Fabrication of amorphous pharmaceutical materials by electrospraying into reduced pressure

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Abstract— **It is estimated that more than 95 % of new drug candidates suffer from limited bioavailability [1]. For poorly soluble drugs, the rate of oral absorption and bioavailability is often controlled by the dissolution rate in the gastrointestinal tract. Particle size reduction and formation of high-energy amorphous form are commonly used techniques in modifying bioavailability of poorly soluble drugs. In the present study, electrospraying was used in drug particle production. Three poorly soluble drugs, indomethacin, piroxicam and budesonide, were electrosprayed. Particle size was reduced down to 1 – 5 micrometers. Electrospraying was carried out in both atmospheric and reduced pressure, the latter in order to improve the drying process. This causes the formation of amorphous material due to the fast solidification. The aim of the study was to determine the degree of crystallinity of the produced particles for both methods.**

I. INTRODUCTION

Electrospraying is a method of liquid atomisation using electrostatic forces. In this process, liquid flows out from a capillary nozzle which is maintained at a high potential. A high electric field is generated at the capillary tip, which causes the meniscus to form a jet. The jet disrupts into droplets which are highly charged and relatively uniform in size. The charge of the same sign prevents the coalescence of the droplets [2,3].

Electrospraying of solutions or suspensions provides a method for production of fine particles, in certain conditions even down to nanometer size [2,4]. In the fabrication of drug particles, a drug powder is dissolved in a convenient solvent. The solution is atomised using electrostatic forces. The solvent is evaporated from the formed droplets in a drying medium, and a dense cluster of the dissolved drug is remained. Hence it is possible to produce solid and dense particles uniform in size.

Most experiments on electrospraying are performed at atmospheric pressure [5]. Nevertheless, there are several studies of electrospraying into vacuum mainly, but not exclusively considering electrical colloidal thrusters [5-9]. In the present study, drug particles

produced by electrospraying were dried in a reduced pressure to improve the drying process.

Three poorly soluble drugs, indomethacin, piroxicam and budesonide were investigated. For such poorly soluble and highly permeable drugs, the rate of oral absorption is often controlled by the dissolution rate in the gastrointestinal tract [10]. Therefore together with the permeability, the solubility and dissolution behaviour of a drug are key determinants of its oral bioavailability. There have been several attempts to improve the solubility of these poorly soluble drugs, for example by cogrinding, microcrystallization, using different additives and dispersion to a polymer matrix [10-16].

There are several materials which are temperature sensitive or highly resistant to migration of moisture from within the solid, such as some pharmaceuticals and foods [17]. The structures of these materials may be damaged in increased temperature, which is often required when drugs are modified by spraydrying or electrospraying [18,19]. By spray-drying in a reduced pressure, electrospraying of also these kinds of materials is possible, since it is a sensitive way to remove the solvent from the solid. Due to the fast solidification, molecules do not have enough time to arrange in the crystal lattice, which causes the formation of amorphous material [18]. Formation of amorphous form is a commonly used technique in improving bioavailability of poorly soluble drugs [20]. In the present study, electrospraying was carried out in both atmospheric and reduced pressure. The degree of crystallinity of the produced indomethacin, piroxicam and budesonide particles was determined for both methods using DSC and XRD. Size distribution of the particles was determined by SEM imaging.

II. Materials and Methods

A. Materials

Indomethacin (IMC) is a light-sensitive drug which is used to relieve pain and inflammatory conditions. It is poorly soluble to water and highly permeable [10]. This drug has also been cited as a possible chemotherapeutic agent for treating colorectal cancer [21]. Indomethacin exists in two polymorphic crystalline forms: metastable α-form and more stable γ-form [22]. Amorphous form is known to be the most soluble of the solid forms of indomethacin [23]. Indomethacin was dissolved into ethanol at room temperature using drug material concentrations of 10 or 15 g/dm^3. Piroxicam is one of the most potent non-steroidal anti-inflammatory drugs. It is poorly soluble to water and shows dissolution-rate-limited low oral bioavailability in the crystalline state [14]. Piroxicam exhibits polymorphism with the form designated as I being the most stable crystal structure [24]. Budesonide is an anti-inflammatory corticosteroid. It is so chemically and physically stable that it is almost insoluble to water at physiological pH [16]. Piroxicam and budesonide were dissolved into chloroform at room temperature using drug material concentration of 15 g/dm^3. All of the prepared solutions were stored at room temperature and protected from light.

The studies were carried out using indomethacin and piroxicam delivered by Hawkins Pharmaceuticals (Minneapolis, USA). It was determined by XRD that indomethacin was originally in γ-form and piroxicam in I-form. Budesonide was acquired from Orion Pharma (Finland).

In electrospraying the most important properties of the solution are its conductivity, surface tension and viscosity [25-27]. The solvents were chosen optimising these properties and taking into account that the drug materials have to be soluble, but do not degrade in the chosen solvents. Thus, ethanol and chloroform were chosen as solvents in the present study. Essential physical properties of these solvents are presented in Table I.

TABLE I: SURFACE TENSION γ, VISCOSITY η AND DENSITY ρ OF ETHANOL AND CHLOROFORM [28]

	γ (20 °C) [10^{-3} N/m]	η (25 °C) [10^{-6} Ns/m^2]	ρ (20 °C) [kg/m^3]
ethanol	22.75	1074	789.3
chloroform	27.14	537	1483.2

B. Particle fabrication

In the electrospraying studies, stainless steel capillaries (manufactured by EFD, USA) with inner diameters of 0.15 or 0.10 mm (ethanol solutions) and 0.33 mm (chloroform solutions) were used. Electrostatic atomisation was carried out using Alpha Series II high voltage source (UK) set to positive potential and connected to the stainless steel capillary. A grounded plate electrode with a hole in the center was placed beneath it. The distance between these electrodes was set to approximately 1 cm. A circular metal plate was attached to HV-conductor above the capillary. This was to make the electric field near the capillary tip more uniform, and to prevent some external electric disturbances [29]. Electrospraying equipment was presented in more detail in a previous paper [30].

In the present study, atomisation voltages ranged between 2.5 – 4.5 kV. Highly charged droplets were neutralized in order to avoid adhesion to grounded surfaces. This was done using a corona neutralizer, which was connected to E.H.T. unit Type 532/D voltage source (The Isotope Developments, UK) and set to negative potential. Liquid flow to the capillary was controlled using a syringe pump (NE-500, New Era Pump Systems, USA). Liquid flow rates ranged from 1.0 to 2.0 ml/h. Atomised droplets were spraydried at room temperature in a pressure of 0.5 – 1 atm. When atomisation was done in a reduced pressure, the solutions were set in a vacuum prior to electrospraying. This was in order to prevent formation of bubbles in the capillary while electrospraying.

C. Particle characterisation

Produced particles were studied using SEM (Cambridge S200, UK). SEM samples were prepared by collecting particles on a nylon filter. The samples were coated with a 20-30 nm layer of gold to improve their conductivity. Samples were stored in room temperature in desiccator containing silica gel for two days before the imaging.

Calorimetric measurements were carried out using differential scanning calorimeter (DSC, PerkinElmer Instruments, USA). The apparatus was controlled with a computer using Pyris DSC Software -program (PerkinElmer Instruments, USA). The equipment was cooled with Intracooler 2D (PerkinElmer Instruments, USA). Nitrogen was used as scavenging gas using mass flow rate controller (B.V. 5850S, Brooks Instruments, USA), which was monitored using *0152* -control unit (Brooks Instruments, USA). Data was collected and analysed using Pyris Software Version 4.02 -software.

Crystallinity of the samples was measured using x-ray diffraction using PW 1830 generator, PW 1820 goniometer and PW 1710 diffractometer controller (Philips Electronics, Netherlands). Data was analysed with X'Pert HighScore Version 1.0 -program. The samples were measured between 3 - 45 º values of *2θ* with scanning speed of 0.02 º/s.

III.   RESULTS

A.   *Particle size*

Produced indomethacin, piroxicam and budesonide particles were studied with an electron microscope. During the fabrication of the SEM samples, liquid flow rate was set to 2.0 ml/h on all samples. Atomisation voltages of 3.0 – 3.5 kV to chloroform solutions and 3.5 kV to ethanol solutions were used, which are in the stable cone-jet mode range for these solutions with the current electrospraying setup [30]. Indomethacin particles were spraydried in the pressure of 0.5 atm, while piroxicam and budesonide were dried in 0.7 – 0.8 atm. SEM images of the fabricated particles are presented in Fig. 1.
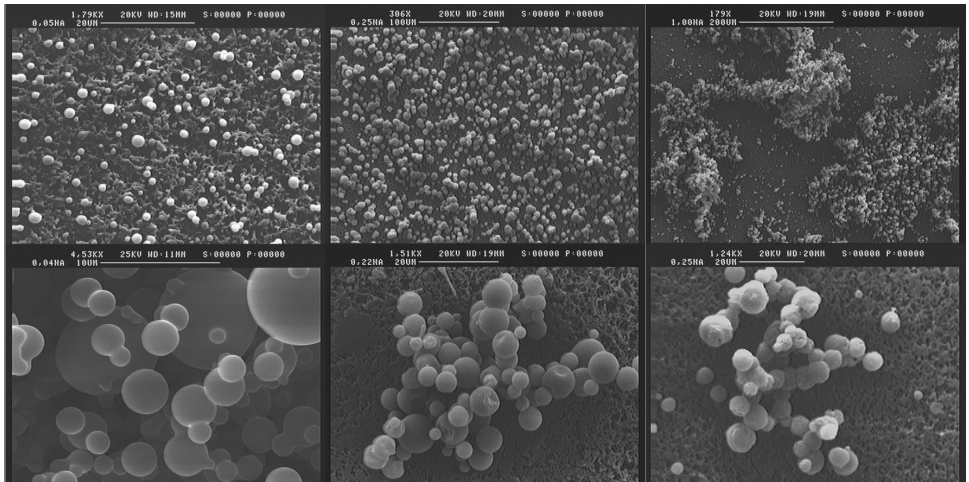

Fig. 1. SEM images of the electrosprayed indomethacin (on the left), piroxicam (in the middle) and budesonide (on the right) particles. Below are magnifications of the corresponding particles.

All the particles presented in Fig. 1 are quite regularly shaped. Based on the images, it can be noted that budesonide microparticles tend to form cluster-like structures. They were also harder to handle than indomethacin or piroxicam particles while preparing the samples. This is due to charging of the particles. Of these materials, budesonide particles seem to charge most. The structure on the background of the images in Fig.1 is the nylon filter, on which the particles were collected.

Based on the SEM images, particle size distribution was determined for each material using Image-Pro Plus (Version 1.3) -program. At least 600 particles were analysed per sample. The size distributions are presented in Fig. 2.
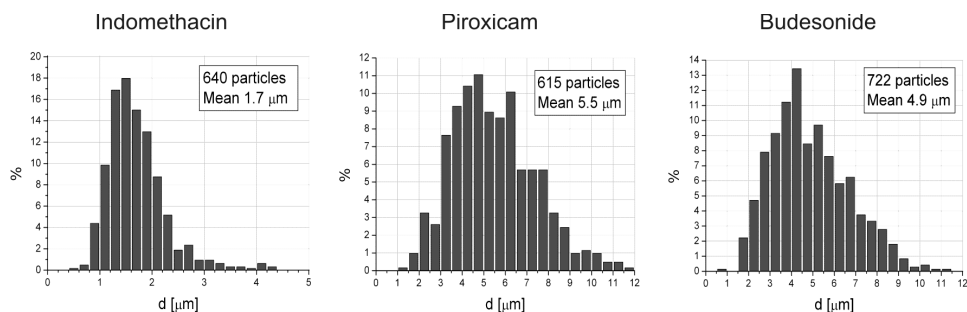
Fig. 2. Particle size distributions of the electrosprayed samples. The amount of analysed particles and the mean particle diameter is mentioned for each sample.

Particle size distribution is especially narrow for indomethacin/ethanol solution. Mean particle size is 1.7 µm, which is somewhat smaller than that of chloroform based solutions (5.5 µm for piroxicam and 4.9 µm for budesonide). One "adjustable" parameter that was different in the atomisation of ethanol and chloroform solutions was the inner diameter of the capillary, which was 0.15 mm for the former and 0.33 mm for the latter. Naturally, the electric field strength was also different, but both solutions were atomised using stable cone-jet mode. The electrical conductivities of the solvents might be somewhat different for chloroform and ethanol. Generally, the conductivities of these sorts of organic solvents are low (~ 10^{-4} S/m), and the addition of low concentrations of drug powder does not affect the conductivity significantly [30]. On the other hand, the viscosity of ethanol is greater than that of chloroform's. This indicates that atomised ethanol droplets (and indomethacin particles) would be larger than chloroform droplets (and piroxicam and budesonide particles), if the atomisation was done using otherwise similar values of parameters.

B.   Degree of crystallinity

The produced indomethacin, piroxicam and budesonide particles were studied with DSC and XRD in order to examine the crystallinity of the samples. In order to determine the degree of crystallinity in a sample, a crystalline reference which is assumed to be 100 % crystalline, is required for both methods. By DSC measurements, the heat of fusion ΔH_f can be determined for both the crystalline reference and the atomised samples. By comparing these, one can determine the proportion of amorphous content in the sample directly. By XRD measurements, areas of the chosen diffraction peaks of the crystalline and atomised samples can be compared, respectively. By these methods, one can determine a minimum value for the proportion of amorphous form in the sample. For DSC and XRD measurements, indomethacin, piroxicam and budesonide were atomised both in the pressure of 1 atm and 0.5 atm.

For budesonide samples, ΔH_f and the areas of the diffraction peaks can be directly compared to those of original crystalline budesonide, since this drug does not exhibit polymorphism. Instead, based on the XRD measurements, the atomised indomethacin particles turned out to be in the metastable α-form. This was expected, since α-indomethacin is obtained when indomethacin is recrystallised from ethanol solutions [22]. A crystalline reference was fabricated by crystallisation from ethanol in room temperature. It was veri-

fied by XRD measurements, that this reference consisted mainly of α-indomethacin. The fabrication of a crystalline reference, which consists of such metastable form is complicated, since the sample begins to reform towards the most stable crystal form before it is fully crystallised. Hence, for indomethacin, the determined absolute values of the degree crystallinity are somewhat greater than the true ones. When piroxicam is recrystallised from chloroform at room temperature, a mixture of two polymorphic forms is obtained: a stable (I) and a metastable form (II) [24]. Hence as expected, the atomised piroxicam particles turned out to be a mixture of these forms. This sort of crystalline reference sample cannot be fabricated. Nevertheless for all samples, polymorphism of the drugs does not disturb the basic concern: comparison of the degree of crystallinity between samples atomised in the pressures of 0.5 atm and 1 atm.

The electrosprayed DSC and XRD samples were measured within 1 - 3 hours after preparation. Samples were prepared using slightly different values for the atomisation parameters for each material and pressure. These are presented in Table II.

TABLE II: ATOMISATION PARAMETERS ON PREPARATION OF DSC AND XRD SAMPLES

Sample	c_{drug} [g/l]	Q [ml/h]	U [kV]	d_n [mm]	p [atm]
IMC	10	1.0	2.7	0.15	1.0
IMC	15	2.0	2.8	0.15	0.5
Piroxicam	15	1.0	3.0	0.33	1.0
Piroxicam	15	1.5	3.2	0.33	0.5
Budesonide	15	2.0	3.3	0.33	1.0
Budesonide	15	2.0	3.2	0.33	0.5

1) DSC measurements

DSC measurements were carried out using 30 μl aluminium crucibles. Nitrogen was used as scavenging gas with flow rate of 40 ml/min. Heating rate of the samples was set to 10.0 °C/min. Two separate samples were prepared and studied for each drug and atomisation pressure (except for piroxicam and budesonide in 1 atm, for which only one sample was obtained). As an example, the heat flow curves of atomised piroxicam in the pressures of 1.0 atm and 0.5 atm are presented in Fig. 3.

For piroxicam and budesonide, an exothermic crystallisation peak occurred in the heat flow curve before the melting (Note: two exothermic peaks for piroxicam), when atomisation was carried out in the reduced pressure. This exothermic peak did not occur for either material for the samples atomised in the atmospheric pressure. This peak is because of the crystallisation of the amorphous content in the sample due to temperature increase. When calculating degrees of crystallinity, the enthalpy of crystallisation must be subtracted from the enthalpy of fusion ΔH_f. For indomethacin samples, this exothermic peak did not occur. For each sample, heat of fusion ΔH_f was determined from the area of the melting peak. These are presented in Table III.
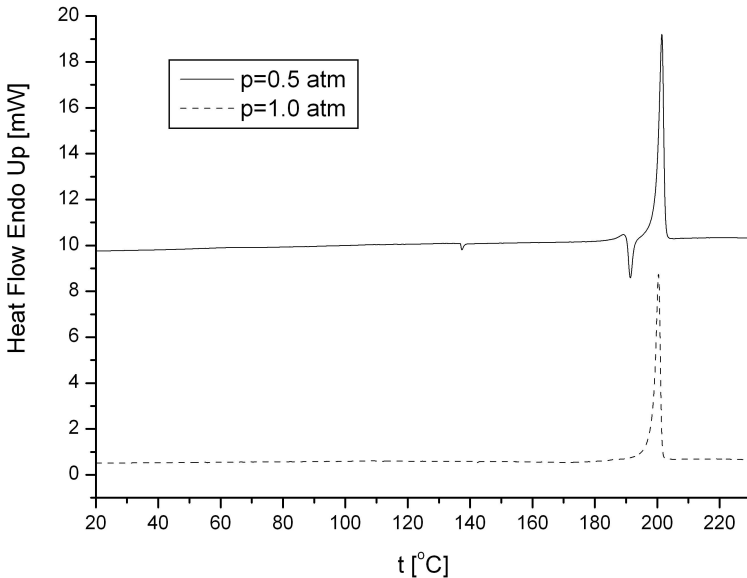
Fig. 3. DSC scans of piroxicam electrosprayed in atmospheric pressure 1 atm and reduced pressure 0.5 atm.

2) XRD measurements

Electrosprayed samples were collected from the drying chamber to small XRD sample holders. The intensities of the measured diffraction peaks are relatively low due to the small sample amounts. Atomisation parameters used in the preparation of XRD samples were presented in table II. As an example, the diffractograms of atomised budesonide and crystalline reference are presented in Fig. 4.

Crystallinity of the electrosprayed indomethacin, piroxicam and budesonide samples can be examined by comparing areas of the diffraction peaks of the electrosprayed samples. Because of the small sample amounts and low peak intensities, four or five peaks which were most distinguishable were chosen to the analysis for each sample. For indomethacin, these peaks were detected on diffraction angles (2θ) of 8.5 °, 12.0 °, 14.3 ° and 28.5 °. For piroxicam, the peaks on 14.2 °, 14.9 °, 17.4 °, 21.1 ° and 24.3 ° were chosen to analysis. For budesonide, the corresponding peaks were on 6.0 °, 14.6 °, 15.5 °, 16.0 ° and 23.0 °. The areas of these peaks were determined with HighScore Version 1.0 - program in units of [cts·2θ]. The sum of the areas of the peaks was calculated for each sample. In Table III, the heat of fusion ΔH_f described in the previous paragraph and the areas of the chosen diffraction peaks are presented for each of the electrosprayed drug material and atomisation pressure. These numbers give a direct indication of the amount of crystalline material in the samples and, for each drug material, can be compared.
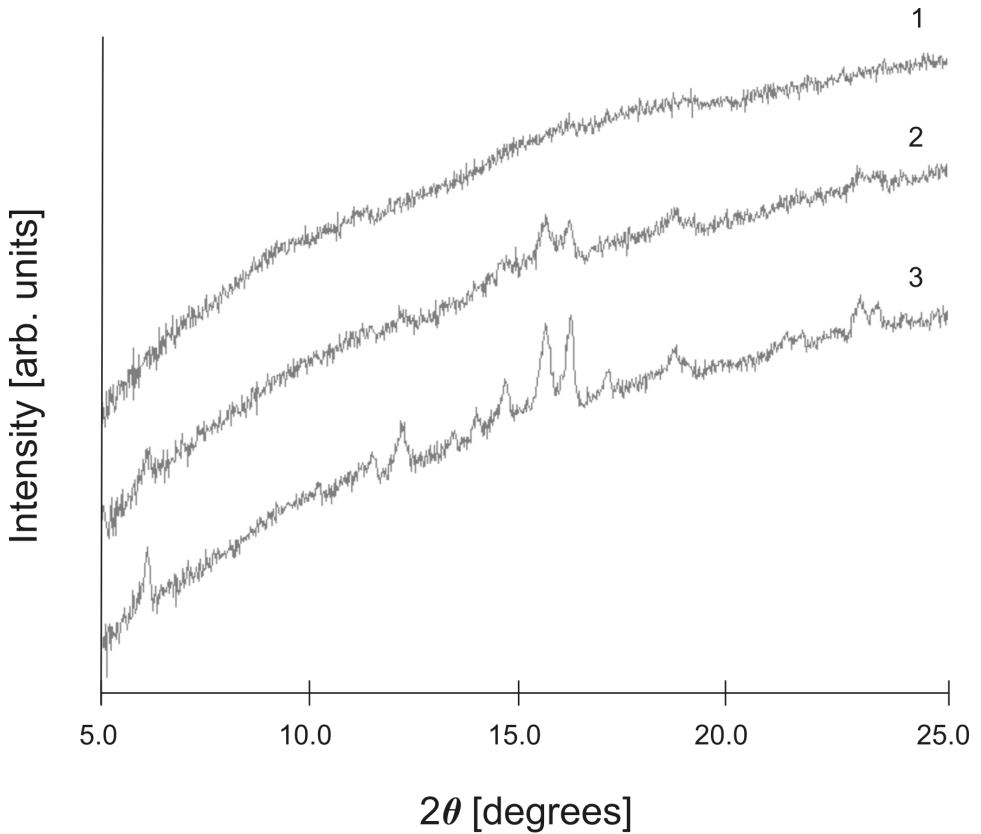
Fig. 4. Powder X-ray diffractograms of electrosprayed and crystalline budesonide: (1) atomised in 0.5 atm, (2) atomised in 1 atm and (3) crystalline reference.

TABLE III: Determined values of heat of fusion ΔH_F and the areas of the chosen diffraction peaks for electrosprayed indomethacin (IMC), piroxicam and budesonide

Sample	p [atm]	DSC ΔH_f [J/g]	XRD Peak Area [cts·2θ]
IMC	1.0	90	189
IMC	0.5	92	181
Piroxicam	1.0	75	347
Piroxicam	0.5	58	184
Budesonide	1.0	50	230
Budesonide	0.5	22	0

Based on the DSC measurements, piroxicam atomised in 1 atm turned out to be 29 % more crystalline than piroxicam atomised in 0.5 atm. Based on the XRD measurements, the corresponding result is 89 %. DSC results indicate that, budesonide atomised in 1 atm

was 127 % more crystalline than that atomised in 0.5 atm. It can be noted from the presented diffractograms (Fig. 4) that for budesonide atomised in 0.5 atm diffraction peaks were not observed, or at best, a few peaks are barely observable. The sample can be interpreted as x-ray amorphous. Instead in 1 atm, the characteristic peaks of budesonide occurred, and the sample turned out to be 65 % crystalline. However, for indomethacin/ethanol solution, the atomisation pressure did not seem to have an effect to the degree of crystallinity.

For indomethacin, the DSC and XRD results of the electrosprayed samples were compared to those of the fabricated crystalline reference. For budesonide, a corresponding comparison was done between atomised samples and original budesonide powder. For these materials, the averages of the maximum degrees of crystallinity (DOC) obtained by DSC and XRD were calculated. These are presented in Table IV.

TABLE IV. AVERAGE MAXIMUM DEGREES OF CRYSTALLINITY (DOC) OF THE ELECTROSPRAYED INDOMETHACIN (IMC) AND BUDESONIDE SAMPLES. PIROXICAM NOT INCLUDED DUE TO LACK OF RELIABLE CRYSTALLINE SAMPLE.

Sample	p [atm]	DOC [%]
IMC	1.0	79
IMC	0.5	79
Budesonide	1.0	64
Budesonide	0.5	14

IV. CONCLUSIONS AND DISCUSSION

By electrospraying, the size of indomethacin, piroxicam and budesonide particles was reduced down to micrometer range. This improves the solubility, since microparticles have a larger specific surface area than conventional drug powder. For poorly soluble drugs, amorphous form is usually notably more soluble to water than the crystalline forms. Besides the improvement of the solubility by size reduction, also the structure of the drugs was converted to more soluble form by electrospraying both in atmospheric and reduced pressure.

For chloroform solutions, electrospraying in a reduced pressure lead to formation of notably more amorphous drug particles than electrospraying in atmospheric pressure. This was verified by DSC and XRD measurements. For ethanol, there was no significant difference in the degree of crystallinity when the pressure was varied. Chloroform is more volatile than ethanol, its relative evaporation rate is 11.6 while that of ethanol's is 2.8 (BuAc reference 1.0). The reduction of pressure seems to have an effect on the degree of crystallinity only if the solution is volatile enough.

In the future, the solubility and dissolution behaviour of the produced drug particles will be tested *in vitro*. This gives more insight to the extent of improvement in the bioavailability of poorly soluble drugs, gained by electrospraying into reduced pressure.

V. ACKNOWLEDGEMENTS

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