Anti-solvent Precipitation Method Coupled Electrospinning Process to Produce Poorly Water-Soluble Drug-Loaded Orodispersible Films

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1 **Introduction**

2 Tablets and capsules are preferred dosage forms for most patients. However, paediatric,
3 geriatric patients and other patients who suffer from dysphagia or central nervous
4 system diseases may have difficulties in swallowing these dosage forms (1). Over the
5 past two decades, orally disintegrating dosages forms, such as orodispersible tablets
6 (ODTs) and orodispersible films (ODFs), have been developed to circumvent these
7 challenges. Orally disintegrating dosage forms are designed to dissolve or disintegrate
8 rapidly in mouth without usage of water. Most drugs released from these dosage forms
9 are absorbed through gastrointestinal tract, and they may exhibit a rapid onset of action
10 and enhanced bioavailability (2).

11
12 There are a few techniques and methods available to produce ODTs, including freeze
13 drying, molding, sublimation, compaction and 3D printing (3, 4). Many ODTs launched
14 on the market, e.g. Zofran® and Aricept®, were produced by freeze drying and
15 compaction (5, 6). One problem with ODTs is their fragility (7). ODFs are relatively
16 new. They are film-like polymeric matrix carrying drug cargo and could be cut into
17 various shapes. ODFs are more flexible and portable as compared to ODTs and they
18 are more precise in dosing than liquid formulations (8). Unlike those mentioned above,
19 the methods for manufacturing ODFs include casting, hot melt extrusion and rolling (9,
20 10). Most of these methods require heating process, so thermal sensitive drugs are
21 normally excluded. Recently, electrospinning has been attempted to prepare oral films
22 because it is versatile and easy to operate (11, 12). The diameter of electrospun fibers
23 ranges from nano to micro scales, providing a high surface to volume ratio so as to
24 promote fast dissolving of active substances (13, 14).

25
26 Electrospun ODFs are generally composed of film forming polymers, plasticizers,
27 saliva simulating agents, and flavouring agents besides active pharmaceutical
28 ingredients (API) (10, 15). Among these excipients, film forming polymers constitute
29 a large proportion. Typically, they are water-soluble polymers, such as maltodextrins,
30 hydroxypropyl methylcellulose, pullulan, polyvinyl alcohol and polyvinyl pyrrolidone
31 (16-20). To formulate water-soluble drugs in ODFs, water will be the obvious solvent
32 to dissolve both film forming polymers and APIs (11, 21, 22). However, for poorly
33 water-soluble drugs, it is always necessary to find a common solvent to dissolve both

hydrophobic/lipophilic APIs and the hydrophilic polymers to form ODFs. As poorly water-soluble drugs are more likely to dissolve in organic solvent, most works used organic solvent for film preparation (23-26). This is a troublesome problem as many water-soluble polymers are not able to dissolve in organic solvent and the massive usage of organic solvent would form an environment burden. Some works directly suspended the poorly water-soluble drugs in aqueous polymer solution to cast films (17, 27), while others micronized the drug into micro/nano particles through milling or high-pressure homogenization technique before casting (28-30). Noticeably, the reports about loading micronized poorly water soluble drugs on electrospun ODFs are still rare.

In our work, we investigated the feasibility to combine the anti-solvent precipitation method and the electrospinning process to prepare poorly water-soluble drug loaded ODFs. We proposed to micronize poorly water-soluble drugs in a size range of less than 25 microns, subsequently suspend the micronized drugs in hydrophilic film forming polymer solutions, and then process the suspension into orodispersible fibrous films through electrospinning. The 25 microns of the particle size was chosen mainly for the sake of patient compliance, as the minimum particle size that could be detected by the human palate was 25 microns (31). Piroxicam (PX), a poorly water-soluble drug, catalogued as class II, was chosen as the model drug. **The stabilizers and their optimal concentrations for precipitating PX in anti-solvent precipitation method were selected among poloxamer, copovidone and hydroxypropyl methylcellulose at three different concentrations, respectively.** Initial investigations were focused on the development of plain ODF platforms, and therefore, various compositions of PVA and PVP polymer solution were electrospun into films for satisfactory tensile behaviour and disintegration time screen. Polyethylene glycol 300 was added in the formulations to evaluate the influence of plasticizer on the properties of electrospun ODFs. Finally, three PX loaded formulations were prepared, and assessed with various techniques.

Material and Method

Material

Piroxicam was purchased from Chr. Olesen Pharmaceuticals A/S (Gentofte, Denmark). Polyvinyl alcohol 26-88 (PVA, Mw 160000) was obtained from Merck (Darmstadt, German) and polyvinylpyrrolidone 360 (PVP, Mw 360000) were purchased from

Sigma–Aldrich (Schnelldorf, German). Poloxamer (Pluronic F127) was purchased from Sigma–Aldrich (Saint Louis, USA). Polyethylene glycol 300 (PEG300) and hydroxypropyl methylcellulose (HPMC, 15 Cps) were received from Sigma–Aldrich (Steinheim, German). Copovidone (Kollidon VA64) was purchased from BASF (Ludwigshafen, Germany). Distilled water was used in all experiments. All solvents used were of HPLC grade with the purity $\geq 99.8\%$.

Method

Preparation of PX microcrystal suspensions

PX microcrystal suspensions were prepared with anti-solvent precipitation method. Piroxicam (175 mg) was first dissolved in 2.0 mL of N, N-Dimethylformamide (DMF) to make an 8.75% (w/v) drug solution. Subsequently, the solution was injected into the 13.0 mL distilled water **containing, either 0.2%, 0.5% or 1.0% (w/v) of each stabilizer (i.e. HPMC E15, Kollidon VA64, and Pluronic F127) with a magnet-stirring rate at 1500 rpm in a cold room (4.0 °C).**

Particle size measurement

The particle size distribution of PX microcrystal suspension was measured using a laser diffraction technique with Mastersizer through Hydrosizer 2000S module (Malvern Instruments, Worcestershire, UK). A certain amount of the suspension was dispersed in the sample chamber (distilled water as dispersion medium) at 2500 rpm until the obscuration reached 8%-15%. The background was measured for 10 s. The chamber was washed with water after each measurement. The broadness of size distribution (span) was calculated by $(D_{90} - D_{10})/D_{50}$, where d_{90} is defined as the equivalent particle size for which 90% of the particles are smaller, with definitions of d_{50} and d_{10} derived similarly.

Preparation of orodispersible films

A 10%(w/v) PVA 26-88 was prepared by dissolving 1500mg of the polymer into 15mL distilled water under stirring until the polymer was completely dissolved. A 10%(w/v) PVA/PVP (2/1, w/w) was prepared by dissolving 1000mg of PVA26-88 and 500mg of PVP 360 into 15mL distilled water under stirring until the polymers were completely dissolved. Other polymer solutions containing different

compositions of PVA 26-88/PVP 360 were prepared by keeping the total concentration at 10% (w/v) with various mass ratios between the two polymers (presented in Table 2 manuscript body). Plain ODFs were obtained by directly electrospinning of the prepared polymer solutions. Drug-loaded ODFs were obtained by dissolving the appropriate amount of film forming polymer into PX microcrystal suspensions produced with anti-solvent method, followed by electrospinning.

After dissolving polymers in PX microcrystal suspensions, the resulting suspensions were kept still in a glass vial for 48 h in order to evaluate the sedimentation rate of the PX microcrystals in the hydrophilic polymer solutions. At pre-determined time intervals, 10.0 μ L of the liquid were taken from the top layer of the suspension. The samples were diluted with a binary mixture of water and ethanol (1:1, v:v), and analysed through UV analysis.

The electrospinning device were customized, equipping with a syringe pump (Pump 11 Elite, Harvard Apparatus, Quebec, Canada), a glass syringe (2.5 mL) attached with a 16 gauge needle (1.19 mm inner diameter and 1.65 mm outer diameter, Hamilton, Bonaduz, Switzerland), a high-voltage supply (PS/FC20R06, Glassman High Voltage, NJ, USA), and a collector wrapped with aluminium foil connected to the ground electrode. The following process parameters were used during electrospinning: electrical high voltage of ca. 9.5-11.5 kV, the distance of 10 cm between the needle and the collector, and the feeding rate of 0.39 mL/h. The collected ODFs were further dried in a vacuum oven to remove residue solvent and stored in a desiccator for further analyses. The whole setting of the preparation of PX microcrystal suspensions and orodispersible films was illustrated in Figure 1.

Fig.1 Preparation of PX-loaded electrospun ODFs.

Morphology

The morphology of the samples was observed using a scanning electron microscope (SEM) (Hitachi High-Tech HITACHI, Tokyo, Japan). Prior to observation, samples were stuck on aluminium stubs with double-sided adhesive tape and coated with gold under argon atmosphere. The diameter distribution of the prepared fibers was determined by measuring 200 fibers with the instrument software (TM3030, Hitachi High-Tech HITACHI, Tokyo, Japan).

Folding endurance

Folding endurance is a parameter reflecting brittleness. A 2 x 2 cm rectangular section of each formulation was repeatedly folded at the same line for 50 times to see whether there was visible damage.

Tensile strength

Tensile strength was measured with Dynamic Mechanical Analysis (DMA) (Q800, TA Instruments, New Castle, USA). Samples were cut into 0.4 x 4 cm rectangular shape with the auxiliary mould. The test mode was DMA Strain Rate with a displacement rate of 200.00 $\mu\text{m}/\text{min}$ at room temperature. Prior to tensile strength tests, the thickness of the film was measured using a digital vernier caliper. In brief, three sections from the middle part of the electrospun ODFs were cut for thickness measurement. As fibrous films were porous, certain pressure needed to be applied during measurements. The samples with similar thickness were chosen for the tensile strength measurements.

X-ray powder diffraction (XRPD) analysis

The X-ray diffractograms of the electrospun ODFs were collected from PANalytical X'Pert PROMPD X-ray diffractometer (PANalytical, Almelo, The Netherlands) using $\text{CuK}\alpha$ radiation with a wavelength of 1.5418 Å. Each sample was put into a zero diffraction plate with a cavity of 10 mm in diameter and 0.2 mm in depth. Samples were measured in a spinning mode with a starting angle of $2\theta=5^\circ$ and an end angle of

158 $2\theta=35^\circ$. The scan speed was $0.6565^\circ 2\theta / \text{min}$. Data was collected and processed with
159 X'Pert Data Collector (PANalytical, Almelo, The Netherlands)

160

161 **Fourier Transform infrared spectroscopy (FTIR)**

162 Infrared spectra of the electrospun ODFs were collected with Horizon MB 300 FT-IR
163 spectrometer (MB300, ABB Ltd, Zurich, Switzerland) coupled with a MIRacle™
164 Single Reflection ATR accessory (PIKE Technologies, Fitchburg, USA) to attenuate
165 total reflectance. The scanning range was $4000\text{--}400\text{ cm}^{-1}$ and the resolution was set at
166 4 cm^{-1} .

167

168 **Disintegration**

169 The disintegration of electrospun ODFs was evaluated using a customized method (25,
170 26). In brief, $2 \times 2\text{ cm}$ film sections of different formulations were placed into 15 mL
171 of distilled water in a petri dish with a diameter of 10 cm at room temperature. **The**
172 **disintegration process was recorded with the video function of a smart phone and**
173 **the parameter for video recording was 60fps. The disintegration processes of the**
174 **films were observed afterwards and screenshots were made and reported. The**
175 **seconds in the results were the time of the screenshots were made.**

176 **Drug Loading**

177 The actual amount of PX was measured by dissolving certain amount of the films in 10
178 mL of a mixture of ethanol and water ($1:1$; v:v), and analysed through UV analysis.
179 **The drug loading and the entrapment efficiency were calculated through following**
180 **equations.**

$$181 \quad \text{Drug loading (\%)} = \frac{\text{Amount of drug in electrospun ODFs}}{\text{Amount of electrospun ODFs}} \times 100$$

$$182 \quad \text{Entrapment efficiency (\%)} = \frac{\text{Amount of drug in electrospun ODFs}}{\text{Amount of drug initially added}} \times 100$$

183

184 **Residue Solvent**

185 **The percentage of the residue solvent was measured by using thermogravimetric**
186 **analysis (TGA) (TGA Discovery, TA Instruments, New Castle, USA). Around**
187 **2.5mg of the samples were put on platinum pans and heated from 25°C to 250°C**

188 **at 10 °C/min. The percentage of weight loss was calculated as the percentage of**
189 **the residue solvent.**

190

191 **In-vitro dissolution study**

192 The dissolution tests were conducted using a USP Apparatus 2 with 250 mL of
193 dissolution cup and rotating mini paddles (Erweka DT70, Heusenstamm, Germany).
194 Around 12 mg of fibrous films with similar dimension (3 x 3 cm) were cut and
195 immobilized on an aluminium foil in order to ensure that the same surface areas were
196 accessible to the dissolution media. The dissolution media was 150 mL of distilled
197 water kept at 37 ± 0.2 °C and the rotation speed of the paddle was 100 rpm. At each
198 predetermined time point, 2 mL samples were withdrawn and then mixed with 2 mL of
199 ethanol for UV analysis. After the samples were taken, 2 mL of fresh dissolution media
200 were added to keep the total volume unchanged.

201

202 **UV analysis**

203 The quantification of PX in the formulations was analysed using a UV
204 spectrophotometer (Evolution 300, ThermoScientific, Cambridge, UK). In brief, PX
205 standard solutions were prepared by diluting a stock solution (0.5 mg/mL PX in
206 ethanol) into the concentrations between 1.0 µg/mL-5.0 µg/mL with a mixture of
207 ethanol/water (1:1; v:v). The absorption wavelength was selected by scanning the
208 standard solution of 5.0 µg/mL in the UV spectrophotometer. A standard curve was
209 constructed by plotting the concentration of standard solutions as x-axis with its
210 absorbance at 357 nm as y-axis. The correlation coefficient value (R) was above 0.999.
211 The experiments were performed at a room temperature.

212

213 **Statistics**

214 Measurements were performed in triplicate, unless otherwise stated. Results were
215 reported as means $\pm$ S.D. Statistically significant differences were evaluated by one
216 way of an analysis of variance (ANOVA) and t-test using GraphPad Prism version 6
217 (GraphPad Software, San Diego, USA).

218

219 **Results and Discussion**

220 **Preparation and characterization of PX suspension**

The minimum particle size that could be detected by the human palate was 25 μm (31), so the intended particle size of PX microcrystals in the suspension was decided to be less than 25 μm for the sake of patients' compliance. Three common polymeric stabilizers, i.e. HPMC E15, Kollidon VA64, and Pluronic F127, were used to prepare PX microcrystals, and the effect of different concentrations of these stabilizers on the particle size distribution of PX microcrystals were studied. It has been reported that the polymers and surfactants adhesive to the drug particles would form steric barrier or electric barrier to prevent the particles growth (32-34). As shown in Table 1, the D_{90} of the formations containing HPMC were exceeding 30 μm , whereas the D_{90} was around 10-20 μm for Kollidon or Pluronic formulations. The 0.5% (w/v) and 1.0% (w/v) Pluronic formulations had narrower span than the other formulations. In the subsequent studies, the 0.5% (w/v) Pluronic formulation was selected for the further studies.

Table 1 Particle sizes of PX suspensions prepared using different polymeric stabilizers (n=3).

Material	Concentration % (w/v)	Particle Size (μm)			
		d_{10}	d_{50}	d_{90}	Span
HPMC E15	0.2	7.9 $\pm$ 0.6	16.8 $\pm$ 0.8	31.9 $\pm$ 1.5	1.5 $\pm$ 0.02
	0.5	9.9 $\pm$ 1.0	21.4 $\pm$ 0.9	39.1 $\pm$ 0.6	1.4 $\pm$ 0.14
	1.0	7.4 $\pm$ 0.5	18.6 $\pm$ 0.5	36.1 $\pm$ 0.2	1.5 $\pm$ 0.06
Kollidon VA64	0.2	3.5 $\pm$ 0.3	11.6 $\pm$ 0.3	23.5 $\pm$ 0.4	1.7 $\pm$ 0.04
	0.5	2.7 $\pm$ 0.3	9.4 $\pm$ 0.2	20.3 $\pm$ 0.2	2.0 $\pm$ 0.23
	1.0	1.7 $\pm$ 0.2	7.4 $\pm$ 0.2	17.7 $\pm$ 0.2	2.2 $\pm$ 0.07
Pluronic F127	0.2	4.0 $\pm$ 0.2	9.4 $\pm$ 0.2	17.2 $\pm$ 0.4	1.4 $\pm$ 0.02
	0.5	3.0 $\pm$ 0.2	6.3 $\pm$ 0.1	10.6 $\pm$ 0.4	1.2 $\pm$ 0.08
	1.0	2.9 $\pm$ 0.2	6.2 $\pm$ 0.03	10.3 $\pm$ 0.2	1.2 $\pm$ 0.07
None	—	3.3 $\pm$ 0.4	13.2 $\pm$ 0.4	25.6 $\pm$ 0.3	1.7 $\pm$ 0.06
Raw PX	—	4.6 $\pm$ 0.1	19.2 $\pm$ 0.2	46.3 $\pm$ 0.5	2.2 $\pm$ 0.01

Prior to the electrospinning process, the sedimentation rate of the aforementioned PX microcrystals (prepared with 0.5% (w/v) Pluronic formulation) in hydrophilic film forming polymer solutions, i.e. PVA and PVA/PVP mixture (2/1; w/w) were investigated. As shown in Figure 2, no obvious sedimentation of PX microcrystals occurred within the first 5 hours. This could be attributed to the viscous nature of the

241 film forming polymer solutions. In the subsequent electrospinning process, 1.5 mL of
 242 the feed was electrospun at a flow rate of 6.5 $\mu\text{L}/\text{min}$. The course of the electrospinning
 243 process was ca. 3.9 h. This implied that no obvious sedimentation of PX microcrystals
 244 would occur during the electrospinning process, which may ensure the uniformity of
 245 the dosage forms.

246

247 **Fig. 2** Sedimentation rate of PX suspensions in film forming polymer solutions (n=3).

248

249 Preparation and characterization of plain electrospun ODFs

250 Initially, many polymer solutions (Table 2) were electrospun into films to construct a
 251 suitable film platform for loading PX microcrystals. As shown in Table 2, with more
 252 PVP in films, both the tensile strength and disintegration time dropped profoundly. The
 253 mixture of PVA/PVP at mass ratio of 2/1 was selected for the following study due to
 254 its short disintegration time without compromising the tensile strength much.

255

256 **Table 2** Tensile strength and disintegration time of films containing PVA/PVP at various ratios
 257 (n=3).

PVA/PVP (w/w)	Tensile strength (Mpa)	Disintegration time(s)
1/0	14.5±0.9	~180

4/1	12.5±1.0*	~25
2/1	12.0±0.8*	~5
1/1	10.0±0.4 ***	<1

Levels of significance are *P< 0.05 as compared to the films prepared from pure PVA; ***P<0.001 as compared to the films prepared from pure PVA.

260

Table 3. Formulation composition of electrospun ODFs with and without loading of PX.

No.	Code	Ingredient (mg)				
		API	Stabilizer	Film forming polymers		Plasticizer
		PX	Pluronic F127	PVA26-88	PVP360	PEG300
1	PVA	0	0	1500	0	0
2	PX/PVA	175	65	1500	0	0
3	PX/PVA+PVP	175	65	1000	500	0
4	PX/PVA+PEG	175	65	1500	0	56.5

262

Preparation of PX loaded electrospun ODFs

Three PX microcrystal loaded ODFs were prepared (Table 3). The total concentration of electrospun polymers solutions was kept at 10% (w/v). The ODFs without PX was also produced as a reference group. These formulations were successfully electrospun into fibrous films. The plain films without PX were white, while films containing PX were yellow by visual inspection. Besides PVP and PVA, an addition of PEG300 as a plasticizer was attempted to investigate its effect on the physicochemical properties of the ODFs.

271

Characterization of PX microcrystals loaded electrospun fibrous films

Morphology

274 As seen from the SEM images in Figure 3, the diameters of most electrospun fibers
 275 were under micron size. There were some particles on the surface or entrapped between
 276 the fibers in PX loaded films, but not in the reference PVA films, which were most
 277 likely due to the PX microcrystals. The mean diameters of the fibers in the PX/PVA
 278 film were 290 ± 58 nm. When PVP was added, the mean diameters of the fibers in film
 279 were decreased to 206 ± 55 nm, while the mean diameter of the fibers in the film
 280 containing PEG 300 was increased to 415 ± 58 nm. The possible reasons for diameter
 281 changing could be the different rheology properties of the polymer solutions in various
 282 compositions. Some researchers also reported that the diameters of electrospun fibers
 283 changed with an addition of drugs and varying excipients (35-37).
 284

Fig.3 SEM images of PVA (a); PX/PVA (b); PX/PVA+PVP (c); PX/PVA+PEG (d) films and its corresponding diameter distribution.

Mechanical properties

ODFs are expected to possess certain mechanical properties to prevent them from damage during handling and transportation. In general, the tensile strength and folding endurance of ODFs are two vital parameters to assess the mechanical properties of ODFs. The curves in Fig. 4 showed that the electrospun ODFs exhibited typical stress-strain behaviours of plastic materials with ductility. It contained a short elastic phase that the strain increased in the proportion with stress and a long plastic phase when the film could no longer return to its original shape. All formulations showed similar curve patterns exhibiting the characteristic of ductility of the films (Fig. 4a). It was obvious from Fig. 4b that the films containing PVP were unlikely to deform (less strain with the same stress) which would be a better product as compared to others with regard to film handling.

Fig.4 Stress-strain curves of PX/PVA films in three measurements (a); Representative stress-strain curves of three PX loaded formulations (b).

308

309 Based on the curves, three indexes were calculated, namely Young's modulus (an index
310 of stiffness), tensile strength and yield strength. The plain PVA films had significant
311 higher Young's modulus 108.3 ± 22.1 MPa than the PX microcrystals loaded
312 **electrospun ODFs** (Fig.5). It implied that an addition of PX microparticles in PVA
313 films compromised the stiffness. This may be because the addition of PX microparticles
314 disrupted the continuation of fibers and their arrangements, and thus weakened the
315 intermolecular strength of the fibers.

316

317 The PX/PVA+PVP films exhibited significant higher yield strength among all PX
318 loaded formulations showing superior ability to keep original shapes. It showed that an
319 addition of PEG300 as the plasticizer in the film formulations resulted in a decrease in
320 Young's modulus and yield strength, but was not statistically significant from the other
321 two formulations. Interestingly, the mean tensile strengths of the three electrospun
322 ODFs were similar. It was probably due to the fact that PVA constituted a large
323 proportion of all formulations, and therefore, tensile strength, the maximum tension
324 that the films could withstand before breaking, was mainly depended on the PVA
325 mechanical property. All the formulations could be folded more than 50 times without
326 break.

327

328

329 **Fig.5** Mechanical properties of plain PVA film and PX loaded films, *P<0.05, **P<0.01, ***P<0.001.

330

Solid state of PX in the film

PX was reported to have four polymorphic forms, namely Form I, II- α 1, II- α 2, III and monohydrate form (38). As shown in the XRPD diffractograms in Fig.6a, the starting material, bulk PX powder, had intense diffractions at 8.93°, 18.06° and 27.08° (2 θ), which were the characteristic diffractions of Form I. The PX microcrystals obtained from the anti-solvent precipitation method was upper-concentrated by centrifugation and analysed immediately with the X-ray diffractometer. The characteristic diffraction of this sample at around 26.61° (Fig. 6b) suggested that it was in the monohydrate form (39). **These indicated that PX transformed from Form I to monohydrate form after anti-solvent precipitation. The physical mixtures were prepared with monohydrate form PX and the polymers in the same ratios as in the drug-loaded ODFs. Comparing the XRPD patterns of the physical mixtures with their corresponding formulation diffractograms, PX microparticles kept their monohydrate form, which suggested that the electrospinning process did not distort the solid state of PX microparticles.**

Fig. 6 Representative XRPD patterns of bulk PX, ball-milled PVA, and physical mixtures of monohydrate form PX and polymers (a); Representative XRPD patterns of d upper-concentrated PX microparticles, and the three formulations (b).

To investigate potential molecular interactions between PX and the excipients, the films were subjected to FTIR. The FTIR spectrum of PX in monohydrate form was used as

a reference. The infrared spectrum of PX monohydrate form had the characteristic peaks at 1641cm^{-1} of C=O stretching and 1326 cm^{-1} of symmetrical O=S=O stretching (Fig. 7a). The spectrum of PVP powder showed C-H stretch between $2826\text{--}3023\text{ cm}^{-1}$, C-N stretch at 1265 cm^{-1} and C=O at 1654 cm^{-1} , while the very broad band at $3650\text{--}3150\text{ cm}^{-1}$ could be assigned to O-H stretching from water absorbed by PVP (40). Similarly, The bands between $2920\text{ cm}^{-1}\text{--}2940\text{ cm}^{-1}$ in PVA spectrum were attributed to -CH₂- and -CH₃- stretching (Fig. 7a). The peak at 1092 cm^{-1} in PVA was C-O stretching. The very broad band at around 3319cm^{-1} was partly attributed to hydroxyl group in PVA structure and partly because of water absorption. In spectrum of PX loaded films, most of PX characteristic signals were overlapped with the polymer signals (Fig.7b). The main functional groups of polymers remained at the same place in Fig.7, suggesting no molecular interaction between PX and polymers occurred.

Fig.7 FT-IR spectra of bulk materials (a) and the three formulations (b).

Disintegration

Disintegration time is one of the key quality attributes of ODFs. The disintegration of the electrospun ODFs ranged from a few seconds to 200 seconds depending on the formulation compositions. During the disintegration process, PX/PVA films first got wet, and then shrank to gel-like small pieces. Eventually, the film disappeared within 200 seconds. The similar phenomenon was also observed for PX/PVA+PEG films. In contrast, PX/PVA+PVP films rapidly disintegrated into small pieces when contacted

377 with water. It exhibited much shorter disintegration time (~3s) as compared to the other
 378 two formulations. The shorter disintegration time of PX/PVA+PVP films may be
 379 because that PVP dissolved faster than PVA in water. It has been reported that the
 380 disintegration of PVP films usually was very fast, however, the disintegration time
 381 tended to be prolonged, when other excipients such as PVA were formulated (41, 42).
 382 The screen shots of the disintegration of various films were presented in Table 4. As
 383 shown, the electrospun ODFs disappeared by the time of disintegration.

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 385
 386
 387
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 389
 390

Table 4 Disintegration times of three formulations (n=3).

Disintegration Time (s)		Disintegration process		
PX/PVA	163±34	0s	10s	150s

PX/PVA+PVP	3±1	0s	0.5s	2s

PX/PVA+PEG	129±10	0s	10s	133s

391

392 *Drug loading and Residue solvent*

393 Prior to dissolution tests, the drug loading and entrapment efficiency of the films were
 394 calculated and listed in Table 5. There was significant difference in drug loading
 395 between PX/PVA and PX/PVA+PEG films. This may be attributed to the instability of
 396 the electrospinning process with PX/PVA+PEG formulation. In fact, it was observed
 397 that the addition of PEG300 as a plasticizer greatly disturbed the electrospinning
 398 process. In addition, the entrapment efficiency of PX in the PX/PVA+PEG films was
 399 significant lower than PX/PVA and PX/PVA+PVP films.

400

401

402

403

Table 5. Characterizing data for electrospun ODFs (n=3).

Formulation	Drug load (%)	Entrapment efficiency (%)	Residue solvent (%)	Thickness (μm)	Weight (mg)
PX/PVA	8.88 $\pm$ 0.26	88.25 $\pm$ 2.59	3.8 $\pm$ 0.2	43.0 $\pm$ 4.0	11.5 $\pm$ 0.2
PX/PVA+PVP	8.80 $\pm$ 0.50	87.51 $\pm$ 5.00	3.4 $\pm$ 0.1	45.0 $\pm$ 1.0	12.8 $\pm$ 0.1
PX/PVA+PEG	7.94 $\pm$ 0.29*	81.54 $\pm$ 2.95	3.1 $\pm$ 0.4	46.0 $\pm$ 3.1	12.5 $\pm$ 0.4

404

Levels of significance are *P< 0.05 as compared to drug loading of PX/PVA films.

405

406 **DMF was used as one of the solvents for electrospun films preparation and it**
 407 **constituted 12.6% (w/v) in the initial PX microcrystal suspension. To measure the**
 408 **percentage of the residue solvent, the obtained ODFs were subjected to TGA**
 409 **measurement. The weight loss until 180 °C was calculated for residue solvent (the**
 410 **boiling point of DMF is 153 °C). It was found that most of the weight loss occurred**
 411 **before 100 °C and the rate of weight loss became slow between 100 °C and 160 °C.**
 412 **After 180 °C a dramatic weight loss was observed, which was assigned to**

413 **decomposition of the chemicals. The results showed that the residue solvents of the**
414 **three formulations were between 3%-4% (Table 5).**

415

416

417 *In-vitro* dissolution profile

418 Different *in-vitro* dissolution methods were reported to test ODFs, such as small
419 volume of media (mimic oral cavity) in container coupled with magnet stirring (25, 26)
420 and shaker incubator (42). The method used in this study was traditional paddle method
421 (Erweka apparatus) as our EEFs based ODFs were designed to disintegrate or dissolve
422 fast in mouth for ease of swallowing, and the dissolved PX was intended to be absorbed
423 through gastrointestinal tract.

424

425 **The thicknesses of the film cuts for dissolution study were measured and the**
426 **corresponding weights of the films were presented in Table 5.** As shown in Figure
427 8, the dissolution rates of the three electrospun ODFs were faster than that of the
428 physical mixture (PM). Within the first 15 minutes, $79.7 \pm 1.4\%$, $88.0 \pm 1.4\%$ and 87.1
429 $\pm 2.0\%$ of PX were dissolved from the PX/PVA, PX/PVA+PVP and PX/PVA+PEG
430 films, respectively, whereas only $9.8\% \pm 5.7\%$ of PX was dissolved from the physical
431 mixture of PVA and PX. In addition, PX in the films composed of PVA and PVP
432 dissolved faster than the films composed of only PVA or PVA+PEG. These results
433 were in consistent with the disintegration study, where PX/PVA+PVP film
434 disintegrated much faster than PX/PVA film. PEG300 demonstrated no obvious effects
435 on PX dissolution profile, as shown in Figure 8.

436

Fig. 8 Dissolution profiles of PX-loaded electrospun ODFs and the physical mixture (PM) of PX/PVA.

Compared to the physical mixture of PX and PVA, the faster dissolution rate of PX in electrospun ODFs can be attributed to the high surface area of PX microcrystals as compared to the bulk powder. Size reduction by the anti-solvent precipitation method enlarged the surface area of PX, thereby improved dissolution rate as illustrated by Noyes-Whitney equation (43). Moreover, as shown in XRPD results (Fig.6), PX in the formulations was monohydrate, while raw API was form I. PX in different crystalline forms exhibited distinct dissolution rates (44), with the highest value for monohydrate, followed by form II, form III and form I. In addition, as electrospun ODFs were porous with high surface area, the PX microparticles entrapped between the fibers was more likely to get access to dissolution media. Finally, the hydrophilic excipients, PVA, PVP and PEG 300 in the formulation could not only contribute to fast disintegration and dissolution of films, but also facilitate wetting of the particles.

Conclusion

In this study, piroxicam, a poorly water-soluble model drug, was loaded into ODFs by electrospinning of micronized piroxicam particles obtained from the anti-solvent precipitation method. The electrospun orodispersible films exhibited satisfactory

mechanical properties, folding endurance and *in-vitro* disintegration performance. This study demonstrated the feasibility of formulating poor water-soluble drug into ODFs without using massive organic solvents by coupling the electrospinning process with the anti-solvent precipitation method.

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635 Dear Prof. Robert O. Williams III,

636 Thank you very much for the valuable feedback and forwarding the comments from the
637 reviewers. We have carefully processed the comments, and addressed their concerns. Our
638 detailed responses are listed below (in *italics* and **green**). In addition, we have also edited the
639 revised manuscript with the changes made during the revision clearly highlighted in boldface
640 type.

641

642 Yours sincerely

643 Mingshi Yang

644

645 Reviewers' Comments:

646

647 Reviewer #1: This manuscript presents a method for preparing ODFs using the electrospinning
648 approach. The API is first crystallized into microparticles using the antisolvent method. The

649 resulting microparticle suspension is stabilized and the used as part of the mixture subjected to
650 electrospinning. The investigation covers the physical, mechanical and performance
651 characterization of ODFs obtained using different polymeric materials as fiber-forming
652 matrices.

653 This manuscript suffers from very poor writing. It needs improvements on the flow and clarity
654 of the presentation before it gets published. Especially in the description of the methods. For
655 example, the use of stabilizers for the suspension is mentioned under the Methods section.
656 However, the specific stabilizers used are not identified until much later, in the Results and
657 Discussion section.

658 *We thank the reviewer for the comments. The manuscript has been checked again and we*
659 *improved the flow and presentation, especially the section of Materials and Methods. The*
660 *information of stabilizers have been supplemented in the section of Martials and Methods:*
661 *“Piroxicam (175 mg) was first dissolved in 2.0 mL of N, N-Dimethylformamide (DMF) to make*
662 *an 8.75% (w/v) drug solution. Subsequently, the solution was injected into the 13.0 mL distilled*
663 *water containing, either 0.2%, 0.5% or 1.0% (w/v) of each stabilizer (i.e. HPMC E15, Kollidon*
664 *VA64, and Pluronic F127) with a magnet-stirring rate at 1500 rpm in a cold room (4.0 °C). ”*

665
666 The Methods section informs the reader that "various film-forming polymers (10%, w/v)
667 were ..." However, it is not until 9 (nine) subsections later in the manuscript, that such film
668 forming polymers are actually identified. This is unacceptable.

669 *We thank the reviewer for the comments. The film forming polymers were presented in the*
670 *section of Martials and Methods: “A 10%(w/v) PVA 26-88 was prepared by dissolving 1500mg*
671 *of the polymer into 15mL distilled water under stirring until the polymer was completely*
672 *dissolved. A 10%(w/v) PVA/PVP (2/1, w/w) was prepared by dissolving 1000mg of PVA26-88*
673 *and 500mg of PVP 360 into 15mL distilled water under stirring until the polymers were*
674 *completely dissolved. Other polymer solutions containing different compositions of PVA 26-*
675 *88/PVP 360 kept the total concentration at 10% (w/v) with various mass ratios of the two*
676 *polymers (presented in Table 2 manuscript body). Plain ODFs were obtained by directly*
677 *electrospinning of the prepared polymer solution. Drug-loaded ODFs were obtained by*
678 *dissolving the appropriate amount of film forming polymer into PX microcrystal suspensions*
679 *produced with anti-solvent method, followed by electrospinning. ”*

680

681 Table 3 would be more useful if it is made more informative. I suggest to include the function
682 of the components listed (e.g., stabilizer or fiber forming polymer) on the header of the column
683 where they are listed.

684 *We thank the reviewer for the comments. The Table 3 in the revised manuscript has been*
685 *supplemented with the information of functionality of the excipients.*

686

687 The discussion about Figure 6 should also be made clearer. It is not that the information is not
688 there, but the reader has to go back and forth between the figure and the text to get a clear idea
689 of the information provided.

690 *We thank the reviewer for the comments. The discussion on Figure 6 has been improved in the*
691 *revised manuscript. We have updated the figure and the text in the section of the discussion.*

692

693 I strongly suggest that the authors use the help of a first time reader in the preparation of their
694 revised manuscript, in order to make the improvements in clarity required.

695 *We thank the reviewer for the comments. We have asked one of our colleagues to help with*
696 *editing the revised manuscript.*

697

698 Table 5 presents the results for drug loading and entrapment efficiency. However, there is no
699 information regarding the procedures used to obtain these numbers. This type of information
700 needs to be included in the manuscript.

701 *We thank the reviewer for the comments. The equation of calculation of the drug loading and*
702 *entrapment efficiency has been added in the revised manuscript in the in the section of Martials*
703 *and Methods.*

704

705 There is one point that will inevitably be present in just about every reader's mind that is not
706 mentioned at all in the manuscript. It is the fate of the dimethylformamide (DMF) present in
707 the obtained films. The initial PX suspension contains about 13% DMF. This mixture is then
708 combined with 10% polymer solution. I suppose I could go back to Table 3 and back-calculate
709 the amounts used to make the mixtures eventually subjected to electrospinning, but this is a
710 burden that the reader should not be expected to carry. The description of the methods is so
711 poor that it forces the reader to either try to figure things not provided by the authors, or to
712 brush it off altogether. The DMF content of the films is something that must be spelled loud
713 and clear. DMF has very low volatility and it is almost impossible to expect it to dry off even

714 with the high surface are provided by the fibers. If the authors do not have information on the
715 residual content of DMF, they should at least provide the nominal content, instead of expecting
716 the reader to do those calculations. DMF is likely to have a plasticizer effect on the fibers. On
717 the other hand, the content of DMF in the ODFs is something no reader will be able to ignore
718 when looking at this manuscript.

719 *We thank the reviewer for the comments. We have added the Residue solvent results in the*
720 *method and discussion part.*

721

722 *In the section of Materials and Method: "Residue solvent was conducted through*
723 *thermogravimetric analysis (TGA) (TGA Discovery, TA Instruments, New Castle, USA).*
724 *Samples around 2.5mg were put on platinum pans and heated from 25 °C to 250 °C at*
725 *10 °C/min. The obtained percentage of weight loss with temperature data was calculated as*
726 *residue solvent."*

727

728 *In the section of Discussion: "DMF was used as one of the solvents for electrospun films*
729 *preparation and it constituted 12.6% (w/v) in the initial PX microcrystal suspension. The*
730 *obtained ODFs were slowly heated to 250 °C and the weight loss until 180 °C was calculated*
731 *for residue solvent (the boiling point of DMF is 153 °C). Most of the weight loss occurred*
732 *before the temperature reached 100 °C and the rate of weight loss with temperature became*
733 *slowly between 100 °C and 160 °C. After 180 °C a dramatic weight loss was observed, which*
734 *was assigned to decomposition of the chemicals. The results showed that the residue solvents*
735 *of the three formulations were between 3%-4% (Table 5) and the most solvent remained in the*
736 *electrospun films was more likely to be water."*

737

738 Minor comments

739 Page 16, Line 294 "loaded EEFs (Fig.5)" => Spell out each acronym when first used Page 3,
740 Line 80 "gradients" => correct or clarify Page 14, Line 337 "stretches" => stretching

741 *We thank the reviewer for the comments and correction. We have edited and corrected these in*
742 *the revised manuscript.*

743

744

745 Reviewer #3: The authors wrote a well structured manuscript. It is just needs some clarifications
746 in some points to be ready for publishing.

747 *We thank the reviewer for the positive comments*

748

749 1. In Page 3 line 78: please mention the stabilizers used.

750 *We thank the reviewer for the comments. We have added the names of the stabilizers used in*
751 *the section of Materials and Method in the revised manuscript as follow: “Piroxicam (175 mg)*
752 *was first dissolved in 2.0 mL of N, N-Dimethylformamide (DMF) to make an 8.75% (w/v) drug*
753 *solution. Subsequently, the solution was injected into 13.0 mL distilled water containing either*
754 *0.2%, 0.5% or 1.0% (w/v) of each stabilizer (i.e. HPMC E15, Kollidon VA64, and Pluronic*
755 *F127) with a magnet-stirring rate at 1500 rpm in a cold room (4.0 °C).”*

756

757 2. In Page 3 line 85: please mention the polymers used.

758 *We thank the reviewer for the comments. We have added the names of the polymers used in the*
759 *section of Materials and Method in the revised manuscript as follow: “A 10%(w/v) PVA 26-88*
760 *was prepared by dissolving 1500mg of the polymer into 15mL distilled water under stirring*
761 *until the polymer was completely dissolved. A 10%(w/v) PVA/PVP (2/1, w/w) was prepared by*
762 *dissolving 1000mg of PVA26-88 and 500mg of PVP 360 into 15mL distilled water under*
763 *stirring until the polymers were completely dissolved. Other polymer solutions containing*
764 *different compositions of PVA 26-88/PVP 360 kept the total concentration at 10% (w/v) with*
765 *various mass ratios of the two polymers (presented in Table 2). Plain ODFs were obtained by*
766 *directly electrospinning of the prepared polymer solution. Drug-loaded ODFs were obtained*
767 *by dissolving the appropriate amount of film forming polymer into PX microcrystal suspensions*
768 *produced with anti-solvent method, followed by electrospinning.”*

769

770 3. In Page 4 line 103: please mention the wavelength used in UV spectrometer measurement.

771 *We thank the reviewer for the comments. We have added the wavelength used in UV*
772 *spectrometer measurement in the section of Materials and Method: “with its absorbance at*
773 *357 nm as y-axis for linear fitting.”*

774

775 4. It is not clear in the part of preparation using different polymers, do you add the polymer to
776 the aqueous solution before or after the addition of piroxicam solution.

777

778 *We thank the reviewer for the comments. To clarify this we have revised in the section of*
 779 *Materials and Methods: “Drug-loaded ODFs were obtained by dissolving the appropriate*
 780 *amount of PVA 26-88/PVP 360 into PX microcrystal suspensions produced with anti-solvent*
 781 *method, followed by electrospinning”. In addition, we moved Fig.1 forward to make sure the*
 782 *clarity of preparation method.*

Fig.1 Preparation of PX-loaded ODFs.

5. In Page 6 line 166, What do you mean by 'The disintegration process was recorded at 60 frame/s and shown as screenshot.' Results submitted are shown in seconds.

We thank the reviewer for the comments. This sentence has been deleted and revised as follow:
“The disintegration process was recorded with the video function of a smart phone and the
parameter for video recording was 60fps. The disintegration process of the films were observed
afterwards and screenshots were made and reported in the manuscript. The seconds in the
results were the time of the screenshots were made” in the revised manuscript.

6. In Page 6 line 181, do you take the part of ODF according to its weight or dimension.

We thank the reviewer for the comments. We took part of the ODFs according to its dimension.
Meanwhile but we have measured their thickness before cutting. As shown in the table below,
their thickness were similar, and their weights were also similar. We have commented on the
thickness and weight of the films in the revised manuscript.

Formulation	Thickness	Weight (mg)
	(μm)	

PX/PVA	43.0±4.0	11.5±0.2
PX/PVA+PVP	45.0±1.0	12.8±0.1
PX/PVA+PEG	46.0±3.1	12.5±0.4

799

800

801 7. Why did you choose distilled water as dissolution medium. The film is supposed to
802 disintegrate in the mouth (simulated saline solution) and also as you said in page 17 line 383
803 'disintegrate or dissolve fast in mouth for ease of swallowing, and the dissolved PX was
804 intended to be absorbed through gastrointestinal tract.' I think dissolution should be repeated in
805 simulated saliva solution.

806 *We thank the reviewer for the comments. We chose distilled water based on the following*
807 *considerations. Due to fast dissolving of film forming polymer, ODFs will disintegrate fairly*
808 *quick and then the microcrystal suspension would be swallowed. The dissolution of PX in*
809 *simulated saliva solution (rather than gastric media) would not be relevant. Dissolution was a*
810 *rough experiment to reflect the in-vitro dissolution rate of the drug in the formulations. No*
811 *dissolution medium (even bio-relevant dissolution medium) would realize the real dissolution*
812 *of the formulations in vivo. We chose distilled water as it is an easy and efficient dissolution*
813 *medium, which could also be reasonable medium to investigate and reflect the in-vitro*
814 *dissolution profile of PX. It also has been used as dissolution media for PX dissolution before*
815 *(Lai F et al. 2014). In addition, we chose water to see whether fibrous electrospun ODFs would*
816 *have any influence on the PX dissolution, a poorly water-soluble drug.*

817

818 *Lai F, Pini E, Corrias F, et al. Formulation strategy and evaluation of nanocrystal piroxicam*
819 *orally disintegrating tablets manufacturing by freeze-drying[J]. International journal of*
820 *pharmaceutics, 2014, 467(1-2): 27-33.*

821

822 8. It is clear in page 9 that you tried different ratios of PVA/PVP and PVA alone was the worst
823 formulation. Then after adding PX, you did not make use of your result, you tried effect of PEG
824 on PVA alone (it is clear to be the worst formulation) and not on the best formula. Please clarify
825 your point of view of comparing with PVA while your screening results showed it is the worst
826 formulation. Also you continued on 2:1 PVA:PVP and not 1:1 ratio, how did you discriminate
827 your results.

828 *We thank the reviewer for the comments. Firstly, It has been reported that PEG could act as a*
829 *plasticizer to facilitate formation of casting film and facilitate disintegration. Therefore, we*
830 *added PEG to PVA film to see whether PEG would facilitate the disintegration of PVA films*
831 *obtained from electrospinning. Secondly, PVA/PVP (2:1/w:w) films exhibited very fast*
832 *disintegration in the test (less than 5 seconds). It would not be easy to see the influence of PEG*
833 *on ODFs if we add PEG into PVA/PVP (2:1/w:w) films. In fact, we tried to add PEG into*
834 *PVA/PVP (2:1/w:w) film formulations (data not shown). However, the electrospinning process*
835 *became unstable. Therefore, we did not continue with this formulation.*

836 *As for PVA/PVP (1:1/w:w), we did not continue this formulation because the electrospun films*
837 *became more soft with an increase in the proportion of PVP. In addition, the film became more*
838 *hygroscopic with an increase in the proportion of PVP. In the disintegration tests with PX*
839 *loaded electrospun PVA/PVP (2:1/w:w) film, the disintegration of the films was ca. 2 seconds.*
840 *We postulated that PVA/PVP (1:1/w:w) film could be faster. However, the difference might not*
841 *be obvious. Lastly, we observed that the electrospinning process of PVA/PVP (2:1/w:w) was*
842 *more stable as compared to that of 1:1. Beads could be observed with an increase in PVP in*
843 *the formulation (Table I. Fig. I below).*

844 Table 1. Experimental observation of electrospinning of PVA and PVP binary mixture solutions

PVA/PVP mass ratio	Electrospinnability	Beads	Process stability
4:1	Yes	Uniform	Yes
2:1	Yes	Uniform	Yes
1:1	Yes	+	No
1:2	Yes	++	No

845

Fig. 1 SEM images of electrospun films composed of PVA and PVP at mass ratios of 4:1; 2:1, 1:1, and 1:2.

The changes in the revised manuscript:

Page 2, Line 53. Adding sentence “The stabilizers and their optimal concentrations for precipitating PX in anti-solvent precipitation method were selected among poloxamer, copovidone and hydroxypropyl methylcellulose at three different concentrations, respectively.”

Page 3, Line 78. Adding sentence “PX microcrystal suspensions were prepared with anti-solvent precipitation method.”

858 Page 3, Line 81. Adding sentence “either 0.2%, 0.5% or 1.0% (w/v) of each stabilizer (HPMC
859 E15, Kollidon VA64, and Pluronic F127) with a magnet-stirring rate at 1500 rpm in a cold
860 room (4.0 °C).”

861

862 Page 3, Line 97. Adding sentence “A 10%(w/v) PVA 26-88 was prepared by dissolving
863 1500mg of the polymer into 15mL distilled water under stirring until the polymer was
864 completely dissolved. A 10%(w/v) PVA/PVP (2/1;w/w) was prepared by dissolving 1000mg
865 of PVA26-88 and 500mg of PVP 360 into 15mL distilled water under stirring until the polymer
866 was completely dissolved. Other polymer solutions containing different compositions of PVA
867 26-88/PVP 360 kept the total polymer concentration at 10%(w/v) with various mass ratios of
868 the two polymers (Table 2). Plain ODFs were obtained by directly electrospinning the prepared
869 polymer solutions. Drug-loaded ODFs were obtained by dissolving the appropriate amount of
870 PVA 26-88/PVP 360 into PX microcrystal suspensions produced with anti-solvent method,
871 followed by electrospinning.”

872

873 Page 6, Line 173. Adding sentence “The disintegration process was recorded with the video
874 function of a smart phone and the parameter for video recording was 60fps. The disintegration
875 process of the film was observed afterwards and screenshots were made and reported. The
876 seconds in the results were the time of the screenshots was made.”

877

878 Page 6, Line 182. Adding sentence “The entrapment efficiency was calculated by using the
879 actual amount of PX in the formulation /the theoretical amount of PX in the formulation.”

880

881 Page 7, Line 186. Adding Residue Solvent part

882 “Residue solvent was conducted through thermogravimetric analysis (TGA) (TGA Discovery,
883 TA Instruments, New Castle, USA). Samples around 2.5mg were put on platinum pans and

884 heated from 25 °C to 250 °C at 10 °C/min. The obtained percentage of weight loss with
885 temperature data was calculated as residue solvent.”

886

887 Page 10, Line 263. Adding function of the components in table 5.

888

889 Page 13, Line 314. Updating with “electrospun ODFs”

890

891 Page 14, Line 341. Adding sentence “These indicated that PX transformed from Form I to
892 monohydrate form after anti-solvent precipitation. The physical mixtures were prepared with
893 monohydrate form PX and the polymers in the same ratios as in the drug-loaded ODFs.
894 Comparing the XRPD patterns of the physical mixtures with their corresponding formulation
895 diffractograms, PX microparticles kept their monohydrate form, which suggested that the
896 electrospinning process did not distort the solid state of PX microparticles.”

897

898 Page 15, Line 360 Updating with stretching.

899

900 Page 18, Line 409. Adding sentence “DMF was used as one of the solvents for electrospun
901 films preparation and it constituted 12.6% (w/v) in the initial PX microcrystal suspension. The
902 obtained ODFs were slowly heated to 250 °C and the weight loss until 180 °C was calculated
903 for residue solvent (the boiling point of DMF is 153 °C). Most of the weight loss occurred
904 before the temperature reached 100 °C and the rate of weight loss with temperature became
905 slowly between 100 °C and 160 °C. After 180 °C, a dramatic weight loss was observed, which
906 was assigned to decomposition of the chemicals. The results showed that the residue solvents
907 of the three formulations were between 3%-4% (Table 5).”

908

909 Page 18, Line 427. Adding sentence “Thet hicknesses of the film cuts for dissolution study
910 were measured and the corresponding weights of the films were presented in Table 5.”

911