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Fast dissolving paracetamol/caffeine nanofibers prepared by electrospinning

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17 Abstract

18 A series of polyvinylpyrrolidone (PVP) fibers loaded with paracetamol (PCM) and caffeine
19 (CAF) was fabricated by electrospinning and explored as potential oral fast-dissolving films.
20 The fibers take the form of uniform cylinders with smooth surfaces, and contain the drugs in
21 the amorphous form. Drug-polymer intermolecular interactions were evidenced by infrared
22 spectroscopy and molecular modelling. The properties of the fiber mats were found to be
23 highly appropriate for the preparation of oral fast dissolving films: their thickness is around
24 120 – 130 μm , and the pH after dissolution in deionized water lies in the range of 6.7 to 7.2.
25 Except at the highest drug loading, the folding endurance of the fibers was found to be > 20
26 times. A flavoring agent can easily be incorporated into the formulation.

27 The fiber mats are all seen to disintegrate completely within 2 s when added to simulated
28 saliva solution. They release their drug cargo within around 150 s in a dissolution test, and
29 to undergo much more rapid dissolution than is seen for the pure drugs. The data reported
30 herein clearly demonstrate that the electrospun PCM/CAF fibers comprise excellent
31 candidates for oral fast-dissolving films, which could be particularly useful for children and
32 patients with swallowing difficulties.

33

34 Keywords

35 Electrospinning, nanofiber, paracetamol, caffeine, fast-dissolving drug delivery system

36 Chemical compounds

37 Caffeine (PubChem CID: 2519); paracetamol (PubChem CID: 1983); polyvinylpyrrolidone
38 (PubChem CID: 6917).

39 Abbreviations

40 CAF – caffeine; PCM – paracetamol.

41

42 1. Introduction

43 Fast-dissolving drug delivery systems (FD-DDSs) were first developed in the late 1970s and
44 rapidly gained interest in the pharmaceutical industry (Chaudhary et al., 2013; Hoffmann et
45 al., 2011). These delivery systems either dissolve or disintegrate in the mouth very rapidly,
46 without requiring any water to aid in swallowing. By releasing their drug cargo directly in the
47 mouth, they enhance bioavailability and deliver rapid onset of action (Seager, 1998). FD-
48 DDSs are available in the form of tablets (Pathan et al., 2013), films (Yu et al., 2009), wafers
49 (Boateng et al., 2010; El-Mahrouk et al., 2014) and buccal (Dinge and Nagarsenker, 2008) or
50 sublingual patches (Vrbata et al., 2013). Examples currently in the market include Zuplenz®
51 (an oral soluble film used for the prevention of chemotherapy-induced, radiotherapy-
52 induced, and postoperative nausea and vomiting) and Suboxone® (a sublingual film for the
53 treatment of opioid dependence). Various other oral dissolving film formulations are in the
54 pipeline, for example to treat central nervous system conditions such as Parkinson's disease,
55 schizophrenia or Alzheimer's disease (Hoffmann et al., 2011).

56 Sublingual films in particular have many advantages compared to other dosage forms: these
57 include rapid onset of action, avoidance of first past metabolism, and convenient and non-
58 invasive administration (Dixit and Puthli, 2009; Hearnden et al., 2012). There are however
59 also some limitations. The relatively small surface area in the sublingual mucosa means that
60 it is possible for the drug to be washed away with saliva before it can permeate the mucosal
61 membrane. In addition, the tendency for involuntary swallowing of liquids greater in volume
62 than 200 µL can lead to the dissolved drug entering the gastro-intestinal tract rather than
63 being absorbed in the mouth. Dislodging of the formulation due to tongue movements can
64 also lead to ineffective drug delivery (Squier and Wertz, 1993; Vrbata et al., 2013).
65 Sublingual dosage forms are nevertheless highly beneficial for paediatric and geriatric
66 patients, and also for any other patients with swallowing or digestion problems (Lam et al.,
67 2014).

68 There are several classical methods used to formulate fast dissolving thin films: solvent
69 casting, semi-solid casting, hot melt extrusion, solid dispersion extrusion and rolling have all
70 been investigated (Hoffmann et al., 2011; Liang and Chen, 2001; Low et al., 2013; Nagy et
71 al., 2012; Ramineni et al., 2013). In recent years, the electrospinning technique has begun to
72 be explored as an alternative route to such systems (Illangakoon et al., 2014; Luo et al.,
73 2012; Williams et al., 2012). Electrospinning is a simple, rapid, inexpensive and easily
74 scalable technique (Persano et al., 2013). It uses an electric field to create a charged jet of
75 polymer solution. As this jet travels in air, the solvent evaporates leaving behind a charged
76 fiber that can be collected on a metal screen (Doshi and Reneker, 1995). Electrospun fibers
77 show great promise for developing many types of novel drug delivery systems (DDS) owing
78 to their high surface area, high porosity, and ability to encapsulate high drug loadings (Cui et
79 al., 2010; Raghavan et al., 2012; Reneker and Chun, 1996). The electrospinning technique
80 can also easily be used to encapsulate more than one active pharmaceutical ingredient (API)
81 (Natu et al., 2010; Wang et al., 2010; Xu et al., 2009).

82 The combination of paracetamol (PCM) and caffeine (CAF) was first approved for medical
83 use by the UK Medicines and Healthcare Regulatory Authority (MHRA) in 1991 (MHRA,
84 1991). PCM is a centrally acting analgesic, which is used to relieve mild to moderate pain in
85 the body; it also acts as an antipyretic to help reduce body temperature. CAF is a mild
86 stimulant which is often used in combination with analgesics, augmenting their effect
87 (Diamond et al., 2000; Migliardi et al., 1994). Renner *et al.* showed that in humans the
88 analgesic effects of PCM or PCM/CAF together, but not CAF alone, caused a significant
89 reduction of pain-related cortical potentials from 30 minutes after medication (Renner et
90 al., 2007). The PCM/CAF combination demonstrated greater effects than PCM alone
91 throughout the 3 hour observation period.

92 Recently Li *et al.* have fabricated poly(vinyl alcohol) fibers loaded with CAF or riboflavin by
93 electrospinning (Li et al., 2013). In a dissolution study both drugs were released from the
94 fiber matrices in a burst manner, with 100 % of the embedded CAF and 40 % of the
95 riboflavin released within 60 s. Yu *et al.* have electrospun PCM with poly(vinyl pyrrolidone)
96 (PVP) and compared the dissolution rate of the drug between electrospun, freeze dried,
97 vacuum dried and heat dried membranes (Yu et al., 2010b). *In vitro* dissolution tests showed
98 that the electrospun fibers released 93.8% of PCM within 2 minutes, with the dissolution
99 rates observed being as follows: electrospun membrane > vacuum-dried membrane ≈
100 freeze-dried membrane > heat-dried membrane.

101 Paediatric oral formulations can be scientifically challenging to develop, and the twin
102 necessities of both preparing a measurable dosage form which can be administered based
103 upon body weight, and also of taste-masking, are key challenges unique to such
104 formulations (Strickley et al., 2008). PCM poisoning has also been increasingly recognised in
105 children (Heubi et al., 1998). In this work therefore, we set out to prepare PCM-containing
106 oral fast dissolving films which could be safely and effectively administered to children.

107 PVP K90 was selected as a film forming agent because it is a non-ionic, biocompatible, and
108 biodegradable polymer featured on the FDA “generally regarded as safe” list (Bühler, 2005).
109 The application of such polymers in DDSs is attractive because they have relatively well
110 defined molecular weights and physicochemical characteristics (Ignatious et al., 2010). PVP
111 also has mucoadhesive properties (Abdel-Hamid et al., 2007; Salamat-Miller et al., 2005). It
112 has been widely used to prepare solid dispersions to improve the dissolution rates of poorly
113 water-soluble drugs (Yu et al., 2009; Yu et al., 2010a). A range of commercial products such
114 as Panadol ActiFast soluble tablets, Beechams’ cold relief orange flavor effervescent tablets,
115 Hedex Extra, and Panadol Extra Advance all contain PVP.

116 In this paper, we report the fabrication of PCM and CAF loaded electrospun PVP fibers. The
117 resultant materials underwent detailed physicochemical characterization, and their
118 dissolution properties were explored. A flavoring agent was also incorporated to enhance
119 palatability.

120

121 2. Materials and methods

122 2.1 Materials

123 Paracetamol (PCM; batch 096K0072), caffeine (CAF; lot 38H0147), and polyvinylpyrrolidone
124 (MW 360 000; PVP K90) (see Fig. 1) were purchased from Sigma Aldrich (Gillingham, UK).
125 Concentrated raspberry flavor was purchased from Cottes' Cordial (Tullamarine, Australia).
126 All other chemicals used were of analytical grade and used as provided.

127

128 2.2 Preparation of the composite nanofibers

129 Anhydrous ethanol was selected as a spinning solvent, because it rapidly evaporates during
130 electrospinning and both PCM and CAF are freely soluble in it. Ethanol is also classified by
131 the FDA as a "Class 3" solvent, recommended for the formulation of oral fast dissolving thin
132 films.

133 A 10 % (w/v) PVP K90 solution was prepared by dissolving the appropriate amount of
134 polymer in ethanol under stirring overnight. The desired amounts of PCM and CAF were pre-
135 dissolved in 1.4 mL of ethanol and added to 8.6 mL of the PVP solution. A series of solutions
136 with varied PCM/CAF contents was prepared as listed in Table 1. The ratio of PCM to CAF
137 was selected to match that in commercial formulations (Laska et al., 1983). Mechanical
138 stirring was applied for at least 20 min at room temperature to obtain homogeneous
139 solutions. The conductivities of the spinning solutions were recorded using a PRIMOS
140 conductivity meter (Hanna Instruments, Woonsocket, RI, USA).

141 The spinning solutions were carefully placed into a plastic syringe (5 mL, BD, Sunderland,
142 UK), with great care taken to avoid any air bubbles. A metal dispensing tip (spinneret; gauge
143 20, 0.61 mm inner diameter, Nordson EFD, Dunstable, UK) was attached to the syringe. The
144 positive electrode of a high voltage power DC supply (HCP35-35,000, FuG Elektronik,
145 Rosenheim, Germany) was then connected to the spinneret. The grounded electrode was
146 connected to a metal collector (17 x 17 cm²) wrapped with aluminum foil. Electrospinning
147 was carried out under ambient conditions (22 ± 1°C and relative humidity 35 ± 3%). An
148 electrical potential of 15 kV was applied across a fixed distance of 12 cm between the
149 spinneret and the collector. The polymer solution was dispensed from the syringe at a feed
150 rate of 1.2 mL/h using a syringe pump (78-9100C, Cole-Parmer, London, UK). Fibers were
151 stored in a vacuum desiccator post-synthesis to facilitate the removal of residual organic
152 solvents and moisture.

153

154

155 2.3 Characterization

156 2.3.1 Thickness of the fiber mat

157 2 mL of each spinning solution was spun onto Al foil, and three circular sections of 3 cm
158 diameter cut out using a biopsy punch. The thickness of each section was measured by using
159 a digital Vernier calliper. Results are reported as mean \pm S.D.

160

161 2.3.2 Folding endurance

162 The folding endurance gives a measure of the brittleness of a film. 3 cm diameter circular
163 sections of each mat (produced as detailed in 2.3.1) were repeatedly folded by hand at the
164 same line until they broke or a visible crack was observed. The number of times a film can
165 be folded without breaking or visibly cracking is defined as the folding endurance (Mundargi
166 et al., 2007). Experiments were performed in triplicate, and data reported as mean \pm S.D.

167

168 2.3.3 pH of the fiber solution

169 A 3 cm diameter section from each formulation was dissolved in 10 mL of distilled water and
170 the pH was measured (pH 211 meter, Hanna Instruments, Woonsocket, RI, USA). Each
171 experiment was carried out in triplicate and data are reported as mean \pm S.D.

172

173 2.3.4 Morphology

174 The fiber morphologies were assessed using a scanning electron microscope (Quanta 200
175 FEG ESEM, FEI, Hillsborough, OR, USA). Prior to examination, the samples were gold sputter-
176 coated (20 nm) under argon to render them electrically conductive. Images were then
177 recorded at an excitation voltage of 5 kV. The average fiber size was determined by
178 measuring their diameters at over 50 points in SEM images, using the ImageJ software
179 (National Institutes of Health, Bethesda, MD, USA). The porosities of the fiber mats were
180 calculated using the method of Ghasemi-Mobarakeh et al. (Ghasemi-Mobarakeh et al.,
181 2007).

182

183 2.3.5 X-ray diffraction

184 X-ray diffraction (XRD) patterns were obtained on a MiniFlex 600 diffractometer (RigaKu,
185 Tokyo, Japan) with Cu K α radiation ($\lambda = 1.5148 \text{ \AA}$). Data were recorded over the 2θ range 5 -
186 45° at 40 mV and 15 mA.

187

188 2.3.6 Differential scanning calorimetry

189 Differential scanning calorimetry (DSC) analyses were carried out using a DSC Q2000
190 calorimeter (TA Instruments, New Castle, DE, USA). Sealed samples were heated at 10 °C /
191 min from 40 °C to 300 °C under a 50 mL / min flow of nitrogen. Recorded data were
192 analysed using the TA Instruments Universal Analysis software.

193

194 2.3.7 Fourier transform infrared spectroscopy

195 Fourier transform infrared (FTIR) spectroscopy was conducted using a Spectrum 100 FTIR
196 spectrometer (Perkin Elmer, Massachusetts, USA) fitted with an ATR attachment. The
197 scanning range was 4000 – 600 cm⁻¹, and the resolution set at 1 cm⁻¹.

198

199 2.4 HPLC analysis

200 A high-performance liquid chromatography (HPLC) method was developed in order to
201 detect PCM and CAF simultaneously. HPLC was performed using an Agilent 1260 Infinity
202 instrument (Agilent Technologies, Santa Clara, CA, USA). The mobile phase consisted of 20 %
203 v/v acetonitrile, 0.8 % v/v trifluoroacetic acid, and 79.2 % v/v distilled water. Analysis was
204 carried out under isocratic conditions using a C18 column (00G-4326-60, Phenomenex,
205 Macclesfield, UK). The column temperature was set to 40 °C, and the flow rate at 1 mL /
206 min. 10 µL of each sample was injected, and chromatograms were recorded at 254 nm for 6
207 min (to detect PCM) and at 276 nm for another 4 min (to detect CAF). The percentage drug
208 loading was calculated using a pre-determined calibration curve prepared using a mixture of
209 PCM and CAF.

210

211 2.5 Wetting assays

212 3 cm diameter circular sections were cut from the fiber mats using a biopsy cutter and
213 placed in a Petri dish containing 15 mL of simulated saliva (NaCl 8.00g, KH₂PO₄ 0.19g,
214 Na₂HPO₄ 2.38g, in 1L of distilled water: pH 6.8) at room temperature. The disintegration and
215 dissolution of the fiber mats was recorded at 1000 frames per second using a high speed
216 video camera (Fastcam SA3, Photron, Tokyo, Japan).

217

218

219 2.6 Dissolution studies

220 The standard British Pharmacopoeia dissolution test is performed in 900 mL of a dissolution
221 medium. However, this does not reflect the volume of the oral cavity (Hoffmann et al.,
222 2011). Therefore a modified dissolution study was performed using a 1 cm long magnetic
223 stirrer in a 7 cm diameter glass Petri dish. 15 mL of simulated saliva pre-warmed to 37 °C
224 was placed in the Petri dish and stirred at 150 rpm on a multipoint stirrer (Cimarec™ iPoly
225 15, ThermoScientific, Loughborough, UK). 200 µL of the supernatant was removed at pre-
226 determined time points and replaced with 200 µL of pre-warmed simulated saliva to
227 maintain a constant volume. Experiments were carried out in triplicate and results reported
228 as mean ± S.D. The temperature remained at 37 ± 2 °C throughout the experiment.

229

230 2.7 Molecular modelling

231 Molecular mechanics *in vacuo* calculations were undertaken using HyperChem version
232 8.0.10 (a molecular modelling software package). The structures of each of the compounds
233 (Figure 1) were first generated with Accelrys Draw 4.1. A decameric PVP species was chosen
234 to represent the polymer. Each structure was individually imported into HyperChem, and a
235 3-D structure using preset bond angles and lengths produced (all hydrogen atoms were
236 explicitly included). Initial geometric minimisation was next performed with the MM+ force
237 field followed by a full energetic minimisation using the AMBER 3 (Assisted Model Building
238 and Energy Refinement) force field. Nonbonded electrostatic interactions were calculated
239 using bond dipole interactions in MM+ optimisation. For AMBER 3 minimisations, the
240 distance-dependent dielectric constant was assigned a scale factor of 1, and the 1-4 scale
241 factors (representing the nonbonded interactions between atoms separated by three
242 atoms) were: electrostatic 0.5, and van der Waals 0.5. Both MM+ and AMBER 3 force fields
243 were computed using a Polak-Ribiere conjugate gradient method finishing when the root
244 mean square gradient reached 0.001 kcal / (Å mol). No cut-offs were applied. The energetic
245 contributions to the total steric energy of the structures by bond stretching / compressing,
246 bond angle deformations, torsional strain, van der Waals repulsions, hydrogen bonding and
247 electrostatic repulsions were all considered. Combinations of the energetically minimised
248 structures were then merged to create drug-polymer complexes. These complexes then
249 underwent the same minimisation procedures.

250

251 3. Results and discussion

252 3.1 Electrospinning

253 The polymer/active pharmaceutical ingredient (API) spinning solutions for used to make the
254 fiber materials F0, F1, F2 and F3 were transparent and clear. For F4, concentrated raspberry
255 flavor (2 µL / mL spinning solution) was also added to fabricate flavored nanofibers. The

256 raspberry flavor is expected to act as a taste masking agent, hiding the bitter taste of PCM,
257 and also as a colouring agent: the spinning solution turned slightly pink upon addition of
258 raspberry flavor. Details of the solutions and resultant fibers are presented in Table 1. The
259 conductivities of the spinning solutions were measured, and found to be approximately the
260 same regardless of the drug content and the presence or absence of the raspberry flavor
261 (see Table 2).

262

263 3.2 Thickness of the fiber mat

264 The mean thicknesses of 3 cm diameter circular sections cut from electrospun mats of each
265 formulation lie between *ca.* 121 μm and 131 μm (data are given in Table 2). This is entirely
266 appropriate for an oral fast-dissolving film, and can be adjusted very easily by varying the
267 collection time (i.e. the volume of solution processed). These values are comparable with
268 those in the literature; for instance, Londhe has reported films of *ca.* 50 μm (Londhe and
269 Umalkar, 2012), while Cilurzo et al. have generated films of 120 – 131 μm thickness (Cilurzo
270 et al., 2011; Cilurzo et al., 2010) and systems of 88 – 420 μm were prepared by Ibrahim's
271 team (Sayed et al., 2013).

272

273 3.3 Folding endurance

274 The folding endurance of 3 cm diameter circular sections of each fiber mat was assessed by
275 hand-folding the sections along a fixed line, and the results are provided in Table 2. The
276 folding endurance is seen to decrease as the drug loading is increased, indicating that the
277 fiber mat becomes more brittle with increasing drug loading. F3 has a folding endurance of
278 only 6.67 times, and hence is very likely to be too brittle for use as an oral film. However,
279 the other fibres have high folding endurance of > 20 times, thus indicating their high
280 potential in this area.

281

282 3.4 pH of the fiber solution

283 Solutions prepared by dissolving a 3 cm diameter circular section of the fiber mats in 10 mL
284 deionised water were found to have pH values lying in the range 6.7 – 7.2 (see Table 2).
285 Acidic or alkaline pHs may cause damage to the oral mucosa, and so the pH of the dissolved
286 oral fast dissolving film should be close to the neutral pH of the mucosa (El-Mahrouk et al.,
287 2014). Mucosal pH values have been found to vary between 6.28 (buccal mucosa) and 7.34
288 (palate) (Aframian et al., 2006). The materials fabricated here hence give solutions with pHs
289 close to those observed for the oral mucosa, and can be expected not to cause mucosal
290 damage upon administration.

291

292 3.5 Fiber morphology

293 Scanning electron microscopy (SEM) images of the electrospun products are given in Figure
294 2. The SEM data show that the composite fibers were cylindrical in shape, with smooth
295 surfaces and no secondary particles visible. No bead-on-string morphology can be observed.
296 This indicates that both PCM and CAF are encapsulated homogeneously in the PVP fiber
297 matrices. The fabricated fibers are oriented in a random manner. The mean fiber diameters
298 (Table 3) are F1: 443 ± 93 nm; F2: 750 ± 222 nm; F3: 1553 ± 435 nm and F4: 518 ± 175 nm
299 respectively. The fiber diameter thus appears to increase with the drug loading [F1 contains
300 PCM 10.27 % / CAF 1.37 % (w/w), while F3 is PCM 35.10% / CAF 4.56% (w/w)]. Both F2 and
301 F4 comprise 21.87 % PCM and 2.89 % CAF (w/w), but the latter also incorporates a
302 raspberry flavoring. Since the F4 fibers are somewhat narrower than the F2 material, it
303 seems that the incorporation of even a small amount of flavoring causes a decrease in fiber
304 diameter. The complex nature of the raspberry flavour, which is not a single chemical entity
305 but rather a mixture of compounds, makes it difficult to ascertain the precise cause of this
306 size variation. The porosities of the fiber mats were calculated to lie in the range of 81.8 –
307 83.6 %, being largely invariant with API loading and the presence or absence of flavor.

308 The fiber mats were found to be very resilient to cutting, and could be formed into a range
309 of different shapes appropriate for use as oral films. Photographs of the F4 fiber mat cut
310 into different shapes are shown in Figure 3.

311

312 3.6 X-ray diffraction

313 X-ray diffraction was undertaken to examine the physical state of the components of the
314 composite nanofibers. Characteristic reflections [see Figure 4 (a)] of CAF appear at
315 diffraction angles 2θ of 11.24° , 25.64° and 26.24° , and for PCM distinct reflections can be
316 observed at 17.18° , 22.66° , and 25.58° . A physical mixture of PVP, PCM and CAF in the same
317 ratios as F2 (F5) shows the diffraction features of both PCM and CAF superimposed on a
318 broad background from the amorphous PVP polymer. The pattern of fibers containing only
319 PVP [F0; Figure 4 (b)] was characterized by the absence of any diffraction peaks, with only a
320 broad halo observed: this confirms the PVP to be amorphous after electrospinning. In the
321 patterns of the drug-loaded nanofibers, the characteristic reflections of PCM or CAF cannot
322 be seen, while the characteristic humps of amorphous materials are observed. This suggests
323 that both active ingredients were present in amorphous form in the fibers.

324

325

326 3.7 Differential scanning calorimetry

327 The differential scanning calorimetry (DSC) curves of pure PCM and CAF [see Figure 5(a)]
328 each show a clear melting endothermic peak. The PCM form I melt can be seen at 169.4 °C.
329 For CAF, the principal feature in the thermogram is the melting of form I of the API at 238.4
330 °C. There is however a small additional endothermic peak at around 160 °C, attributed to
331 the presence of a small amount of caffeine form II in the material provided (Hubert et al.,
332 2011). The physical mixture (F5) shows a broad shallow endothermic peak below 100 °C due
333 to the dehydration of PVP, followed by a broad peak believed to correspond to melting of
334 PCM centred at around 150 °C. The CAF melting point cannot be observed, probably
335 because of its low loading in the physical mixture.

336 The DSC thermograms of the composite nanofibers do not show any melting peaks, only a
337 broad dehydration endothermic peak ranging from 40 to 110 °C, with a peak at 80 - 83 °C.
338 This suggested that PCM and CAF were not present as crystalline materials, but had been
339 converted into an amorphous state in the fibers.

340

341 3.8 FTIR spectroscopy

342 Compatibility between the drug and polymer is important for the formation of nanofibers
343 during electrospinning and for the stability of the resultant materials. If the drug is not
344 compatible with the polymer, then solid phase separation will be observed. The interactions
345 between the drug and the polymer can be probed using IR spectroscopy.

346 The FTIR spectrum of pure PCM is shown in Figure 6(a). The broad peak between around
347 3000 and 3700 cm^{-1} is assigned as H-bonded O-H and N-H stretching vibrations.
348 Absorptions at *ca.* 2880 and 2950 cm^{-1} denote C-H stretches. The peaks at 1644, 1560 and
349 1511 are assigned to the C=O stretching and N-H bending vibrations of the amide group. A
350 very sharp peak at 835 cm^{-1} is also visible. The infrared spectra of CAF [see Figure 6(a)]
351 shows an absorbance at 1650 cm^{-1} corresponding to the C=O stretch of the amide group.
352 There are also peaks at around 1435 and 1504 cm^{-1} (C=C stretching), and between 1330 and
353 1105 cm^{-1} which may be ascribed to the C-N amide stretches. Sharp bands at 835, 807, and
354 796 cm^{-1} are present in the fingerprint region. The spectrum of the pure PVP fibers F0
355 [Figure 6 (b)] shows broad bands at 3650 – 3050 cm^{-1} (H-bonded O-H stretches from residual
356 water) and 2840 – 3010 cm^{-1} (C-H stretching), as well as peaks at 1643 cm^{-1} (C=O) and at
357 1290 cm^{-1} (C-N stretch) (Borodko et al., 2006).

358 The FTIR spectra of the medicated fibers comprise a composite of those from PVP and the
359 drugs. They show two main peaks at around 1645 – 1650 cm^{-1} and 1290 cm^{-1} due to the
360 C=O and C-N stretch from PVP. Peaks can also be seen corresponding to the PCM and CAF,
361 for instance at 1550 cm^{-1} (PCM N-H bend), 833 cm^{-1} (PCM/CAF fingerprint), and 793 cm^{-1}
362 (CAF fingerprint). It is hard to unambiguously assign peaks because of the complexity of the
363 spectra, but small shifts in peak positions (*e.g.* from 1643 in pure PVP to 1650 cm^{-1} in the
364 fibers) indicate that there may be intermolecular interactions between the drugs and PVP.

365

366 3.9 Molecular modelling

367 Although interactions between the APIs and polymer are suggested by the IR spectra, the
368 complexity of the spectra mean that it is impossible to characterise these in detail.
369 Molecular models of PCM, CAF, PVP, and the API-polymer complexes were constructed
370 using the Hyperchem software. The geometric preferences for the energetically minimised
371 API-polymer systems are depicted in Figure 7. The energetic contributions to the overall
372 steric energy for the drug-polymer complexes and the individual API molecules and PVP
373 decamer are given in Table 4. Stabilisation of the complexes is indicated by a negative
374 difference (ΔE) between the total steric energy of the complex and the sum of the total
375 steric energies of the individual molecules. The ΔE values for PVP-PCM, and PVP-CAF, and
376 PVP-PCM-CAF are -19.126, -13.105, and -30.451 kcal mol⁻¹ respectively. These negative
377 values clearly confirm that there are interactions between the PVP polymer and the APIs.
378 The ΔE value is more negative for PCM than CAF, indicating stronger interactions with the
379 former.

380

381 3.10 Drug loading

382 The percentage drug loadings in the fibers were determined by HPLC. A bespoke method
383 was devised to permit the observation of both APIs in the same experiment (see Section
384 2.4). The resultant data are given in Figure 8. Solutions of PCM and CAF were first run
385 separately and PCM observed at an elution time of 4.87 min, and CAF at 7.92 min. Similar
386 results were observed for the mixture of PCM and CAF, where elution was noted at 4.78 min
387 for PCM and at 7.62 min for CAF. The dissolved fiber formulations show peaks at the same
388 retention times as the pure drug materials (see Figure 8), confirming that neither API was
389 degraded during the electrospinning process. Following construction of a calibration curve,
390 the drug loading was determined for the fibers: the results are presented in Table 5. It can
391 be seen that the formulations generally show very high (> 90 %) loading of both drugs, with
392 the exception of F3 where the PCM loading is slightly below 90 %.

393

394

395 3.11 Wetting assays and dissolution studies

396 The PCM/CAF-loaded fiber mats were found to be wetted and to disintegrate very rapidly in
397 simulated saliva. The process was recorded using a standard video camera for all
398 formulations, and using high-speed camera for F2 and F4. All the formulations appeared to
399 disintegrate within < 3 s when recorded using the standard camera, but it proved impossible
400 to discern the disintegration time more precisely. Further observations were thus carried

401 out using a high-speed camera for the F2 and F4 fiber mats. Both were seen to disintegrate
402 within around 320 ms. This is clearly visible from the photographs given in Figure 9
403 (depicting F4). These disintegration times are exceptionally rapid, and eminently suitable for
404 the preparation of oral fast-dissolving films: other researchers preparing such systems
405 report disintegration times of 10 – 20 s (Cilurzo et al., 2011; Cilurzo et al., 2010; Londhe and
406 Umalkar, 2012).

407 Dissolution studies (see Figure 10) demonstrated that with the physical mixture (F5), $48 \pm$
408 5.6 % of the incorporated PCM and 87 ± 2.5 % of the CAF were released within 6 minutes.
409 Within 30 s, fibers F1, F4 and F5 respectively released 38 ± 12 %, 66 ± 7.0 % and 4.5 ± 1.8 %
410 of their PCM loading, and 52 ± 12 %, 72 ± 11 % and 37 ± 5.7 % of the incorporated CAF. The
411 poor folding endurance of the F3 fibers indicated that they were not suitable for oral films,
412 and thus dissolution studies were not performed.

413 The rapid dissolution observed with the PCM/CAF loaded fiber mats can be attributed to the
414 amorphous physical state of the APIs in the formulations, the high surface area and high
415 porosity of the of the drug loaded fibers, and the exceptional hydrophilicity of PVP. API
416 release from a formulation occurs at its interface with the buffer solution; the high surface
417 area to volume ratio of the fiber mats ensures that this contact area is very high, thus
418 accelerating release. The amorphous nature of the API removes the need to overcome any
419 crystalline lattice enthalpy, again facilitating dissolution. Finally, the hygroscopicity of PVP
420 also encourages the mat to disintegrate, dissolve, and free its drug loading into solution.
421 Attempts were made to fit various kinetic models to the experimental data, but these were
422 unsuccessful owing to the very rapid nature of the release processes.

423 For all the formulation studied, the CAF release is seen to be more rapid. This is consistent
424 with its higher solubility under the dissolution conditions [the respective solubilities for CAF
425 and PCM are *ca.* 21.6 mg ml^{-1} vs. 14.0 mg ml^{-1} in water at $25 \text{ }^\circ\text{C}$ (<http://www.drugbank.ca/>)].
426 It is also consistent with the molecular modelling results (Section 3.9) which show stronger
427 interactions between PCM and PVP than between the polymer and CAF. The difference in
428 release rate between PCM and CAF is very much less for the fiber formulations than for the
429 physical mixture, presumably a result of the amorphous nature of the APIs in the former
430 ameliorating any differences in lattice enthalpy. Similar results were recorded by Khan and
431 Craig when they performed dissolution studies on solid dispersions of PCM and CAF (Khan
432 and Craig, 2003).

433 Overall, the systems prepared in this work have great potential as oral fast dissolving films.
434 Both drugs can be successfully loaded into the fibers in amorphous physical form, and very
435 rapid disintegration (< 0.5 s) and release of drug (< 150 s) are observed. The pH of the fiber
436 solution is close to neutral, and hence no mucosal irritation is to be expected. A flavoring
437 can be incorporated into the fibers to ameliorate issues of bitterness. Such formulations
438 could thus have great utility as paediatric medicines. The British National Formulary for
439 Children (BNF-C) suggests that an appropriate dose of paracetamol for the treatment of pain
440 a child of 8 years old is between 240 and 375 mg four times a day (BNF-C, 2014). The loading

441 in F4 is 21.87 % w/w; thus a mass of formulation of between 1100 and 1715 mg would be
442 needed for each dose. For a child of six months to two years in age, the required dose is 120
443 mg, demanding a fiber mass of *ca.* 550 mg. This mass of formulation could easily be
444 prepared and applied by mouth, particularly at the lower end of the dosage regimen. In
445 addition, further optimisation could increase the drug loading to reduce the formulation
446 mass required.

447

448 4. Conclusions

449 In this study we successfully produced fast-dissolving drug delivery systems for the
450 simultaneous release of paracetamol (PCM) and caffeine (CAF). This was achieved by
451 processing them into electrospun fibers using polyvinylpyrrolidone (PVP) as the filament
452 forming agent. Scanning electron microscopy showed that the composite nanofibers had
453 smooth surfaces and average fiber diameters between 400 – 1600 nm. IR spectroscopy
454 results combined with molecular modelling demonstrated that there were clear
455 intermolecular interactions between paracetamol, caffeine, and PVP. X-ray diffraction and
456 differential scanning calorimetry studies indicated that both drugs were fully converted into
457 the amorphous form in the fibers. Both APIs were observed to remain intact after spinning,
458 with drug loadings close to 100 % of the theoretical value. In wetting tests, the drug loaded
459 fiber mats disintegrated within 0.5 s, and dissolution studies revealed that all the embedded
460 drug was freed into solution in less than 150 s: a significant improvement over the pure APIs
461 and the physical mixture. A flavoring agent can easily be incorporated into the fibers to
462 overcome problems with bitterness. These flavored fibers can be used as potential drug
463 delivery systems, especially for the paediatric population.

464

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470

471 6. Author contributions

472 UEI prepared and characterised fibers, undertook functional performance assays, and
473 analysed experimental data. HG developed the HPLC protocol and analysed the resultant
474 data. GCS performed molecular modelling simulations. MP and SM recorded the high-speed
475 camera videos. NPC and GRW provided strategic guidance to the project and support to
476 data analysis. All authors contributed to the writing of the manuscript.

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