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

Song, Qingchun; Guo, Xiong; Sun, Yi; Yang, Mingshi

Published in:
AAPS PharmSciTech

Link to article, DOI:
10.1208/s12249-019-1464-2

Publication date:
2019

Document Version
Peer reviewed version

Introduction

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

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

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

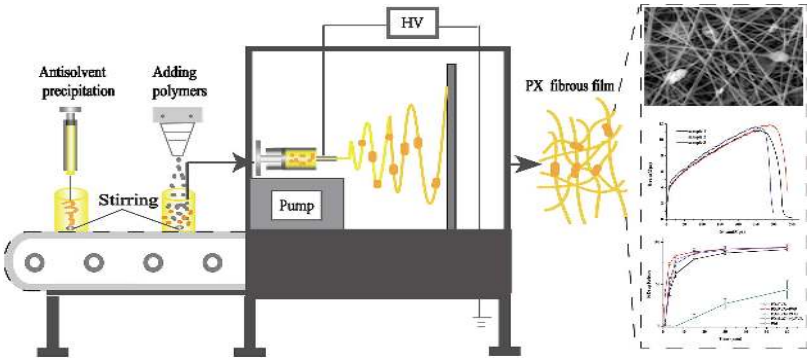


Fig.1 Preparation of PX-loaded electrospun ODFs.

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 μm/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 fiberous 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 PANanalytical X'Pert PROMPD X-ray diffractmeter (PANalytical, Almelo, The Netherlands) using CuKα 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 2Θ=5° and an end angle of

158 2Θ=35°. The scan speed was 0.6565°2 Θ /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 4000–400 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 x 2 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 $$\text{Drug loading } (\%) = \frac{\text{Amount of drug in electrospun ODFs}}{\text{Amount of electrospun ODFs}} \times 100$$

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

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**

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

In-vitro dissolution study

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

UV analysis

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

Statistics

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

Results and Discussion

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±0.6	16.8±0.8	31.9±1.5	1.5±0.02
	0.5	9.9±1.0	21.4±0.9	39.1±0.6	1.4±0.14
	1.0	7.4±0.5	18.6±0.5	36.1±0.2	1.5±0.06
Kollidon VA64	0.2	3.5±0.3	11.6±0.3	23.5±0.4	1.7±0.04
	0.5	2.7±0.3	9.4±0.2	20.3±0.2	2.0±0.23
	1.0	1.7±0.2	7.4±0.2	17.7±0.2	2.2±0.07
Pluronic F127	0.2	4.0±0.2	9.4±0.2	17.2±0.4	1.4±0.02
	0.5	3.0±0.2	6.3±0.1	10.6±0.4	1.2±0.08
	1.0	2.9±0.2	6.2±0.03	10.3±0.2	1.2±0.07
None	—	3.3±0.4	13.2±0.4	25.6±0.3	1.7±0.06
Raw PX	—	4.6±0.1	19.2±0.2	46.3±0.5	2.2±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

film forming polymer solutions. In the subsequent electrospinning process, 1.5 mL of the feed was electrospun at a flow rate of 6.5 μL/min. The course of the electrospinning process was ca. 3.9 h. This implied that no obvious sedimentation of PX microcrystals would occur during the electrospinning process, which may ensure the uniformity of the dosage forms.

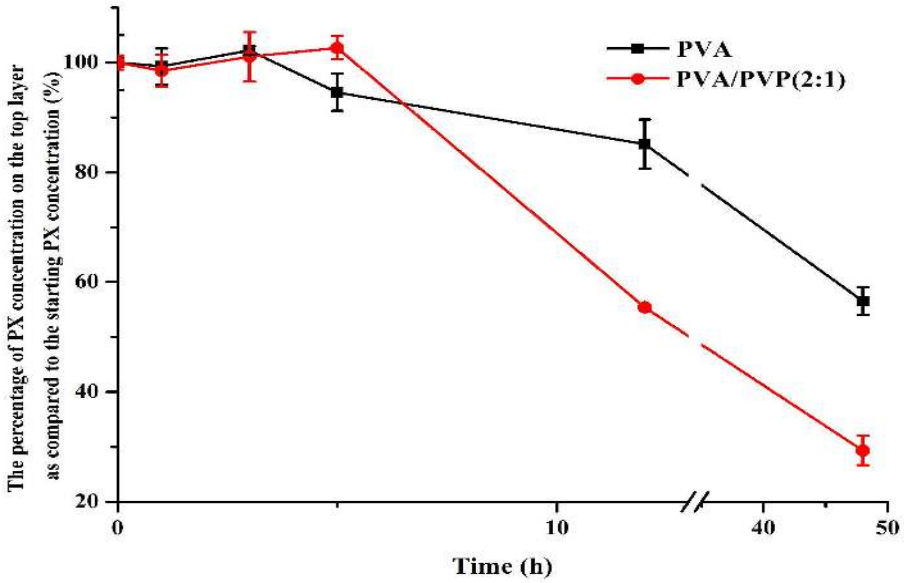


Fig. 2 Sedimentation rate of PX suspensions in film forming polymer solutions (n=3).

Preparation and characterization of plain electrospun ODFs

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

Table 2 Tensile strength and disintegration time of films containing PVA/PVP at various ratios (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.

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

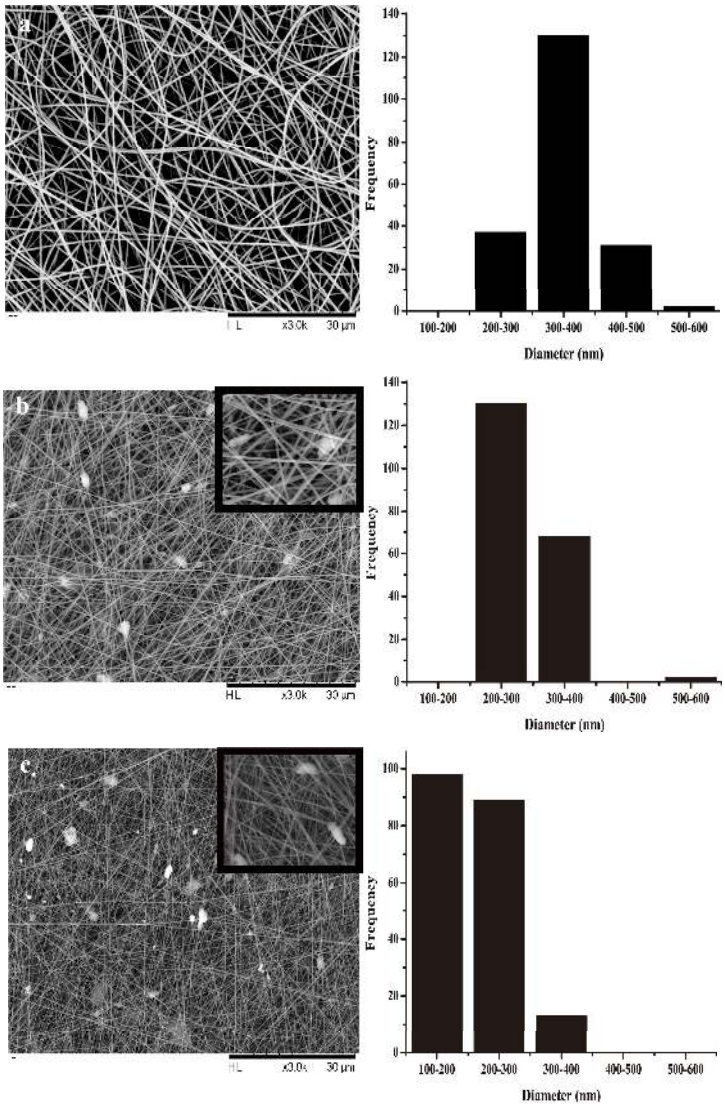
Preparation of PX loaded electrospun ODFs

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 fiberous 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.

Characterization of PX microcrystals loaded electrospun fiberous films

Morphology

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



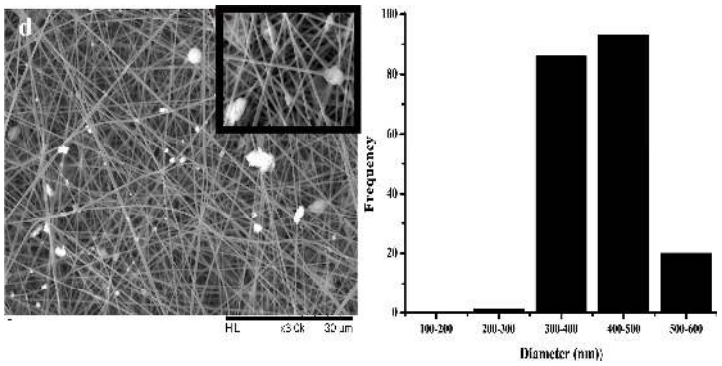


Fig.3 SEM images of PVA (a); PX/PVA (b); PX/PVA+PVP (c); PX/PVA+PEG (d) films and its corresponding diameter distribution.

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.

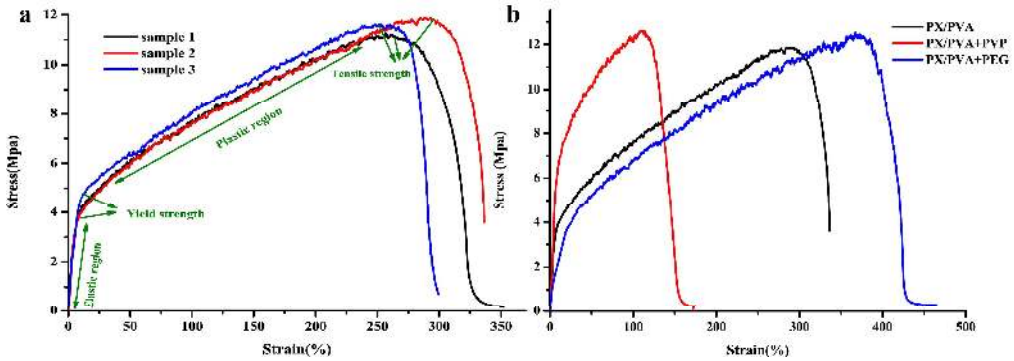


Fig.4 Stress-strain curves of PX/PVA films in three measurements (a); Representative stress-strain curves of three PX loaded formulations (b).

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

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

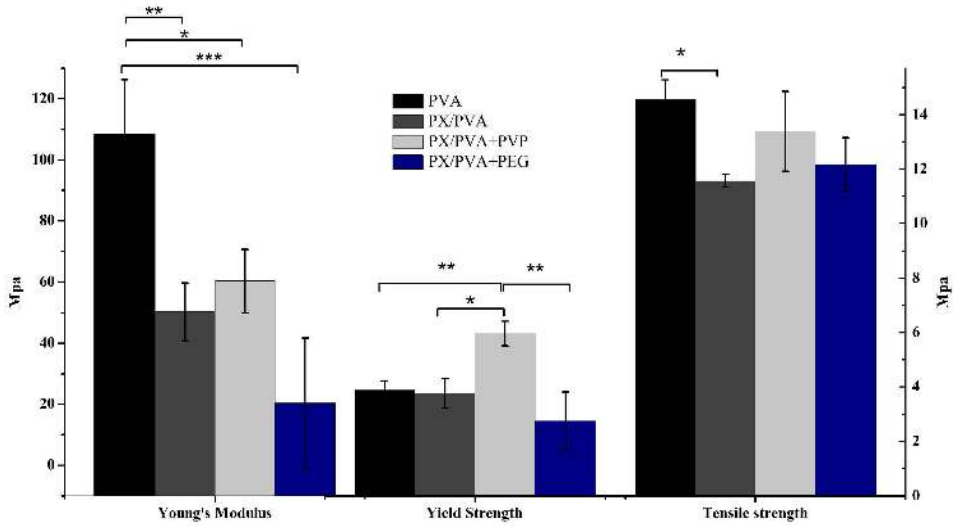


Fig.5 Mechanical properties of plain PVA film and PX loaded films, *P<0.05, **P<0.01, ***P<0.001.

Solid state of PX in the film

PX was reported to have four polymorphic forms, namely Form I, II-α1, II-α2, III and monohydrate form (38). As shown in the XRPD diffractgrams in Fig.6a, the starting material, bulk PX powder, had intense diffractions at 8.93°, 18.06° and 27.08° (2θ), 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 diffractgrams, PX microparticles kept their monohydrate form, which suggested that the electrospinning process did not distort the solid state of PX microparticles.**

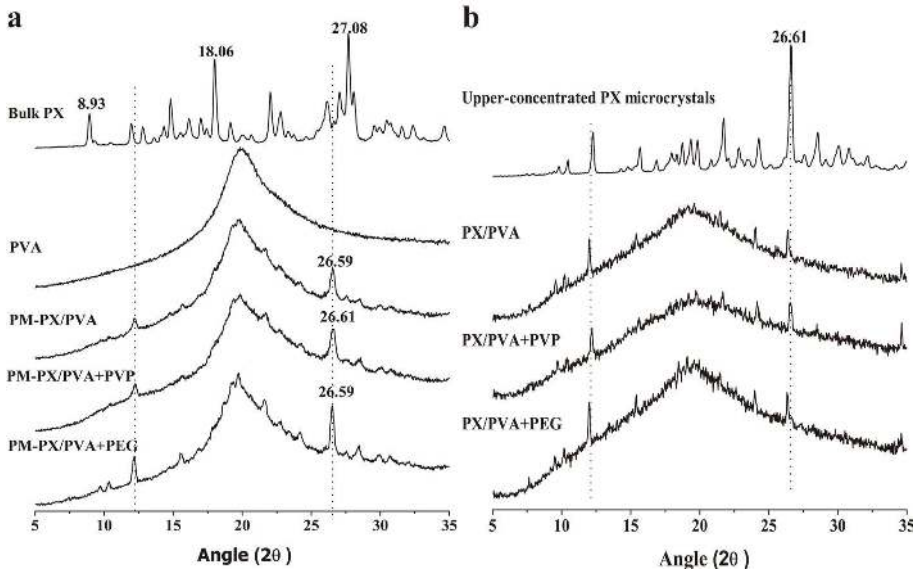


Fig. 6 Representative XRPD patterns of bulk PX, ball-milled PVA, and physical mixtures of monohydrate form PX and polymers (a); Representative XRPD patterns of d upper-concentrated PX microparticles, and the three formulations (b).

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 2826–3023 cm^{-1}, C-N stretch at 1265 cm^{-1} and C=O at 1654 cm^{-1}, while the very broad band at 3650–3150 cm-^{1} could be assigned to O-H **stretching** from water absorbed by PVP (40). Similarly, The bands between 2920 cm^{-1}-2940 cm^{-1} in PVA spectrum were attributed to -CH_2- and -CH_3- 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.

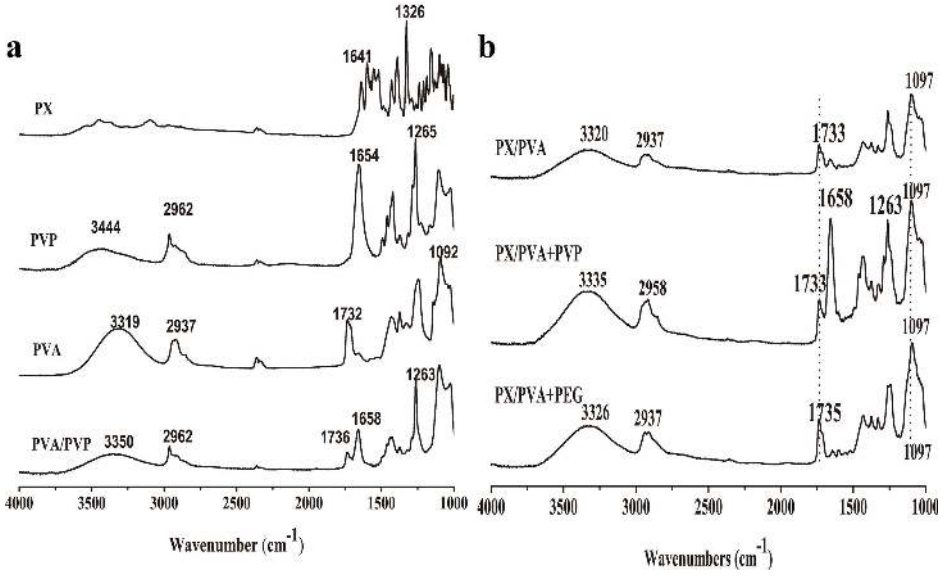


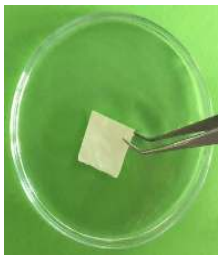
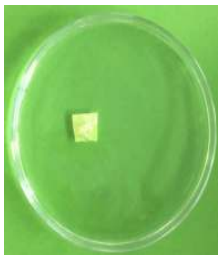
Fig.7 FT-IR spectra of bulk materials (a) and the three formulations (b).

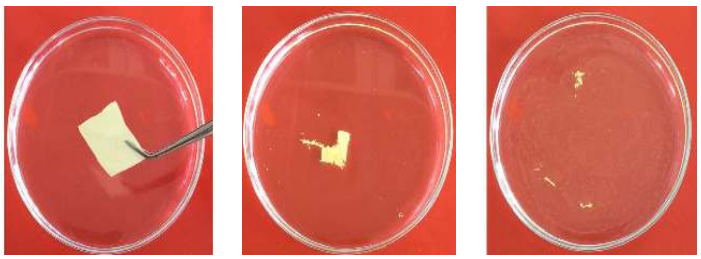
Disintegration

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

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

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



Drug loading and Residue solvent

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

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

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

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

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

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

In-vitro dissolution profile

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

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

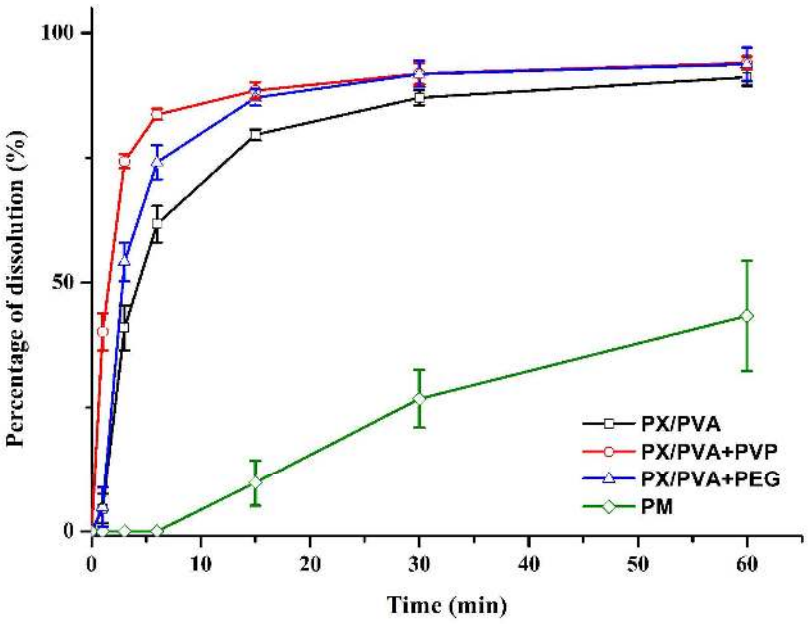


Fig. 8 Dissolution profiles of PX-loaded electrospun ODFs and the physical mixture (PM) of PX/PVA.

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 praticles obtained from the anti-solvent precipitation method. The electrospun orodispersible films exhibited satisfactory

457 mechanical properties, folding endurance and *in-vitro* disintegration performance. This
458 study demonstrated the feasibility of formulating poor water-soluble drug into ODFs
459 without using massive organic solvents by coupling the electrospinning process with
460 the anti-solvent precipitation method.
461
462
463 **References**

464 1. Bhosle M, Benner JS, Dekoven M, Shelton J. Difficult to swallow: patient
465 preferences for alternative valproate pharmaceutical formulations. Patient
466 preference and adherence. 2009;3:161-71.
467 2. Borges AF, Silva C, Coelho JF, Simoes S. Oral films: Current status and future
468 perspectives: I - Galenical development and quality attributes. Journal of controlled
469 release : official journal of the Controlled Release Society. 2015;206:1-19. doi:
470 10.1016/j.jconrel.2015.03.006.
471 3. Hirani JJ, Rathod DA, Vadalia KR. Orally disintegrating tablets: a review.
472 Tropical journal of pharmaceutical research. 2009;8(2).
473 4. Douroumis D. Orally disintegrating dosage forms and taste-masking
474 technologies; 2010. Expert Opin Drug Deliv. 2011;8(5):665-75. doi:
475 10.1517/17425247.2011.566553.
476 5. Venkatesh GM. Ondansetron Orally Disintegrating Tablet Compositions for
477 Prevention of Nausea and Vomiting. Google Patents; 2011.
478 6. Pilgaonkar P, Rustomjee M, Gandhi A, Bagde PM. Orally disintegrating tablets.
479 Google Patents; 2013.
480 7. Elnaggar YS, El-Massik MA, Abdallah OY, Ebian AE. Maltodextrin: a novel
481 excipient used in sugar-based orally disintegrating tablets and phase transition
482 process. AAPS PharmSciTech. 2010;11(2):645-51. doi: 10.1208/s12249-010-9423-
483 y.
484 8. Arya A, Chandra A, Sharma V, Pathak K. Fast dissolving oral films: an
485 innovative drug delivery system and dosage form. International Journal of Chem
486 Tech Research. 2010;2(1):576-83.
487 9. Repka MA, Gutta K, Prodduturi S, Munjal M, Stodghill SP. Characterization of
488 cellulosic hot-melt extruded films containing lidocaine. European journal of
489 pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur

490 Pharmazeutische Verfahrenstechnik eV. 2005;59(1):189-96. doi:
491 10.1016/j.ejpb.2004.06.008.
492 10. Irfan M, Rabel S, Bukhtar Q, Qadir MI, Jabeen F, Khan A. Orally disintegrating
493 films: A modern expansion in drug delivery system. Saudi pharmaceutical journal :
494 SPJ : the official publication of the Saudi Pharmaceutical Society. 2016;24(5):537-
495 46. doi: 10.1016/j.jsps.2015.02.024.
496 11. Vuddanda PR, Mathew AP, Velaga S. Electrospun nanofiber mats for ultrafast
497 release of ondansetron. Reactive and Functional Polymers. 2016;99:65-72. doi:
498 10.1016/j.reactfunctpolym.2015.12.009.
499 12. Krstic M, Radojevic M, Stojanovic D, Radojevic V, Uskokovic P, Ibric S.
500 Formulation and characterization of nanofibers and films with carvedilol prepared
501 by electrospinning and solution casting method. European journal of pharmaceutical
502 sciences : official journal of the European Federation for Pharmaceutical Sciences.
503 2017;101:160-6. doi: 10.1016/j.ejps.2017.02.006.
504 13. Yu DG, Yang JM, Branford-White C, Lu P, Zhang L, Zhu LM. Third generation
505 solid dispersions of ferulic acid in electrospun composite nanofibers. Int J Pharm.
506 2010;400(1-2):158-64. doi: 10.1016/j.ijpharm.2010.08.010.
507 14. Wang Q, Yu DG, Zhang LL, Liu XK, Deng YC, Zhao M. Electrospun
508 hypromellose-based hydrophilic composites for rapid dissolution of poorly water-
509 soluble drug. Carbohydr Polym. 2017;174:617-25. doi:
510 10.1016/j.carbpol.2017.06.075.
511 15. Sakellariou P, Rowe R. Interactions in cellulose derivative films for oral drug
512 delivery. Progress in polymer science. 1995;20(5):889-942.
513 16. Mahesh A, Shastri N, Sadanandam M. Development of taste masked fast
514 disintegrating films of levocetirizine dihydrochloride for oral use. Current drug
515 delivery. 2010;7(1):21-7.
516 17. Cilurzo F, Cupone IE, Minghetti P, Selmin F, Montanari L. Fast dissolving films
517 made of maltodextrins. European journal of pharmaceutics and biopharmaceutics :
518 official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik eV.
519 2008;70(3):895-900. doi: 10.1016/j.ejpb.2008.06.032.
520 18. Kunte S, Tandale P. Fast dissolving strips: A novel approach for the delivery of
521 verapamil. Journal of pharmacy & bioallied sciences. 2010;2(4):325-8. doi:
522 10.4103/0975-7406.72133.

19. El-Setouhy DA, Abd El-Malak NS. Formulation of a novel tianeptine sodium orodispersible film. AAPS PharmSciTech. 2010;11(3):1018-25. doi: 10.1208/s12249-010-9464-2.
20. Tayel SA, El Nabarawi MA, Amin MM, Abou Ghaly MH. Sumatriptan succinate sublingual fast dissolving thin films: formulation and in vitro/in vivo evaluation. Pharm Dev Technol. 2016;21(3):328-37. doi: 10.3109/10837450.2014.1003655.
21. Sagban TH, Ismail KY. Formulation and evaluation of orodispersible film of sildenafil citrate. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6(2):81-6.
22. Shimoda H, Taniguchi K, Nishimura M, Matsuura K, Tsukioka T, Yamashita H, et al. Preparation of a fast dissolving oral thin film containing dexamethasone: a possible application to antiemesis during cancer chemotherapy. European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik eV. 2009;73(3):361-5. doi: 10.1016/j.ejpb.2009.08.010.
23. Kumar GP, Phani AR, Prasad RG, Sanganal JS, Manali N, Gupta R, et al. Polyvinylpyrrolidone oral films of enrofloxacin: film characterization and drug release. Int J Pharm. 2014;471(1-2):146-52. doi: 10.1016/j.ijpharm.2014.05.033.
24. Dinge A, Nagarsenker M. Formulation and evaluation of fast dissolving films for delivery of triclosan to the oral cavity. AAPS PharmSciTech. 2008;9(2):349-56. doi: 10.1208/s12249-008-9047-7.
25. Li X, Kanjwal MA, Lin L, Chronakis IS. Electrospun polyvinyl-alcohol nanofibers as oral fast-dissolving delivery system of caffeine and riboflavin. Colloids and surfaces B, Biointerfaces. 2013;103:182-8. doi: 10.1016/j.colsurfb.2012.10.016.
26. Illangakoon UE, Gill H, Shearman GC, Parhizkar M, Mahalingam S, Chatterton NP, et al. Fast dissolving paracetamol/caffeine nanofibers prepared by electrospinning. Int J Pharm. 2014;477(1-2):369-79. doi: 10.1016/j.ijpharm.2014.10.036.
27. Tomar A, Sharma K, Chauhan NS, Mittal A, Bajaj U. Formulation and evaluation of fast dissolving oral film of dicyclomine as potential route of buccal delivery. International Journal of Drug Development Research. 2012;4(2):408-17.

556 28. Susarla R, Sievens-Figueroa L, Bhakay A, Shen Y, Jerez-Rozo JI, Engen W, et
557 al. Fast drying of biocompatible polymer films loaded with poorly water-soluble
558 drug nano-particles via low temperature forced convection. Int J Pharm. 2013;455(1-
559 2):93-103. doi: 10.1016/j.ijpharm.2013.07.051.
560 29. Shen BD, Shen CY, Yuan XD, Bai JX, Lv QY, Xu H, et al. Development and
561 characterization of an orodispersible film containing drug nanoparticles. European
562 journal of pharmaceutics and biopharmaceutics : official journal of
563 Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik eV. 2013;85(3 Pt
564 B):1348-56. doi: 10.1016/j.ejpb.2013.09.019.
565 30. Sievens-Figueroa L, Bhakay A, Jerez-Rozo JI, Pandya N, Romanach RJ,
566 Michniak-Kohn B, et al. Preparation and characterization of hydroxypropyl methyl
567 cellulose films containing stable BCS Class II drug nanoparticles for pharmaceutical
568 applications. Int J Pharm. 2012;423(2):496-508. doi:
569 10.1016/j.ijpharm.2011.12.001.
570 31. Hinton C, Pratt C, De Vadetzsky E, Landwill K, McCloskey K, Schuemann H.
571 Twenty years of confectionery and chocolate progress. Twenty years of
572 confectionery chocolate progress. 1970;111.
573 32. Won DH, Kim MS, Lee S, Park JS, Hwang SJ. Improved physicochemical
574 characteristics of felodipine solid dispersion particles by supercritical anti-solvent
575 precipitation process. Int J Pharm. 2005;301(1-2):199-208. doi:
576 10.1016/j.ijpharm.2005.05.017.
577 33. Zimmermann A, Millqvist-Fureby A, Elema MR, Hansen T, Mullertz A,
578 Hovgaard L. Adsorption of pharmaceutical excipients onto microcrystals of
579 siramesine hydrochloride: effects on physicochemical properties. European journal
580 of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur
581 Pharmazeutische Verfahrenstechnik eV. 2009;71(1):109-16. doi:
582 10.1016/j.ejpb.2008.06.014.
583 34. Cho E, Cho W, Cha KH, Park J, Kim MS, Kim JS, et al. Enhanced dissolution
584 of megestrol acetate microcrystals prepared by antisolvent precipitation process
585 using hydrophilic additives. Int J Pharm. 2010;396(1-2):91-8. doi:
586 10.1016/j.ijpharm.2010.06.016.

587 35. Zong X, Kim K, Fang D, Ran S, Hsiao BS, Chu B. Structure and process
588 relationship of electrospun bioabsorbable nanofiber membranes. Polymer.
589 2002;43(16):4403-12.
590 36. Liu X, Nielsen LH, Klodzinska SN, Nielsen HM, Qu H, Christensen LP, et al.
591 Ciprofloxacin-loaded sodium alginate/poly (lactic-co-glycolic acid) electrospun
592 fibrous mats for wound healing. European journal of pharmaceutics and
593 biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische
594 Verfahrenstechnik eV. 2018;123:42-9. doi: 10.1016/j.ejpb.2017.11.004.
595 37. Liu X, Aho J, Baldursdottir S, Bohr A, Qu H, Christensen LP, et al. The effect
596 of poly (lactic-co-glycolic) acid composition on the mechanical properties of
597 electrospun fibrous mats. Int J Pharm. 2017;529(1-2):371-80. doi:
598 10.1016/j.ijpharm.2017.06.086.
599 38. Upadhyay PP, Bond AD. Crystallization and disorder of the polytypic α 1 and
600 α 2 polymorphs of piroxicam. CrystEngComm. 2015;17(28):5266-72.
601 39. Lai F, Pini E, Corrias F, Perricci J, Manconi M, Fadda AM, et al. Formulation
602 strategy and evaluation of nanocrystal piroxicam orally disintegrating tablets
603 manufacturing by freeze-drying. Int J Pharm. 2014;467(1-2):27-33. doi:
604 10.1016/j.ijpharm.2014.03.047.
605 40. Borodko Y, Habas SE, Koebel M, Yang P, Frei H, Somorjai GA. Probing the
606 interaction of poly(vinylpyrrolidone) with platinum nanocrystals by UV-Raman and
607 FTIR. The journal of physical chemistry B. 2006;110(46):23052-9. doi:
608 10.1021/jp063338+.
609 41. Koland M, Sandeep V, Charyulu N. Fast dissolving sublingual films of
610 ondansetron hydrochloride: effect of additives on in vitro drug release and mucosal
611 permeation. Journal of young pharmacists: JYP. 2010;2(3):216.
612 42. Samprasit W, Akkaramongkolporn P, Ngawhirunpat T, Rojanarata T,
613 Kaomongkolgit R, Opanasopit P. Fast releasing oral electrospun PVP/CD nanofiber
614 mats of taste-masked meloxicam. Int J Pharm. 2015;487(1-2):213-22. doi:
615 10.1016/j.ijpharm.2015.04.044.
616 43. Noyes AA, Whitney WR. The rate of solution of solid substances in their own
617 solutions. Journal of the American Chemical Society. 1897;19(12):930-4.
618 44. Vrecer F, Vrbinc M, Meden A. Characterization of piroxicam crystal
619 modifications. Int J Pharm. 2003;256(1-2):3-15.

Dear Prof. Robert O. Williams III,

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

Yours sincerely

Mingshi Yang

Reviewers' Comments:

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

649 resulting microparticle supension 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

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

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

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

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

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

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

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

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

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

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

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

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

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

Minor comments

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

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

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

We thank the reviewer for the positive comments

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

We thank the reviewer for the comments. We have added the names of the stabilizers used in the section of Materials and Method in the revised manuscript as follow: "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 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)."

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

We thank the reviewer for the comments. We have added the names of the polymers used in the section of Materials and Method in the revised manuscript as follow: "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 kept the total concentration at 10% (w/v) with various mass ratios of the two polymers (presented in Table 2). Plain ODFs were obtained by directly electrospinning of the prepared polymer solution. 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."

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

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

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

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

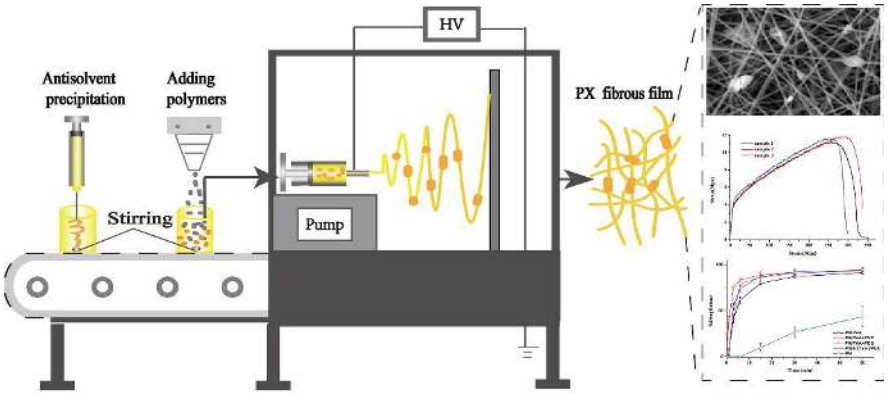


Fig.1 Preparation of PX-loaded ODFs.

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 (μm)	Weight (mg)

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

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

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

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

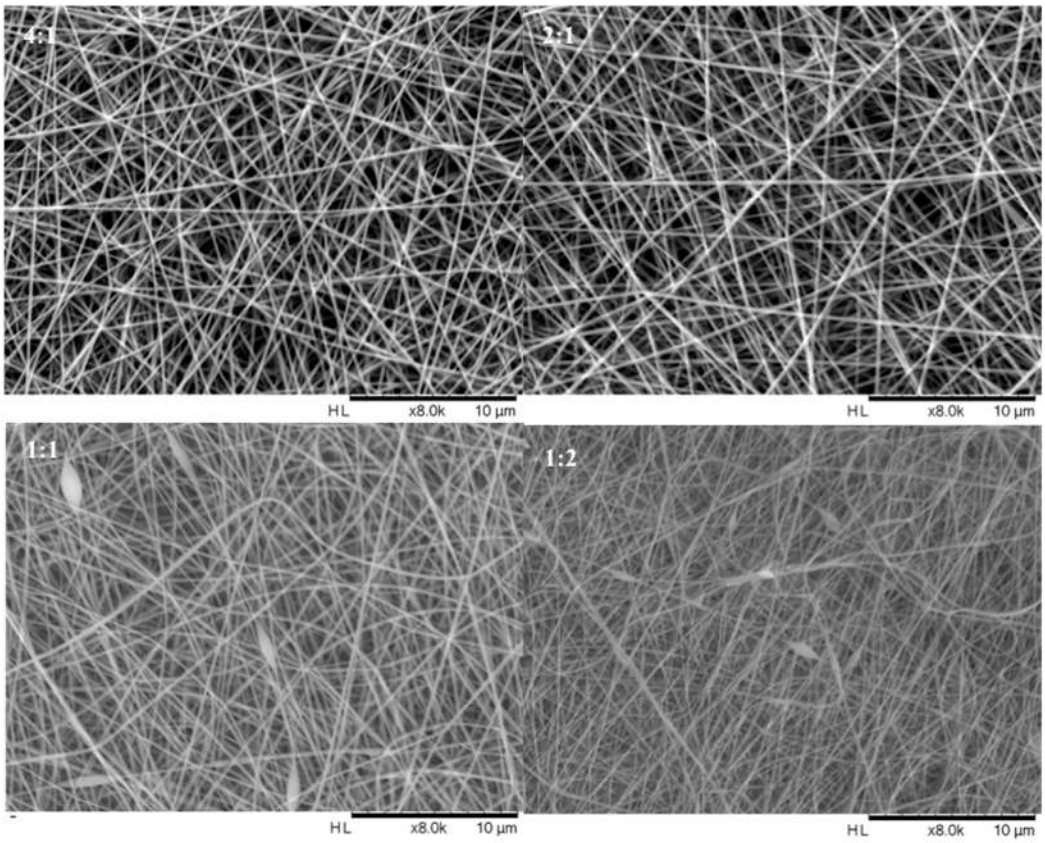
8. It is clear in page 9 that you tried different ratios of PVA/PVP and PVA alone was the worst formulation. Then after adding PX, you did not make use of your result, you tried effect of PEG on PVA alone (it is clear to be the worst formulation) and not on the best formula. Please clarify your point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

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

As for PVA/PVP (1:1/w:w), we did not continue this formulation because the electrospun films became more soft with an increase in the proportion of PVP. In addition, the film became more hygroscopic with an increase in the proportion of PVP. In the disintegration tests with PX loaded electrospun PVA/PVP (2:1/w:w) film, the disintegration of the films was ca. 2 seconds. We postulated that PVA/PVP (1:1/w:w) film could be faster. However, the difference might not be obvious. Lastly, we observed that the electrospinning process of PVA/PVP (2:1/w:w) was more stable as compared to that of 1:1. Beads could be observed with an increase in PVP in the formulation (Table1. Fig.1 below).

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

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



846

847 **Fig. 1** SEM images of electrospun films composed of PVA and PVP at mass ratios of 4:1; 2:1, 1:1, and
848 1:2.

849

850 **The changes in the revised manuscript:**

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

854

855 Page 3, Line 78.Adding sentence "PX microcrystal suspensions were prepared with anti-solvent
856 precipitation method."

857

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

Page 3, Line 97. Adding sentence “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 polymer was completely dissolved. Other polymer solutions containing different compositions of PVA 26-88/PVP 360 kept the total polymer concentration at 10%(w/v) with various mass ratios of the two polymers (Table 2). Plain ODFs were obtained by directly electrospinning the prepared polymer solutions. Drug-loaded ODFs were obtained by dissolving the appropriate amount of PVA 26-88/PVP 360 into PX microcrystal suspensions produced with anti-solvent method, followed by electrospinning.”

Page 6, Line 173. Adding sentence “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 film was observed afterwards and screenshots were made and reported. The seconds in the results were the time of the screenshots was made.”

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

Page 7, Line 186. Adding Residue Solvent part

“Residue solvent was conducted through thermogravimetric analysis (TGA) (TGA Discovery, 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 diffractgrams, 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

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