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Abstract

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

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

Keywords

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

Chemical compounds

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

Abbreviations

CAF – caffeine; PCM – paracetamol.

1. Introduction

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

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

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

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

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

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

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

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

2. Materials and methods

2.1 Materials

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

2.2 Preparation of the composite nanofibers

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

A 10 % (w/v) PVP K90 solution was prepared by dissolving the appropriate amount of polymer in ethanol under stirring overnight. The desired amounts of PCM and CAF were pre-dissolved in 1.4 mL of ethanol and added to 8.6 mL of the PVP solution. A series of solutions with varied PCM/CAF contents was prepared as listed in Table 1. The ratio of PCM to CAF was selected to match that in commercial formulations (Laska et al., 1983). Mechanical stirring was applied for at least 20 min at room temperature to obtain homogeneous solutions. The conductivities of the spinning solutions were recorded using a PRIMO5 conductivity meter (Hanna Instruments, Woonsocket, RI, USA).

The spinning solutions were carefully placed into a plastic syringe (5 mL, BD, Sunderland, UK), with great care taken to avoid any air bubbles. A metal dispensing tip (spinneret; gauge 20, 0.61 mm inner diameter, Nordson EFD, Dunstable, UK) was attached to the syringe. The positive electrode of a high voltage power DC supply (HCP35-35,000, FuG Elektronik, Rosenheim, Germany) was then connected to the spinneret. The grounded electrode was connected to a metal collector (17 x 17 cm^2) wrapped with aluminum foil. Electrospinning was carried out under ambient conditions (22 ± 1°C and relative humidity 35 ± 3%). An electrical potential of 15 kV was applied across a fixed distance of 12 cm between the spinneret and the collector. The polymer solution was dispensed from the syringe at a feed rate of 1.2 mL/h using a syringe pump (78-9100C, Cole-Parmer, London, UK). Fibers were stored in a vacuum desiccator post-synthesis to facilitate the removal of residual organic solvents and moisture.

2.3 Characterization

2.3.1 Thickness of the fiber mat

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

2.3.2 Folding endurance

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

2.3.3 pH of the fiber solution

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

2.3.4 Morphology

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

2.3.5 X-ray diffraction

X-ray diffraction (XRD) patterns were obtained on a MiniFlex 600 diffractometer (RigaKu, Tokyo, Japan) with Cu Kα radiation (λ = 1.5148 Å). Data were recorded over the 2θ range 5 - 45° at 40 mV and 15 mA.

2.3.6 Differential scanning calorimetry

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

2.3.7 Fourier transform infrared spectroscopy

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

2.4 HPLC analysis

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

2.5 Wetting assays

3 cm diameter circular sections were cut from the fiber mats using a biopsy cutter and placed in a Petri dish containing 15 mL of simulated saliva (NaCl 8.00g, KH_2PO_4 0.19g, Na_2HPO_4 2.38g, in 1L of distilled water: pH 6.8) at room temperature. The disintegration and dissolution of the fiber mats was recorded at 1000 frames per second using a high speed video camera (Fastcam SA3, Photron, Tokyo, Japan).

2.6 Dissolution studies

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

2.7 Molecular modelling

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

3. Results and discussion

3.1 Electrospinning

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

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

3.2 Thickness of the fiber mat

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

3.3 Folding endurance

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

3.4 pH of the fiber solution

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

3.5 Fiber morphology

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

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

3.6 X-ray diffraction

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

3.7 Differential scanning calorimetry

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

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

3.8 FTIR spectroscopy

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

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

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

3.9 Molecular modelling

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

3.10 Drug loading

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

3.11 Wetting assays and dissolution studies

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

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

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

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

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

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

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

4. Conclusions

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

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6. Author contributions

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

7. References

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