Department of Pharmaceutical Sciences, Johnson & Johnson Pharmaceutical Research and Development[1], Beerse, Belgium, Johnson & Johnson Center for Biomaterials and Advanced Technologies[2], Somerville, New Jersey, USA

The use of polymer-based electrospun nanofibers containing amorphous drug dispersions for the delivery of poorly water-soluble pharmaceuticals

M. E. Brewster[1], G. Verreck[1], I. Chun[2], J. Rosenblatt[2], J. Mensch[1], A. Van Dijck[1], M. Noppe[1], A. Ariën[1], M. Bruining[1], J. Peeters[1]

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Marcus E. Brewster, Ph. D., Johnson & Johnson Pharmaceutical Research and Development, Pharmaceutical Sciences, Drug Evaluation, Turnhoutseweg 30, 2340 Beerse, Belgium
mbrewste@prdbe.jnj.com

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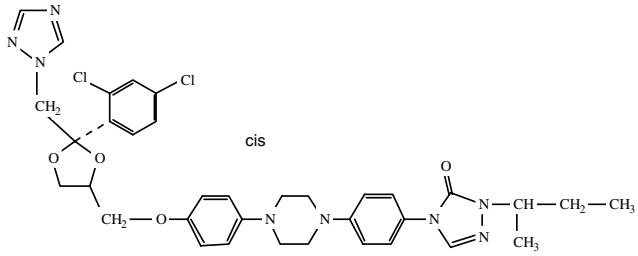
Electrostatic spinning was applied to the preparation of drug-laden nanofiber for potential use in oral and topical drug delivery. While this technique is in its infancy with regard to pharmaceutical applications, a number of recent publications suggest that it may be of high value in the formulation of poorly water-soluble drugs by combining nanotechnology and solid solution/dispersion methodologies. The purpose of this article is to describe some of these recently published applications. For immediate release oral application, a water-soluble cellulose polymer was selected (i.e., hydroxypropylmethylcellulose, HPMC) while for topical application, a nonbiodegradable, water-insoluble polymer was investigated (i.e., a segmented polyurethane, SPU). Solutions of the polymer and the drugs in appropriate solvents could be spun across various potentials (16–24 kV) generating nanofibers with diameters ranging from 300 to 2000 nm. Dissolution studies found that the non-woven fabrics derived from HPMC and containing itraconazole dissolved over a time course of minutes to hours depending on the formulation used as well as the drug/polymer ratios. Drug release from the SPU samples was dependent on the incorporated drug as well as nanostructure obtained.

1. Introduction

The use of high throughput (HT) lead identification in the discovery environment has had a number of consequences in downstream developmental activities. Various retrospective studies have shown that HT techniques tend to select for highly lipophilic derivatives meaning that there is a trend for new drug candidates to be more poorly water-soluble, of higher molecular weight and to possess poorer absorption (Lipinski et al. 1997; Lipinski 2001). The formulation of such compounds into systems that allow for relevant absorption, be that through the GI track or other biological barriers such as the skin, represents a significant challenge to the formulator. Numerous approaches have been suggested to improve the oral bioavailability of poorly water-soluble drug candidates including micronization and nanonozation (particle size reduction), complexation, formation of a solid solution, preparation of amorphous solid oral dosage forms as well as lipid based systems (Liu 2000).

The development of bioavailable formulations of itraconazole is a useful example. While this antifungal agent was the first orally effective drug for the treatment of both candidiasis and *Aspergillus* infection, its physicochemical properties including a log P > 5, a low pKa (pKa ~ 4) and an aqueous solubility at neutral pH estimated at about 1 ng/mL, confounded dosage form design (Peeters et al. 2002). Physicochemical properties are listed in Table 1. Techniques such as salt formation, prodrugs, particle size



reduction (even at nanometer dimensions) and use of surfactants were not successful in generating useful oral systems. Interestingly, the only approaches which appeared to be viable for generating bioavailable solid oral dosage forms were those that gave rise to stable supersaturated solutions. Based on this finding, a marketed solid oral capsule product was generated in which a mixture of itraconazole and hydroxylpropylmethylcellulose (HPMC) was coated onto an inert sugar (Gilis et al. 1997). As the thin

Table 1: Physicochemical characteristics of itraconazole

MW	705.64
Partition coefficient	Log P > 5
Ionization constant	4.0
Melting point	166 °C
Solubility in water (pH 7)	~1 ng/ml
Solubility in 0.1 N HCl	6 µg/ml

film dissolved in gastric fluid, the molecularly dispersed itraconazole is released at supersaturated concentration with the co-dissolving HPMC acting as a stabilizer (i.e., to inhibit recrystallization of itraconazole) (Peeters et al. 2002). The supersaturated solutions of itraconazole were stable for over 24 h allowing for absorption and distribution. The fraction absorbed for itraconazole in this systems is ~85%. Parenteral delivery of itraconazole has been made possible through two techniques including the use of hydroxypropyl-β-cyclodextrin (which has lead to a marketed formulation) (Peeters et al. 2002) as well as through the formation of nanosuspensions (which have been assessed in clinical trials).

Similar challenges have been observed for dermal delivery of itraconazole where topical treatment of fungal infections can offer several advantages over oral dosing. Critical success factors in achieving therapeutically important concentrations of drug in the skin are similar to those in transdermal delivery and include the ability of the drug to be released from the formulation and the permeation of the drug through the barrier elements of the skin (i.e., the stratum corneum). Studies have suggested that release of drug from the formulation is enhanced not simply by increasing the drug concentration in the dosage form but by increasing the thermodynamic activity of the drug, that is to increase its degree of saturation in the delivery vehicle (Hadgraft 1999). Stable supersaturated systems represent an important construct in this context.

These two issues (1) generating oral systems that dissolve rapidly enough to ensure good bioavailability and (2) generating topical delivery systems wherein the drug is present at thermodynamic activities such that entry into the skin is enhanced, may be addressed by several approaches. Based on the success of solid solutions as well as nanonization, the possibility of a hybrid technology seemed attractive (Verreck et al. 2003a, 2003b). In this context, electrostatic spinning of drug-laden polymer fibers was considered. As described, fibers at submicron diameters can be formed when electrical forces overcome the surface tension of the drug/polymer solution at the air interface such that a fiber forms (Reneker and Chun 1996). As the fiber accelerates through the electric field, the continuous, single filament is further stretched based on Coulombic forces and bending instability (Yarin et al. 2001). As the solvent evaporates, the formed fiber can be collected on a screen to give a non-woven fabric or on a spinning mandril to generate a tube. The collected fibers generate a non-woven fabric which can be applied to a number of formulations. In this process, the diameter and morphology of the filaments is determined by solution parameters such as solution dielectric constant, conductivity, polymer type and concentration and surface tension, equipment-controlled parameters including flow rate of the solution, hydrostatic pressure in the spinneret, applied electric field and tip-to-collector distance and environmental parameters (temperature, humidity, air velocity in the spinning chamber) (Leet et al. 2003; Shin et al. 2001). We have, therefore, recently assessed the applicability of electrostatic spinning to oral and topical formulations of itraconazole (Verreck et al. 2003a, 2003b; Brewster et al. 2002; Verreck et al; 2003c).

Table 2: Viscosity of various spinning solutions of itraconazole and segmented polyurethane in DMF

Drug : Polymer Conc. Percent w/v	Drug : Polyer Ratio	Viscosity (Poise, P)
10%	10 : 90	1.08
10%	20 : 80	0.62
10%	30 : 70	0.3
20%	10 : 90	31
20%	20 : 80	12
20%	30 : 70	8
20%	40 : 60	5.5
20%	50 : 50	1.8
20%	60 : 40	1.1

2. Investigations, results and discussion

2.1. Spinning conditions

Electrostatic spinning for the HPMC systems was optimal at concentrations of 12% w/w (drug/polymer) in ethanol/methylene chloride (40/60 w/w). This concentration was selected since at higher values (15% w/w), the viscosity of the solution became limiting while at lower concentrations (10% w/w), nanofibers could not be formed due to "sputtering" of the solution. For the polyurethane polymer, optimal spinning was obtained at polymer/drug concentrations of 20% in DMF. Solution viscosities at various drug/polymer concentrations are given in Table 2. A number of system parameters including molecular weight and the viscosity of the drug/polymer solution determine the optimal polymer concentrations for electrostatic spinning. Previous studies found that for polylactic acid (PLA), poly(ethylene-co-vinyl acetate) (PEVA) and mixtures of the two could be spun at 14% w/v concentration while work with polyethylene oxide (PEO, Polyox) suggested that the polymer could be spun at concentrations ranging from 2.5 to 30% w/v based on the grade and molecular weight (100,000 to 7,000,000 daltons) of the polymer (Kenawy et al. 2002; Ignatious and Baldoni 2001). Working with aqueous solutions of PEO at a MW of 1,450,000 daltons, Doshi and Reneker found that electrostatic spinning was most efficient between a solution viscosity of 0.8 to 4 Poise (Doshi and Reneker 1995).

2.2. Structure of the electrospun fibers

SEM and AFM were used to determine the morphology of the spun fibers and the formed non-woven fabrics. As illustrated in Fig. 1 (spinning at 16 kV), fibers were generated with diameters of 1 to 4 μm when a 40 : 60, itraconazole : HPMC ratio was used. An AFM measurement of a single fiber corroborated this measurement. Increasing the potential to 24 kV resulted in a reduction of the fiber diameter to approximately 300 to 500 nanometers for this same drug/polymer ratio. Spinning of a itraconazole : HPMC (20 : 80) solution (12% w/w) across a 24 kV potential also resulted in the formation of cylindrical fibers with diameters ranging from 500 nm to 3 μm.

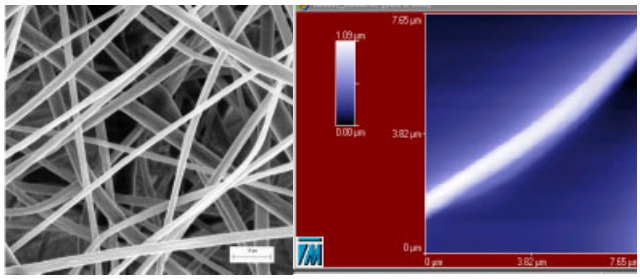


Fig. 1: SEM (Left) and AFM (Right) renderings of 40 : 60 itraconazole : HPMC nanofibers spun across a potential of 16 kV

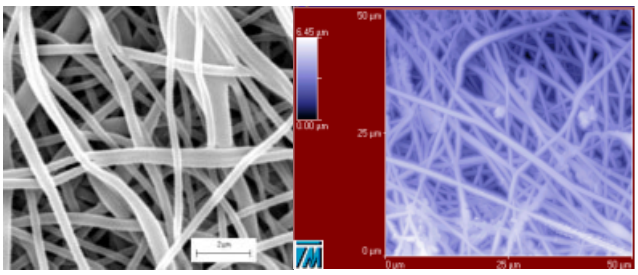


Fig. 2: SEM (Left) and AFM (Right) renderings of 40 : 60 itraconazole : SPU nanofibers spun across a potential of 16 kV

Increasing polymer ratios are often associated with increasing viscosity with a resulting increase in nanofiber diameter (Zong et al. 2002; Demir et al. 2002). Polyurethane fibers (itraconazole : SPU 10 : 90 and 40 : 60) were spun from a drug/polymer solutions of 20% w/w (DMF) and a voltage of 16 kV. SEM and AFM investigation of the 10 : 90 drug : polymer ratio gave fiber diameters in the 2 μm range and, as illustrated in Fig. 2, the 40 : 60 samples gave fiber diameters of 300–700 nm. These data are comparable to those generated for other polymers. PEVA and PLA-PEVA blends (50 : 50), for example generated fibers in the range of 1–3 μm when spun at a potential difference of 15 kV out of a 14% w/v solution while PLA alone resulted in larger fibers (3–6 μm) under similar conditions (Kenawy et al. 2002). The non-woven fabrics possess similar macroscopic characteristics including opacity (as a function of light scattering) although mechanical properties are related both to the processing method as well as the type of polymer used for the spinning.

2.3. *Physical form of the drug in the spun fibers*

Since the non-woven fabrics consist of fibers with diameters in the nano- to micro-range, they possess a large specific surface area which results in fast and efficient evaporation of the organic solvent. Due to this rapid rate of solvent evaporation, the drug/polymer system has limited time to re-crystallize, favoring the formation of non-crystalline solid dispersions or solid solutions. DSC was performed to determine the physical form of itraconazole in the electrospun mats as well as of the unmanipulated materials. The thermogram of crystalline itraconazole shows an endothermic melting peak with its maximum at 172 °C (T_m) and a melting enthalpy (ΔH) of about 85 J/g. During cooling of the melt, two exothermic peaks (T_c) are observed at 87 °C and 69 °C, respectively, both of which are associated with small enthalpies. Reheating these samples results in a glass transition (T_g) at 60 °C and two endothermic peaks at 76 °C and 92 °C, respectively, characterized by a low enthalpy. It has been suggested that the transitions observed during the cooling and the second heating run of itraconazole represent the formation of glassy itraconazole and a monotropic mesophase upon cooling from the melt (Six et al. 2001). HPMC shows a glass transition at 141 °C. Electrospun materials based on HPMC lack a melting peak as well as the thermal events related to monotropic mesophase formation for itraconazole. Likewise, the itraconazole : SPU systems generally lack melting endotherms for itraconazole as well as SPU (unmanipulated SPU shows a broad melting endotherm between 70 °C and 135 °C), although the fibers containing the higher drug load (40 : 60 itraconazole : SPU) do show traces amounts of crystalline itraconazole (the melting endotherm at 162 °C was associated with an enthalpy of $\Delta H = 0.33$ J/g). These results suggest that electrostatic spinning produces an amorphous solid dispersion of itraconazole and the polymers.

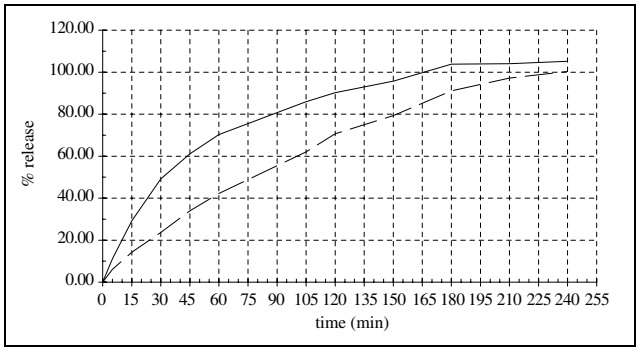


Fig. 3: Dissolution of Itraconazole : HPMC (40 : 60) Electrospun at 16 kV (upper, solid line) and 24 kV (lower, dotted line)

2.4. *Release of drug from the fibers*

The release profiles of itraconazole from the itraconazole : HPMC 40 : 60 w/w electrostatic spun fabric were evaluated as a function of the applied voltage and formulation presentation. Samples spun at either 16 or 24 kV are directly added to the dissolution medium. Complete release is observed for both although there is a tendency for the material spun at 16 kV (i.e., the larger fiber diameter) to release itraconazole faster than the fibers spun at 24 kV (i.e., the smaller fiber diameter) (Verreck et al. 2003). Complete release is obtained at ~160 min for the 16 kV sample and by 240 min for the 24 kV material (Fig. 3). This difference may be due to wettability and solvent accessibility. When the electrospun fabric is folded either in a sinker or hard gelatin capsule, significantly longer dissolution times are generated (i.e., release times of 24 h or greater). In the case of the capsules, the 24 kV samples tended to release drug faster than the 16 kV regimens. The effect of drug/polymer ratio on the *in vitro* release characteristics of itraconazole/HPMC electrostatic spun fabrics is shown in Fig. 4. In this set of comparisons, there tended to be little effect of the polymer-drug ratio for fabric added directly to the dissolution bath. For the hard gelatin capsule and sinker systems, there was a clear

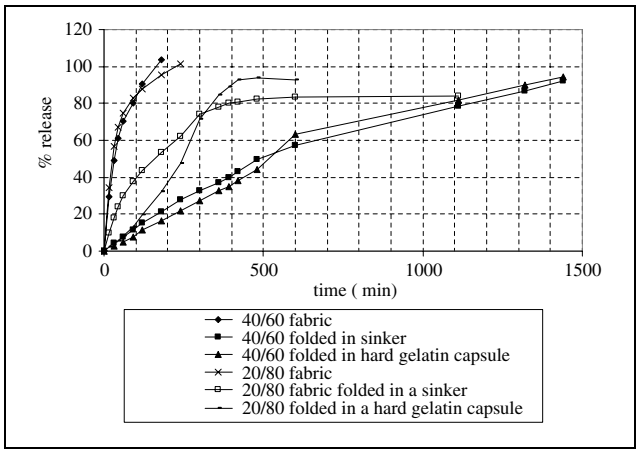


Fig. 4: Dissolution of itraconazole/HPMC electrospun at 24 kV as a function of drug to polymer ratio (40 : 60 versus 20 : 80) and method of measurement (directly added versus a sinker system versus a hard gelatin capsule)

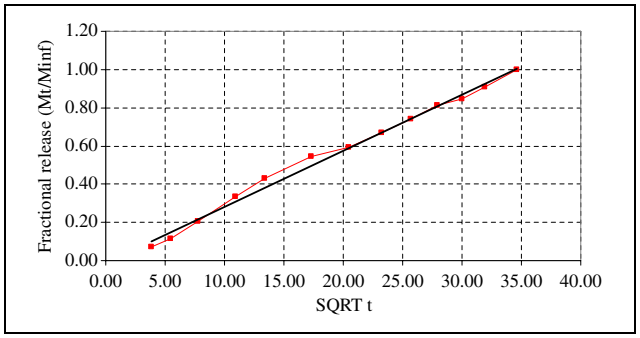


Fig. 5: Fractional release versus the square root of time for itraconazole/PU 10 : 90

trend that a higher polymer to drug ratio resulted in a greater rate or extent of itraconazole release.

For the SPU systems, itraconazole was released without an initial drug burst. Drug release from the 10 : 90 drug : polymer dispersion appeared to reach a plateau after 5–6 h while the 40 : 60 drug : polymer fibers released the drug throughout the time course of the experiment. To assess the mechanism of drug release, a plot of fractional release versus the square root of time was completed (Romero-Cano and Vincent 2002; Miyajima et al. 1997). As illustrated in Fig. 5, the 10 : 90 drug : polymer fabric released drug in a more or less linear mechanism as function of the square root of time ($r = 0.996$) suggesting Fickian diffusion.

2.5. Conclusions

These results indicate a number of potential applications of electrostatically spun fibers in drug delivery based on the following observations: (1) complete release of highly poorly water-soluble drugs can be achieved, (2) the rate of drug release can be tailored, and (3) drug delivery from insoluble polymers is possible opening the possibilities of topical-based formulations. Studies suggest that drug release can be controlled by a variety of parameters. These and other data suggest that electrostatic spinning can be used for the difficult task of controlled drug delivery for poorly water-soluble drugs for both oral and topical dosage forms.

3. Experimental

3.1. Materials

Itraconazole (purity more than 99%) was provided by Janssen Pharmaceutica (Beerse, Belgium). Hydroxypropylmethylcellulose 2910 5 mPa.s (HPMC) and a segmented polyurethane (SPU) were used as provided by the manufacturer (Verreck et al. 2003a, 2003b).

3.2. Electrostatic spinning

The electrostatic spinner used for the experiments was equipped with a Spellman High Voltage DC Supply (Model No: RHR30PN30, Spellman High Voltage Electronics Corporation, Hauppauge, NY). The applied voltage was set at either 16 kV or 24 kV. The distance between the spinneret and the fiber collector was mechanically controlled to 13 cm for the HMPC systems and 10 cm for the SPU formulations. Solutions of itraconazole and HPMC were obtained by dissolving 12% w/w of physical blends of the drug and polymer (ranging from 1 : 9 itraconazole : polymer to 6 : 4 itraconazole:polymer) in a mixture of ethanol and methylene chloride (40/60 w/w). For the polyurethane systems, blends of the drug and polymer were prepared and dissolved in DMF at concentrations of 10 and 20% w/v. The ratio of drug to polymer was varied from 1 : 9 to 6 : 4.

3.3. Scanning electron microscopy (SEM)

The surface topography of the electrostatic spun fibers was assessed using a scanning electron microscope (JSM-5900LV, Japan Electron Optics Laboratory LTD). Electrostatically spun fibers were placed in the center of an aluminum stud. These fibers were fixed to the stud using liquid graphite conductive adhesive 154 (Electron Microscopy Sciences, PA, USA). A very thin layer of gold was applied to the fibers by a sputtering unit (EMS 550, Electron Microscopy Sciences, PA, USA). Gold-coated fibers were placed in the microscope chamber to which a high vacuum was applied. Surface morphological features were obtained in 5–10 kV, usually 5 kV mode.

3.4. Atomic force microscopy

Measurements were carried out using a μTA 2990 Microthermal Analyser (TA Instruments, New Castle, DE, USA) operating in the AFM non-contact mode. An area was scanned with a resolution of 300×300 pixels. The scan rate was 51.7 μm/s in the forward direction.

3.5. Differential scanning calorimetry (DSC)

The DSC measurements were performed using a Perkin-Elmer DSC-7 differential scanning calorimeter with a TAC7/DX thermal analysis controller. Cooling was provided with a Perkin-Elmer refrigerated cooling device (FC-60-PED). Data were treated mathematically using the resident Pyris Software. Calibration was carried out using indium and zinc as reference materials. The samples were analyzed in perforated and covered Aluminum pans under a Nitrogen purge. For the electrospun material, approximately 5 mg were weighed in the DSC pan. The samples were heated from 25 °C to 200 °C with a heating rate of 20 °C/min.

3.6. In vitro drug release measurement

For the soluble polymeric systems, the dissolution of the non-woven fabric was measured directly, manually filled into a hard gelatin capsule size 0 (Capsugel, Morris Plains, NJ) or manually folded and filled in a sinker. The electrostatically spun samples were maintained at a drug dose of 50 mg. Dissolution measurement was performed in 600 ml of 0.1 N HCl (37 °C) using a paddle rotating at 100 rpm (USP II apparatus). Samples were taken at predetermined time intervals up to 24 h. An aliquot of 3 ml was filtered through a Millex HV 0.45 μm filter (Millipore SLHV R04 NL) with the removed solvent not being replaced with fresh solvent. The concentration of itraconazole was quantified with UV at a maximum wavelength of 254 nm.

For the insoluble polymeric systems, discs of 18 mm diameter were punched out of the polymer fabrics (Verreck et al. 2003b). These discs were placed on top of a 20 mm diameter Finn Chamber (True Test, Nashville, TN, USA) which was covered with a 18 mm diameter filter paper disc (True Test, Nashville, TN, USA). A 20 mm ring net (type XK26, Pharmacia Biotech, Uppsala, Sweden) was then placed on top of the sample. The whole system was held together using a rubber band and fixed on top of a star-shaped magnetic bar with double sided adhesive tape. Drug release of samples containing itraconazole was measured in a 20 ml glass beaker containing 10 ml in a 20% HP-β-CD aqueous solution at pH 4 and room temperature. Stirring was performed with a magnetic stirrer at 300 rpm. Samples of 1 ml were taken from the dissolution medium after 15, 30, 60, 120, 180, 300, 420, 540, 660, 780, 900, 1020 and 1200 min. After sampling 1 ml fresh solvent was added. The release was measured in triplicate. The concentration of itraconazole was determined using a Waters HPLC (Waters, Milford, MA, US) with Millennium 32 Software. The column used was a RP Hypersil BDS-C18, 3 μm, 10 cm × 4 mm ID. The mobile phase consisted of 0.01 M tetrabutylammonium hydrogen sulphate in water and acetonitrile using an isocratic elution (33/67 vol%) at a flow rate of 0.7 ml/min. Concentration determination was performed using UV detection at a wavelength of 263 nm.

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