5-Fluorouracil loaded Eudragit fibers prepared by electrospinning

U. Eranka Illangakoon,[1] Deng-Guang Yu,[2]* Bilal S. Ahmad,[1] Nicholas P. Chatterton,[3]* and Gareth R. Williams[1]*

[1] UCL School of Pharmacy, University College London, 29 – 39 Brunswick Square, London, WC1N 1AX, UK

[2] School of Materials Science & Engineering, University of Shanghai for Science and Technology, Shanghai 200093, China.

[3] Department of Life, Health & Chemical Sciences, The Open University, Walton Hall, Milton Keynes, MK7 6AA, UK.

* Authors for correspondence. Email: ydg017@usst.edu.cn (DGY); nicholas.chatterton@open.ac.uk (NPC); g.williams@ucl.ac.uk (GRW). Tel: +86 21 5527 0632 (DGY); +44 (0) 1908 655488 (NPC); +44 (0) 207 753 5868 (GRW). Fax: +44 (0) 20 7753 5942 (GRW).

Abstract

A series of 5-fluorouracil (5-FU) loaded core/shell electrospun fibers is reported. The fibers have shells made of Eudragit S100 (ES-100), and drug-loaded cores comprising poly(vinylpyrrolidone), ethyl cellulose, ES-100, or drug alone. Monolithic 5-FU loaded ES-100 fibers were also prepared for comparison. Electron microscopy showed all the fibers to have smooth cylindrical shapes, and clear core-shell structures were visible for all samples except the monolithic fibers. 5-FU was present in the amorphous physical form in all the materials prepared. Dissolution studies showed that the ES-100 shell was not able to prevent drug release at pH 1.0, even though the polymer is completely insoluble at this pH: around 30 to 80 % of the maximum drug release was reached after 2h immersion at pH 1.0. These observations are ascribed to the low molecular weight of 5-FU permitting it to diffuse through pores in the ES-100 coating, and the high acid solubility of the drug providing a thermodynamic impetus for this to happen. In addition, the fibers were observed to be broken or merged following 2h at pH 1.0, providing additional escape routes for the 5-FU.

Keywords

Coaxial electrospinning; Eudragit; 5-Fluorouracil; colon-targeted drug delivery

Chemical compounds studied in this article

5-Fluoruracil (PubChem CID: 3385)

1. Introduction

Electrospinning is a facile technique which has been widely explored in pharmaceutics (Chakraborty et al., 2009; Williams et al., 2012). In its simplest embodiment, a polymer is dissolved in a volatile solvent and ejected from a syringe fitted with a metal needle (spinneret) towards a metal collector at a controlled rate. The application of a high (kV) voltage between the spinneret and the collector causes the rapid evaporation of solvent, and results in the formation of nanoscale one-dimensional polymer fibers on the collector. If an active pharmaceutical ingredient (API) is co-dissolved with the polymer then drug-loaded fibers can be prepared, and these have been investigated for use as a broad range of drug delivery systems including fast-dissolving (Balogh et al., 2015; Li et al., 2013a; Li et al., 2013b; Nagy et al., 2010; Samprasit et al., 2015), sustained release (Chen et al., 2010b; Okuda et al., 2010; Xie and Wang, 2006; Xu et al., 2011), pulsatile release (Kaassis et al., 2014), and targeted release formulations (Abdullah et al., 2011; Shen et al., 2011; Yu et al., 2014). In recent years, researchers have developed increasing complex electrospinning experiments, and the use of coaxial electrospinning (which uses a concentric spinneret, with one needle nested inside another) to prepare core/shell structures has been very widely reported (Chakraborty et al., 2009; Chen et al., 2010a; Llorens et al., 2015).

A common way to achieve targeted drug release is to use a pH-sensitive polymer to ensure that the API is freed only in a certain part of the gastro-intestinal tract. An enteric coating to a tablet or capsule to preclude drug release in the stomach is perhaps the simplest and most commonly employed embodiment of this. A range of pH-sensitive polymers exists, such as alginates, chitosan, poly(methacrylic acid-grafted-poly(ethylene glycol)) (Lowman et al., 1999), or poly(methacrylic acid-*co*-*N*-vinylpyrrolidone) (Carr and Peppas, 2010). One such family of materials, the Eudragit methacrylate polymers, has been widely used in the formulation of oral dosage forms including as tablet coatings or tablet matrices, and to prepare microspheres and nanoparticles for controlled drug delivery in the gastro-intestinal (GI) tract (Krishnaiah et al., 2002; Momoh et al., 2014; Varshosaz et al., 2015). Eudragit L100, L100-55 and S100 are specifically designed for targeting the lower parts of the GI tract; these fibers are insoluble at low pH, dissolving only at pH 6.0, 5.5, or 7.0 respectively.

Shen et al (Shen et al., 2011) were the first to fabricate electrospun Eudragit fibers, making materials of the Eudragit L100-55 (EL-100-55) polymer and diclofenac sodium. *In vitro* dissolution tests revealed that the fibers had pH-dependent release profiles, with very limited (less than 3%) diclofenac release at pH 1.0, but sustained and complete drug release over 6 hours at pH 6.8. A second study prepared analogous fibers using coaxial spinning with a mixture of ethanol and dimethylacetamide as the sheath fluid, which was reported to yield better quality fibers (Yu et al., 2014); again, very little drug was released in the acidic medium (< 5 %). Similar results have been seen for systems comprising EL-100-55 and ketoprofen (Yu et al., 2013b), helicid (Yu et al., 2013a), and mebeverine hydrochloride (Illangakoon et al., 2014). In other work, Aguilar *et al.* made blend fibers of EL-100-55 and poly(urethane) with paclitaxel, and saw very little release at pH 4 but much greater release at pH 6 (Aguilar et al., 2015). Eudragit S100 (ES-100) fibers containing uranine and nifedipine have been shown to give rapid release of the incorporated drugs at pH 6.8, but no *in vitro* studies were performed at lower pH values (Hamori et al., 2014). Eudragit has also been used to coat electrospun fibers (Nista et al., 2013).

Some authors have also reported successful colon targeting using core/shell fibers made with a ES-100 shell and an ethyl cellulose core (Xu et al., 2013). It appears, however, that in some cases – most likely because of the very high surface-area-to-volume ratio of electrospun fibers – drug release can be seen at low pH even when the polymer filament is not soluble. Karthikeyan and co-workers generated mixed fibers of zein and ES-100 loaded with pantoprazole and aceclofenac and found that after 2 h immersion 0.1 N HCl, while only 6 % of the former was released, some 25 % of the latter was freed into solution (Karthikeyan et al., 2012).

In this work, we were interested in preparing pH-sensitive electrospun drug delivery systems for the anti-cancer drug 5-fluorouracil (5-FU; Figure 1). 5-FU has a very low molecular weight, and is very soluble in acidic media. It has been prescribed for over 55 years, and is widely used for the treatment of colorectal, breast, gastrointestinal, and ovarian cancers (Rejinold et al., 2011). The drug is usually administered intravenously (due to its poor water solubility) or topically as an ointment, especially in the case of skin cancer.

Here we sought to develop systems to deliver 5-FU specifically to the lower reaches of the GI tract.

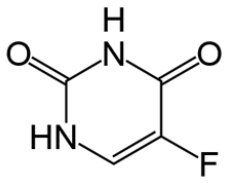

Figure 1. The chemical structure of 5-FU.

2. Materials and methods

2.1 Materials

Eudragit S100 (Mw = 125,000 Da) was a gift from Evonik GmbH (Darmstadt, Germany). Poly(vinylpyrrolidone) (PVP) K60 (Mw = 360,000 Da) was purchased from the Shanghai Yunhong Pharmaceutical Aids and Technology Co., Ltd. (Shanghai, China). Ethyl cellulose (6 mPa·s to 9 mPa·s) was obtained from the Aladdin Chemistry Co., Ltd (Shanghai, China). 5-FU was purchased from Sigma–Aldrich (Gillingham, UK). Basic fuchsin, N,N-dimethylformamide (DMF), and anhydrous ethanol were provided by the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other chemicals and reagents were of analytical grade, and were used as supplied. Water was distilled prior to use.

2.2 Electrospinning

A 13 % w/v Eudragit S100 (ES-100) solution was prepared in a mixture of ethanol and N,N-dimethylformamide (DMF; 8:2 v/v) and used as the sheath solution. A 10 % w/v solution of 5-FU in DMF was also prepared, and used to generate four different core solutions: 1 mL of the 5-FU solution was combined with 1 mL of a polymer solution, as detailed in Table 1. To aid observation of the electrospinning process, 0.2 mg/mL of basic fuchsin was added to the S3 solution.

Table 1. The compositions of the solutions used for coaxial electrospinning.

ID	Core solution prepared from[a]	Core solution composition (w/v)	Sheath solution (w/v)
S1	6 % PVP in ethanol and 10 % 5-FU	3 % PVP and 5 % 5-FU	13 % ES-100
S2	20 % ethyl cellulose in ethanol and 10 % 5-FU	10 % ethyl cellulose and 5 % 5-FU	13 % ES-100
S3	5 % ES-100 in ethanol/DMF and 10 % 5-FU	2.5 % ES-100 and 5 % 5-FU	13 % ES-100
S4	DMF and 10 % 5-FU	5 % 5-FU	13 % ES-100
S5	13 % ES-100 and 5 % FU in ethanol/DMF (8/2 v/v)	13 % ES-100 and 5 % 5-FU	Ethanol

[a] All % ages are as w/v, and the 5-FU solution was prepared in DMF. Core solutions were prepared by combining 1 mL of the appropriate polymer solution with 1 mL of the 5-FU solution for S1 – S4.

Coaxial electrospinning was performed on a setup comprising two syringe pumps (KDS100 and KDS200, Cole-Parmer, Vernon Hills, IL, USA) and a high voltage power supply (ZGF 60Kv/2mA, Shanghai Sute Corp., Shanghai, China). A concentric spinneret was employed for the electrospinning process: the outer needle had an internal diameter (I.D.) of 1.2 mm, and the inner needle an I.D. of 0.3 mm. The electrospinning processes were recorded using a digital camera (PowerShot A490, Canon, Tokyo, Japan). Following a series of optimization experiments, the applied voltage was fixed at 14.5 kV, the core fluid flow rate at 0.1 mL / h (S1 and S2) or 0.2 mL / h (S3, S4, and S5), and the sheath fluid rate at 1.5 mL / h (S1/S2) or 3 mL / h (S3/S4/S5). Fibers were collected on a flat piece of aluminium foil placed 12 cm from the spinneret. All experiments were performed under ambient conditions (25 ± 2 °C; 57 ± 6% relative humidity).

2.3 Characterization

2.3.1 Electron microscopy

The morphology of the fibers was examined using an S-4800 field-emission scanning electron microscope (FESEM, Hitachi, Tokyo, Japan). The average fiber diameter was determined by measuring the fibers (n > 50) in SEM images, using the ImageJ software (National Institutes of Health, Bethesda, MD, USA). Transmission electron microscope (TEM) images of the samples were obtained on a JEM-3000F HR field emission TEM (JEOL, Tokyo, Japan). Fiber samples were collected by fixing a lacey carbon-coated copper grid to the collector and electrospinning directly on to this.

2.3.2 Physical form assessment

X-ray diffraction (XRD) patterns were recorded on a D8 Advance instrument (Bruker, Billerica, MA, USA) using Cu Kα radiation at 40 kV and 25 mA. 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 to 300 °C under a 50 mL / min flow of nitrogen.

2.3.3 IR spectroscopy

Attenuated total reflectance Fourier transform infrared (FTIR) analysis was carried out on a Spectrum 100 FTIR spectrometer (PerkinElmer, Waltham, MA, USA). The scanning range was 650–4000 cm^{-1}, and the resolution was set at 1 cm^{-1}.

2.3.4 *In vitro* dissolution testing

Drug release was quantified using a USP-II test performed on automated apparatus (PTWS instrument, Pharma Test, Hainburg, Germany). 50 mg of the fiber mat was inserted into a size 0 gelatine capsule (SpruytHillen, IJsselstein, Holland) which was in turn loaded into a metal sinker. Each capsule was placed in a vessel containing 750 ml of 0.1 N HCl. After 120 min, 250 ml of 0.2 M tri-sodium phosphate (equilibrated to 37 ± 0.5 °C) was added to each vessel, and the pH of the solution was adjusted to pH 6.8 using 2 N HCl. The vessel was continuously stirred with a paddle at 50 rpm, and throughout the experiment the temperature of the dissolution medium was maintained at 37 ± 0.5 °C. The 5–FU released was assayed at 266 nm using an inline UV spectrophotometer (Cecil 2020, Cecil Instruments Ltd., Cambridge, UK). Data were processed using the Icalis software (Icalis Data Systems Ltd, Wokingham, UK). Experiments were performed in triplicate and data are reported as mean ± S.D. To observe the fibers after 2h immersion at pH 1.0, a separate set of experiments was performed in which 7 – 8 mg of fibers was placed in 10 mL of 0.1 N HCl and incubated at 37 °C for 2 h. The fiber mat was then recovered, dried, and imaged by SEM.

3. Results

3.1 The electrospinning process

Photographs of the electrospinning process for S3 are given in Figure 2. When no voltage was applied, it is evident that the core and sheath solutions did not mix when they came

together. This indicates that the solution parameters were correctly tuned to yield a core/shell structure. When the applied voltage was increased to 14.5 kV a straight thinning jet was ejected from the compound Taylor cone; this then undergoes bending and whipping motions forming loops of increasing size (Figure 2(b) and (c)).

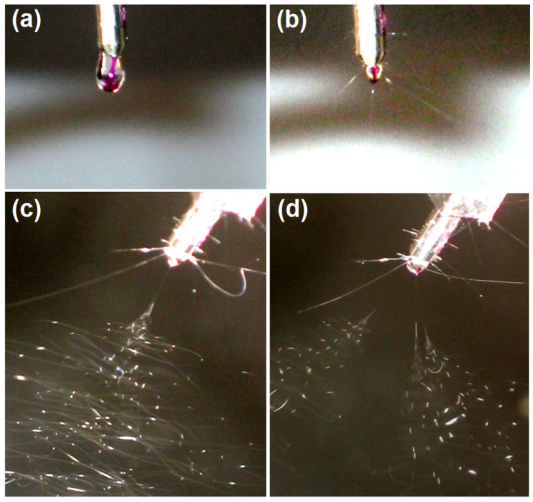

Figure 2. Photographs of the S3 coaxial electrospinning process. **(a)** the liquid droplet at 0 kV; **(b)** the compound Taylor cone showing jets ejecting from the tip at 14.5 kV; **(c)** the bending and whipping movement of the jet at 14.5 keV; and, **(d)** the division of the jet at 16 kV**.**

A further increase in the applied voltage to 16 kV led to branching of the spinning jet, giving two bending and whipping instability regions. Branching of the spinning jet is a complex phenomenon and may give rise to separation of the shell and core parts of the fibers. Therefore, the applied voltage was set as 14.5 kV for all subsequent electrospinning processes.

3.2 Fiber morphology

SEM images of the fibers produced are given in Figure 3. The fibers have smooth surfaces and comprise uniform structures without any 'beads-on-a-string' morphology visible. There is no evidence for any particles or phase separation present, indicating that the multiple components of the formulations are homogeneously mixed. Some precipitation of 5-FU was observed during the solution preparation process for S2, but no drug crystals can be seen in the fibers.

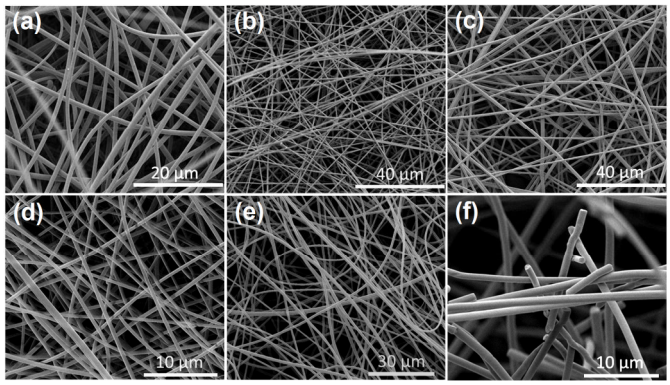

Figure 3. SEM images of the fibers. **(a)** S1; **(b)** S2; **(c)** S3; **(d)** S4; **(e)** S5; and, **(f)** the ends of the S3 fibers.

The fiber diameters are listed in Table 2. It can be seen that all the fibers are around 1 µm in size, with the fibers containing S100 and 5-FU solution only as the core being larger than the PVP and EC containing samples.

Table 2: The diameters of the Eudragit-based fibers.

Formulation	Core	Shell	Fiber diameter / nm
S1	PVP / 5-FU	ES-100	899 ± 208
S2	EC / 5-FU	ES-100	848 ± 215
S3	ES-100 / 5-FU	ES-100	1275 ± 383
S4	5-FU	ES-100	1033 ± 233
S5	ES-100/5-FU	-	873 ± 232

TEM was employed to study in more detail the structures of the fibers, and the results are presented in Figure 4. S1, S2, S3 and S4 possess distinct core-shell structures. The S5 fibers, in contrast, do not show any core-shell structure; this is as expected, since the shell fluid for S5 comprised purely a solvent.

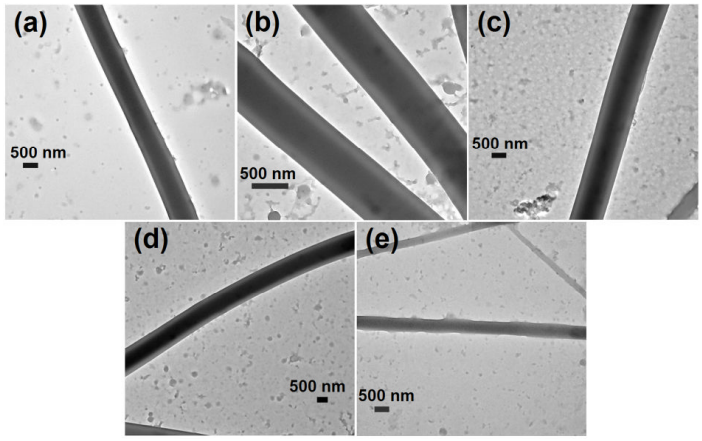

Figure 4. TEM images of **(a)** S1; **(b)** S2; **(c)** S3; **(d)** S4; and, **(e)** S5.

3.3 Physical form

The physical form of the fiber components was studied by X-ray diffraction (XRD) and differential scanning calorimetry (DSC). The resultant data are shown in Figure 5.

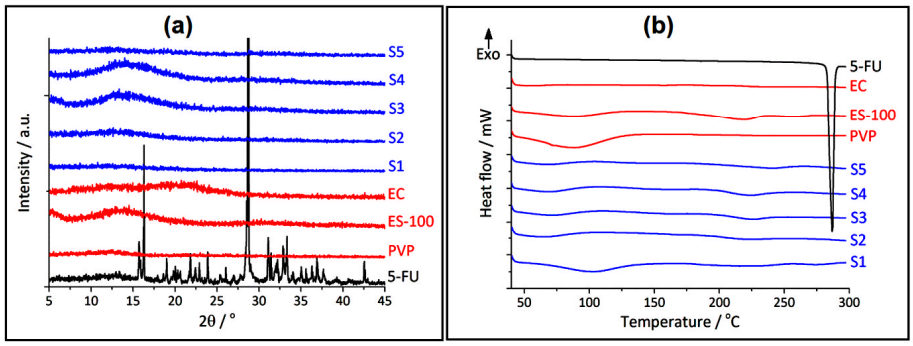

Figure 5. (a) XRD and **(b)** DSC data for the electrospun fibers and raw materials.

From the diffraction data, it is clear that 5-FU is a crystalline material with numerous characteristic reflections present in its XRD pattern. The polymers ES-100, PVP and EC display only broad haloes in their patterns, consistent with their existence as amorphous materials. In all cases, the XRD patterns of the composite fibers do not show any Bragg reflections, only the broad humps typical of amorphous materials.

In DSC, 5-FU shows a sharp endothermic melting peak at 287 °C, in good agreement with the literature value (Krishnaiah et al., 2002). The DSC spectrum of Eudragit S100 showed a broad endothermic band between 55 and 100 ˚C, which can be ascribed to the loss of absorbed and adsorbed water. This is followed by a second endothermic band which begins

at around 150 ˚C, in accordance with the literature (Chawla et al., 2012; Hu et al., 2012). PVP also shows a broad dehydration endotherm between 55 and 125 °C. The EC thermogram contains no distinct features.

The DSC traces of all the fibers do not contain a 5-FU melting endothermic peak, thus demonstrating the absence of crystalline material in the formulations. This is consistent with the XRD data. All the fiber formulations exhibit a shallow endothermic peak below 100 – 150 °C, which is attributed to the loss of water.

3.4 IR spectroscopy

IR spectra of the raw materials and electrospun fibers are depicted in Figure 6.

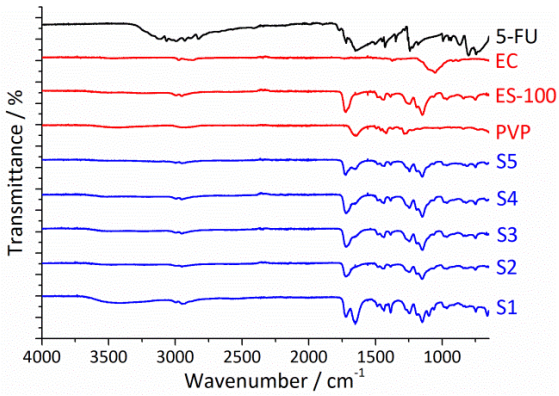

Figure 6. FTIR spectra of the fibers and raw materials.

The IR spectrum of pure 5-FU shows two carbonyl (C=O) stretching at 1720 and *ca.* 1645 cm^{-1} (Gao et al., 2007), a C-N stretch at 1243 cm^{-1} and a C-F stretch at 995 cm^{-1}. The broad stretch between 3150- 2800 cm^{-1} is due to C-H and N-H stretching. The spectrum of ES-100 displays characteristic bands of methyl and methylene C–H stretching vibrations at 2997 and 2952 cm^{-1}, a strong band because of carbonyl groups at 1724 cm^{-1} (C=O stretch) and two bands because of ester linkages (C–O–C stretches) at 1257 and 1148 cm^{-1}. In the spectra of the fibers, the most intense peaks from ES-100 are visible at 1720 – 1724 cm^{-1} (C=O stretch) and a second peak can be seen at 1148 - 1150 cm^{-1} due to C–O–C stretching. The S1 fibers additionally show a strong peak at 1650 cm^{-1}, which may arise either from 5-FU or from the PVP comprising its core. The other fibers show a shoulder to the Eudragit peak at 1720 – 1724 cm^{-1}, which is expected to correspond to a 5-FU carbonyl stretch; this is particularly

marked in S5. The peak positions are little changed in the fibers from the raw materials. The distinct 5-FU phonon vibrations below 1000 cm^{-1} (*e.g.* at 750 cm^{-1}) do not appear to be present, but the picture is confused by the fact that ES-100 has peaks at similar wavenumbers. All in all, these data are consistent with the XRD and DSC data, indicating a molecular dispersion of 5-FU in the polymer carriers.

3.5 *In vitro* drug release

The *in vitro* drug release profiles of the different fibers are given in Figure 7. The S2 fibers were not studied in this assay, because the observation of precipitates in the electrospinning process led to concern about their homogeneity.

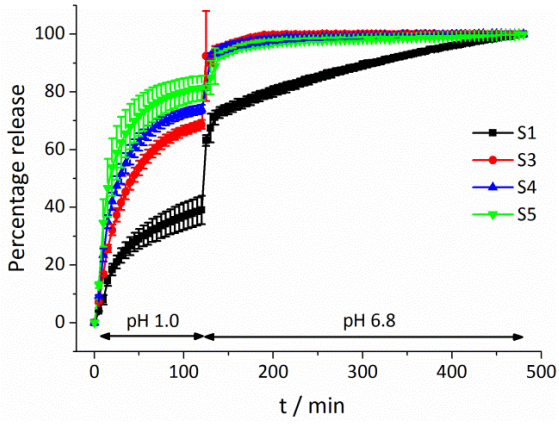

Figure 7. *In vitro* 5-FU release from the fiber formulations. Experiments were performed in triplicate, and the data shown as mean ± S.D. 100% release is defined as the point at which maximum drug release was observed.

These results are unexpected. Since ES-100 is insoluble at pH 1.0, it would intuitively be expected that the drug would release very slowly under these conditions. However, it is clear that in all cases 5-FU release happens rather rapidly, even at such a low pH. The single-fluid S5 fibers release their drug load most quickly, followed by S4 (for which the core fluid was a 5-FU solution only), S3 (ES-100 core), and S1 (PVP core). When the pH is raised to 6.8, a second burst of release is seen for all the fibers, with S3, S4 and S5 then very rapidly reaching maximum drug release. S1, possibly counter-intuitively given the very high solubility of PVP, gives a sustained release of drug over the remaining 6h of the experiment.

The rapid release from S5 at pH 1.0 can be explained by a combination of two factors. First, 5-FU is a low molecular weight and basic drug, and is very soluble at low pH. Second, the

very high surface-area-to-volume ratio of the fibers will result in a large amount of 5-FU being present at the fiber surfaces. This surface drug will be freed rapidly into solution. 5-FU from further inside the fibers may also be able to diffuse out through pores created by earlier departing drug molecules. It was observed that after 2h of immersion in 750 mL of the acid medium, the S5 fiber mat had virtually completely disintegrated, presumably as a result of the loss of a significant amount of its drug loading causing the fibers to collapse and separate.

The rapid release of drug from the core/shell fibers is more puzzling. Although the fibers appear to have clear core/shell structures from TEM images, with a clear interface between these two compartments of the fibers, it may be that some mixing of the core and shell solutions occurred, resulting in some 5-FU being present at the surface of the fibers. The dissolution of this could yield pores through which the remaining drug in the core could escape. This process is somewhat more arduous than the freeing of surface drug into solution, and thus takes longer, leading to slower release from the core/shell fibers. Pores through which drug molecules could escape from the fibers could also be created by swelling of the S100 shell and the permeation of water into the centre of the fibers.

To obtain more insight into the drug release mechanism, the fibers were imaged after 2 h immersion in 0.1 N HCl (see Figure 8).

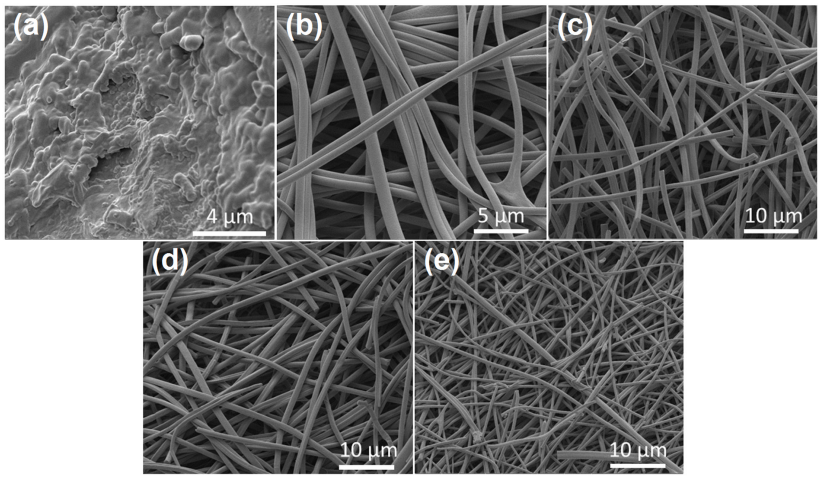

Figure 8. SEM images of the fibers recovered after 2 h immersion in HCl. **(a)** S1; **(b)** S2; **(c)** S3; **(d)** S4; and, **(e)** S5.

The fibers S2, S3, S4 and S5 appear largely unaffected by the acid treatment, as would be expected given the insolubility of Eudragit at this pH. However, for S3, S4 and S5, it is clear that there are a number of broken fibers present. The breaking of the fibers will aid the release of 5-FU, since it will expose sections of the core to the release medium. However, the area revealed by such breakages is relatively small, and thus this is not expected to be a major factor.

In contrast, the S1 fibers are no longer visible as individual entities, having merged and formed an irregular and continuous sheet. This must be ascribed to the very high hydrophilicity of the PVP core in these fibers: water ingress through small pores in the Eudragit shell must have been absorbed by the PVP, causing it to swell and the fibers to "burst", losing their integrity. The formation of this agglomerate will reduce the surface-area-to-volume ratio of the fiber mat, and can perhaps explain the sustained release observed for the S1 material. Attempts to model the first stage of drug release, in the pH 1.0 buffer, were undertaken using the Peppas model (Ritger and Peppas, 1986); the resultant plots were decidedly non-linear, showing that simple Peppas-type release kinetics are not applicable to these systems.

4. Discussion

In this work, a family of core/shell fibers based on Eudragit S100 has been prepared and fully characterised. We find that the fibers have very distinct core/shell structures, but that even when there is no drug in the shell release at pH 1 is nevertheless rapid. In contrast to these findings, previous reports (Aguilar et al., 2015; Illangakoon et al., 2014; Shen et al., 2011; Yu et al., 2014) have shown that monolithic Eudragit fibers can preclude drug release at acidic pHs. In these studies, a range of APIs were used; the properties of these, together with those of 5-FU, are summarised in Table 3. Other than the data reported in this work, only the API aceclofenac showed appreciable release at low pH (Karthikeyan et al., 2012).

401 **Table 3:** A summary of the literature data on electrospun Eudragit fibers where dissolution at low pH has been
402 explored, and the APIs incorporated.

API	Reference	pKa	RMM	Polymer(s) in fiber
Diclofenac sodium	(Shen et al., 2011; Yu et al., 2014)	4.1	318	EL-100-55
Mebeverine hydrochloride	(Illangakoon et al., 2014)	8.1	466	EL-100-55
Paclitaxel	(Aguilar et al., 2015)	10.9	854	EL-100-55 / PU[a]
Aceclofenac	(Karthikeyan et al., 2012)	4.7	354	ES-100 / zein
Pantoprazole	(Karthikeyan et al., 2012)	4.0	383	ES-100 / zein
Ketoprofen	(Yu et al., 2013b)	4.45	254	EL-100-55
Helicid	(Yu et al., 2013a)	-	284	EL100-55
5-FU	This work	8.1	130	ES-100

403 [a] PU = polyurethane.

404

405 From a consideration of the data in Table 3, it is not completely clear why high levels of 5-FU
406 and aceclofenac release are seen at low pH, while for all the other APIs only minimal release
407 is seen. It is not possible to make completely clear comparisons because of differences in
408 the polymer systems used. We have prepared monolithic EL-100-55 fibers with 5-FU and
409 found substantial release at pH 1.0 (data not shown), so we do not believe that the use of
410 EL-100-55 or ES-100 is a major contributory factor to the different behaviours: both are
411 after all insoluble at pH 1.0. Ketoprofen and diclofenac are acidic drugs, and thus the fact
412 that they do not release from EL-100-55 fibers at pH 1.0 can be attributed to their low
413 solubility at this pH. Helicid is non-ionisable and poorly soluble, and thus its lack of release
414 at low pH is also understandable. Paclitaxel has a very high molecular weight, and very low
415 solubility, so again here the data are intuitively understandable. In contrast, 5-FU is basic,
416 and releases substantially at low pH from monolithic ES-100 fibers presumably owing to its
417 high solubility in acidic conditions. However, mebeverine, another basic drug, does not.
418 Looking at the drug properties, the major factor that stands out is the very low molecular
419 weight of 5-FU. We thus believe that it is a combination of small molecule size and high acid
420 solubility which together cause the large amounts of 5-FU release observed from monolithic
421 ES-100 fibers at low pH. The low molecular weight of the drug is expected to aid it diffusing
422 through pores into the fibers and into solution, a situation encouraged by the favourable
423 resultant dissolution energy.

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It should be noted that the results obtained by Karthikeyan *et al.* are contradictory to this explanation. These authors found that aceclofenac (acidic) and pantoprazole (basic) behave differently, with the acidic drug releasing to a greater extent (Karthikeyan et al., 2012). This may be because of the inclusion of zein in their monolithic fibers, and/or suggests that the picture is more complex and the intermolecular interactions between the polymer matrix and the drug also need to be considered, even with the high surface-area-to-volume ratios seen with electrospun fibers.

Considering the core/shell fibers, significant amounts of 5-FU release are still seen at low pH. This may arise for two reasons. It may be there is some mixing of the core and shell solutions during electrospinning (even though clear compartments are observed by TEM), leading to the presence of 5-FU at the surface. This can dissolve easily, leading to pores in the shell through which the 5-FU can escape. Alternatively, it may be that 5-FU from the core can simply diffuse through pores already existing in the shell. The loss of fiber morphology of the S1 fibers after 2h in an acid medium (Figure 8) indicates that it is possible for small molecules to permeate through the shell, since it is believed that water ingress led to this destruction. The fiber breakages observed will accelerate drug release by exposing some of the core to the dissolution medium, but this should be a relatively small effect given the small area of the core revealed in this manner. It should be noted that polymer solubility is no predictor of the rate of release, nor how much will release at pH 1.0: the S1 fibers with a PVP core show much less release in acidic conditions than the S3 fibers (ES-100 core).

Although substantial release is observed in the pH 1.0 medium, the fibers prepared in this work nevertheless show interesting two-stage release profiles. *In vivo*, this would be expected to yield some release in the stomach and more subsequently lower in the GI tract. The balance between these stages can be tuned by varying the polymer composition in the core. Such two-stage release profiles are much sought after in pharmaceutics, and may have utility for the treatment of colorectal cancer.

5. Conclusions

A series of 5-fluorouracil (5-FU) loaded electrospun fibers was prepared in this work: four core/shell materials with a Eudragit S100 (ES-100) shell and a drug-loaded core, and one monolithic fiber material in which ES-100 comprised the filament-forming polymer. The fibers were smooth and cylindrical in shape, and the core/shell materials clearly showed two distinct phases in transmission electron microscopy. The active ingredient existed in the amorphous form in the fibers. In contrast to previous literature reports, very significant amounts of drug release (around 30 – 80 % of maximum release) were seen during immersion in a pH 1.0 medium, despite the insolubility of ES-100 below pH 7.0. Inspection by electron microscopy of the fibers after 2h in pH 1.0 showed that when the core polymer was poly(vinyl pyrrolidone) the individual fibers had merged to form a film, while fibers with cores of ES-100 or drug alone were observed to have snapped and broken up into smaller parts in places. The monolithic ES-100 fibers additionally showed breakages. It is proposed that the low molecular weight of 5-FU permitted it to diffuse through pores in the ES-100 coating, with the high acid solubility of the drug providing a thermodynamic driver for this to happen. In addition, the loss of fiber integrity observed is expected to provide additional escape routes for the 5-FU. The fiber formulations thus show two very distinct phases of release, with burst release immediately after immersion into a stomach-mimicking environment, and a second bust of release upon transfer into a pH 6.8 buffer imitating the small intestine.

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