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ENHANCING THE USABILITY OF DRUG MATERIALS BY ELECTROSTATIC ATOMISATION

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Maija Nyström

Abstract

Electrospraying or electrostatic atomisation is a process of liquid disruption by electrostatic forces. When liquid is brought into an electric field, charge is induced to its surface. Once the repulsive electrostatic force exceeds the liquid surface tension, the liquid disrupts into small highly charged droplets. The size of the electrosprayed droplets can range from hundreds of micrometers down to a few tens of nanometers.

Electrospraying can be used not only to produce droplets, but also solid particles. The research presented in this thesis concentrates on producing drug particles by this method. In the experiments, a drug powder was dissolved in a convenient solvent and the solution was atomised. The solvent was then evaporated from the formed droplets in a drying medium and inside each droplet, a dense cluster of the dissolved drug remained. From the pharmaceutical point of view, the most important characteristics of the produced particles are size distribution, porosity, crystal form and degree of crystallinity. These properties affect the dissolution behaviour and ultimately the drug bioavailability in the body. The effects of electrostatic atomisation on the aforementioned characteristics are generally not well understood. The research focused on studying these particle properties and finding possible correlations with the spraying parameters.

The produced droplets were dried either under atmospheric or reduced pressure, the latter in order to improve the drying process. Special emphasis was put on implementing the spraying under reduced pressure, and the effects of the drying pressure on particle properties. Based on the results, the possibilities to enhance the dissolution of poorly soluble drugs by this method were estimated. In the course of experiments, it was also discovered that electrospraying may have a profound effect on the polymorphic form of the produced drug particles. In the light of the obtained results, it was concluded that electrospraying may offer a valuable tool to overcome some of the challenges met in modern drug development and formulation.

Tiivistelmä

Sähköstaattinen sumutus (sähköstaattinen atomisaatio) on menetelmä, jolla neste voidaan hajottaa pieniksi pisaroiksi. Kun neste altistetaan sähkökentälle, sen pintaan indusoituu varaus. Sähköisen hylkimisvoiman ylittäessä nesteen pintajännityksen hajoaa nestesuihku voimakkaasti varautuneiksi pisaroiksi. Sähköstaattisella atomisaatiolla tuotettujen pisaroiden koko voi vaihdella sadoista mikrometreistä muutamiin kymmeniin nanometreihin.

Nestepisaroiden tuottamisen lisäksi sähköstaattista sumutusta voidaan käyttää kiinteiden partikkeleiden valmistukseen. Väitöskirjatutkimuksessa on keskitytty lääkepartikkeleiden tuottamiseen tällä menetelmällä. Tutkimuksissa lääkejauhe liuotettiin sopivaan liuottimeen ja liuos atomisoitiin. Tämän jälkeen liuotin poistettiin höyrystämällä, ja pisaroiden sisässä liuotetusta lääkeaineesta muodostui tiivis klusteri. Farmaseuttisessa mielessä valmistettujen partikkeleiden tärkeimpiä ominaisuuksia ovat kokojakauma, huokoisuus, kidemuoto ja kiteisyysaste. Nämä vaikuttavat materiaalin liukenemiseen, ja lopulta myös lääkkeen biologiseen hyötyosuuteen elimistössä. Sähköstaattisen atomisaation vaikutukset edellä mainittuihin ominaisuuksiin eivät ole yleisesti tunnettuja. Tutkimuksissa selvitettiin näitä partikkelien ominaisuuksia sekä mahdollista korrelaatiota sumutusparametrien kanssa.

Tuotetut pisarat kuivatettiin joko normaalissa ilmanpaineessa tai alennetussa paineessa. Jälkimmäisellä vaihtoehdolla kuivausta pyrittiin tehostamaan. Erityisesti alennetussa paineessa tapahtuvaa sumutusta sekä kuivauspaineen vaikutusta partikkelien ominaisuuksiin tutkittiin. Tulosten perusteella arvioitiin mahdollisuuksia hyödyntää kuvattua menetelmää niukkaliukoisten lääkeaineiden liukoisuuden parantamisessa. Tutkimuksissa havaittiin myös, että sähköstaattinen sumutus saattaa oleellisesti vaikuttaa tuotettujen partikkeleiden polymorfiseen muotoon. Tulosten perusteella pääteltiin, että sähköstaattinen atomisaatio voi tarjota varteenotettavan keinon ratkaista joitakin moderniin lääkekehitykseen ja formulointiin liittyviä haasteita.

List of Papers

I M. Nyström, M. Murtomaa and J. Salonen
Fabrication and characterization of drug particles produced by electrospraying into reduced pressure
J. Electrostat. 68, 42-48, 2010

II M. Nyström, M. Murtomaa and J. Salonen
Fabrication of amorphous pharmaceutical materials by electrospraying into reduced pressure
J. Electrostat. 69, 351-356, 2011

III M. Nyström, M. Murtomaa, J. Roine, N. Sandler and J. Salonen
Processing of pharmaceutical materials by electrospraying under reduced pressure
Drug Dev. Ind. Pharm. (In press), 2013

IV M. Nyström, J. Roine, M. Murtomaa, R. M. Sankaran, H. A. Santos and J. Salonen
Solid state transformations in consequence of electrospraying – a novel polymorphic form of piroxicam
Eur. J. Pharm. Biopharm. (Accepted for publication), 2014

Contents

1 Background

Regardless of the somewhat misleading designation, atomisation is generally not related to materials in the atomic scale. Atomisation in the scope of this thesis refers to modifying a bulk of liquid into a fine spray. A spray in turn stands for essentially the same as in the colloquial language: a two-phase system of droplets flowing within a gas [1].

Transforming a liquid bulk into a spray requires energy. It is needed to overcome the viscous forces of the liquid and formation of the new surface [2]. In pneumatic and rotary atomisers this energy is mechanical, but it can also be originated from an electric field. The process of liquid disruption by electrostatic forces is known as *electrostatic atomisation*. In this thesis, utilising this phenomenon to produce droplets with a continuous liquid flow is referred to as *electrospraying*. The presented research concentrates on fabricating solid drug particles by this method.

In the pharmaceutical industry, the proportion of drug candidates with relatively poor biopharmaceutical properties is vast. Dissolution is a major problem for drugs with poor aqueous solubility. While there are several approaches available to improve the dissolution of such materials, each process has its limitations and may not be suitable for a certain application. Therefore various approaches to overcome the problem are required. The apparatus generally used for particle production by electrospraying exhibit low-volume throughput, and are adequate only for the laboratory scale. However, the production scale can be increased to meet the requirements of an industrial process.

2 Theory

2.1 Electrostatic atomisation

When a liquid is pumped through a capillary into an electric field, charge is induced to its surface. The charge exerts a repulsive force on the surface, which modifies the shape of the liquid meniscus. Once the electrostatic force exceeds the liquid surface tension, the surface becomes unstable and the jet disrupts into small droplets. In a convenient magnitude of the electric field, the meniscus gets a conical form and small highly charged droplets eject from the cone tip [3]. This process is known as electrostatic atomisation. A typical setup for utilising the phenomenon is presented in Fig. 1.

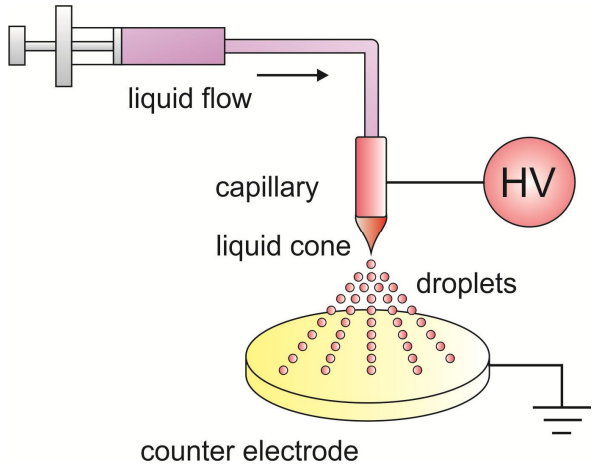

Fig.1. Schematic of the main components of electrostatic atomisation and electrospraying. The liquid flows through a capillary, which is connected to a high voltage (HV) source. The plate electrode beneath is grounded.

The high-intensity electric field necessary for the atomisation can be formed by concentrating the field to a small region, for example to a capillary tip. The most common electrode configuration is the capillary – plate or capillary – ring pair [4–6]. Usually the capillary is connected to a high voltage (HV)

supply and the counter electrode is grounded, but also the inverse connection (with the HV on the counter electrode and ground on the capillary) is possible [1,4]. The electrode configuration can also be modified by other means depending on the application, for example by using a grounded disc with a hole in the centre for sampling or introducing additional electrodes [5,7–11].

2.1.1 Energy requirements

Atomising an initial volume of liquid into smaller droplets requires energy. As reviewed by Cross (1987), energy is expended above all in three processes: formation of a new surface, overcoming the viscous forces of the liquid and energy dissipation [2]. The energy U_S required for the formation of the new surface has been estimated by Bailey (1988) [4]. If an initial spherical liquid volume with radius R is atomised into n pieces of droplets with radius r ($r << R$), the energy required for the surface formation per unit of mass becomes

$$U_S = \frac{n4\pi r^2\gamma}{n\frac{4}{3}\pi r^3\rho} = \frac{3\gamma}{r\rho} \tag{1}$$

where γ is the surface tension and ρ the density of the liquid.

In the atomisation process, the liquid changes from a continuous bulk to a dispersed state, and its dimensions change significantly. Monk (1952) approximated the energy U_V required for the shape change by considering a liquid flow through a conical body: in from the large end (input diameter d_1) with a low velocity v_1 and out of the narrow end (output diameter d_2) with a high velocity v_2. The energy lost per second due to the viscosity η is

$$\frac{U_V}{t} = \frac{8\eta Q^2 d_1^2}{3\pi L d_2^4} \tag{2}$$

where Q is the liquid volume flow rate and L is the length of the cone [12]. The energy required for the formation of the new surface is usually higher than the energy consumed due to the viscous effect [2].

Energy is also lost in the form of Joule heating of the liquid and the possible electrical breakdown processes [2,4]. In electrospraying, the jet deforms and disrupts mainly due to the electrostatic force, and no additional mechanical energy, for example in the form of forcing the liquid through a nozzle with pressure, is needed [13].

2.1.2 Liquid deformation

In electrostatic atomisation, the electric field induces stress on the liquid surface. The total electrically induced force (F_e) consists of components normal (F_{e_n}) and tangential (F_{e_t}) to the surface. The relative magnitude of the components has been reported to depend on the liquid conductivity. On low conductivity organic solvents, the tangential component is dominant. For conductive liquids, metals for instance, the predominant component of the electrostatic force is normal to the surface [14]. This is due to the distinct routes of charge convection in the materials: in a conductive liquid the charge can be transported through the bulk, but in a low conductivity liquid the charge is convected mainly on the surface [15].

On top of the electrostatic and liquid surface tension forces (F_e and F_γ), also the viscous and inertial forces (F_η and F_i) which oppose the liquid movement, gravity (F_G) and electrostatic repulsion between the formed droplets (F_q) act on the liquid cone and jet. These forces are illustrated in Fig. 2. The lengths of the arrows in Fig. 2 do not refer to the force magnitudes, only their directions in the macroscopic view.

2.1.3 Rayleigh limit

In an electrically charged droplet, the limit at which the repulsive electrostatic force becomes larger than the attractive surface tension force, is known as the Rayleigh limit [16]. It sets an upper limit for the charge q that can be carried by a single droplet, and is expressed as follows:

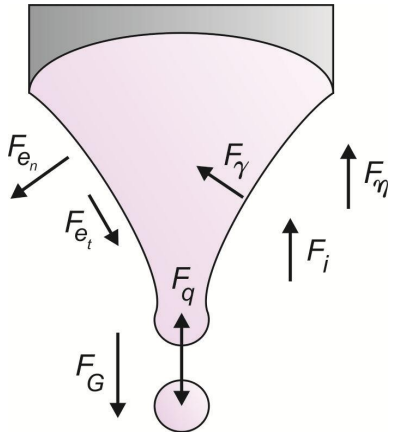

Fig. 2. Forces acting on the liquid cone in electrostatic atomisation: normal and tangential components of the electrically induced force (F_{e_n} and F_{e_t}); surface tension (F_γ); viscous and inertial forces (F_η and F_i); gravity (F_G); and electrostatic repulsion between the formed droplets (F_q).

$$q = \sqrt{64\pi^2 \varepsilon_0 \gamma r^3} \tag{3}$$

Accordingly, the maximum specific charge of the droplet is

$$\frac{q}{m} = \sqrt{\frac{36\varepsilon_0 \gamma}{r^3 \rho}} \tag{4}$$

where ε_0 is the permittivity of free space, γ is the surface tension, r and m are the droplet radius and mass, and ρ is the liquid density. In electrospraying, the disruption takes place already at lower levels of charge, according to some estimates close to one-half of that predicted by the Rayleigh limit [13,17–20].

2.2 Functioning modes

Electrostatic atomisation progresses through several visual and measurable changes, when the capillary voltage is increased and the liquid flow rate maintained constant. The different spraying regimes are called modes. In his pio-

neering work on electrified liquids, this phenomenon was described and illustrated roughly a century ago by Zeleny (1916 and 1917) [21,22].

Since the droplets formed in electrostatic atomisation are highly charged, the stability and functioning mode of the spray can be monitored by measuring the electric current (I) carried by the droplets [19,23,24]. When the voltage at the capillary tip (U) is increased, the dripping frequency and I increase accordingly. The most important functioning modes and I as a function of U on ethanol are presented in Fig. 3. The measurement was done by first increasing the U up to the maximum value, and then decreasing it back to the starting value, using voltage intervals of 0.1 kV and constant liquid flow rate. A more detailed description of the measurement is given in Paper I.

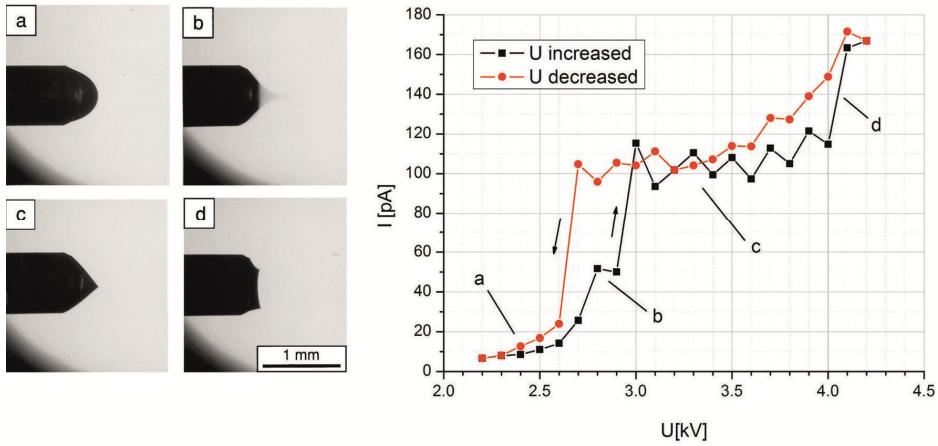

Fig. 3. On the left, photographs of the different spraying modes on ethanol: a) dripping mode, b) pulsating mode, c) cone-jet mode and d) multi-jet mode [24]. On the right, the electric current I carried by ethanol droplets versus the applied voltage U. The letters a, b, c and d correspond to the different spraying modes [25].

The dripping mode (Fig. 3a; U = 2.2 – 2.6 kV) is generally characterised by production of large droplets at low frequency [6]. Therefore also I remains low. This mode is obtained when the electric field is absent, and with low values of U. Depending on the liquid properties, wettability of the capillary and liquid flow rate, the droplet diameter may be smaller or larger than that

of the capillary. The former mode is referred to as micro dripping [26,27]. When *U* is increased, the dripping frequency increases, and the droplets become smaller. The liquid meniscus alternates between a round and a conical form, and an increase in *I* is detected. This phase is called pulsating mode (Fig. 3b; *U* = 2.7 – 2.9 kV). In the dripping and pulsating modes, the main droplets may be accompanied with formation of smaller satellite droplets.

When *U* is increased further up to the critical value of 3.0 kV, *I* rises sharply and the liquid meniscus changes abruptly to a conical shape. This is the usually desired cone-jet mode (Fig. 3c). Taylor (1964) showed that a liquid surface gets a conical form in a strong electric field, which is stable if the half-angle of the cone apex is equal to 49.3 ° [28]. Therefore, the liquid cone is often referred to as Taylor cone [29]. The cone-jet domain with the current setup is between 3.0 and 4.0 kV. A hysteresis phenomenon is detected in the appearance of this mode when *U* is decreased. When *U* is further increased above 4.0 kV, the meniscus becomes unstable and multiple jets appear on the cone. The number of the emitting jets increases with the increasing *U*, and *I* rises sharply again [3]. This mode is called multi-jet mode (Fig. 3d).

The droplet formation mechanism at the liquid meniscus can also be classified more broadly into just two categories: dripping (Fig. 3a and b) and jetting (Fig. 3c and d) [5,26]. Alternatively, a more detailed classification can be done by studying the intermediate forms of the modes, ramification of the jet and the detailed shape of the cone [8,19,26,27]. Moreover, not all of the above-mentioned modes appear on all liquids: for example the multi-jet mode is not detected on water. Although transitions between modes are not always clearly defined and overlap regions exist, different spraying modes may lead to very different characteristics of the produced aerosols. Therefore distinguishing and controlling the regime at which the spray operates, is fundamental [3,5,27].

2.3 Electrospray operation

The size of the electrosprayed droplets can range from hundreds of micrometers down to a few tens of nanometers, and the size distribution is usually nearly monodisperse [6,19]. In addition to the electric field strength,

the droplet formation is affected by the solvent properties (internal conditions) and the geometry and operation of the electrospraying apparatus (external conditions).

2.3.1 Internal conditions

Electrical relaxation time of a liquid is given by the product of its resistivity and absolute permittivity, and it determines the rate at which electric charge relaxes from the liquid bulk to its surface. Regarding the droplet formation and electrospraying, this quantity is of primary importance [4]. While liquid resistivities may range over several orders of magnitudes, the permittivities range within a much narrower region. Therefore the resistivity has a more profound effect on the relaxation time [30]. When the conductivity of an electrospraying solvent increases (with the other solvent properties remaining constant), the amount of charge on the liquid surface can grow higher, and the obtained droplets become smaller [3,6,10,23,30]. The drawback of the increased charge density is that it causes irregular lateral motion inside the cone and wider droplet size distribution [3,30]. Liquids with very low conductivity cannot be atomised using electrostatic forces: estimates of this lower limit vary between 10^{-8} – 10^{-11} S/m [3,23,30,31].

According to Eq. (1), the energy needed for the surface formation during atomisation is proportional to the surface tension of the liquid. Liquids with low surface tension can be atomised in a relatively weak electric field. Instead, liquids with high surface tension such as water, may require electric fields that are high enough to exceed the breakdown threshold of the surrounding gas (Chapter 2.3.2) [3,23,32]. The surface tension of an organic solvent can be reduced by adding surfactants, and the effect of surfactants on the droplet size in electrospraying has been reported to be marginal [10,23].

According to Eq. (2), the energy required for the jet formation depends also on the liquid viscosity, which affects the jet breakup at the capillary tip. On a viscous solvent, the liquid filament forming at the cone apex is long and the disrupted droplets are large [23,30]. However, also contradictorily results have been reported on a setup utilising AC voltage excitation (Chapter 2.3.2): an increase in the viscosity caused a decrease in the jet diameter and droplet size on water/glycol mixtures [33]. AC excited and direct voltage setups are nevertheless quite different, and changing the liquid properties independently from each other may not be possible.

2.3.2 External conditions

As mentioned previously, the droplet size and charging are strongly affected by the mode of spraying. Increasing the capillary voltage leads to formation of smaller droplets, but the electrical breakdown strength of the surrounding gas sets an upper-limit for the voltage. A discharge momentarily reduces the electric field strength, and results in fairly broad droplet size distributions [34]. This problem can be overcome by executing the electrospraying in an atmosphere with a higher electrical breakdown threshold than air, such as vacuum or sheath gas [5,19,21,24,34–39]. In addition to the applied voltage, the electric field strength is affected by the diameter of the capillary and the distance between the high voltage and grounded electrodes. In the cone-jet mode, the size of the capillary has been reported to affect the droplet size [4,6,18].

Various models for the dependency between liquid flow rate and droplet size in the cone-jet mode have been presented. De la Mora and Loscertales (1994) stated that the droplet size is proportional to the cubic root of the liquid flow rate, while Gañan-Calvo (1999) calculated the proportionality to the square root of the flow rate [15,29]. Regardless of the lack of indisputable knowledge, the effect of liquid flow rate on the droplet size is apparent: the smaller the flow rate, the smaller the droplet size. Depending on the liquid properties, there is a minimum flow rate at which electrospraying can be implemented in a steady state [40].

The droplet formation can also be regulated by harmonic jet excitation. The periodic oscillations at the liquid meniscus can be accomplished either by mechanical [41,42] or electrical methods [20,33,43–47]. The jet source can be vibrated mechanically for example by a piezo-electric transducer. With the latter method, a pulsed or AC voltage is superimposed on the DC voltage at the capillary tip, and uniform droplets can be produced synchronously with the voltage oscillations.

2.4 Particle production

The electrospraying process can be used not only to produce liquid droplets, but also solid particles. In drug particle production, the drug powder is dissolved in a convenient solvent and the solution is atomised using

electrostatic forces. The jet disrupts into droplets which are highly charged and relatively uniform in size, and the charge of the same sign prevents the coalescence of the droplets [13]. The solvent is then evaporated from the formed droplets in a drying medium, and a dense cluster of the dissolved drug remains. The size of the produced particles can be controlled by varying the concentration of the dissolved material and the previously mentioned internal and external spraying conditions [6,30].

2.4.1 Neutralisation

The highly charged droplets produced by electrospraying repel each other and drift in the direction governed by the electric field. As the liquid evaporates, the droplets' mass decreases and they may disrupt again at the Rayleigh limit. In order to maintain continuous and stable droplet production, it is often favourable to remove the electric charge from the droplets by introducing a neutralisator to the system. The neutralised droplets can be directed to the collector for example by air flow or gravity.

The functioning of the static charge neutralisators is based on ion production. The ions increase the conductivity of the surrounding gas, which allows the neutralisation of the charged bodies. The ion production can be based for example on corona discharge. The discharge occurs when a high voltage is applied between two electrodes, one of which is a sharp spike or a thin wire. The strong electric field near the sharp electrode causes a discharge in the surrounding gas resulting in an avalanche of electrons and formation of ions [2]. The corona neutralisator can be operated either with direct or alternating voltage. Other appropriate techniques to eliminate the droplets' charge are radioactive neutralisators and electrospraying two liquids from adjacent capillaries with opposite polarities [19,24,48–50].

2.4.2 Drying and collecting

In order to acquire solid particles by electrospraying, the solvent and the dissolved material need to be separated by vaporising the solvent. The drying phase in electrospraying is similar to the spray drying method, which is used in diverse industrial fields [51–53]. In the spray drying process, a solution or dispersion is atomised for example by rotary discs or pneumatic nozzles, after

which it is set in contact with a hot drying gas. Also in applications based on electrostatic atomisation, the drying can be done in an elevated temperature or reduced pressure to enhance the solvent vaporisation [13,54–56]. Naturally, also the distance between the capillary and the collecting interface affects the drying efficiency. The dried particles can be collected depending on the subsequent treatment on grounded surfaces, membrane filters, sample holders or in receiving liquids [10,39,56–60].

2.4.3 Upscaling

A single capillary electrospraying system is mainly adequate for laboratory research scale. Increasing the liquid flow rate or concentration of the dissolved material leads to larger droplet and particle sizes, which in turn would require compensating by increasing the electric field strength to form a stable cone or multi-jet spraying mode. This is possible only up until the electrical breakdown threshold of the surrounding gas. The yield of the process can hence be improved only slightly by increasing the aforementioned parameters. Also, using small capillaries is essential.

The process can be upscaled to a higher level of throughput by using a linear array of capillaries [9,61–63]. Constant liquid flow is delivered to the emitters, and each capillary works similarly to a basic, single nozzle unit. The capillaries can also be replaced by planar array of holes machined on a substrate [64–67]. In such systems, the proximity of the capillaries influences the electric field. In order to attain a product of uniform quality, irregularities in the liquid flow to each capillary and interaction of nozzles need to be taken into account. Also by carefully optimising the internal and external spraying conditions, the particle collection efficiency can be improved [68].

3 Electrospraying of pharmaceutical materials

The main objective of the research presented in this thesis was to study certain properties of the drug particles produced by electrospraying, and the possibilities to tailor these properties. In the pharmaceutical sense, the most important characteristics of the particles are size distribution, porosity, crystal form and degree of crystallinity. These affect the dissolution behaviour of the drug, and ultimately the bioavailability in the body. In the following, the most important particle properties and the role of electrospraying in determining them are discussed.

3.1 Particle properties

3.1.1 Dissolution

The proportion of drug candidates with relatively poor biopharmaceutical properties, particularly in the form of low water solubility, has increased significantly over the past twenty years [69–76]. In drug discovery setting, the compounds found by high throughput screening tend to shift towards more lipophilic and higher molecular weight profile and therefore the new compounds are less soluble [71,77,78]. Dissolution is a major problem for orally administered drugs with poor aqueous solubility, which results in low bioavailability [79–83]. While there are several approaches available to improve the dissolution, such as salt formation, particle size reduction, prodrug formation and complexication, each process has its limitations and may not be suitable for a certain application. Therefore various approaches are required.

The dissolution of a drug from a solid dosage form involves at least two consecutive steps: liberation of the drug from the solid to the liquid phase (disintegration) and migration of the drug molecules to the bulk of the solution (solubilisation). The overall dissolution rate depends on the slower one of these processes [84,85]. Dissolution test determines the cumulative amount of drug that is released into the solution as a function of time [85]. The dissolution rate can be formulated by the well-known Noyes-Whitney

equation (1897), which was modified in the following form by Brunner (1904):

$$\frac{dC}{dt} = \frac{DS}{Vh}(C_s - C_t) \quad (5)$$

where dC/dt is the dissolution rate, D is the diffusion coefficient of the drug in the solution, S is the interfacial surface area of the exposed solid drug, V is the volume of the solution, h is the thickness of the diffusion boundary layer, C_s is the concentration of a saturated solution of the drug at the surface of the solid, and C_t is the concentration of the drug in the bulk medium at time t [86]. Based on equation (5), the dissolution rate is proportional to the surface area of the dissolving particles. Hence it can be concluded that the dissolution can be enhanced for example by decreasing the particle size and increasing the porosity of the particles.

3.1.2 Particle size

By size reduction, the specific surface area of the drug particles can be increased. Also the thickness of the diffusion boundary layer h is small on micronized and nanosized particles [87,88]. This results in a faster transport of the dissolved material from the solid – liquid surface to the bulk of the solution, which in turn enhances the dissolution rate. Hydrophilic drug materials tend to dissolve fast regardless of the size distribution. For poorly water-soluble drugs however, the particle size affects the dissolution rate significantly, and micronized particles ($r < 10$ μm) are often used [78,89]. The most common methods for drug particle size reduction are milling, microemulsification and spray drying [84,89–92]. However, these methods are not free of complications and may not be suitable for certain compounds and applications [83,93–95].

Electrospraying enables fabrication of monodisperse particles in both micro- and nanoscale, with sizes down to 10 nm (according to some reports even 2 - 4 nm) [13,19,48]. The particle size can be controlled by the means described in paragraphs 2.3 and 2.4. In addition, the drying pressure has been reported to affect the particle size during electrospraying [55].

3.1.3 Porosity

Similarly to the particle size reduction, dissolution enhancement by introducing porous particles is based on the growth of the specific surface area. Porous particles also have a small density compared to compact particles. These properties influence the functional properties of the drug remarkably. Porous or shell particles have proved to be very useful for example in the pulmonary drug delivery [56,91].

The porosity of the particles produced by electrostatic and rotating disc atomisation has been reported to depend mainly on the drying conditions [10,56,96,97]. Quick drying in an elevated temperature tends to lead to formation of porous or hollow particles, because the solvent evaporates faster than the concentration gradients inside the particle can equalize. The fast evaporation may result in an increased concentration of drug material on the droplet surface, and formation of a "skin layer". In order to avoid the collapse of the skin layer and to attain solid and dense particles, small values of liquid flow rate have been recommended [98]. As to electrospraying polymer materials, reducing the drying pressure increased the surface roughness of the produced particles. Increasing the dissolved polymer concentration had a similar effect [55].

3.1.4 Amorphous materials

Insolubility of a drug material is caused either by a limited ability to hydrogen bond with water (hydrophobicity) or by difficulty in breaking apart molecules in the solid state (high lattice energy) [99]. Converting crystalline drug compounds to their amorphous counterparts is a promising tool in improving the solubility, since the physical links between the drug molecules are weaker in the amorphous form [100–104]. The long-range order of molecular packing characteristic to a crystalline solid, is absent in the amorphous material.

The drawback of using amorphous materials in pharmaceutical applications is their instability: amorphous solids are generally less stable than the corresponding crystals. Amorphous solids tend to crystallise during manufacturing, storage and administration (dissolution) [102]. In order to avoid crystallisation, amorphous drugs can be formulated as solid

dispersions, corporated in mesoporous materials, treated with stabilising additives and stored in an appropriate temperature and humidity [102,103,105,106].

Similarly with the porosity, the drying phase is most likely a substantial factor regarding the formation of amorphous material during electrospraying. If the drying and solidification take place fast as is the case in spray drying, the molecules may not have enough time to arrange into crystals, which causes formation of amorphous material [84,94].

3.1.5 Crystal forms

Depending on the crystallisation conditions, some solid materials possess more than one potential crystal structure, and the molecules may be aligned in different ways respect to one another in the crystal lattice. The different crystal forms of the same substance are known as polymorphs. A classic example of crystalline polymorphism is the graphite – diamond pair. Since the different polymorphs possess varying lattice energies, the macroscopic properties such as melting point and stability of the material depend on the polymorphic form [84].

In a drug molecule, the presence of many functional groups enables the multitude of hydrogen-bonding regimes. Therefore the majority of drug materials exhibits polymorphism [107]. Often it is of a form known as monotropic polymorphism, meaning that the lowest energy polymorph is the most stable one and will not convert to another form during storage as a drug product. The other polymorphs are described as metastable, and tend to spontaneously convert to the stable form with the rate depending on the storage conditions [84,108]. Crystalline solids can also contain a solvent molecule within the crystal structure. These adducts are known as solvates or pseudo polymorphs. If the incorporated solvent molecule is water, the solvate is termed a hydrate.

During the course of development of a drug, usually the lowest energy polymorph is chosen for development to assure reproducible bioavailability of the product over its shelf life [108]. Any phase change due to polymorph conversion, desolvation of the solvates, formation of the hydrate or a change in the degree of crystallinity may alter the bioavailability. However, also metastable crystalline (or amorphous) forms may be justifiable because of a medical benefit, for example an increase in the dissolution rate. It is often

possible to improve chemical stability of such forms by using appropriate excipients and storage conditions [84,109–113]. Since polymorphism may have such a profound effect on the bioavailability of poorly soluble drugs, it is of great importance for manufacturers to chart the different polymorphs of a potential product. The search for drug polymorphs is a complex empirical exercise, and discrete crystal forms are considered non-obvious and patentable [114].

The surface temperature of the droplets formed in electrospraying decreases due to solvent evaporation, which may be of the essence on temperature dependent crystal transitions. In the study of Wang *et al* (2012), the energy of a drug molecule (carbamazepine) dipole in an electric field was estimated, and it was concluded that the fast evaporation and temperature decrease preponderated over the effect of electric field on the material crystallisation [98]. The produced nanoparticles were determined to be predominantly amorphous immediately after electrospraying, but the crystallisation could be accelerated by annealing at high temperatures. In another study on electrospraying drug (naproxen) nanosuspensions, the polymorph remained the same throughout the process, but slight decrease in the crystallinity and reduction in the size of the primary crystals (grains) were detected [115].

As stated in the previous paragraphs, the drying phase of electrospraying has a profound effect on the drug particle morphology. The spray drying kinetics of biological and pharmaceutical materials and the subsequent particle morphology have been previously studied [116]. However, the effect of electric field and the electrospraying process on the morphology, crystallinity, and polymorphism of pharmaceutical materials during and after the process are not well understood [98].

3.2 Electrospraying of biological materials

When electrospraying is used for processing of biological or other fragile materials, special attention should be paid on the process conditions. The solvent must be chosen carefully, so that the molecules remain chemically stable in it. With drug materials, the main concern should be preserving the therapeutic effect of the material. Ion bombardment and the possible formation of free radicals in an electric discharge could damage the materials.

In a previous spectroscopic study, the production of OH-radicals which could be harmful to biological molecules, was reported to be negligible in the vicinity of an electrospraying nozzle [117]. The liquids used in the study were distilled water, methanol, ethanol and ethylene glycol. Also, the biological activity of insulin was found to endure the electrospraying process, and the secondary structure of a protein (bovine serum albumin) did not go through significant changes either [118,119]. Solutions containing living cells, viruses and bacteria have also been electrosprayed together with a shell or matrix material, and the organisms remained viable in the process [120–125]. According to these findings, the biological molecules are not damaged by electrospraying at fairly low voltages.

3.3 Pharmaceutical applications

Perhaps the best-known biological application of electrospray is its use for ion production in mass spectrometry [126]. This development was recognised by Nobel Prize in Chemistry in 2002. There are a number of other potential biomedical applications that utilise electrospraying, ranging from implant coating to gene therapy. In the following, some promising reports are discussed.

In pulmonary drug administration, small particles with narrow size distribution are required. The first inhaler known to be based on electrospraying was patented in 1989, and later further developed [34,127]. Electrosprays present a promising method for administering also DNA-based therapeutics to the pulmonary epithelium [128]. The potential harm to the respiratory epithelium caused by the highly charged droplets has also been studied, and the effects were negligible [124]. Regardless of the number of academic efforts and encouraging results in designing an electrospray inhalation device, so far no commercial product is available [32,59,130,131].

Electrospraying can also be used for deposition of biological coatings with controlled surface morphology. In this process, the electrosprayed droplets including the coating material are directed towards a grounded substrate. After solvent evaporation, a thin layer is formed onto the substrate surface. This method has been successfully used to fabricate biologically active coatings on metallic implants, which enables the control of the interaction between the implant and the surrounding tissue [132–135].

Nanosized or nanostructured pharmaceutical particles can provide enhanced efficacy, solubility or biocompatibility, and administration at much lower dosages. Electrospraying has been used to produce polymer particles, which can be used as delivery vehicles for bioactive agents in the nanoscale [10,58,136,137]. To prevent irreversible aggregation of the drug nanoparticles during drying, electrospraying has been used to produce redispersable drug nanoparticles [115]. Also solid nanoparticles of pure insulin have been produced and characterized [118].

As to electrospraying polymer materials, different shapes such as spherical, elongated, doughnut-shaped and fibrous particles can also be obtained. The final morphology can be tailored suitable for the drug delivery application in question, by judiciously selecting the polymer molecular weight, concentration and solution flow rate [97,138,139]. Also different shapes of drug (carbamazepine) particles have been produced by varying the dissolved drug concentration [140].

3.4 Electrospray derivatives

Electrostatic atomisation can be utilised not only to produce single component particles, but also capsules and matrix particles. The encapsulation can be implemented by electrospraying colloidal suspension of the core material, and solidifying the shell by solvent evaporation or immersing in a gelatinising or polymerising agent. The core and shell material can be sprayed from single or separate capillaries. This method is referred to in the literature as electroencapsulation, and it can be used for example to deliver a core drug material to its target in the body in a controlled manner [6,54,141–148].

Another derivative of electrospraying that has been widely studied for biomedical applications is electrospinning [120,149–152]. This technique enables the production of polymeric multifunctional fibres in the micro- and nanoscale. In electrospinning, the desired polymer solution is atomised similarly as in electrospraying, but the ejected jet elongates to form a continuous fibre. The ordering of the fibres can be controlled by manipulating the collector or the electric field [149]. For example, a portable electrospinning spraygun has been developed to produce fibrous wound dressing patches [63,153].

4 Aims of the Study

An apparatus for electrospraying pharmaceutical materials was designed and built at the Laboratory of Industrial Physics (Department of Physics and Astronomy, University of Turku) in 2007 by Matti Murtomaa, Olli-Pekka Hämäläinen and Mika Aarnio. Experiments on certain solvents and drug materials were performed at the time. Sustaining droplet and particle output in a scale adequate for process and product characterisation was nevertheless yet to be achieved.

Early on in the project, the study focused on finding suitable parameters of operation (solvents, liquid flow rates, atomisation and neutralisation voltages, drying pressures, drug concentrations etc.) to enable stable droplet production. Determining the spray functioning modes (Chapter 2.2) of appropriate solvents was of the essence. Also, estimating the droplet and particle size as a function of liquid flow rate became an objective. Introducing the reduced drying pressure to the system brought about its own challenges in the form of possible boiling of the solvents, clogging of the capillaries, electric discharges and incomplete droplet neutralisation. Determining the working range of the apparatus was fundamental at the beginning, and it is mostly discussed in paper I.

Once the sample amounts had become adequate for experiments other than electron microscopy, emphasis was put on studying the produced particles (papers II, III and IV). Particularly, the fast evaporation of solvents in the reduced pressure had raised questions about the amorphicity and porosity of the particles. Together with the size distribution, these characteristics are known to substantially affect the dissolution of drug materials. Therefore these properties and the possible correlation with the spraying parameters were studied, and a hypothesis regarding dissolution enhancement of poorly soluble drugs by electrospraying, was stated.

In the course of the studies on the produced particles, some new findings were made. One of the model drug materials exhibited an unrecognised x-ray diffraction pattern after electrospraying. It was unclear whether this crystal structure was due to molecule degradation, formation of a solvate or an unknown polymorphic form of the material in question. The material crystallisation during electrospraying is not similar to slow crystallisation in a dish or even fast crystallisation achieved by spray drying, although the same

drug and solvent combination would be used. The electrospraying process involves strong electric fields, temperature gradients and fast evaporation of the solvent. Under these circumstances, for example polymorphic transitions may take place. At the final stage of the research, the objective was to discover what had caused the unexpected diffraction pattern of the electrosprayed drug.

5 Experimental

5.1 Materials

The studies were performed using three model drugs: *indomethacin*, *piroxicam* and *budesonide*. Indomethacin (delivered by Hawkins Pharmaceutical, Roseville, MN, USA) is a light-sensitive drug used to relieve pain and inflammatory conditions. It is poorly soluble in water, and exists in at least two polymorphic crystalline forms: metastable α-form and a stable γ-form [154,155]. Piroxicam (Hawkins Pharmaceutical, Roseville, MN, USA) is a potent, non-steroidal anti-inflammatory drug. It is also poorly soluble in water and shows dissolution-rate-limited low oral bioavailability in the crystalline state [156]. Piroxicam exhibits polymorphism with the form designated as I being the most stable crystal structure [157,158]. Budesonide (Orion Corporation, Espoo, Finland) is an anti-inflammatory corticosteroid. It is so chemically and physically stable that it is almost insoluble to water at physiological pH, and it is not known to exhibit polymorphism [159].

In electrospraying the most important properties of the solution are its conductivity, surface tension and viscosity. The solvents were chosen optimising these properties and taking into account that the drug materials have to be soluble, nevertheless chemically stable in them. Thus, ethanol and chloroform were chosen for the studies.

5.2 Electrospraying

A schematic of the electrostatic atomisation unit and the drying chamber is presented in Figure 4. Solutions were pumped continuously through a stainless steel capillary with a computer controlled syringe pump. The capillary was connected to a high voltage (HV) source set to positive potential. The atomising electric field formed between the capillary and a grounded plate electrode beneath it. The distance between these electrodes was fixed at ca. 1 cm. A circular metal plate (guard plate) was attached to HV conductor above the capillary, and maintained at the same potential as the capillary. The purpose of the guard plate was to

make the electric field near the capillary tip more uniform, and to prevent external electric disturbances [7].

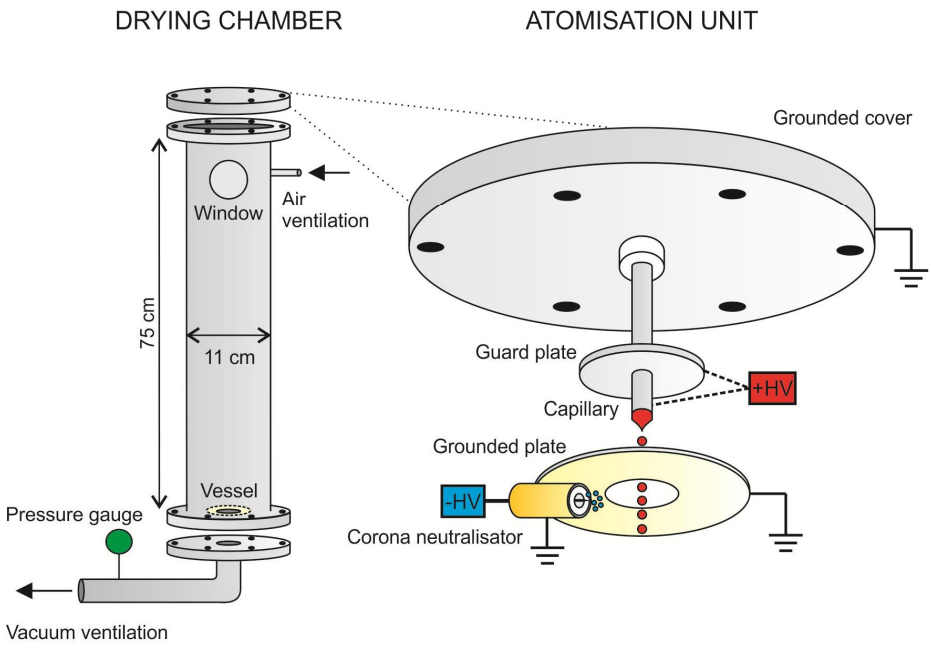

Fig. 4. Depressurisable drying chamber (on the left) and the electrostatic atomisation unit (on the right). The parts connected to high voltage sources are marked with a symbol HV.

When the apparatus was used for particle production, the produced droplets were dried either under atmospheric or reduced pressure, the latter in order to improve the drying process. The drying was carried out in a cylindrical steel chamber under room temperature. The particles were collected on a vessel located at the bottom of the chamber (in paper III, also from the chamber walls).

The electrosprayed, highly charged droplets were neutralised in order to avoid adhesion to grounded surfaces and further disruption at the Rayleigh limit. This was done using a corona neutralisator with the configuration presented in Fig. 5. The corona needle was connected to a HV source set to negative potential, and the cylinder electrode

surrounding the needle was grounded. A stable corona burst was maintained at the needle tip, which caused ionisation of the air and neutralisation of the cascading droplets.

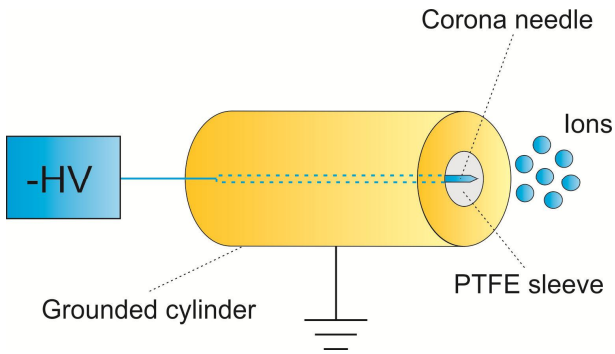

Fig. 5. Configuration of the corona neutralisator. Negative high voltage (HV) was connected to the needle electrode, which was insulated from the grounded cylinder electrode with a PTFE (polytetrafluoroethylene) sleeve.

5.3 Process analysis

In papers I and III, the electric charge carried by the droplets was measured by spraying the solutions directly into a Faraday pail. The pail was located approximately 3 cm below the capillary. The accumulated charge was measured with a Keithley 6517A electrometer (Keithley Instruments, Cleveland, OH, USA).

5.4 Particle characterisation

5.4.1 Scanning electron microscopy

Produced particles were characterised with scanning electron microscope (SEM) for the size distribution, shape, porosity and crystal habit. In papers I – III, the particles were collected on a nylon filter placed to the bottom of the drying chamber. The filter was set on an SEM stub, which was coated with carbon conductive tape. To improve the conductivity, the samples were

coated with a 20-30 nm layer of gold or palladium. The particles were studied with an S200 SEM system (Cambridge Instruments, Cambridge, UK). In paper IV, a part of the samples was collected also from the bottom of the drying chamber, and studied with a Vega SB SEM system (Tescan USA, Warrendale, PA, USA).

An Image-Pro Plus 1.3 image analysis software (Media Cybernetics, Inc., Rockville, MD, USA) was used in parallel with the SEM images to determine the particle size distribution of the fabricated particles.

5.4.2 X-ray diffraction

X-ray diffraction (XRD) was utilised to study the crystallinity and polymorphism of the electrosprayed particles. In papers I and II, the crystallinity was studied with PW 1830/1820/1710 diffractometer (Panalytical, Almelo, Netherlands). In paper IV, the samples were studied with Scintag X-1 XRD system (Scintag Inc., Cupertino, CA, USA); or Philips X'Pert Pro MPD X-ray diffraction system, equipped with a PW3050/60 goniometer (Panalytical, Almelo, Netherlands) and a TTK 450 low temperature chamber (Anton Paar, Graz, Austria) for the variable temperature x-ray diffraction (VTXRD) measurements. Cu-*K*α radiation and scanning speed of 0.01 – 0.1 °/s were used for all measurements, except for the VTXRD measurements where a 255 channel PIXcel detector (Panalytical, Almelo, Netherlands) enabled a high combined scanning speed of 0.66 °/s.

In papers I and II, XRD was exploited to detect and quantify the amorphous proportion in the electrosprayed samples. The presence of amorphous content leads to a decrease in the intensity of the diffraction peaks [160]. The degree of crystallinity (DOC) of the samples was quantified by calculating the ratio of the integrated peak intensities of the sample and a crystalline reference after a programmatic background subtraction. By XRD, disorder below approximately 10 % cannot be accurately detected, but on larger proportions of amorphous material, it is an applicable method [161–163].

5.4.3 Differential scanning calorimetry

Differential scanning calorimetry (DSC) was used to determine the amorphicity in the electrosprayed drug materials (papers I and II). Calorimetric measurements were carried out using Pyris Diamond DSC (PerkinElmer, Waltham, MA, USA) with a scanning speed of 10 °C/min. The calibration was done with an indium standard. The weighed samples were sealed in aluminium pans with holes, and measured under nitrogen gas flow.
In a DSC thermogram, clear indications of amorphous content in the sample are glass transition, exothermic crystallisation peak and a decrease in the enthalpy of fusion [162–165]. In paper I, the degree of crystallinity (DOC) of the electrosprayed samples was calculated from the ratio of the heat of fusion of the sample and a crystalline reference. In paper II, the amorphicity of the samples was estimated qualitatively based on the DSC results, and quantitatively by XRD. The detection limit of DSC is reported to be rather similar to that of XRD: the lower limit of detectable amorphous content is approximately 10 % [166]. In paper IV, DSC was used to study the polymorphic form of the electrosprayed samples.

5.4.4 Nitrogen adsorption

The porosity of the electrosprayed particles was studied quantitatively with nitrogen adsorption in paper III. Nitrogen adsorption measurements at 77 K were done with TriStar 3000 gas sorption apparatus (Micromeritics, Norcross, GA, USA). The specific surface areas of the particles were determined from the obtained adsorption isotherms using the equation by Brunauer, Emmett and Teller (BET equation) [167].

5.4.5 UV spectroscopy

In paper III, the dissolution of the electrosprayed drug particles in PBS (*phosphate buffered saline*, pH = 7.4) medium was studied. The studies were performed at room temperature under *sink conditions*, defined as follows: the volume of the medium must be greater than three times that required to form a saturated solution of the drug substance [85]. The solution was set on a magnetic stirrer in a sealed vessel. At appropriate time intervals, a sample of

the dissolution medium was withdrawn and immediately replaced with fresh medium. Subsequently, the samples were centrifuged to separate the non-dissolved fraction of the drug material. The supernatants were collected, and the UV absorbance of the solutions was measured with a Lambda 25 UV-VIS spectrophotometer (PerkinElmer, Waltham, MA, USA) using quartz cells with a light path of 10 mm. Based on the Beer-Lambert law for liquids, the absorbance is directly proportional to the molar concentration of the absorbing species. For calibration, standard solutions with known values of concentration were fabricated and measured.

5.4.6 Additional methods

In paper IV, some additional techniques were utilised to study the crystalline structure of the electrosprayed piroxicam. To detect the possible molecule degradation products, the total piroxicam content and the stability of the drug were quantified by high performance liquid chromatography (HPLC, Agilent 1100 Series, Agilent Technologies, Waldbronn, Germany). The experimental details are presented in the original paper.

The produced particles were also characterised by Raman and Fourier transform infrared (FTIR) spectroscopies. The Raman spectra were obtained with an InVia spectrometer (Renishaw, Wotton-under-Edge, UK) at a laser excitation wavelength of 785 nm. The FTIR spectra were collected with a Spectrum BX spectrometer (PerkinElmer, Waltham, MA, USA) using MIRacle attenuated total reflectance accessory (Pike Technologies Inc., Madison, WI, USA).

Also a thermogravimetric analysis was performed to detect the possible solvate removal. The measurements were performed with a TGA-7 instrument (PerkinElmer, Waltham, MA, USA) at a heating rate of 10 °C/min under nitrogen gas flow.

6 Results and Discussion on Papers

In the following section, a short discussion of each paper is presented. The experiments have been described in detail in the original papers, and the purpose of this chapter is merely to highlight the most important results.

6.1 Paper I

In paper I, the construction of the apparatus designed for electrospraying pharmaceutical materials was presented. The atomisation unit, electrode geometry and drying system were described. The setup was tested using pure solvent (ethanol) and a solution containing drug material (indomethacin). Experiments were performed both under atmospheric and reduced pressure. The essential variables in the studies were liquid flow rate and atomisation voltage.

In this paper, emphasis was put on the measurements performed by Faraday pail and electrometer. The electric current carried by the electrosprayed ethanol droplets as a function of the atomisation voltage was measured (Fig. 3 of this thesis). The different functioning modes were clearly visible in the graph. The electric current was measured also as a function of the liquid flow rate. The size of the produced droplets was estimated based on their charge, approximating the charge level equal to 50 % of the Rayleigh limit. The results correlated fairly well with the scaling laws presented by De la Mora and Loscertales (1994) and Gañan-Calvo (1999) [15,29]. By this method, also the size of the produced particles was calculated and compared with the experimental results obtained by SEM. The calculated average sizes were consistent with the measured ones. Also, the degree of crystallinity (DOC) and the polymorphic form of the produced indomethacin particles were evaluated and discussed briefly.

Paper I was more or less technical in nature. The electric charge measurements were informative and useful regarding the forthcoming research. At the time, the yield of the process was low, and mainly microscopy and DSC analysis of the particles was feasible. The polymorphic form was studied also by XRD, but the measurements were performed with a very small amount of sample. It was evident that the throughput had to be increased in order to study the produced particles

more profoundly. The results gave however a good indication of the nature of the particles that could be produced by the setup: partly amorphous, very regularly shaped with diameters less than 10 μm and a narrow size distribution (Fig. 6).

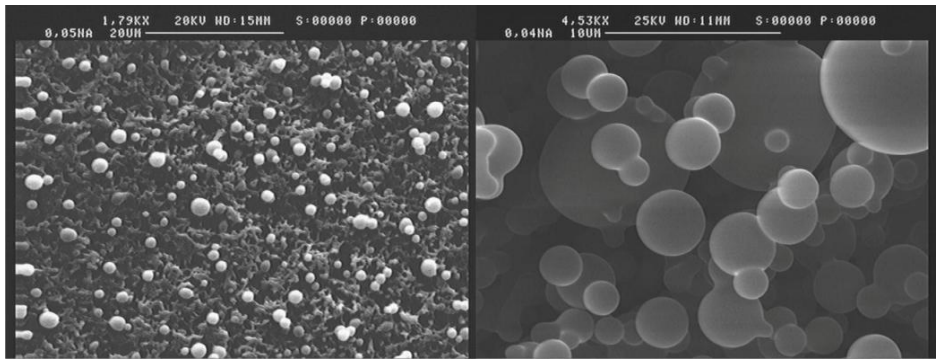

Fig. 6. SEM images of the indomethacin particles produced by electrospraying. (The structure on the background is the nylon filter on which the particles were collected.)

6.2 Paper II

By the time of the research related to paper II, some modifications had been made to the setup to improve the yield. The corona neutralisator had been moved closer to the jet and it was operated at slightly higher voltages. Liquid flow rates were also increased. The samples were collected not only on a nylon filter, but also from the bottom of the drying chamber. Though appearing minor, these changes increased the particle production rate remarkably.

Since producing adequate sample amounts for XRD measurements was possible, the studies of paper II focused on the amorphicity of the produced particles. The experiments were performed with three model drugs: indomethacin (dissolved in ethanol), piroxicam and budesonide (dissolved in chloroform). Special attention was paid on electrospraying under reduced pressure and the effect of pressure on the product crystallinity. All the experiments were performed using stable cone-jet mode.

Regarding the particle shape and size distribution, the results were consistent with those presented in paper I; all materials produced spherical, regularly shaped particles. The determined particle size distributions are presented in Fig. 7.

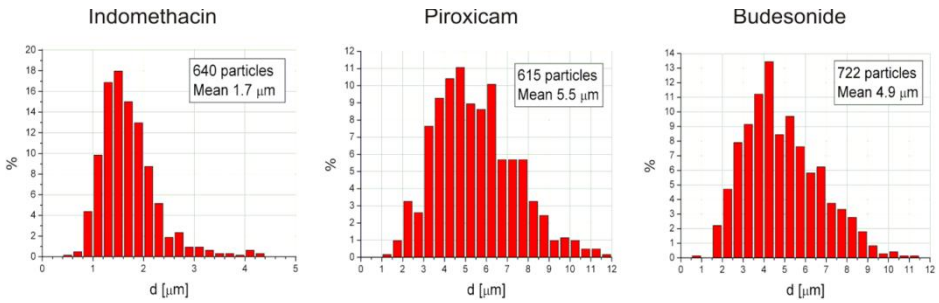

Fig. 7. Particle size distributions of the electrosprayed indomethacin, piroxicam and budesonide particles. The number of analysed particles and the mean particle diameter are also included.

The fabricated particles were analysed with DSC and XRD to study their crystallinity. Based on the XRD results, the maximum degree of crystallinity (DOC) was calculated for the electrosprayed indomethacin and budesonide particles. The samples contained a notable proportion of amorphous material. (For piroxicam, this sort of absolute degree of crystallinity could not be calculated due to the lack of crystalline reference.) The XRD measurement of the electrosprayed budesonide is presented in Fig. 8.

For chloroform solutions (budesonide and piroxicam particles) electrospraying in the reduced pressure, led to formation of more amorphous particles than electrospraying in the atmospheric pressure. Instead for ethanol solutions (indomethacin particles), the results were quite the opposite: the particles produced in the atmospheric pressure were more amorphous. It was concluded that this was due to the fast evaporation of chloroform compared to ethanol: the pressure reduction at this range increased the amount of amorphous material only if the solution was volatile enough. With ethanol solutions, the droplet formation in the reduced pressure was too irregular after all, to gain a more amorphous product.

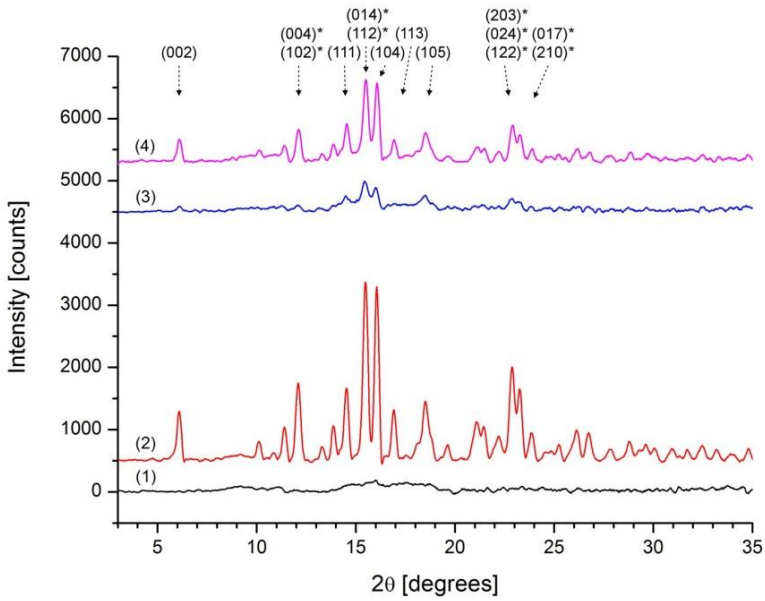

Fig. 8. The diffractograms of budesonide particles: electrosprayed in the pressure of 0.5 atm (1) and 1.0 atm (3). Diffractogram (2) is that of a crystalline reference measured with the same sample holder as (1). Similarly, diffractogram (4) represents the crystalline reference for (3). The measured peak positions of diffractograms (2)–(4) match with the referenced peak positions of budesonide. The Miller indices for some of the identified diffraction peaks are shown above the diffractograms (an asterisk denotes overlapping peaks).

Determining the DOC from the particles produced by this method is quite coarse and dependent on the XRD data processing techniques. The possible crystallisation during sample preparation and different crystal size of the sample and the reference may cause error to the absolute values of DOC. However, the peak areas obtained for atmospheric and reduced pressure samples can be reasonably compared, because the measurements include similar sources of error. The quantification of amorphous proportion by DSC can also be somewhat inaccurate, so it was used only to detect the amorphous content in the present study. The results proved to be useful regarding the

possible dissolution enhancement of poorly soluble drugs, achieved by electrospraying. They can also be beneficial for scientists designing electrospraying experiments: in some cases amorphous material is the desired outcome, in other applications its formation is disadvantageous.

6.3 Paper III

Also in paper III emphasis was put on the properties of the produced particles, yet also the technical features of the apparatus were supplemented through an experiment regarding the corona neutralisator. This experiment will be discussed first.

The functioning of the neutralisator was tested by electrospraying tap water directly into a Faraday pail. Four different values of liquid flow rate (Q) were used, and the accumulation of electric charge (q) into the pail was measured with an electrometer. The high voltage of the neutralisator was switched on at t = 180 s point of time (Fig. 9).

It can be noted in Fig. 9 that q increased linearly for all values of Q until the neutralisator was switched on. After this point, q remained at the same level or even decreased slightly. The results indicated that the neutralisator worked efficiently.

In paper III, the effect of pressure reduction on particle morphology was further studied. In addition to amorphicity, the fast solvent evaporation may affect also other important particle properties: the size distribution, porosity and ultimately dissolution. These attributes were studied using budesonide as a model drug. It was dissolved in chloroform and electrosprayed using stable cone-jet mode under pressures of 0.5 – 1.0 atm.

The fabricated budesonide particles were studied firstly with SEM. Based on the images, the particle size distributions for both the atmospheric and reduced pressure samples were determined. These deviated very slightly. In the SEM images, the particles produced in the reduced pressure seemed more porous and hollow than the particles produced in atmospheric pressure (Fig. 10).

To quantify the porosity, the specific surface areas of the particles were determined by nitrogen adsorption. For the particles produced in pressure of 0.7 atm, the specific surface area was 1.6 times larger than that of the particles produced in the atmospheric pressure.

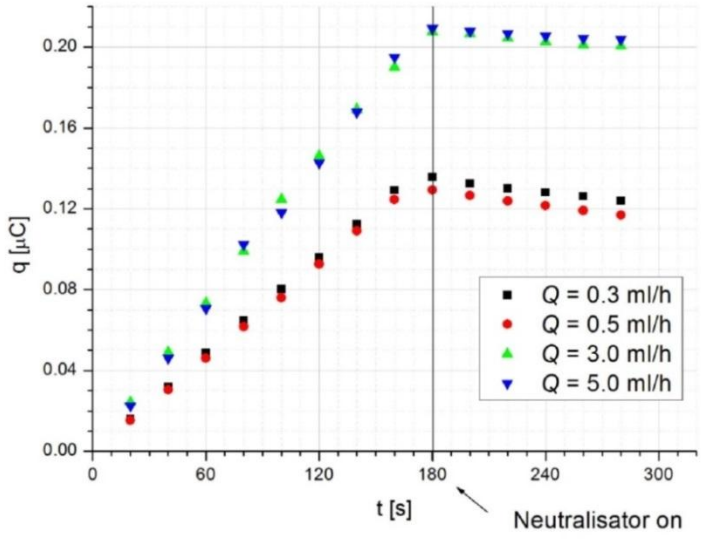

Fig. 9. Electrospraying of water into a Faraday pail using four different values of liquid flow rate (Q). The amount of cumulative charge (q) increased constantly until the neutralisator was switched on at t = 180 s.

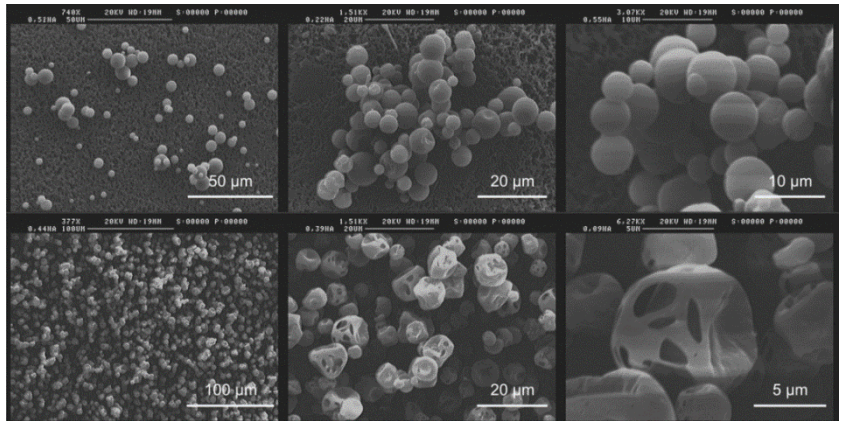

Fig. 10. Budesonide particles fabricated by electrospraying in atmospheric pressure (upper row) and reduced pressure (lower row).

To summarise, in paper II it was concluded that the budesonide particles produced in the reduced pressure were more amorphous than those produced in the atmospheric pressure. Also the porosity increased due to the pressure reduction, but the particle size remained roughly the same. Finally, the dissolution of the particles was studied with UV-spectrometry. The results indicated the following: for particles produced in the pressure of 0.7 atm, the dissolution was faster than for the particles produced in the pressure of 1.0 atm. However, no further improvement in the dissolution rate was detected as the pressure was reduced below 0.7 atm. This was most likely due to the spraying instabilities occurring at low pressures, which were discussed in more detail in the original paper.

When designing the experiments of paper III, the dissolution test was considered to summarise the benefits of electrospraying under reduced pressure. However, the dissolution rate was improved only when the pressure was reduced to 0.7 atm, and the increase was not as remarkable as was expected. The wettability of the amorphous and crystalline samples may be different, and therefore comparing the dissolution results directly may not be the most descriptive method. Therefore, if the increased dissolution rate is the main concern, using composite particles would perhaps be more advisable than pure drug materials.

Nevertheless, when pharmaceutical particles with certain morphology are required, for example for pulmonary drug delivery, electrospraying is a potential method. As demonstrated in papers I-III, the particle properties can be controlled quite comprehensively by adjusting the aforementioned process parameters. Since electrospraying in the reduced pressure does not require elevated temperatures, it may be an appropriate technique to process also temperature sensitive materials.

6.4 Paper IV

The basis for the research related to paper IV was formed during the preparation of paper II. In the studies, piroxicam was dissolved in chloroform and electrosprayed. It was noted that the diffractogram measured for the electrosprayed particles did not match with any of the known piroxicam crystal structures (*Cambridge Crystallographic Data Centre*). Three polymorphic forms of piroxicam (known as I, II and III)

and a monohydrate have been found without dispute [157,158]. In a previous study by another group, piroxicam was crystallised from chloroform and the obtained polymorph was either I or II depending on the cooling rate of the solution [168]. The x-ray diffractograms of the reported forms of piroxicam and the electrosprayed sample are presented in Fig. 11.

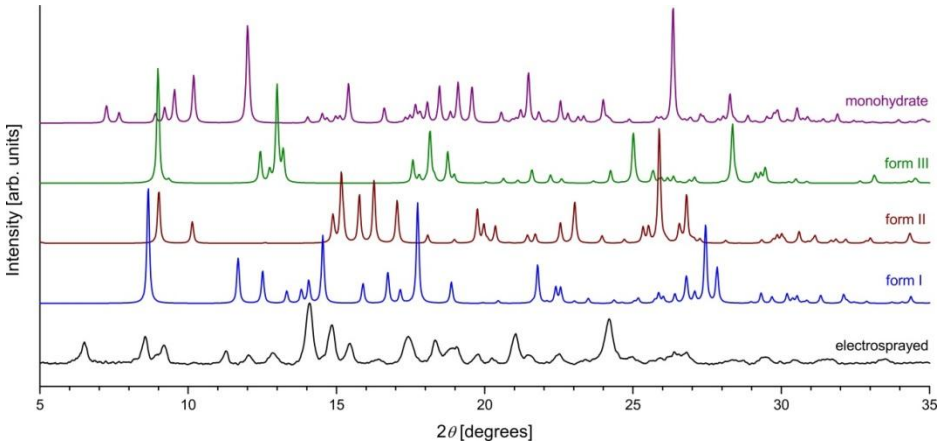

Fig. 11. Calculated x-ray diffractograms of the known crystal forms of piroxicam, and the measured diffractogram of electrosprayed piroxicam.

It was concluded that the piroxicam molecule may had degraded during the electrospraying process, and therefore it crystallised in an unrecognised form. Another possibility was formation of a solvate. Therefore the electrosprayed piroxicam particles were studied with HPLC, which however did not reveal any degradation products. No solvate removal was detected in the calorimetric and thermogravimetric measurements either (Fig. 12).

It can be noted in Fig. 12, that the melting of the as-received piroxicam batch (form I) takes place at a slightly higher temperature (202.9 °C) than that of the electrosprayed samples (199.6 °C). Also, the melting of the electrosprayed samples is preceded by exothermic recrystallisation.

Formation of a novel polymorphic form of piroxicam during electrospraying appeared possible. Therefore the electrosprayed particles were studied extensively by XRD. The unrecognised crystal structure remained intact even after 127 days of storage at room conditions.

A strong implication of a novel polymorphic form was obtained by variable temperature XRD: the material recrystallised completely into piroxicam form I as a result of heating. Also, the FTIR and Raman spectra of the electrosprayed piroxicam were compared to those of the form I, and some notable differences in peak positions, shapes and intensities were detected. Overall, the results indicated that electrospraying produced an as yet unreported polymorph of piroxicam. Crystallisation of this form was independent of the values of atomisation voltage, liquid flow rate and drying pressure at the applied range.

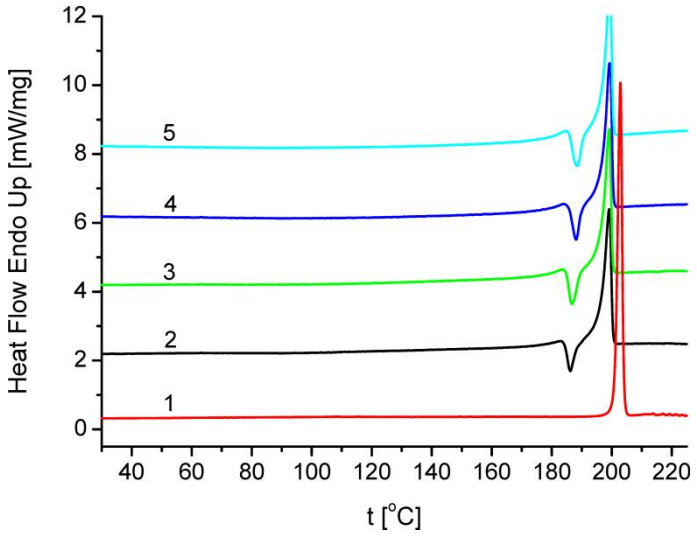

Fig. 12. DSC scans of the original piroxicam powder (1) and piroxicam electrosprayed at atmospheric pressure (2 – 5).

In electrospraying, the conditions for material crystallisation are drastic: formation of small highly charged droplets in a strong electric field, followed by fast evaporation of the solvent. Under the influence of a strong electric field, the drug molecules may be forced to align in the direction of the field in the solution. Due to the small droplet size and fast solvent evaporation, the molecules may remain in this position after exiting the field, which may enable the crystallisation of new polymorphic forms.

7 Conclusions

In this thesis, a setup for electrospraying pharmaceutical materials was presented. The produced droplets were dried both under atmospheric pressure, and in order to improve the drying process, under reduced pressure. To avoid adhesion to grounded surfaces and further disruption, the electrosprayed droplets were neutralised with a corona neutralisator. By this method, microscale particles with narrow size distribution were produced using indomethacin, piroxicam and budesonide as model drugs.

The effects of electrospraying on the morphology, crystallinity, and polymorphism of pharmaceutical materials during and after the process are generally not well understood. Several parameters can be varied during the process, each of which may have an effect on the particle properties. In this thesis the size distribution, degree of crystallinity, polymorphic form and porosity of the produced particles were studied. Special emphasis was put on the effects of drying pressure on these properties. The results of the samples obtained in reduced pressure were compared to those of the atmospheric pressure batches. When a volatile solvent (chloroform) was used, the amorphicity and porosity of the product increased due to the pressure reduction. The particle size remained roughly the same. In addition, the possibilities to improve the dissolution of poorly soluble drugs by electrospraying were estimated. By using a moderate pressure reduction, the dissolution was slightly improved.

Since electrospraying in the reduced pressure does not require elevated temperatures, it may be an appropriate technique to process temperature sensitive materials. The electrostatic atomisation itself has proved to be a non-destructive method for processing biological materials. With drug materials, the main concern should be preserving the therapeutic effect of the material. As demonstrated in papers I-III of this thesis and previous studies by other groups, the particle properties can be controlled quite comprehensively by adjusting the process parameters. When pharmaceutical particles with certain morphology are required, electrospraying is a very potential method.

The search for drug polymorphs is a complex empirical exercise, and discrete crystal forms are considered non-obvious. In paper IV, it was shown that by utilising electrostatic atomisation, production of new polymorphs is

feasible. To the best of author's knowledge, electrospraying has previously been used in drug particle production mainly to fabricate micro- (and nano-) particles with appropriate properties for certain drug delivery application. Therefore, the scope presented in paper IV and this thesis is novel, and the technique should be applicable to other drug materials as well. The material crystallisation during electrospraying is not similar to slow crystallisation in a dish or even fast crystallisation processes (spray drying), although the same drug and solvent combination would be used. The process involves strong electric fields, possible temperature gradients and fast evaporation of the solvent. Under these conditions, formation of new exiting crystal forms is possible, and electrospraying may offer a valuable tool in the search of new drug polymorphs in the future.

References

[1] A. Krupa, A. Jaworek, A.T. Sobczyk, A. Marchewicz, M. Szudyga, T. Antes, Charged spray generation for gas cleaning applications, J. Electrostat. 71 (2013) 260–264.

[2] J. Cross, Electrostatics principles, problems and applications, Adam Hilger, Bristol, England, 1987.

[3] M. Cloupeau, B. Prunet-Foch, Electrostatic spraying of liquids in cone-jet mode, J. Electrostat. 22 (1989) 135–159.

[4] A.G. Bailey, Electrostatic spraying of liquids, Research Studies Press Ltd. / John Wiley & Sons Inc., Somerset, England, 1988.

[5] J.M. Grace, J.C.M. Marijnissen, A review of liquid atomisation, J. Aerosol Sci. 25 (1994) 1005–1019.

[6] A. Jaworek, A.T. Sobczyk, Electrospraying route to nanotechnology: An overview, J. Electrostat. 66 (2008) 197–219.

[7] H. Park, K. Kim, S. Kim, Effects of a guard plate on the characteristics of an electrospray in the cone-jet mode, J. Aerosol Sci. 35 (2004) 1295–1312.

[8] A. Jaworek, A. Krupa, Jet and drops formation in electrohydrodynamic spraying of liquids. A systematic approach, Exp. Fluids. 27 (1999) 43–52.

[9] A. Jaworek, M. Lackowski, A. Krupa, T. Czech, Electrostatic interaction of free EHD jets, Exp. Fluids. 40 (2006) 568–576.

[10] J. Xie, L.K. Lim, Y. Phua, J. Hua, C.-H. Wang, Electrohydrodynamic atomization for biodegradable polymeric particle production., J. Colloid Interface Sci. 302 (2006) 103–12.

[11] G. Kim, J. Park, H. Han, Production of microsized PMMA droplets using electrospraying with various auxiliary fields., J. Colloid Interface Sci. 299 (2006) 593–8.

[12] G. Monk, Viscous energy dissipated during the atomization of a liquid, J. Appl. Phys. 23 (1952) 288.

[13] A. Jaworek, Micro- and nanoparticle production by electrospraying, Powder Technol. 176 (2007) 18–35.

[14] R.G. Forbes, Liquid-Metal Ion Sources and Electrosprays Operating in Cone-Jet Mode: Some Theoretical Comparisons and Comments, J. Aerosol Sci. 31 (2000) 97–120.

[15] A.M. Gañan-Calvo, The surface charge in electrospraying: its nature and its universal scaling laws, J. Aerosol Sci. 30 (1999) 863–872.

[16] J. Rayleigh, On the Equilibrium of Liquid Conducting Masses charged with Electricity, Phil. Mag. 5 (1882) 184–186.

[17] C. Hendricks, Charged droplet experiments, J. Colloid Sci. 17 (1962) 249–259.

[18] T. Williams, A. Bailey, Anomalous charging of droplets during electric-field-accelerated dripping, J. Electrostat. 17 (1985) 47–55.

[19] D. Chen, D.Y.H. Pui, S.L. Kaufman, Electrospraying of conducting liquids for monodisperse aerosol generation in the 4 nm to 1.8 μm diameter range, J. Aerosol Sci. 26 (1995) 963–977.

[20] S.. Sample, R. Bollini, Production of Liquid Aerosols by Harmonic Electrical Spraying, J. Colloid Interface Sci. 41 (1972) 185–193.

[21] J. Zeleny, On the conditions of instability of electrified drops, with applications to the electrical discharge from liquid points, Proceeding Cambridge Philos. Soc. 18 (1916) 71–83.

[22] J. Zeleny, Instability of electrified liquid surfaces, Phys. Rev. 10 (1917) 1–6.

[23] D.P.H. Smith, The Electrohydrodynamic Atomization of Liquids, IEEE Trans. Ind. Appl. IA-22 (1986) 527–535.

[24] I.W. Lenggoro, K. Okuyama, J. Fernández de la Mora, N. Tohge, Preparation of ZnS nanoparticles by electrospray pyrolysis, J. Aerosol Sci. 31 (2000) 121–136.

[25] M. Nyström, M. Murtomaa, J. Salonen, Fabrication and characterization of drug particles produced by electrospraying into reduced pressure, J. Electrostat. 68 (2010) 42–48.

[26] M. Cloupeau, B. Prunet-Foch, Electrohydrodynamic spraying functioning modes: a critical review, J. Aerosol Sci. 25 (1994) 1021–1036.

[27] M. Cloupeau, B. Prunet-Foch, Electrostatic spraying of liquids: Main functioning modes, J. Electrostat. 25 (1990) 165–184.

[28] G. Taylor, Disintegration of water drops in an electric field, Proc. R. Soc. London. Ser. A. 280 (1964) 383–397.

[29] J. De La Mora, I. Loscertales, The current emitted by highly conducting Taylor cones, J. Fluid Mech. 260 (1994) 155–184.

[30] A.G. Bailey, W. Balachandran, The disruption of electrically charged jets of viscous liquid, J. Electrostat. 10 (1981) 99–105.

[31] V. Drozin, The electrical dispersion of liquids as aerosols, J. Colloid Sci. 10 (1955) 158–164.

[32] J.C. Ijsebaert, K.B. Geerse, J.C. Marijnissen, J.W. Lammers, P. Zanen, Electro-hydrodynamic atomization of drug solutions for inhalation purposes., J. Appl. Physiol. 91 (2001) 2735–41.

[33] A. Jaworek, W. Machowski, A. Krupa, W. Balachandran, Viscosity effect on EHD spraying using AC superimposed on DC electric field, 35th IEEE Ind. Appl. Conf. Rome, 8–12 Oct. 2000. (2000) 770–776.

[34] K. Tang, A. Gomez, Generation by electrospray of monodisperse water droplets for targeted drug delivery by inhalation, J. Aerosol Sci. 25 (1994) 1237–1249.

[35] A.G. Bailey, A.E. Borzabadi, Natural periodic electrostatic spraying of liquids, IEEE Trans. Ind. Appl. IA-14 (1978) 162–167.

[36] B.K. Ku, S.S. Kim, Electrohydrodynamic spraying characteristics of glycerol solutions in vacuum, J. Electrostat. 57 (2003) 109–128.

[37] M. Gamero-Castaño, The structure of electrospray beams in vacuum, J. Fluid Mech. 604 (2008) 339–368.

[38] J.C. Swarbrick, J. Ben Taylor, J.N. O'Shea, Electrospray deposition in vacuum, Appl. Surf. Sci. 252 (2006) 5622–5626.

[39] C.J. Hogan, K.M. Yun, D.-R. Chen, I.W. Lenggoro, P. Biswas, K. Okuyama, Controlled size polymer particle production via electrohydrodynamic atomization, Colloid Surf. A. 311 (2007) 67–76.

[40] A. Barrero, I.G. Loscertales, Micro- and Nanoparticles via Capillary Flows, Annu. Rev. Fluid Mech. 39 (2007) 89–106.

[41] J. Schneider, N. Lindblad, C. Hendricks, J. Crowley, Stability of an Electrified Liquid Jet, J. Appl. Phys. 38 (1967) 2599–2605.

[42] L. Ström, The Generation of Monodisperse Aerosols by Means of a Disintegrated Jet of Liquid, Rev. Sci. Instrum. 40 (1969) 778–782.

[43] B. Vonnegut, R. Neubauer, Production of monodisperse liquid particles by electrical atomization, J. Colloid Sci. 7 (1952) 616–622.

[44] M. Sato, The production of essentially uniform-sized liquid droplets in gaseous or immiscible liquid media under applied a.c. potential, J. Electrostat. 15 (1984) 237–247.

[45] Z.A. Huneiti, W. Balachandran, S. Member, W.W. Machowski, Harmonic Spraying of Conducting Liquids Employing AC – DC Electric Fields, 34 (1998) 279–285.

[46] J. Li, On the stability of electrohydrodynamic spraying in the cone-jet mode, J. Electrostat. 65 (2007) 251–255.

[47] J.L. Li, EHD sprayings induced by the pulsed voltage superimposed to a bias voltage, J. Electrostat. 65 (2007) 750–757.

[48] K.C. Lewis, D.M. Dohmeier, J.W. Jorgenson, S.L. Kaufman, F. Zarrin, F.D. Dorman, Electrospray-condensation particle counter: a molecule-counting LC detector for macromolecules., Anal. Chem. 66 (1994) 2285–92.

[49] M. Cloupeau, Recipes for use of EHD spraying in cone-jet mode and notes on corona discharge effects, J. Aerosol Sci. 25 (1994) 1143–1157.

[50] J. Borra, D. Camelot, J. Marijnissen, B. Scarlett, A new production process of powders with defined properties by electrohydrodynamic atomization of liquids and post-production electrical mixing, J. Electrostat. 40-41 (1997) 633–638.

[51] A. Paudel, Z.A. Worku, J. Meeus, S. Guns, G. Van den Mooter, Manufacturing of solid dispersions of poorly water soluble drugs by spray drying: formulation and process considerations., Int. J. Pharm. 453 (2013) 253–84.

[52] K. Okuyama, M. Abdullah, I.W. Lenggoro, F. Iskandar, Preparation of functional nanostructured particles by spray drying, Adv. Powder Technol. 17 (2006) 587–611.

[53] K. Cal, K. Sollohub, Spray Drying Technique. I: Hardware and Process Parameters, J. Pharm. Sci. 99 (2010) 575–586.

[54] J. Roine, M. Murtomaa, M. Myllys, J. Salonen, Dual-capillary electroencapsulation of mesoporous silicon drug carrier particles for controlled oral drug delivery, J. Electrostat. 70 (2012) 428–437.

[55] Y. Wu, S.J. Kennedy, R.L. Clark, Polymeric particle formation through electrospraying at low atmospheric pressure, J. Biomed. Mater. Res. B. Appl. Biomater. 90 (2009) 381–7.

[56] T. Ciach, Microencapsulation of drugs by electro-hydro-dynamic atomization, Int. J. Pharm. 324 (2006) 51–55.

[57] J. Yao, L. Kuang Lim, J. Xie, J. Hua, C.-H. Wang, Characterization of electrospraying process for polymeric particle fabrication, J. Aerosol Sci. 39 (2008) 987–1002.

[58] Y. Wu, J.A. MacKay, J.R. McDaniel, A. Chilkoti, R.L. Clark, Fabrication of elastin-like polypeptide nanoparticles for drug delivery by electrospraying, Biomacromolecules. 10 (2009) 19–24.

[59] S.W. Li, S.N. Jayasinghe, M.J. Edirisinghe, Aspirin particle formation by electric-field-assisted release of droplets, Chem. Eng. Sci. 61 (2006) 3091–3097.

[60] M. Sato, H. Takahashi, M. Awazu, T. Ohshima, Production of ultra-uniformly-sized silica particles by applying ac superimposed on dc voltage, J. Electrostat. 46 (1999) 171–176.

[61] J.C. Almekinders, Multiple jet electrohydrodynamic spraying and applications, J. Aerosol Sci. 30 (1999) 969–971.

[62] A.J. Rulison, R.C. Flagan, Scale-up of electrospray atomization using linear arrays of Taylor cones, (1993) 683–687.

[63] P. Sofokleous, E. Stride, W. Bonfield, M. Edirisinghe, Design, construction and performance of a portable handheld electrohydrodynamic multi-needle spray gun for biomedical applications, Mater. Sci. Eng. C. 33 (2013) 213–223.

[64] R. Bocanegra, D. Galán, M. Márquez, I. Loscertales, A. Barrero, Multiple electrosprays emitted from an array of holes, J. Aerosol Sci. 36 (2005) 1387–1399.

[65] W. Deng, J.F. Klemic, X. Li, M.A. Reed, A. Gomez, Increase of electrospray throughput using multiplexed microfabricated sources for the scalable generation of monodisperse droplets, J. Aerosol Sci. 37 (2006) 696–714.

[66] W. Deng, C.M. Waits, B. Morgan, A. Gomez, Compact multiplexing of monodisperse electrosprays, J. Aerosol Sci. 40 (2009) 907–918.

[67] K. Tang, Y. Lin, D.W. Matson, T. Kim, R.D. Smith, Generation of multiple electrosprays using microfabricated emitter arrays for improved mass spectrometric sensitivity, Anal. Chem. 73 (2001) 1658–63.

[68] A. Rezvanpour, A.B.E. Attia, C. Wang, Enhancement of particle collection efficiency in electrohydrodynamic atomization process for pharmaceutical particle fabrication, Ind. Eng. Chem. Res. 49 (2010) 12620–12631.

[69] D. V Patel, E.M. Gordon, Applications of small-molecule combinatorial chemistry to drug discovery, Drug Discov. Today. 1 (1996) 134–144.

[70] A. Fahr, X. Liu, Drug delivery strategies for poorly water-soluble drugs, Expert Opin. Drug Deliv. 4 (2007) 403–16.

[71] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Deliv. Rev. 46 (2001) 3–26.

[72] P.H.-L. Tran, T.T.-D. Tran, K.-H. Lee, D.-J. Kim, B.-J. Lee, Dissolution-modulating mechanism of pH modifiers in solid dispersion containing weakly acidic or basic drugs with poor water solubility, Expert Opin. Drug Deliv. 7 (2010) 647–61.

[73] S.T. Buckley, K.J. Frank, G. Fricker, M. Brandl, Biopharmaceutical classification of poorly soluble drugs with respect to "enabling formulations," Eur. J. Pharm. Sci. 50 (2013) 8–16.

[74] D.N. Bikiaris, Solid dispersions, part I: recent evolutions and future opportunities in manufacturing methods for dissolution rate enhancement of poorly water-soluble drugs, Expert Opin. Drug Deliv. 8 (2011) 1501–19.

[75] B.E. Rabinow, Nanosuspensions in drug delivery, Nat. Rev. Drug Discov. 3 (2004) 785–96.

[76] C. Leuner, J. Dressman, Improving drug solubility for oral delivery using solid dispersions, Eur. J. Pharm. Biopharm. 50 (2000) 47–60.

[77] C.A. Lipinski, Drug-like properties and the causes of poor solubility and poor permeability, J. Pharmacol. Toxicol. Methods. 44 (2001) 235–49.

[78] K.R. Chu, E. Lee, S.H. Jeong, E.-S. Park, Effect of particle size on the dissolution behaviors of poorly water-soluble drugs, Arch. Pharm. Res. 35 (2012) 1187–95.

[79] F. Kesisoglou, S. Panmai, Y. Wu, Nanosizing - oral formulation development and biopharmaceutical evaluation, Adv. Drug Deliv. Rev. 59 (2007) 631–44.

[80] N. Blagden, M. de Matas, P.T. Gavan, P. York, Crystal engineering of active pharmaceutical ingredients to improve solubility and dissolution rates, Adv. Drug Deliv. Rev. 59 (2007) 617–30.

[81] J.-U.A.H. Junghanns, R.H. Müller, Nanocrystal technology, drug delivery and clinical applications, Int. J. Nanomedicine. 3 (2008) 295–309.

[82] S. Janssens, G. Van den Mooter, Review: physical chemistry of solid dispersions, J. Pharm. Pharmacol. 61 (2009) 1571–86.

[83] Y. Kawabata, K. Wada, M. Nakatani, S. Yamada, S. Onoue, Formulation design for poorly water-soluble drugs based on biopharmaceutics classification system: basic approaches and practical applications, Int. J. Pharm. 420 (2011) 1–10.

[84] M.E. Aulton, Pharmaceutics, 3rd ed., Elsevier, Amsterdam, Netherlands, 2007.

[85] C.K. Brown, H.P. Chokshi, B. Nickerson, R.A. Reed, B.R. Rohrs, P.A. Shah, Acceptable analytical practices for dissolution testing of poorly soluble compounds, Pharm. Technol. Dec 2004 (2005) 56–65.

[86] A. Dokoumetzidis, P. Macheras, A century of dissolution research: from Noyes and Whitney to the biopharmaceutics classification system, Int. J. Pharm. 321 (2006) 1–11.

[87] M. Bisrat, C. Nyström, Physicochemical aspects of drug release . VIII . The relation between particle size and surface specific dissolution rate in agitated suspensions, Int. J. Pharm. 47 (1988) 223–231.

[88] J.P. Möschwitzer, Drug nanocrystals in the commercial pharmaceutical development process, Int. J. Pharm. 453 (2013) 142–56.

[89] N. Rasenack, B.W. Müller, Micron-size drug particles: common and novel micronization techniques, Pharm. Dev. Technol. 9 (2004) 1–13.

[90] A.O. Nornoo, H. Zheng, L.B. Lopes, B. Johnson-Restrepo, K. Kannan, R. Reed, Oral microemulsions of paclitaxel: in situ and pharmacokinetic studies, Eur. J. Pharm. Biopharm. 71 (2009) 310–7.

[91] L.M. Nolan, L. Tajber, B.F. McDonald, A.S. Barham, O.I. Corrigan, A.M. Healy, Excipient-free nanoporous microparticles of budesonide for pulmonary delivery, Eur. J. Pharm. Sci. 37 (2009) 593–602.

[92] K. Sollohub, K. Cal, Spray drying technique: II. Current applications in pharmaceutical technology, J. Pharm. Sci. 99 (2010) 587–597.

[93] A. Bohr, J. Kristensen, E. Stride, M. Dyas, M. Edirisinghe, Preparation of microspheres containing low solubility drug compound by electrohydrodynamic spraying, Int. J. Pharm. 412 (2011) 59–67.

[94] M. Murtomaa, M. Savolainen, L. Christiansen, J. Rantanen, E. Laine, J. Yliruusi, Static electrification of powders during spray drying, J. Electrostat. 62 (2004) 63–72.

[95] S. Zgoulli, V. Grek, G. Barre, G. Goffinet, P. Thonart, S. Zinner, Microencapsulation of erythromycin and clarithromycin using a spray-drying technique, J. Microencapsul. 16 (1999) 565–71.

[96] Y. Senuma, S. Franceschin, J.G. Hilborn, P. Tissières, I. Bisson, P. Frey, Bioresorbable microspheres by spinning disk atomization as injectable cell carrier: from preparation to in vitro evaluation, Biomaterials. 21 (2000) 1135–44.

[97] B. Almería, W. Deng, T.M. Fahmy, A. Gomez, Controlling the morphology of electrospray-generated PLGA microparticles for drug delivery, J. Colloid Interface Sci. 343 (2010) 125–33.

[98] M.A.O. Wang, G.C. Rutledge, A.S. Myerson, B.L. Trout, Production and Characterization of Carbamazepine Nanocrystals by Electrospraying for Continuous Pharmaceutical Manufacturing, J. Pharm. Sci. 101 (2012) 1178–1188.

[99] J.E. Kipp, The role of solid nanoparticle technology in the parenteral delivery of poorly water-soluble drugs, Int. J. Pharm. 284 (2004) 109–22.

[100] R. Laitinen, K. Löbmann, C.J. Strachan, H. Grohganz, T. Rades, Emerging trends in the stabilization of amorphous drugs, Int. J. Pharm. 453 (2013) 65–79.

[101] E. Dudognon, J.F. Willart, V. Caron, F. Capet, T. Larsson, M. Descamps, Formation of budesonide/α-lactose glass solutions by ball-milling, Solid State Commun. 138 (2006) 68–71.

[102] J.A. Baird, L.S. Taylor, Evaluation of amorphous solid dispersion properties using thermal analysis techniques, Adv. Drug Deliv. Rev. 64 (2012) 396–421.

[103] L. Yu, Amorphous pharmaceutical solids: preparation, characterization and stabilization, Adv. Drug Deliv. Rev. 48 (2001) 27–42.

[104] K. Adrjanowicz, D. Zakowiecki, K. Kaminski, L. Hawelek, K. Grzybowska, M. Tarnacka, et al., Molecular dynamics in supercooled liquid and glassy states of antibiotics: azithromycin, clarithromycin and roxithromycin studied by dielectric spectroscopy. Advantages given by the amorphous state, Mol. Pharm. 9 (2012) 1748–63.

[105] J. Salonen, L. Laitinen, a M. Kaukonen, J. Tuura, M. Björkqvist, T. Heikkilä, et al., Mesoporous silicon microparticles for oral drug delivery: loading and release of five model drugs, J. Control. Release. 108 (2005) 362–74.

[106] D.Q. Craig, P.G. Royall, V.L. Kett, M.L. Hopton, The relevance of the amorphous state to pharmaceutical dosage forms: glassy drugs and freeze dried systems, Int. J. Pharm. 179 (1999) 179–207.

[107] H.G. Brittain, X-ray diffraction II: Using single-crystal X-ray diffraction to study polymorphism and solvatomorphism, Spectroscopy. 15 (2000) 34–39.

[108] D. Singhal, W. Curatolo, Drug polymorphism and dosage form design: a practical perspective, Adv. Drug Deliv. Rev. 56 (2004) 335–47.

[109] R.J. Davey, N. Blagden, G.D. Potts, R. Docherty, Polymorphism in molecular crystals: stabilization of a metastable form by conformational mimicry, J. Am. Chem. Soc. 7863 (1997) 1767–1772.

[110] X. He, J.G. Stowell, K.R. Morris, R.R. Pfeiffer, H. Li, G.P. Stahly, et al., Stabilization of a metastable polymorph of 4-methyl-2-

nitroacetanilide by isomorphic additives, Cryst. Growth Des. 1 (2001) 305–312.

[111] I. Weissbuch, L. Addadi, L. Leiserowitz, Molecular recognition at crystal interfaces, Science. 253 (1991) 637–45.

[112] M. Kitamura, Polymorphism in the crystallization of L-glutamic acid, J. Cryst. Growth. 96 (1989) 541–546.

[113] R.W. Lancaster, P.G. Karamertzanis, A.T. Hulme, D.A. Tocher, T.C. Lewis, S.L. Price, The polymorphism of progesterone: stabilization of a "disappearing" polymorph by co-crystallization, J. Pharm. Sci. 96 (2007) 3419–3431.

[114] S.L. Morissette, O. Almarsson, M.L. Peterson, J.F. Remenar, M.J. Read, A. V Lemmo, et al., High-throughput crystallization: polymorphs, salts, co-crystals and solvates of pharmaceutical solids, Adv. Drug Deliv. Rev. 56 (2004) 275–300.

[115] H. Ho, J. Lee, Redispersible drug nanoparticles prepared without dispersant by electro-spray drying, Drug Dev. Ind. Pharm. 38 (2012) 744–51.

[116] J. Sloth, K. Jørgensen, P. Bach, A.D. Jensen, S. Kiil, K. Dam-Johansen, Spray drying of suspensions for pharma and bio products: drying kinetics and morphology, Ind. Eng. Chem. Res. 48 (2009) 3657–3664.

[117] A. Jaworek, A. Sobczyk, T. Czech, A. Krupa, Corona discharge in electrospraying, J. Electrostat. 72 (2014) 166–178.

[118] A. Gomez, D. Bingham, R.L. De Juan, K.S. Tang, Production of protein nanoparticles by electrospray drying, J. Aerosol Sci. 29 (1998) 561–574.

[119] R. Pareta, A. Brindley, M.J. Edirisinghe, S.N. Jayasinghe, Z.B. Luklinska, Electrohydrodynamic atomization of protein (bovine serum albumin), J. Mater. Sci. Mater. Med. 16 (2005) 919–25.

[120] A. Townsend-Nicholson, S.N. Jayasinghe, Cell electrospinning: a unique biotechnique for encapsulating living organisms for generating active biological microthreads/scaffolds, Biomacromolecules. 7 (2006) 3364–9.

[121] H. Park, P.-H. Kim, T. Hwang, O.-J. Kwon, T.-J. Park, S.-W. Choi, et al., Fabrication of cross-linked alginate beads using electrospraying for adenovirus delivery, Int. J. Pharm. 427 (2012) 417–25.

[122] Y. Zhou, T. Sun, M. Chan, J. Zhang, Z. Han, X. Wang, et al., Scalable encapsulation of hepatocytes by electrostatic spraying, J. Biotechnol. 117 (2005) 99–109.

[123] J. Xie, C.-H. Wang, Electrospray in the dripping mode for cell microencapsulation, J. Colloid Interface Sci. 312 (2007) 247–55.

[124] K. Kim, W. Kim, S. Hwa Yun, J. Hyun Lee, S. Kim, B.U. Lee, Use of an electrospray for the generation of bacterial bioaerosols, J. Aerosol Sci. 39 (2008) 365–372.

[125] K. Kim, B.U. Lee, G.B. Hwang, J.H. Lee, S. Kim, Drop-on-demand patterning of bacterial cells using pulsed jet electrospraying, Anal. Chem. 82 (2010) 2109–12.

[126] J.B. Fenn, M. Mann, C.K.A.I. Meng, S.F. Wong, C.M. Whitehouse, Electrospray Ionization for Mass Spectrometry of Large Biomolecules, Science (80-.). 246 (1989) 64–71.

[127] T.J. Noakes, I.D. Pavey, D. Bray, R.C. Rowe, Apparatus for producing a spray of droplets of a liquid (Patent), US4829996, 1989.

[128] L.A. Davies, K. Hannavy, N. Davies, A. Pirrie, R.A. Coffee, S.C. Hyde, et al., Electrohydrodynamic comminution: a novel technique for the aerosolisation of plasmid DNA, Pharm. Res. 22 (2005) 1294–304.

[129] M.G. Zeles-Hahn, Y.K. Lentz, T.J. Anchordoquy, C.S. Lengsfeld, Effect of electrostatic spray on human pulmonary epithelial cells, J. Electrostat. 69 (2011) 67–77.

[130] O. Lastow, J. Andersson, A. Nilsson, W. Balachandran, Low-voltage electrohydrodynamic (EHD) spray drying of respirable particles, Pharm. Dev. Technol. 12 (2007) 175–81.

[131] C.U. Yurteri, R.P.A. Hartman, J.C.M. Marijnissen, Producing pharmaceutical particles via electrospraying with an emphasis on nano and nano structured particles - A review, Kona. 28 (2010) 91–115.

[132] S.C.G. Leeuwenburgh, M.C. Heine, J.G.C. Wolke, S.E. Pratsinis, J. Schoonman, J.A. Jansen, Morphology of calcium phosphate coatings for biomedical applications deposited using electrostatic spray deposition, Thin Solid Films. 503 (2006) 69–78.

[133] L.T. de Jonge, J. Ju, S.C.G. Leeuwenburgh, Y. Yamagata, T. Higuchi, J.G.C. Wolke, et al., A comparative study of two advanced spraying techniques for the deposition of biologically active enzyme coatings onto bone-substituting implants, Thin Solid Films. 518 (2010) 5615–5621.

[134] C. Schouten, G.J. Meijer, J.J.J.P. van den Beucken, S.C.G. Leeuwenburgh, L.T. de Jonge, J.G.C. Wolke, et al., In vivo bone response and mechanical evaluation of electrosprayed CaP nanoparticle coatings using the iliac crest of goats as an implantation model, Acta Biomater. 6 (2010) 2227–36.

[135] A.W.G. Nijhuis, J.J.J.P. van den Beucken, J. a Jansen, S.C.G. Leeuwenburgh, In vitro response to alkaline phosphatase coatings immobilized onto titanium implants using electrospray deposition or polydopamine-assisted deposition, J. Biomed. Mater. Res. A. 102 (2014) 1102–9.

[136] N. Arya, S. Chakraborty, N. Dube, D.S. Katti, Electrospraying: a facile technique for synthesis of chitosan-based micro/nanospheres for drug delivery applications, J. Biomed. Mater. Res. B. Appl. Biomater. 88 (2009) 17–31.

[137] L.Y. Yeo, Z. Gagnon, H.-C. Chang, AC electrospray biomaterials synthesis, Biomaterials. 26 (2005) 6122–8.

[138] F. Meng, Y. Jiang, Z. Sun, Y. Yin, Y. Li, Electrohydrodynamic liquid atomization of biodegradable polymer microparticles: effect of electrohydrodynamic liquid atomization variables on microparticles, J. Appl. Polym. Sci. 113 (2009) 526–534.

[139] J. Xie, J.C.M. Marijnissen, C.-H. Wang, Microparticles developed by electrohydrodynamic atomization for the local delivery of anticancer drug to treat C6 glioma in vitro, Biomaterials. 27 (2006) 3321–32.

[140] E. Scholten, H. Dhamankar, L. Bromberg, G.C. Rutledge, T.A. Hatton, Electrospray as a tool for drug micro- and nanoparticle patterning, Langmuir. 27 (2011) 6683–8.

[141] A. Jaworek, Electrostatic micro- and nanoencapsulation and electroemulsification: A brief review, J. Microencapsul. 25 (2008) 443–468.

[142] G. Langer, G. Yamate, Encapsulation of liquid and solid aerosol particles to form dry powders, J. Colloid Interface Sci. 29 (1969) 450–455.

[143] J. Xie, W.J. Ng, L.Y. Lee, C.-H. Wang, Encapsulation of protein drugs in biodegradable microparticles by co-axial electrospray, J. Colloid Interface Sci. 317 (2008) 469–76.

[144] L. Ding, T. Lee, C.-H. Wang, Fabrication of monodispersed Taxol-loaded particles using electrohydrodynamic atomization, J. Control. Release. 102 (2005) 395–413.

[145] Y. Xu, M. a Hanna, Electrospray encapsulation of water-soluble protein with polylactide. Effects of formulations on morphology, encapsulation efficiency and release profile of particles, Int. J. Pharm. 320 (2006) 30–6.

[146] J. Xie, J.U.N.C. Tan, C. Wang, Biodegradable films developed by electrospray deposition for sustained drug delivery, J. Pharm. Sci. 97 (2008) 3109–3122.

[147] M. Enayati, Z. Ahmad, E. Stride, M. Edirisinghe, J.R.S. Interface, Size mapping of electric field-assisted production of polycaprolactone particles Size mapping of electric field-assisted production of polycaprolactone particles, J. R. Soc. Interface. June (2010).

[148] M. Enayati, Z. Ahmad, E. Stride, M. Edirisinghe, One-step electrohydrodynamic production of drug-loaded micro- and nanoparticles, J. R. Soc. Interface. October (2009).

[149] S. Chakraborty, I.-C. Liao, A. Adler, K.W. Leong, Electrohydrodynamics: A facile technique to fabricate drug delivery systems, Adv. Drug Deliv. Rev. 61 (2009) 1043–54.

[150] J.S. Choi, Y. Kim, J. Kang, S.Y. Jeong, H.S. Yoo, Electrospun chitosan microspheres for complete encapsulation of anionic proteins: controlling particle size and encapsulation efficiency, AAPS PharmSciTech. 14 (2013) 794–801.

[151] Y. Il Cho, J.S. Choi, S.Y. Jeong, H.S. Yoo, Nerve growth factor (NGF)-conjugated electrospun nanostructures with topographical cues for neuronal differentiation of mesenchymal stem cells, Acta Biomater. 6 (2010) 4725–33.

[152] A. Saraf, L.S. Baggett, R.M. Raphael, F.K. Kasper, A.G. Mikos, Regulated non-viral gene delivery from coaxial electrospun fiber mesh scaffolds., J. Control. Release. 143 (2010) 95–103.

[153] P. Sofokleous, E. Stride, M. Edirisinghe, Preparation, characterization, and release of amoxicillin from electrospun fibrous wound dressing patches, Pharm. Res. 30 (2013) 1926–38.

[154] A. V. Ewing, G.S. Clarke, S.G. Kazarian, Stability of indomethacin with relevance to the release from amorphous solid dispersions

studied with ATR-FTIR spectroscopic imaging, Eur. J. Pharm. Sci. 60 (2014) 64–71.

[155] A. Heinz, M. Savolainen, T. Rades, C.J. Strachan, Quantifying ternary mixtures of different solid-state forms of indomethacin by Raman and near-infrared spectroscopy, Eur. J. Pharm. Sci. 32 (2007) 182–92.

[156] A. Forster, J. Hempenstall, The potential of small-scale fusion experiments and the Gordon-Taylor equation to predict the suitability of drug/polymer blends for melt extrusion, Drug Dev. Ind. Pharm. 27 (2001) 549–560.

[157] F. Vrečer, M. Vrbinc, a Meden, Characterization of piroxicam crystal modifications, Int. J. Pharm. 256 (2003) 3–15.

[158] A.R. Sheth, S. Bates, F.X. Muller, D.J.W. Grant, Polymorphism in Piroxicam, Cryst. Growth Des. 4 (2004) 1091–1098.

[159] N. Bandi, W. Wei, C.B. Roberts, L.P. Kotra, U.B. Kompella, Preparation of budesonide- and indomethacin-hydroxypropyl-beta-cyclodextrin (HPBCD) complexes using a single-step, organic-solvent-free supercritical fluid process, Eur. J. Pharm. Sci. 23 (2004) 159–68.

[160] B. Cullity, Elements of X-ray Diffraction, Second Edi, Addison - Wesley Publishing Company Inc., Boston, USA, 1978.

[161] J.H. Wakelin, H.S. Virgin, E. Crystal, Development and comparison of two x-ray methods for determining the crystallinity of cotton cellulose, J. Appl. Phys. 30 (1959) 1654–1662.

[162] A. Saleki-Gerhardt, C. Ahlneck, G. Zografi, Assessment of disorder in crystalline solids, Int. J. Pharm. 101 (1994) 237–247.

[163] V.-P. Lehto, M. Tenho, K. Vähä-Heikkilä, P. Harjunen, M. Päällysaho, J. Välisaari, et al., The comparison of seven different methods to quantify the amorphous content of spray dried lactose, Powder Technol. 167 (2006) 85–93.

[164] D. Giron, Thermal analysis and calorimetric methods in the characterisation of polymorphs and solvates, Thermochim. Acta. 248 (1995) 1–59.

[165] D. Giron, Applications of thermal analysis in the pharmaceutical industry, J. Pharm. Biomed. Anal. 4 (1986) 755–70.

[166] H. Ahmed, G. Buckton, D.A. Rawlins, The use of isothermal microcalorimetry in the study of small degrees of amorphous content of a hydrophobic powder, Int. J. Pharm. 130 (1996) 195–201.

[167] S. Brunauer, P.H. Emmett, E. Teller, Adsorption of gases in multimolecular layers, J. Am. Chem. Soc. 60 (1938) 309–319.

[168] P. Taddei, A. Torreggiani, R. Simoni, Influence of environment on piroxicam polymorphism: vibrational spectroscopic study, Biopolymers. 62 (2001) 68–78.