientific perspective with a view to exploiting potentially exciting applications in terms of drug formulation and performance. Based on literature studies, conditions which appear to favour oriented nanoparticle assembly mechanisms are; high supersaturation to achieve high nanoparticle numbers [9, 10], and a temporary stabilisation layer on the nanoparticles to prevent immediate fusion of adjacent particles before they mutually align [2].
This chapter investigates the potential mesocrystal formation of polymer-stabilised fenofibrate nanoparticles, prepared in conditions of extremely high supersaturation, during the freeze-drying process. The effect of freeze-drying and the effect of stabiliser presence during freeze-drying on the final size, morphology and observed growth pathway of the drug are discussed. These isolation-related effects were found to be particularly critical when trying to obtain nanoparticles in the solid form.

5.3 Experimental Details

5.3.1 Materials. Fenofibrate (FF) (99.7% purity) was generously gifted from Abbvie Laboratories. Ethanol (99.8% purity) was purchased from Merck Millipore. PVA (9-10 kDa) was purchased from Sigma Aldrich. Liquid nitrogen (99.99%) was obtained from BOC Gases. Distilled water was used for sample preparations.

5.3.2 Particle Preparation and Drying. An organic solution of FF in ethanol (1 mL, 50 mg/mL) was quickly introduced by Eppendorf pipette to a 10 mL aqueous solution of PVA (0.2% w/v) antisolvent under rapid agitation (800 rpm) at 25°C to precipitate the FF. For general isolation conditions, precipitated suspensions were flash frozen in liquid nitrogen at 0.5, 11, 21 and 31 min aging time and freeze-dried under vacuum (< 27 Pa) for 48 h using a Dura-Dry Microprocessor Control freeze-dryer. For comparison purposes, an aliquot of the precipitated drug suspension (1 drop) was quickly oven-dried at 55°C.

5.3.3 Scanning Electron Microscopy. Sizes and morphologies of particles in oven and freeze-dried samples were analysed using a Hitachi SU-70 high-resolution scanning electron microscope (SEM). Freeze-dried particles in powder form were placed on carbon tape on an aluminium stub. Oven-dried particles were prepared by drying the suspension directly on the carbon tape in an oven at 55°C. For both preparation methods, samples were coated with an ~8 nm gold deposit using an EMITECH K55 and the particles were imaged in field-free mode at a voltage of 10 kV and a working distance of 10 mm. The image analysis software, ImageJ, was used to analyse particle sizes (maximum dimension) from SEM images. All SEM images show particles which were fully representative of the entire sample analysed in each case.
5.3.4 Transmission Electron Microscopy and Electron Diffraction. Surface morphology analysis and selected area electron diffraction (SAED) of freeze-dried PVA-precipitated FF particles were conducted using a transmission electron microscope (TEM, Joel JEM-2011 equipped with GATAN Multiscan CCD camera, model 794) at an accelerating voltage of 200 kV. The dried samples were suspended in deionised water and placed in a sonication bath for 30 min before applying a drop of the suspension to a holey carbon-supported TEM grid and air-drying overnight. Diffraction patterns were obtained before TEM imaging to minimise the effects of beam damage on the diffraction results.

5.3.5 Focused Ion Beam. A focused ion beam (FIB) system (FEI 200, 30 kV, Ga+ ions) was employed to cut through the freeze-dried FF particles. Particles were mounted on an aluminium stub and gold-coated before analysis. The part of the particle to be protected from the beam was coated with a 1 µm layer of platinum. The cut particles were tilted through 45° and their cross-sections were imaged.

5.4 Results

Figure 5.2 shows that PVA-precipitated FF particles, which were quickly oven-dried from a drop of suspension at various aging times, gradually increased from nano (~380 nm) to micron (4-5 µm) size at a time scale comparable to that of the suspended particles as seen in Chapter 3, Figure 3.6c (laser diffraction). Figure 5.2a and 5.2b depict immature crystals with rounded edges which are still in the growth stage. Image 5.2c shows highly-defined parallelepiped-shaped crystals which represent the final observed particle size and morphology. Particles dried in this way were taken as being representative of the particles in suspension and served as a comparison to freeze-dried particles.

Figure 5.2. SEM images showing size evolution of oven-dried PVA-precipitated FF at 40 second intervals from aging time (a) ~12 to (b) ~12.7 to (c) 13.3 min (drying: ~ 12 min).
Figure 5.3, and the higher magnification images in Figure 5.4, shows the growth pattern observed for freeze-dried, PVA-precipitated FF particles. The growth pattern appeared different to that of oven-dried, and consequentially suspended FF particles. Over time, particles developed from smooth-surfaced but rounded, immature, and irregular particles at 0.5 min (3.7 µm), to roughly surfaced particles at 11 and 21 min, to smooth surfaced parallelepiped-shaped particles with defined edges at 31 min (4.0 µm). No evidence of nanoparticles was observed at the shortest aging time of 0.5 min, but some nanoparticle subunits were visible within or attached to a larger core particle at 11 and 21 min aging time (Figures 5.3 and 5.4).

Figure 5.3. Time-resolved SEM images of freeze-dried PVA-precipitated FF particles after different aging times in suspension (0.5, 11, 21 and 31 min).

Figure 5.4. Higher magnification SEM images of freeze-dried PVA-precipitated FF at aging times of (a) 11 min, (b) 21 min and (c) 31 min.

As outlined in Chapter 3, dissolution testing of the freeze-dried material showed that, apart from the sample with 0.5 min aging time, all other samples followed the expected trend of dissolution rate reduction with increased aging time (due to particle enlargement). However, the fastest dissolution was obtained for a sample at 4 min aging time and the slowest was obtained for a sample at 0.5 min aging time, Figure 5.5.
Figure 5.5  Dissolution profiles comparing the effects of aging time on dissolution rate for PVA-precipitated, freeze-dried FF

XRD analysis, in combination with data from the Cambridge Structural Database, showed that all freeze-dried samples precipitated from PVA crystallised in the polymorphic form I (centro-symmetric triclinic space group P-1), Figure 5.6 [18].

Figure 5.6  X-ray diffraction patterns of (A) theoretical form I (triclinic), (B) blank background, and (C) ‘as received’ FF; and of freeze-dried, PVA-precipitated FF at aging times of (D) 0.5 min, (E) 11 min, (F) 21 min, and (G) 31 min.
TEM images and corresponding SAED patterns of the 11, 21 and 31 min PVA-precipitated samples (from Figure 5.4) are shown in Figure 5.7. Diffraction patterns obtained from the aggregates in the 11 min sample appeared as rings depicting their polycrystalline nature (Figure 5.7a). The diffraction patterns obtained from particles in both the 21 min and 31 min samples appeared as discrete, ordered diffraction spots, portraying the particles as single crystals (Figure 5.7b-c).

![Figure 5.7 TEM micrographs and SAED patterns of freeze-dried, PVA-precipitated FF particles at aging times of (a) 11 min, (b) 21 min, and (c) 31 min](image)

The inner structures of the roughly-surfaced 21 min particles were examined by cutting along the length of the crystals with a focused ion beam. Despite the rough surface on the particles’ exterior, the interior was predominantly solid, compact and void of cavities, apart from a porous/spongy region at each tip, Figure 5.8. Similar analysis of the 31 min smooth-surfaced particles showed a solid interior with no inner porosity (not shown).
Figure 5.8  FIB image of a freeze-dried PVA-precipitated particle at an aging time of 21 min (a) before, and (b) after cutting by focused ion beam. The particle in (b) is tilted by 45° and the red line designates the external surface of the original crystal inside a layer of re-deposited material.

5.5 Discussion

Unlike their oven-dried equivalents (Figure 5.2a), PVA-precipitated nanoparticles (at 0.5 min aging time, Chapter 3, Figure 3.7) were not preserved as individual nanoentities during freeze-drying (Figure 5.3). SEM images showed no evidence of nanoparticle content in the freeze-dried sample at 0.5 min aging time. However, interestingly, some nanoscale subunits were visible on the surface of larger micro scale particles in the dried 11 min and 21 min particles, giving the particles a rough appearance. By 31 min aging time in suspension, the surface roughness of the particles in the corresponding freeze-dried sample had disappeared.

The oven-dried particles in Figure 5.2 gradually evolved from submicron to small micron size in a manner corresponding to Ostwald ripening. However, the same cannot be clearly said for freeze-dried particles. SEM analysis of freeze-dried particles presented a visually different morphological development of the particles over time, which appeared inconsistent with the classical Ostwald ripening mechanism.

During the precipitation process, conditions of high supersaturation and polymer stabilisation were used, which are reported to favour the formation of mesocrystals. Based on this consideration, and on the unusual growth pathway observed in time-resolved SEM imaging of the freeze-dried particles, the potential formation of self-assembled
mesocrystals was investigated. SEM analysis alone was insufficient in distinguishing if the visible nanoparticles in the 11 and 21 min samples in Figure 5.4 were a surface event or if they extended throughout the entire structure in an assembled fashion (i.e. as a mesocrystal), as per Figure 5.1.

FIB analysis revealed that the interior of the 21 min particles was predominantly solid and free of the cavities which could be associated with nanoparticle assembly into mesocrystals. The porous region at the particle tips (consistent for all 21 min particles analysed) was caused by the visible coating of submicron particles in these areas, Figure 5.8. This porous region was not observed for the smooth-surfaced 31 min particles.

SAED analysis designated the 11 min particles as polycrystals indicating that there was no crystallographic order between the assembled subunits. The 21 and 31 min particles, on the other hand, were designated single crystals, despite their rough surface. A likely explanation as to how a polycrystalline aggregate could develop into a highly-crystalline single crystal (confirmed by XRD, Figure 5.6) is through a conventional Ostwald ripening pathway, whereby the inner particle gradually grows at the expense of the remaining nanoparticles. Both of these analyses advocate a classical molecular-addition based growth pathway for freeze-dried FF particles, as opposed to an oriented-assembly mechanism. The unusual growth pathway which was observed for freeze-dried particles in Figure 5.3 can be explained by aggregation and fusion of insufficiently stabilised nanoparticles at 0.5 min, followed by surface-attachment of PVA-coated FF nanoparticles onto larger FF crystals at 11 and 21 min during the destabilising drying process.

While we know from Chapter 3 that FF nanoparticles exist in suspension at 0.5 min aging time (Figure 3.7), they were not observed in the corresponding freeze-dried sample (Figure 5.3). It is proposed that this short contact time was insufficient for the PVA to bond to, and stabilise the suspended nanoparticles. The subsequent drying step caused aggregation, followed by fusion of the unstabilised 0.5 min nanoparticles. At 11 min, freeze-drying again forced aggregation of the nanoparticles, but the PVA-coating which was attached to the FF surface at the longer contact time prevented the fusion step from occurring (Figure 5.9). During the period of accelerated growth between 11 and 21 mins, nano and small micron particles coexisted in suspension (Ch 3, Figure 3.6c), but drying forced the nanoparticles to surface-attach to the larger particles.
Based on time-resolved particle sizing in Chapter 3 and SEM, TEM, FIB and SAED analysis conducted here, it is hypothesised that PVA-precipitated particles grow by Ostwald ripening in suspension followed by forced aggregation, attachment and/or fusion during the freeze-drying step. The change in morphologies of the freeze-dried particles from irregular immature crystals at 0.5 min, to aggregated nanoparticles at 11 min, to roughly-surfaced and then smooth-surfacd parallelepiped crystals at 21 and 31 min respectively was determined to be an artefact of the freeze-drying process. Ostwald ripening induces a bimodal size distribution of nano and micron particles when the suspension is in the growth stages, which, when freeze-dried results in a nanoparticle coating on an inner core micron-sized crystal. As the aging time progresses, depletion of the nanoparticles in suspension prevents further growth from occurring through Ostwald ripening, resulting in a plateaued stable monodispersion of micron-sized particles. The absence of nanoparticles at this time point explains the preservation of the smooth-surface on the 31 min particles during drying. It must be clarified that the morphological development of the freeze-dried particles with time is not the result of a continuous growth process, but a knock-on effect from isolation of the growing particles at various time points by freeze-drying (Figure 5.9).

![Figure 5.9. Schematic description of the hypothesised growth mechanism of particles in suspension, highlighting the drying-induced morphologies of freeze-dried particles](image)

Dissolution testing of the dried samples with various aging times showed a trend which complimented this hypothesis. Poor dissolution of the 0.5 min samples resulted from the
insufficient stabilisation at that short time. An aging time of 4 min was required for PVA-FF interactions to occur, thus preventing fusion of the particles during drying, and preserving their higher surface area and dissolution rate. However, as the nanosized fraction in the sample disappeared (through Ostwald ripening), the reduction in surface area induced a reduction in dissolution rate as the aging time increased.

Therefore, despite the appearance of an unusual growth trend for freeze-dried PVA-precipitated FF from time-resolved SEM analysis, results from FIB and SAED indicated that the growth mechanism was no different to that of the suspended and oven-dried particles. The visual differences were found to be related to the drying process. For this reason, examination of the crystal habits alone can be misleading in determining the growth mechanism, leading to frequent misinterpretation. Results have shown that the effect of the drying process on the system is quite significant and must also be considered.

5.6 Conclusion

While freeze-drying proved successful in isolating small-micron sized particles, it was unsuccessful in preserving the pre-dried nanoparticles as individual nanoentities during the drying process. The isolation process was found to destabilise high energy nanoparticle systems so that the nanoparticles were not preserved as individual entities in the solid form.

During the course of this work, it was demonstrated that the additive and isolation method used impacted on the final size and shape of the crystals. The growth pathway of FF was probed due to considerable differences in the crystal habits and observed growth patterns of quickly oven-dried vs. freeze-dried particles. Despite visual differences, both processes were found to adhere to the conventional growth mechanism of molecular addition by Ostwald ripening. No evidence of orientated attachment or mesocrystal particle assemblies was found. Results indicated that due to a bimodal particle size distribution, single crystals became coated in a layer of nanoparticles during the freeze-drying process. These results demonstrate the importance of considering the effect of the drying process on the observed growth mechanism.
5.7 References


Chapter 6: Conclusions and Future Directions

6.1 Conclusions

The primary objective of the research presented in this thesis was to develop an approach to improve the therapeutic response of drugs with limited bioavailability, which stems from their poor dissolution rates in aqueous environments (specifically BCS Class II drugs). Antisolvent precipitation was selected as a viable approach to generate micro and nano range drug particles, with improved dissolution rates resulting from their reduced particle size.

In Chapter 2, micron range particles of salicylic acid were prepared following an antisolvent precipitation process. The effects of changes in process conditions (and thus changes in supersaturation and mass available for crystallisation) on the particle size were analysed. Concurrent changes in both supersaturation and available mass, which accompany changes in process conditions, can have competing influences on the particle size. The factor which has the biggest dependence on changes in the process conditions (ie. m or S) governs the change in particle size which is expected to occur with changes in process conditions. For the pure system, salicylic acid crystals ranging from 20 to 150 µm were prepared by controlling the precipitation conditions. Furthermore, for the pure salicylic acid system, supersaturation was influential on the shape of precipitated crystals. Partial or complete hollowing of the rod-shaped crystals occurred under certain supersaturation conditions with the indentations being more prominent at higher supersaturation. The hollowing effect was attributed to the packing geometry of salicylic acid molecules into centrosymmetric carboxylic acid dimers in the centre of the crystal lattice, which induced a hydrophilic channel down the centre of the rods. This central hydrophilic location was more susceptible to dissolution than the hydrophobic outer surfaces. Dissolution and concomitant recrystallisation facilitated the hollowing process.
Its effect was intensified at higher supersaturation levels since crystal defects, which are more likely to occur at higher supersaturation, can aid in the dissolution process.

The use of additives provided additional control over the size and the shape of salicylic acid particles in Chapter 2. Polymers (CMC and HPMC) were the most effective in preserving the smallest particle size (e.g. 6 µm with HPMC). The change in induction time in the presence of the additives was an indicator of interactions between the additives and the drug. HPMC, and to a lesser extent CMC, increased the induction time indicating an ability to disrupt the nucleation/growth processes, resulting in a reduced particle size compared to a control sample. Furthermore, interaction between the polymeric additives and the drug induced a crystal shape modification from rod-like to needle-like. It is understood that the exposed phenolic and oxygen groups on the \{110\} surfaces of salicylic acid crystals are capable of face-selective hydrogen bonding with HPMC and CMC molecules, thus influencing the crystal habit.

The relatively low supersaturation levels utilised in Chapter 2 (<8) facilitated control over the particle size within the micron scale range. However, for particle size control at the nanoscale, much higher supersaturation conditions were required. In Chapter 3, a supersaturation level of >3000 was employed to prepare nanoparticles of the BCS Class II drug, fenofibrate. However, stabilisation, isolation and characterisation of these nanosized particles became more difficult as a result of their reduced length scale. PVA provided the most effective (albeit temporary) stabilisation for the nanoparticles in suspension due to the characteristic hydrogen bonding capabilities of both PVA and FF, compared to the other additives tested. Nanoparticle isolation and drying which was required for prolonged stabilisation proved problematic, and preservation of the original particle size and dissolution rate was not always achievable. Freeze-drying, as discussed in Chapter 5, induced agglomeration and/or fusion of insufficiently stabilised fenofibrate nanoparticles, with the fused or agglomerated outcome depending on the attachment of PVA to fenofibrate at the time of drying. Of the drying techniques tested, only evaporation at small volume (1 drop) enabled size preservation of individual nanoparticles during the drying stage.

Following the nanoparticle isolation problems which were encountered using the conventional isolation and drying techniques reported in Chapter 3 (such as direct filtration, centrifugation, freeze-drying and spray-drying), a novel approach to
nanoparticle stabilisation and isolation was developed. Chapter 4 describes a method by which nanoparticles can be isolated into the dried, solid powder form, while retaining dissolution rates which are comparable to the nanoparticles in suspension. In a one-step antisolvent precipitation process, nanoparticles of fenofibrate and mefenamic acid were generated in the presence of insoluble, micro scale, surface-functionalised carrier particles, which attracted the nanoparticles to their surface (up to 9% loading). Once captured, the nanoparticles were stable in suspension for 24 hrs and were easily filtered to produce solid-state nanoparticle composites with fast dissolution rates. The process outlined in Chapter 4 was validated for two BCS Class II drugs, but has generic potential for application to a far greater range of drug substances.

As outlined, the primary objective of the research presented in this thesis was to develop an approach to improve the bioavailability of drugs with limited dissolution rates (ie. BCS Class II drugs). By generating nanoparticles of such drugs from a supersaturated solution, and by coupling the nanoparticle preparation step with a novel nanoparticle stabilisation and isolation process, fast-dissolving, solid-state drug nanoparticles could be prepared, thus meeting the original project goals which were outlined at the outset.

6.2 Recommendations for Further Work

The literature is inundated with examples where nanoparticle generation has been used to improve the dissolution behaviour of drug particles. Regardless of whether the particles are produced through crystallisation or disintegration approaches, the vast majority of cases require isolation and drying of the nanoparticles from the liquid state. However, this important final step in the overall nanoparticle production process is relatively neglected.

The research reported in this thesis has scratched the surface in this area and has set out guidelines of a novel approach to nanoparticle production which spans the entire production process, from their preparation, to their stabilisation to their unencumbered and scalable isolation to solid state. The process incorporates a stabilisation mechanism which obviates the need for selecting specific stabilisers using the traditional trial and error stabiliser-screening approach. While the process was successful for two drug substances, a key area for future work is the application of the system to a range of other drug substances, with varying properties (ie. surface charge and hydrophobicity). Based
on the results obtained in this work (Chapter 4), and based on a logical explanation of the mechanism of action, it is hypothesised that the process is applicable to both negatively charged (as demonstrated) and positively charged drugs by changing the nature of the carrier and its surface functionalising agent. Cinnarizine and carvedilol are examples of BCS class II drugs which, based on their pKa values, are expected to possess a positive charge at neutral pH [1].

In this work, a negatively charged carrier, functionalised with a positively charged surface modifier was used to capture and stabilise negatively charged drug nanoparticles. Using the same rational, a positively charged carrier, functionalised with a negatively charged surface modifier could be used to capture and stabilise positively charged drug nanoparticles.

Based on the knowledge gained throughout the course of this research, the following approach can be followed for the preparation of drugs with improved therapeutic response, Figure 6.1.
Figure 6.1 Flow diagram of a proposed approach to prepare fast-dissolving, solid-state drug nanoparticles.

1. Use Log P values to help identify hydrophobic, BCS Class II drugs with dissolution-limited bioavailability.
2. Select water as the antisolvent. The lower the solubility in water, the higher the supersaturation which can be achieved.
3. Determine the solubility of the drug in a range of water-miscible solvents at a fixed temperature (eg. 25°C). Higher solubility will facilitate higher yields per
experiment. Where possible select a less hazardous solvent that is acceptable in
the pharmaceutical industry, as outlined by the ACS guidelines [2]. Consider the
boiling point of the solvent, since solvents with high boiling points may be
difficult to remove from the sample.

4. Determine if the drug precipitates as nanoparticles from a supersaturated solution
when a small amount of a solution of the drug (eg. 1 mL) is quickly added to an
excess amount of antisolvent (water, eg. 10 mL) under rapid agitation (eg. 1000
rpm). Measure the particle size of the precipitated particles as quick as is practical
after precipitation (eg. at an aging time of 0.5 min). If the precipitated particles are
not in the nanoparticle size range, vary the precipitation conditions (eg. drug
concentration, antisolvent to solvent volume ratio, temperature, agitation rate) in
order to tune the particle size to the nanoscale.

5. Select a medium for dissolution testing (eg. a 0.1 M HCl solution at 37°C is
representative of the environmental conditions in the stomach. A small amount of
Tween-80 can be added to improve the wettability and mixing of the hydrophobic
drug with the dissolution medium)

6. Generate a calibration curve of drug concentration vs absorption by UV-visible
spectroscopy, or HPLC if necessary. Determine the solubility of the drug in the
dissolution medium. Conduct dissolution testing at approx. 1/3 of the saturation
concentration to achieve sink conditions.

7. Determine the dissolution rate of freshly precipitated nanoparticles in suspension
(eg. at 0.5 min aging time). For example, measure the time taken to reach 90%
dissolved, 50% dissolved or measure the amount dissolved per second over the
first 30 seconds. This value can be used as a comparison, to determine if
nanoparticles are preserved as individual nano-entities during the isolation and
drying processes.

8. Monitor the size evolution of the nanoparticles in suspension over time (eg. by
laser diffraction) to determine the time window available for isolation.

9. Try to isolate the nanoparticles eg. by freeze-drying or filtration (filtration should
be finished within the stable period determined in Step 8). Determine if the
dissolution rate of the dried material is preserved compared to that of the
suspended nanoparticles. If it is not, proceed to Step 10.
10. Determine the zetapotential of the nanoparticles in suspension. If the nanoparticles are negatively charged, follow the procedure outlined in Chapter 4. (ie. using sparsely protamine-functionalise montmorillonite clay, and precipitating the drug in the presence of the functionalised clay). If the nanoparticles are positively charged, a positively charged carrier with a negatively charged functionalising agent should be considered.

11. Filter the composites using a filter paper with a pore size somewhere in between that of the nanoparticle size and the carrier particle size (eg. 2.7 µm if using montmorillonite clay as the carrier) and ensure that the drug is retained by the filter. Vacuum dry the filter cake.

12. Determine if the dissolution rate of the drug is preserved compared to that of the suspended nanoparticles.

The steps above and the flow diagram in Figure 6.1 provide a guideline of the logical steps which should be undertaken to prepare and characterise solid-state nanoparticles with fast dissolution rates, which could help to save many potential drug candidates from developmental discontinuation.

6.3 References


Appendix

A.1 Supplementary Data

Appendix 3.1
Particle size distribution of the nanosized fraction in ground TriCor tablets, after removing the larger insoluble excipients by filtration.

Appendix 3.2
Determination of the solubility of FF, and the effect of additives on the solubility of FF in the dissolution medium.
Appendix 3.3

SEM images of FF precipitated in varying antisolvent/solvent volume ratios, showing no significant influence of the AS/S ratio on the particle size.

Appendix 3.4

Calculation of the supersaturation level present during the antisolvent precipitation of FF at optimum conditions (50 mg/mL drug solution, 1 mL addition to 10 mL antisolvent, 25°C)

\[ SS = \frac{c}{c^*} = \frac{\text{Solute concentration in } \text{AS/S mixture}}{\text{Solubility in } \text{AS/S mixture}} = \frac{4.545 \text{mg/mL}}{1.169 \times 10^{-3} \text{mg/mL}} = 3888 \]

Appendix 3.5

Particle size distribution of FF precipitated from a PVA-solution at 50 mL scale (0.5 min aging time).
Appendix 4.1  NMR spectra of (a) as received and (b) converted fenofibrate

Appendix 4.2  FTIR spectra of as received and converted fenofibrate

Appendix 4.3  XRD spectra of as received and converted fenofibrate
Appendix 4.4  SEM images of (a) as received and (b) converted fenofibrate at magnifications x200 and x1000

Appendix 4.5  Dissolution profiles of Ponstan and of MEF from MEF-PA-MMT composites at 4.8% and 9.1% drug loading.
Appendix 4.6  (a) Evolution of the particle size distributions over time for FF precipitated in water with no stabilising additives, including the average particle size from each distribution, and (b) their corresponding dissolution profiles.

Appendix 4.7 Dissolution profiles of TriCor and FF-PA-MMT samples (9.1% loading) which were aged for 1 min in suspension and various times after drying.

Appendix 4.8 Particle size distribution of montmorillonite clay
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Investigation of the particle growth of fenofibrate following antisolvent precipitation and freeze-drying

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