

The Impact of the Core-Shell Fiber Composition on the Properties and Stability of the Electrospun Films

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Purpose: This study aimed to develop and evaluate core-shell electrospun orodispersible films (ODFs) containing lopinavir (LPV) and ritonavir (RTV) for pediatric HIV therapy. The investigation focused on the impact of fiber composition and storage conditions on film morphology, physicochemical stability, mechanical properties, disintegration time, and drug dissolution profiles.

Patients and Methods: Core-shell ODFs were prepared via co-axial electrospinning using LPV and RTV solutions in Eudragit[®] E100 and Kollidon[®] VA64 matrices, respectively. Two configurations were tested, ie LPV in the core and RTV in the shell (LPV/RTV), and vice versa (RTV/LPV). Films were characterized using SEM, DSC, XRD, mechanical testing, disintegration and dissolution studies, and uniformity of content analysis. Stability was assessed under long-term (25 °C/60% RH) and accelerated (40 °C/75% RH) conditions over six months.

Results: LPV/RTV films were more homogeneous in their morphology and showed superior stability during storage compared to RTV/LPV films. SEM analysis revealed compact, well-aligned fibers in LPV/RTV mats, while RTV/LPV mats showed heterogeneous, ribbon-like structures. LPV/RTV films disintegrated within 100 ± 37 s, meeting requirements of very fast disintegration, whereas RTV/LPV films remained intact for 180 s. Compared to RTV/LPV films, LPV/RTV films showed greater uniformity in API content and stability over time, while both formulations exhibited slight, non-significant shifts in LPV/RTV ratios during storage. Dissolution profiles indicated enhanced release from RTV/LPV films, though structural degradation limited their stability. After storage, for both types of films, partial recrystallization of API was observed. The LPV/RTV films maintained their dissolution performance, whereas the RTV/LPV films showed significant deterioration.

Conclusion: Core-shell electrospun ODFs with LPV in the core and RTV in the shell demonstrated more homogeneous and resistant to storage-related changes, although the release of the active ingredients was characterized by slower dissolution. These findings support the potential of co-axial electrospinning for developing pediatric-friendly antiretroviral formulations.

Keywords: co-axial electrospinning, core-shell fibers, orodispersible films, stability studies, lopinavir, ritonavir

Introduction

Despite progress in antiretroviral therapy (ART) and a global decline in AIDS-related mortality, the human immunodeficiency virus (HIV) remains a significant public health concern. According to the World Health Organization (WHO),¹ an estimated 40.8 million people lived with HIV in 2024, including 1.4 million children under 15. Sub-Saharan Africa bears the greatest burden, home to approximately 90% of the 2.8 million children and adolescents living with HIV worldwide. Among this pediatric population, only 52% had access to ART, largely due to the limited availability of child-appropriate drug formulations.^{2–6}

Pediatric AIDS treatment currently includes protease inhibitors, which inhibit the viral protease enzyme, crucial to the generation of mature functional viral proteins, by binding to its active site. Examples of active pharmaceutical ingredients

(APIs) that belong to this class include lopinavir (LPV) and ritonavir (RTV), both listed on the WHO Model List of Essential Medicines for Children.^{2,3,7-9} They are co-administered in an LPV:RTV 4:1 ratio. Lopinavir is extensively metabolized by CYP3A4, which limits its efficacy, while ritonavir, a potent inhibitor of CYP3A4, reduces lopinavir metabolism, thus increasing its plasma concentration and increasing its efficacy. Currently available formulations combining lopinavir and ritonavir include coated tablets (100/25 mg and 200/50 mg), pellets (40/10 mg), and oral solution (80/20 mg per mL).^{7,10-12} Although multiple dosage forms exist, pediatric HIV therapy is hindered by poor palatability and limited swallowability. Large tablets are difficult for young children to swallow, and crushing them significantly reduces the bioavailability of both drugs. Best et al¹³ have observed a reduction of approximately 45% in the median area under the curve (AUC) for lopinavir and 47% for ritonavir in HIV-infected children when the tablets were crushed, as compared to their administration in whole form. This phenomenon may result from decreased drug exposure and irregular absorption, potentially leading to therapeutic failure.^{14,15} In the case of liquid dosage forms, which are generally recommended for pediatric use, the poor taste of syrups and the presence of alcohol and propylene glycol, toxic especially to infants, negatively affect adherence for both patients and caregivers.^{2,7,16-19} Thus, several attempts have been made to formulate pediatric-friendly dosage forms with lopinavir and ritonavir, such as 3D printed tablets and orodispersible tablets (ODTs).^{17,19}

It is widely recognized that the convenience and ease of administration associated with orodispersible formulations can improve patient acceptance and improve adherence to therapy. Due to fast disintegration in the mouth without the need for water, they are suitable for specific subpopulations, including pediatric patients and individuals with swallowing difficulties or dysphagia.²⁰ Among them can be distinguished orodispersible tablets and minitables, orodispersible granules and orodispersible films (ODFs).

The ODFs single or multilayer thin polymeric strips represent a novel orodispersible formulation suitable even for infants due to their precise and flexible dosing, ease of administration, and high acceptability. Studies conducted by Rodd et al.²¹ Orlu et al²² and Klingmann et al²³ have shown that ODFs were more convenient and easier to administer compared to oral drops or syrup, both in groups of young children, including infants, as well as their caregivers or medical personnel.

They can be manufactured by different methods, such as solvent casting – the most common technique, electrospinning, hot melt extrusion and printing technologies (ink-jet, flexographic and 3D printing).²⁴⁻²⁶

Electrospinning is a widely studied technique for producing fibrous orodispersible films from polymer solutions using a high-voltage electric field. When electrostatic forces overcome surface tension, a Taylor cone forms, and the resulting jet elongates, allowing solvent evaporation and fiber deposition on a collector. This method offers advantages such as high surface-to-volume ratio, improved drug solubility and dissolution through amorphous solid dispersions, incorporation of multiple APIs, and scalability.²⁷⁻³²

Conventional electrospinning typically utilizes a single-needle configuration to generate homogeneous nanofibers incorporating a single API. To facilitate the fabrication of more complex architectures capable of encapsulating two distinct APIs, coaxial electrospinning was introduced. This technique employs a concentric dual-needle spinneret that concurrently delivers different polymer solutions, thereby enabling the continuous formation of core-shell nanofibers.^{33,34} Co-axial electrospinning is utilized in drug delivery systems to mask the bitter taste^{35,36} of APIs, control release^{37,38} profiles, co-deliver multiple^{33,38,39} therapeutics within a single fiber, and produce orally disintegrating films. So far, core-shell electrospun ODFs have been formulated with a range of active compounds, including nifedipine and atorvastatin,⁴⁰ helcid and sucralose,⁴¹ chlorpheniramine maleate,³⁶ quercetin,⁴² and diclofenac sodium.⁴³

Electrospun orodispersible films demonstrate notable advantages over conventional manufacturing techniques, including faster disintegration and dissolution rates compared to cast and 3D-printed films. Nevertheless, their unique structural morphology, ie reduced fiber diameter, the high surface area and porosity of nanofibers make them more susceptible to moisture uptake, renders them more susceptible to environmental influences, potentially affecting their long-term stability. Literature reports confirm that these factors can affect fiber morphology, mechanical integrity, and API content uniformity over time, leading to variability in performance.^{25,44}

Therefore, stability studies are essential to evaluate the impact of temperature and humidity on physicochemical properties and dissolution performance. Without such testing, the risk of recrystallization, fiber fusion, and loss of

mechanical integrity cannot be adequately assessed, which may compromise product quality and therapeutic efficacy. Furthermore, regulatory guidelines such as ICH Q1A(R2)⁴⁵ require stability data for dosage forms. These studies are particularly critical for electrospun systems due to their hygroscopic nature and high surface area, which accelerate degradation processes compared to conventional solid dosage forms.

Considering the limitations associated with conventional electrospun orodispersible films, this study explores the development of core-shell electrospun films incorporating lopinavir and ritonavir in ratio 4:1. Electrospinning has previously been shown to facilitate the formation of amorphous solid dispersions, thereby enhancing the solubility and dissolution of poorly water-soluble drugs.⁴⁶ Based on these findings, LPV and RTV were incorporated into Eudragit E100 (E100) and Kollidon VA64 (KVA64) matrices, respectively. Core-shell fibers were fabricated using coaxial electrospinning, which employs a concentric dual-needle to simultaneously deliver two polymer solutions, enabling continuous formation of fibers with a distinct core-shell architecture.

The study evaluated the impact of core-shell composition and storage conditions on quality attributes, including fiber morphology, physicochemical stability of LPV and RTV, content uniformity, mechanical properties, disintegration time, and drug dissolution profiles. To the best of our knowledge, this is the first report on the development of orodispersible films containing LPV and RTV using the coaxial electrospinning technique, as well as on the evaluation of their long-term stability.

Materials and Methods

Materials

Lopinavir (LPV, (2S)-N-[(2S,4S,5S)-5-[[2-(2,6-dimethylphenoxy)acetyl]amino]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-(2-oxo-1,3-diazinan-1-yl)butanamide) and ritonavir (RTV, 1,3-thiazol-5-ylmethyl N-[(2S,3S,5S)-3-hydroxy-5-[[[(2S)-3-methyl-2-[[methyl-[(2-propan-2-yl-1,3-thiazol-4-yl)methyl]carbamoyl]amino]butanoyl]amino]-1,6-diphenylhexan-2-yl]carbamate) were purchased from Wuhan ChemNorm Biotech Co., Ltd., Wuhan, China. Polymers used as fiber matrices were Eudragit[®] E100 (cationic copolymer based on dimethylaminoethyl methacrylate, butyl methacrylate, and methyl methacrylate with a ratio of 2:1:1, E100), kindly donated by Evonik Industries AG, Essen, Germany, and poly(vinylpyrrolidone) (Kollidon VA64, KVA64), purchased from BASF, Ludwigshafen am Rhein, Germany.

Polyoxyethylene (10) lauryl ether (Brij-35; Merck Life Science, Darmstadt, Germany) was used as the dissolution medium component. Acetonitrile of gradient-grade purity was used as a component of the mobile phase for high-performance liquid chromatography (HPLC) analysis. Methanol and ethanol 96% (v/v) were utilized as solvents. Ultrapure water utilized in all experiments was obtained from an Elix 15UV Essential reverse osmosis system (Merck KGaA, Darmstadt, Germany).

Methods

Preparation of Solution for Electrospinning

Based on previously published⁴⁶ studies, Eudragit E100 and Kollidon VA64 were selected as polymeric carriers for the preparation of core-shell electrospun films containing lopinavir and ritonavir, respectively. Both polymers were dissolved in 96% (v/v) ethanol at ambient temperature. A 25% (w/w) E100 solution was prepared using a Heidolph MR Hei-Tec magnetic stirrer at stirring rate 250 rpm, while a 35% (w/w) KVA64 solution was obtained by manual dispersion of the polymer in ethanol using a glass rod in a beaker.

Drug-loaded electrospun solutions were prepared ex tempore by dissolving the appropriate amounts of LPV and RTV in polymer solutions. The concentrations of the APIs were selected to achieve an LPV:RTV mass ratio of 4:1 in the dry electrospun films. Consequently, the final concentrations of LPV and RTV in the solutions were set at 20% and 4.75% (w/w), respectively. The dissolution process was carried out in beakers with manual stirring using a glass rod to ensure homogeneity.

Coaxial Electrospinning

Electrospun films containing LPV and RTV were prepared using co-axial electrospinning in two distinct configurations, ie LPV/RTV – LPV solution in the core and RTV solution in the shell, and RTV/LPV – RTV solution in the core and LPV solution in the shell.

Drug-loaded polymer solutions were transferred into 20 mL syringes and connected through silicone tubing to a co-axial needle (SKE E-Fiber Accessories, SKE Research Equipment, Italy) consisting of an inner Gauge needle 20G (inner diameter: 0.584 mm; wall thickness: 0.152 mm) and an outer Gauge needle 14G (inner diameter: 1.6 mm, wall thickness: 0.254 mm), with the inner needle retracted by 1 mm relative to the outer needle.

The syringes were mounted on Ascor AP14I syringe pumps, which maintained a constant flow rate of 4 mL/h per channel. The positive electrode of a high-voltage power supply (SKE E-Fiber EF020) was connected to the co-axial needle, while the ground electrode was attached to a custom-made rotating drum collector covered with aluminum foil. The drum rotation speed was 50 rpm. The co-axial electrospinning scheme process is presented in Figure 1.

Electrospinning was performed at 30 kV with a 25 cm tip-to-collector gap. The process was conducted for a duration of two hours at an ambient temperature of $22 \pm 2^\circ\text{C}$ and a relative humidity of $35 \pm 6\%$. The total mass of fibers obtained in the process was approximately 4.1 g and 3.7 g for LPV/RTV and RTV/LPV formulations, respectively. The films were cut into rectangular strips of 2×3 cm, 1.5×1.5 cm or the dumbbell-shaped samples (Type 5), in accordance with the DIN EN ISO 527–3⁴⁷ standard for the determination of tensile properties of polymeric films, and placed in aluminum sachets.

Viscosity of the Electrospinning Solutions

The rheological properties of the drug-loaded polymer solutions were evaluated using a Haake VT 550 viscometer (Thermoscientific, Waltham, Massachusetts, USA) equipped with concentric cylinders, with an NV cylinder for a sample volume of 9 mL at 22°C . Dynamic viscosity was measured over a range of shear rates $10\text{--}300\text{ s}^{-1}$ to characterize the flow behavior. The viscosity of the tested samples at a shear rate of 300 s^{-1} was determined based on the formula:

$$\eta = k \times \gamma^{(n-1)}$$

where:

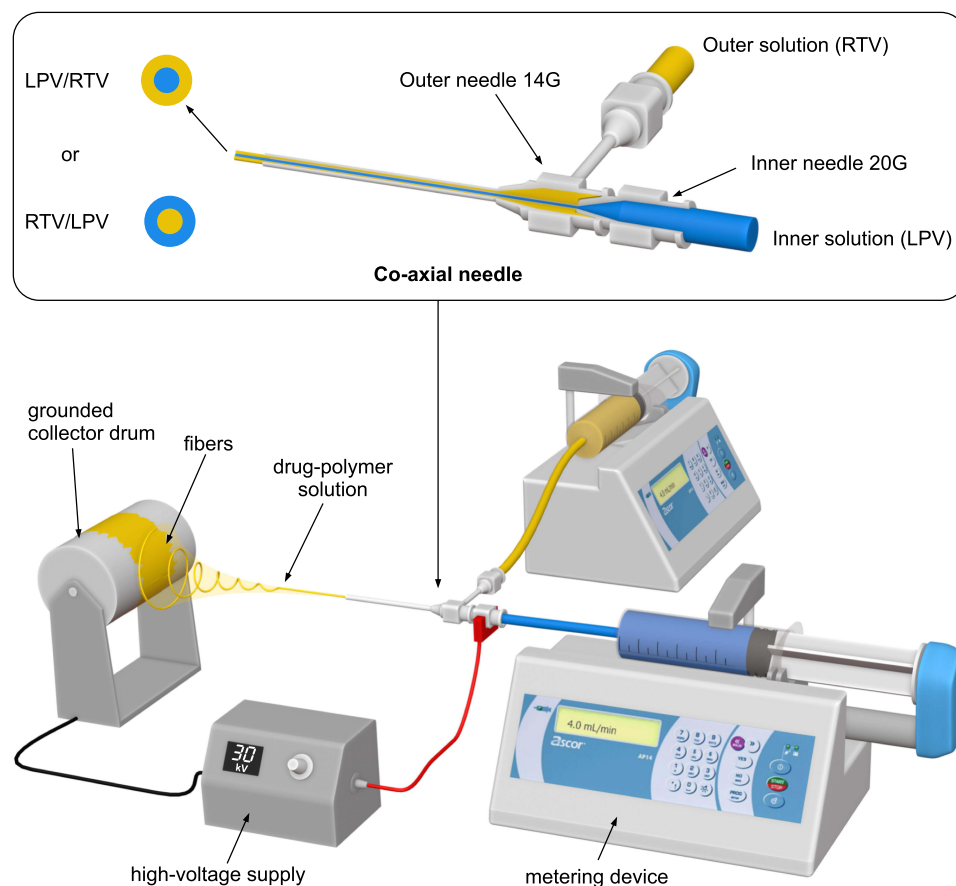


Figure 1 Scheme of the co-axial electrospinning process.

η – sample viscosity (mPa·s)

k – consistency coefficient (Pa·s ^{n})

n – characteristic flow index, which for shear-thinning liquids is <1

Each formulation was tested in triplicate, and the results are presented as mean values with the corresponding SDs.

Morphology Assessment

The morphological characteristics of the electrospun films were analyzed using a scanning electron microscope (SEM; Hitachi S-4700, Tokyo, Japan). The imaging was performed at an accelerating voltage of 20 kV. Film specimens, approximately 0.5×0.5 cm in size, were mounted onto aluminum stubs using conductive carbon adhesive tape. Prior to imaging, the samples were sputter-coated with a thin layer of gold to enhance conductivity and image quality. SEM micrographs were acquired at magnifications of 500×, 5000×, and 10,000×. The thickness of the fibers was determined from the SEM images obtained, utilizing the scaling tools available in the CorelDRAW software (Corel Corp., Ottawa, Canada). The mean fiber diameter was determined by measuring the thickness of 20 randomly selected individual fibers and calculating the arithmetic average.

Differential Scanning Calorimetry (DSC)

The thermodynamic behavior of raw APIs and electrospun films was investigated using a Mettler-Toledo DSC 3+ System (Greifensee, Switzerland). The precisely weighed samples were subjected to thermal analysis under an argon flow of 50 cm³/min within the temperature range of 20°C and 200°C at a constant heating rate of 10°C/min. All measurements were conducted in aluminum pans equipped with perforated lids. The melting temperature was identified as the onset of the endothermic peak during the initial heating cycle, while the glass transition temperature was determined from the second heating run. Measurements were carried out once per sample without replication.

X-Ray Diffraction (XRD)

The crystalline structure of raw APIs and electrospun films was analyzed using an Empyrean diffractometer (Malvern Panalytical). The measurements were conducted in Bragg-Brentano reflection geometry, employing Cu K α radiation generated by an LFF-type X-ray tube operating at 40 kV and 30 mA. The analysis was carried out for crystalline lopinavir and ritonavir, as well as for electrospun films. The diffraction patterns were recorded in a 2 θ range of 3° to 43°. Measurements were carried out once per sample without replication.

Mechanical Properties

The mechanical properties of the electrospun films were evaluated using a texture analyzer (EZ-SX, Shimadzu, Kyoto, Japan) equipped with a 20 N load cell. The samples were prepared in accordance with the DIN EN ISO 527–3 standard,⁴⁷ using Type 5 geometry. Each film sample was positioned between the instrument's grips and subjected to uniaxial tensile testing at a constant extension rate of 5 mm/min until it broke. Young's modulus, tensile strength (TS), and elongation at break (%E) were calculated using Trapezium X software (Shimadzu, Kyoto, Japan). Mechanical testing was performed on five replicates per formulation.

Disintegration Time

The disintegration time of the electrospun films was assessed using the slide frame and ball method.⁴⁸ Square film samples (3 × 3 cm) were mounted in a holder with a circular aperture with a diameter of 10 mm. A stainless-steel ball (diameter = 10 mm, mass = 3.5 g) was carefully placed in the center of each film. Subsequently, 900 μ L of distilled water, preheated to 37 °C, was applied to the film surface. The time required for the ball to penetrate through the film was recorded as the disintegration time. The test was conducted in six replicates for each formulation. The slide frame and ball method scheme is presented in Figure 2.

High-Performance Liquid Chromatography (HPLC)

For the quantitative assessment of LPV and ritonavir (RTV), HPLC was used, using a Jasco LCNetII/ADC system (JASCO Corporation, Tokyo, Japan) featuring a diode array detector, a built-in autosampler, and an InfinityLab Poroshell 120 EC-C18 column (100 × 4.6 mm, 4 μ m particle size; Agilent Technologies). Chromatographic separation was

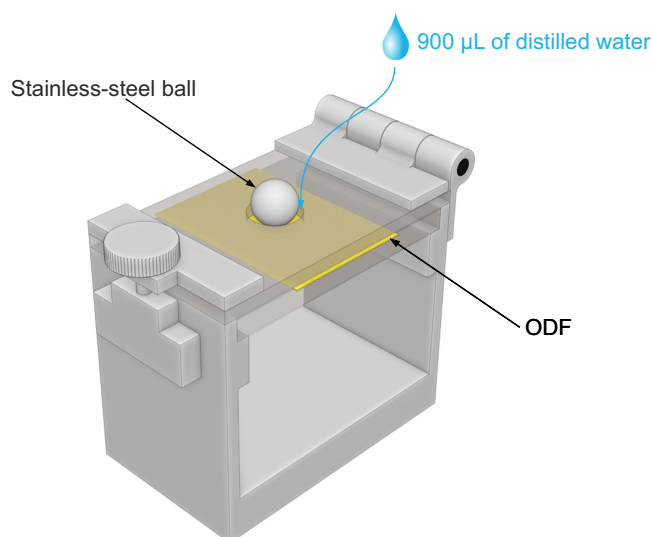


Figure 2 Scheme of the slide frame and ball method.

conducted with a mobile phase composed of water and acetonitrile in a 45:55 (v/v) proportion, maintained at a flow rate of 1.0 mL/min. The column was set at room temperature (around 22 °C). Each sample, with a volume of 10 µL, was injected, and the entire process took 5 minutes. Retention times were approximately 3.5 minutes for RTV and 4.0 minutes for LPV. UV detection occurred at 240 nm for RTV and 210 nm for LPV. The calibration curves exhibited linearity within the concentration ranges of 0.8 to 20.0 ng/µL for RTV and 4.0 to 30.0 ng/µL for LPV, achieving correlation coefficients (R^2) of 0.9998 for both substances.

Uniformity of Content

Precisely weighed samples of the electrospun films were measured using an analytical balance (MS105DU Mettler Toledo, Greifensee, Switzerland) and transferred to 15 mL Falcone tubes. Each sample was dissolved in a binary solvent system consisting of 5 mL of methanol and 5 mL of the relevant dissolution medium, specifically 0.06 M polyoxyethylene (10) lauryl ether (Brij-35) (3.756% w/w). The solutions were subjected to vortex mixing for 2 minutes at 2000 rpm using a Heidolph Reax Control vortex shaker (Schwabach, Germany). The samples were filtered through 0.22 µm nylon syringe filters, 0.22 µm, and subsequently analyzed by HPLC. The reported results represent the mean values of ten independent measurements, accompanied by standard deviations (SD).

Dissolution Study

Dissolution testing was conducted according to the USP 43–NF38 guidelines for lopinavir/ritonavir tablets,⁴⁹ using a Type II paddle apparatus (Hanson Vision G2 Elite 8, Chatsworth, CA, USA) equipped with a VisionG2 AutoPlus autosampler. The electrospun films containing 20 mg of lopinavir and 5 mg of ritonavir were placed on stainless-steel sinkers and immersed in 900 mL of a 0.06 M polyoxyethylene (10) lauryl ether solution (3.756% w/w) (pH 6.4), maintained at 37 °C. The paddle rotation speed was set to 75 rpm. Aliquots were withdrawn at predetermined time intervals (1, 3, 5, 10, 15, 30, 45, 60 and 90 minutes), filtered through 0.22 µm nylon syringe filters and analyzed using a validated HPLC method. Each formulation was tested in triplicate, and the results are presented as mean values with the corresponding SDs.

Analysis of Release Kinetics

Kinetic analysis was performed with the software RKinetDS 1.2⁵⁰ under R statistical environment 4.5.1⁵¹ using selected library of the models with possible approach to mechanistic interpretation, namely diffusion-based mechanisms, ie Higuchi, Korsmeyer-Peppas, Peppas-Sahlin; and erosion-based mechanisms: Hixson-Crowell and Hopfenberg. Fitting procedure was performed with nlopt optimization engine⁵² and with fitting tolerance criterion 1e-20.

Stability Study

The electrospun film samples were stored in hermetically sealed aluminum sachets and subjected to stability testing under both long-term (25 °C/60% RH) and accelerated (40 °C/75% RH) conditions using climate-controlled chambers (HP 105, Memmert, Schwabach, Germany) for a period of six months. The stability of the formulations was evaluated by monitoring changes in fiber morphology, physicochemical characteristics, mechanical properties, disintegration time, content uniformity, and dissolution profiles.

Statistical Analysis

Statistical analysis was conducted using Statistica 13 (StatSoft Polska Sp. z o.o., Kraków, Poland). The normality of the data distribution was assessed with the Shapiro–Wilk test and the homogeneity of the variances with the Levene test. Differences in fiber thickness and content uniformity in fresh and stored samples were evaluated using an unpaired two-tailed Student's *t*-test. Statistical significance was set at $p < 0.05$.

Results and Discussion

Based on previous published research,⁴⁶ particularly those concerning the improvement of drug solubility, and in alignment with the therapeutic 4:1 LPV to RTV ratio, the solutions selected for the formulation of core-shell electrospun films were 20% LPV in a 25% ethanolic solution of E100 and 4.75% RTV in a 35% ethanolic solution of KVA64. The viscosities of the solutions were adjusted to conform with the Ostwald–de Waele model, resulting in values of 111.1 ± 0.2 mPa·s for the LPV solution and 185.9 ± 1.5 mPa·s for the RTV solution at a shear rate of 200 s^{-1} , respectively. Those differences affected the efficacy of the electrospinning process. When the lower-viscosity LPV solution was positioned in the core layer (LPV/RTV configuration), the process operated smoothly without interruptions. In contrast, placing the higher-viscosity RTV solution in the core layer (RTV/LPV configuration) led to frequent needle clogging. This issue likely resulted from the combination of the higher viscosity of the RTV solution and the smaller diameter of the inner needle compared to the outer needle, which restricted flow and promoted fiber accumulation at the tip.

Morphology Assessment

All electrospun polymer mats used for film preparation exhibited a white, soft morphology. The LPV/RTV films showed a compact, layered structure with uniformly aligned fibers and no visible loose ends, indicating high deposition quality. In contrast, mats obtained from the RTV/LPV formulation displayed a non-layered, cotton-like morphology, characterized by loosely entangled, low-density fibers with a random spatial distribution.

The morphology and fiber thickness of the fabricated core-shell electrospun films were assessed using SEM imaging. As illustrated in the micrographs of the electrospun films, the formulations displayed fibers oriented in a random manner (Figure 3). The LPV/RTV fibers demonstrated greater thickness compared to the RTV/LPV formulation, with an average thickness measured at 1123 ± 389 nm and 986 ± 345 nm, respectively. This observation can be ascribed to the application of a shell layer solution exhibiting higher viscosity, and it is consistent with the existing literature, which indicates that increased viscosity of the shell solution typically results in thicker fibers.^{53,54}

The LPV/RTV films exhibited a smooth cylindrical structure of fibers with no observable bends or flattening. The resulting fibrous mat formed a dense and continuous network, although with a moderate degree of structural disorder. Only sporadic beads were observed within the fiber structure, in contrast to single fibers based on Kollidon, where the presence of the beads was much more frequent.⁴⁶ These findings confirm the positive influence of the coaxial electrospinning technique on fiber morphology.

In contrast, the RTV/LPV formulation produced more heterogeneous fibers, comprising smooth straight fibers with consistent diameters and tangled, irregular, and flattened fibers resembling ribbons. The ribbon-like shape of the fibers can be attributed to the presence of Eudragit in the outer layer of the fiber.^{46,55} A distinct delamination, with thin fragments branching from the main fiber, was also observed. In addition, localized fiber adhesion and fracture were observed within densely packed regions, indicating compromised structural integrity.

Following a six-month storage period, the LPV/RTV fibers demonstrated significant deformation and damage, particularly visible in samples kept under accelerated conditions. Observations revealed irregular linkages between

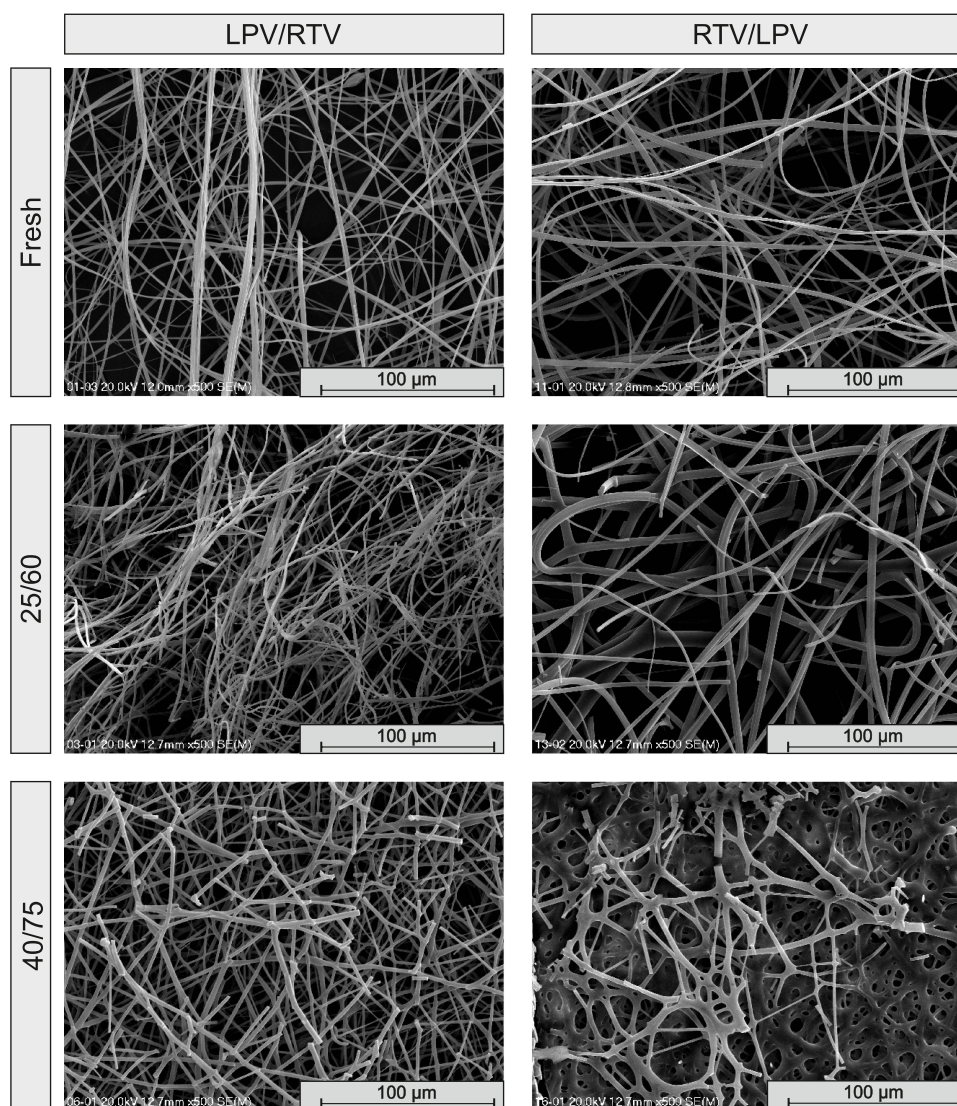


Figure 3 Scanning electron microscopy images of the LPV/RTV and RTV/LPV films directly after preparation and after six months of storage under long-term (25°C and 60% RH) and accelerated (40°C and 75% RH) conditions (magnification 500x).

residual fragments, characterized by incoherence and a deficiency in structural integrity. Certain fibers were destroyed, clearly indicating deterioration in material integrity and a notable influence of storage conditions. The average fiber thickness increased to 1588 ± 326 nm and 1774 ± 654 nm for the films LPV/RTV_25/60 and LPV/RTV_40/75, respectively.

Regarding the RTV/LPV_25/60 films, the samples showed a heterogeneous fibrous microstructure, characterized by the presence of thick and thin fibers forming an interwoven, albeit discontinuous, network. SEM imaging revealed fragmented and randomly oriented fibers, suggesting partial mechanical degradation and deterioration of structural integrity. Their average thickness increased 2.4 times to 2330 ± 713 nm. In contrast, samples exposed to accelerated conditions exhibited complete disruption of fiber continuity and architectural structure. The swollen fiber residues and the melted segments formed an irregular matrix, indicating structural degradation. The thickness of the remaining fibers was 2210 ± 740 nm. The increased thickness of the fibers after storage could be due to water absorption. The physical degradation of electrospun films under accelerated conditions may result from moisture-induced plasticization and crystallization. Their highly porous structure and large surface area promote rapid moisture uptake, which plasticizes amorphous polymer regions, lowers the glass transition temperature, and increases chain mobility. This facilitates

recrystallization of the amorphous drug into a more stable form, weakening the fibrous network and resulting in fiber fusion, structural collapse, or caking, as observed in degraded samples.

Differential Scanning Calorimetry

The DSC profiles of raw LPV, RTV, polymers E 100 and KVA64 as well as LPV/RTV and RTV/LPV are presented in Figure 4a. The DSC curve of LPV illustrates an endothermic melting with minima at 85 and 100 °C.^{17,56,57} For RTV the profile shows the melting at 127°C, which stays in agreement with the literature data.^{17,58} In case of E100 the small step observed in the DSC at c.a. 65°C can be attributed to glass transition.^{57,59} The DSC thermogram of KVA64 shows a shallow, broad endotherm between 37 °C and 105 °C due to loss of moisture.⁶⁰

It can be seen from the DSC curves of the LPV/RTV and RTV/LPV that during preparation of the mixture the properties of the ingredients are not fully preserved. The broad peaks with minima above 60 °C originate from LPV and RTV. The enthalpy of the transition observed in LPV/RTV (40 J/g) accounts for 30% of the total enthalpy of the processes observed for raw LPV and RTV (130 J/g). This suggests transformation of LPV and RTV from their crystalline to amorphous forms during sample preparation.

In Figure 4b, the DSC profiles of LPV/RTV and RTV/ LPV registered just after preparation compared to those measured after 6 months of storage in 25°C and 60%RH and in 40°C and 75%RH are presented. Regardless the composition of the films (LPV/RTV or RTV/LPV) storing for 6 months change significantly their thermal properties. It is clearly seen that after storing in long-term conditions in both samples probably partial recrystallization of LPV appears while in accelerated conditions it is not seen, probably because of higher humidity. In both cases melting enthalpy increases of about 60 J/g while storing in 25°C/60%RH (from 40 to 102 J/g in case of LPV/RTV and from 30 to 97 J/g in case RTV/LPV). It additionally confirms recrystallization. Storing in 40°C/75% also changes the melting enthalpy but in this case increase of enthalpy is different: for LPV/RTV it is 45 J/g (from 40 to 85 J/g) while for RTV/LPV it of 72 J/g (from 30 to 102 J/g). This observation suggests that the influence of temperature and humidity of storage is higher for the RTV/LPV films.

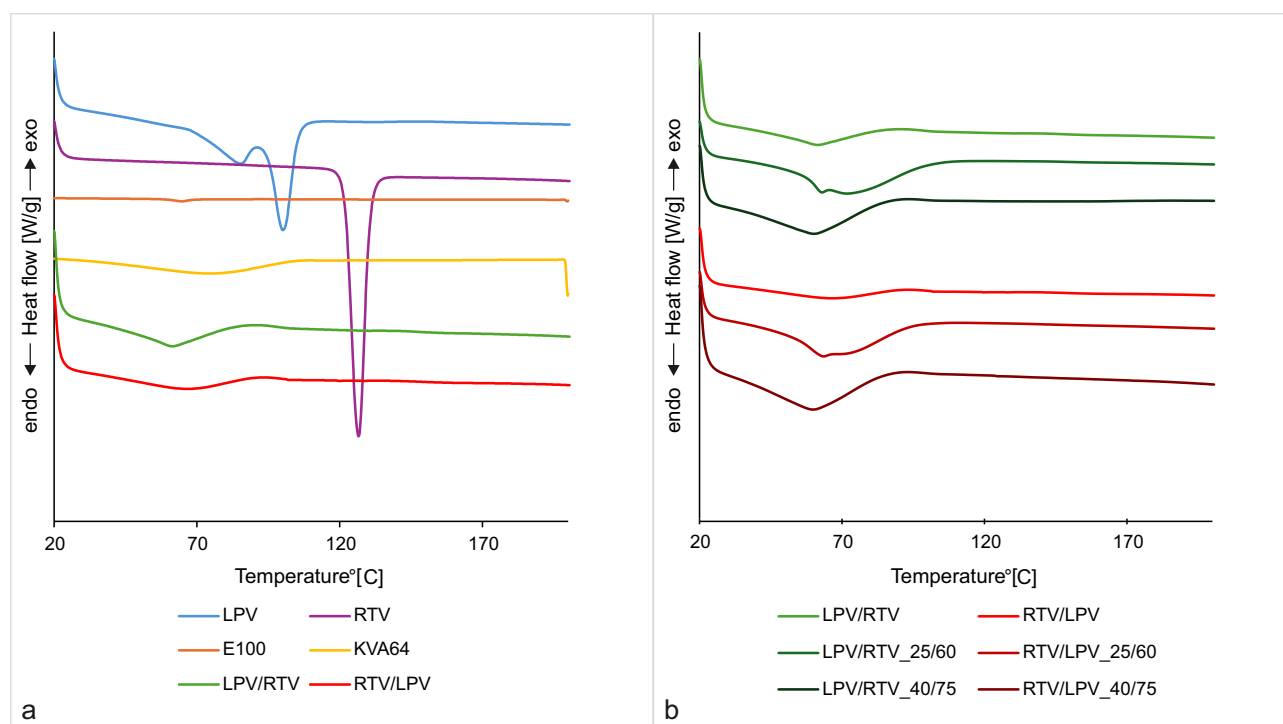


Figure 4 DSC thermograms of (a) individual components (LPV, RTV, E100, KVA64) and freshly prepared core-shell electrospun films (LPV/RTV, RTV/LPV), and (b) core-shell electrospun films after six months of storage under long-term (25°C and 60% RH) and accelerated (40°C and 75% RH) stability testing conditions.

X-Ray Diffraction

The XRD analysis was conducted on raw LPV and RTV, along with electrospun films, to characterize the molecular structure of the drug substances (Figure 5). This analysis also assessed how the electrospinning process and storage conditions influenced their crystallinity.

Lopinavir in its raw form manifests a crystalline arrangement, as demonstrated by its XRD pattern, which reveals distinct and intense peaks at 2θ angles of 6.6° , 7.7° , 8.6° , 9.7° , 10.6° , 12.5° , 14.8° , 15.6° , 16.5° , 18.3° , 19.0° , 19.8° , 21.7° , 22.7° and 26.4° (Figure 5 – blue line), indicating a well-ordered crystal lattice.^{56,57} Ritonavir is recognized for its conformational polymorphism, and the distinct Bragg peaks at 2θ values of 8.6° , 9.4° , 9.8° , 10.9° , 13.7° , 16.1° , 16.6° , 17.3° , 17.7° , 18.3° , 20.0° , 21.6° , 22.2° and 25.3° (Figure 5 – purple line) signify the existence of polymorphic form II, which aligns with previous research findings.^{58,61}

The broad halo and lack of Bragg peaks in XRD patterns confirm that co-axial electrospinning effectively induces amorphization of LPV and RTV. Additionally, stability assessments under both long-term and accelerated storage conditions revealed that the amorphous state maintained its physical stability over time, with no observable indications of recrystallization.

The discrepancies observed between XRD and DSC findings stem from their differing sensitivity levels and approaches to sample analysis. In the case of DSC, the sample undergoes complete melting, allowing for the assessment of the material's entire volume. Conversely, XRD evaluates only a small surface area using an X-ray beam, which can miss non-uniform features like crystalline domains. The sensitivity of XRD to crystalline substances is also lower than that of DSC, often failing to detect crystallinity present in amounts below a few percent, a challenge that does not impact DSC. Consequently, while DSC identifies a minor melting peak of lopinavir, XRD does not reveal any Bragg peaks indicative of crystalline phases in its diffraction pattern.

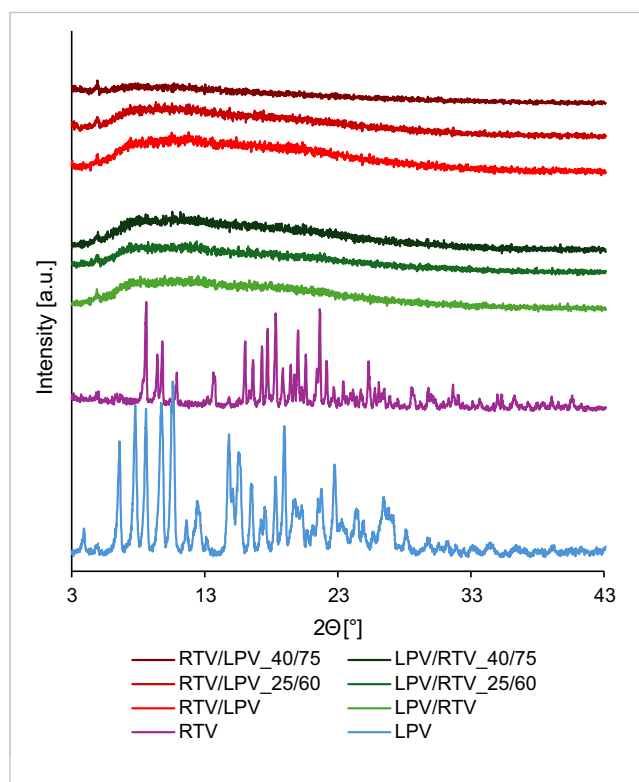


Figure 5 XRD patterns of LPV, RTV, and core-shell electrospun films (LPV/RTV and RTV/LPV), obtained immediately after preparation and after six months of storage under long-term (25°C and 60% RH) and accelerated (40°C and 75% RH) stability conditions.

Mechanical Properties

Mechanical characterization of the core-shell electrospun films indicated limited tensile strength and ductility across all electrospinning configurations. This was reflected in low values for the maximum breaking force, the nominal stress ($\sim 0.20 \text{ N/mm}^2$), and the elongation at the breaking. ($< 5\%$). Young's modulus values were $16.81 \pm 0.73 \text{ N/mm}^2$ for LPV/RTV and $11.96 \pm 5.92 \text{ N/mm}^2$ for RTV/LPV, confirming the films' low stiffness and mechanical robustness. These values are consistent with other reports on electrospun ODFs and nanofibrous mats.^{62,63}

Although the films did not meet the mechanical thresholds proposed by Visser et al⁶⁴ (tensile strength $> 2 \text{ N/mm}^2$, elongation $> 10\%$), they exhibited satisfactory handling during experiments, suggesting potential ease of use in patient administration. It is important to note that these criteria were originally proposed for solvent-cast ODFs, which possess a dense, continuous structure. Yao et al⁶⁵ emphasized that electrospun mats generally exhibit inferior mechanical performance compared to conventional films, electrospun films consist of randomly oriented nanofibers forming a highly porous network. This morphology inherently results in lower tensile strength compared to dense films, as mechanical failure primarily occurs through disruption of fiber-to-fiber junctions and fiber separation rather than fracture of a bulk material.

Mechanical evaluation of the stored films was performed only for the LPV/RTV_25/60 formulation. These samples exhibited reduced stiffness and durability, reflected by lower Young's modulus and tensile strength, ie $12.53 \pm 1.73 \text{ N/mm}^2$ and $0.12 \pm 0.07 \text{ N/mm}^2$, respectively, while ductility remained unaffected by storage conditions. LPV/RTV films stored under accelerated conditions and RTV/LPV films stored in both climatic chambers due to sample degradation and partial disintegration, as confirmed by morphological analysis. The partial dissolution observed in RTV/LPV samples after six months may be attributed to the presence of E100 in the shell layer, consistent with previous findings of structural deterioration in Eudragit-based fibers after just one month of storage at 40°C and $75\% \text{ RH}$.⁶⁶

Disintegration Time

In order to assess the disintegration time of the prepared core-shell orodispersible films, and given the lack of established pharmacopoeial methods and acceptance criteria for this form of dosage, a literature-based technique, specifically the slide frame and ball method, was utilized.⁴⁸ This method was selected due to improved sensitivity compared to PharmaTest®, Petri dishes and slide frames methods and the endpoint is clearly defined and independent of the operator. Moreover the slide frame and ball method is particularly recommended for research and development purposes, as it allows precise differentiation between ODFs formulations with minor variations in composition. The pharmacopoeial criterion for ODTs, ie disintegration within 3 minutes, was adopted as a reference.⁶⁷

The LPV/RTV films disintegrated in 100 ± 37 seconds on average. Rapid surface wetting, driven by the hydrophilic shell polymer KVA64,^{46,68} initiated immediate film disintegration upon contact with water and the steel ball. The complete disintegration of the ODFs was delayed, likely due to the presence of water-insoluble Eudragit in the fiber core.

In contrast, the RTV/LPV films remained intact throughout the 180-second test, what is critical drawback for ODFs. The absence of surface wetting and limited water penetration prevented mechanical disintegration, indicating that the hydrophobic fiber shell effectively restricted water access.^{46,69}

After six months of storage under long-term conditions, the disintegration time of the LPV/RTV films decreased to $58 \pm 8 \text{ s}$, likely due to structural changes in the electrospun fibrous mat observed in SEM images (Figure 3). Partial fiber degradation may have compromised the mechanical integrity of the films, accelerating disintegration. In contrast, in the case of the RTV/LPV_25/60 formulation, the disintegration time exceeded 180 seconds due to the absence of the surface wetting of the films by a water droplet, despite the decreased mechanical strength.

The disintegration time test was not conducted for films stored under accelerated conditions because of the inability to obtain suitable samples, resulting from partial dissolution of the samples, and the occurrence of the structural changes that rendered the films brittle and shell-like.

Uniformity of Content

It is crucial to guarantee consistent content in APIs to uphold the quality, safety, and therapeutic efficacy of pharmaceutical products. Additionally, for formulations containing lopinavir and ritonavir, the mass ratio of these two drug substances plays a significant role in ensuring the treatment's effectiveness.

The findings on the uniformity of LPV and RTV content analysis in freshly prepared and stored films are presented in Table 1. The LPV content was significantly higher in the LPV/RTV films compared to the RTV/LPV films ($p = 0.0000$). In contrast, the RTV content in both formulations did not show significant differences ($p = 0.6838$). Consequently, the LPV to RTV ratio was 4.4:1 in the LPV/RTV films and 3.7:1 in the RTV/LPV films. Furthermore, the LPV/RTV films exhibited a lower variability in the content of both APIs compared to the RTV/LPV formulation, as evidenced by lower values of relative standard deviation (RSD). Higher variability in content of the RTV/LPV films can potentially be ascribed to the obstruction of the core needle during the electrospinning process. Despite the observed differences in content and variability among the analyzed formulations, the content of LPV and RTV in individual units of both types of film remained within $\pm 15\%$ of the mean content.

After six months of storage under long-term and accelerated conditions, the LPV content in the LPV/RTV films increased to $332.7 \pm 15.2 \mu\text{g/mg}$ ($p = 0.0896$) or decreased to $301.7 \pm 26.7 \mu\text{g/mg}$ ($p = 0.0565$), respectively. Nevertheless, these differences were not statistically significant. Regarding the RTV, its content in films stored at 25°C and 60% RH has remained nearly the same as that of fresh samples ($p = 0.3524$). However, a significant reduction was observed in films subjected to accelerated conditions, with concentration diminishing to $66.3 \pm 5.9 \mu\text{g/mg}$ ($p = 0.0073$). As a result of alterations in the content of both APIs, the LPV:RTV ratio exhibited a slight increase, reaching values of 4.7:1 in films stored under long-term conditions and 4.6:1 in those subjected to accelerated conditions. Variability within the samples also increased, as confirmed by higher RSD values (Table 1).

For the RTV/LPV_25/60 and RTV/LPV_40/75 film samples, the LPV content significantly increased by more than 10% (p values 0.0076 and 0.0058, respectively), whereas the RTV content significantly decreased by 18% ($p = 0.0000$) and 13% ($p = 0.010$), respectively. Consequently, the LPV:RTV ratio experienced an increase relative to the fresh samples, achieving ratios of 5.0:1 in films stored under long-term conditions and 4.6:1 in those maintained under accelerated conditions. The variability among samples of films stored at 25°C and 60% RH was consistent with that of

Table 1 Results of Content Uniformity Test in Fresh Core-Shell Films ($n=10$)

Formulation	API	Parameters				
		Content [$\mu\text{g/mg}$]	SD [$\mu\text{g/mg}$]	Content [$\mu\text{g/cm}^2$]	SD [$\mu\text{g/cm}^2$]	RSD [%]
LPV/RTV	LPV	321.1	13.8	1412.8	60.5	4.3
	RTV	72.5	2.7	319.0	11.9	3.7
LPV/RTV_25/60	LPV	332.7	15.2	1463.9	66.9	5.2
	RTV	71.3	3.0	313.7	13.2	4.1
LPV/RTV_40/75	LPV	301.7	26.7	1327.5	117.5	8.1
	RTV	66.3	5.9	291.7	26.0	8.8
RTV/LPV	LPV	262.6	21.2	1050.4	84.8	8.1
	RTV	71.6	6.1	286.4	24.4	8.6
RTV/LPV_25/60	LPV	291.5	23.4	1166.0	93.6	8.0
	RTV	58.7	6.3	234.8	25.2	10.7
RTV/LPV_40/75	LPV	288.5	17.1	1154.0	68.4	5.9
	RTV	62.4	4.2	249.6	16.8	6.8

fresh films, whereas the variability of films stored under accelerated conditions was reduced, as indicated by lower RSD values.

Dissolution Study

A comprehensive examination of the release profiles of LPV and RTV from electrospun films, along with the dissolution rate of crystalline API, demonstrated the influence of the core-shell composition on the dissolution profiles (Figure 6).

In the RTV/LPV formulation, the release of LPV after a mere 3 minutes was observed to be three times greater compared to that of the LPV/RTV formulation and the dissolved drug, and after 15 minutes, the amounts released amounted to 25.1%, 8.8%, and 14.3%, respectively. By the end of the test, LPV release from RTV/LPV films attained a level of 34.3%, which represents an increase of 50.9% and 13.3% over the LPV/RTV films and the dissolved drug, respectively (Figure 6a).

An analogous trend was observed for RTV, in which the maximum release was recorded from RTV/LPV films. After 90 minutes, 32.0% of RTV was released, which is approximately threefold higher than the release from LPV/RTV films and exceeds by over 10% compared to the dissolved crystalline form (Figure 6b). The release profile of RTV from RTV/LPV films was comparable to the dissolution curve of the crystalline drug, with a similarity factor (f_2) of 82.25.

The results of the dissolution studies appeared to contradict those of the disintegration study. RTV/LPV films exhibited a longer disintegration time due to the poor wettability of insoluble Eudragit E; however, a higher amount of dissolved APIs was observed in the RTV/LPV formulation. It should be noted that the disintegration tests were conducted in the presence of water, whereas the dissolution media contained Brij 35. As a surfactant, Brij 35 acts in two ways: it enhances the solubility of the APIs and increases film wettability. The RTV shell layer, which contains KVA64—a water-soluble polymer—transforms into a viscous hydrogel during dissolution, thereby reducing the dissolution rate of the APIs. In contrast, the LPV shell layer behaves like an insoluble network that can be penetrated by the dissolution medium containing surfactant, which facilitates API dissolution. For these reasons, in both formulations, film residues persisted in the sinkers after the test, and undissolved crystalline LPV and RTV were observed at the base of the vessels, potentially contributing to incomplete drug release. After a storage period of six months under both long-term and accelerated conditions, the dissolution test conducted on the LPV/RTV films demonstrated that the dissolution profiles of LPV and RTV remained comparable to those of the films evaluated soon after preparation (Figure 7), as evidenced by the values of f_2 between 77.2 and 89.5. The amounts of both APIs released from the LPV/RTV_25/60 and LPV/RTV_40/75 films after 90 minutes were similar to those of fresh films, averaging 10.8% and 16.4% for LPV, and 8.4% and 14.8% for RTV, respectively. The increase in release observed from samples stored under accelerated conditions may be ascribed to the partial degradation of fibers.

For the RTV/LPV formulation, films exposed to accelerated storage conditions experienced partial dissolution, resulting in the formation of a crust-like layer, which impeded the feasibility of collecting testing samples. Consequently, dissolution studies were conducted on films stored under long-term conditions. The amount of LPV released from the stored films after a 90-minute period showed approximately a 40% decrease when compared to freshly prepared films (Figure 7a). In contrast, the amount of the RTV released from RTV/LPV_25/60 films after 90 minutes was nearly 1.3 times greater than that from films assessed shortly after preparation (Figure 7b).

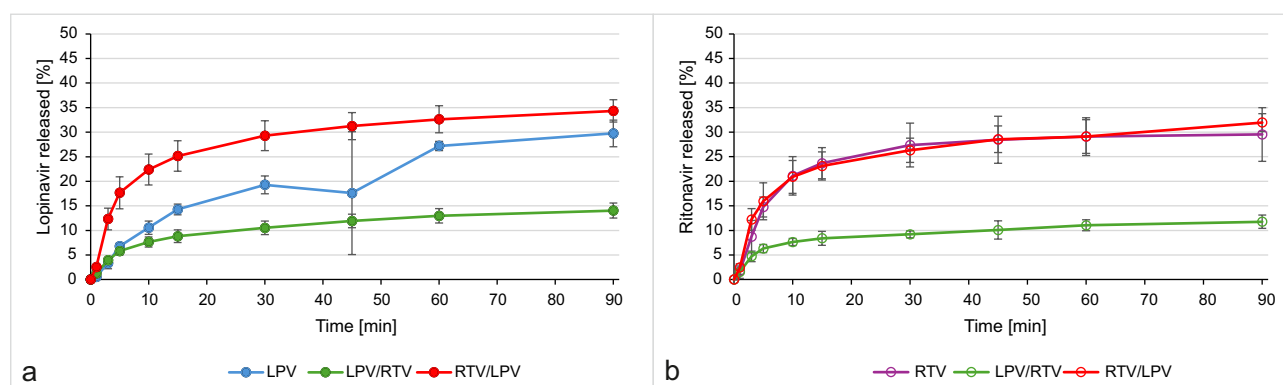


Figure 6 Dissolution and release profiles of (a) lopinavir and (b) ritonavir from LPV/RTV and RTV/LPV films directly after preparation.

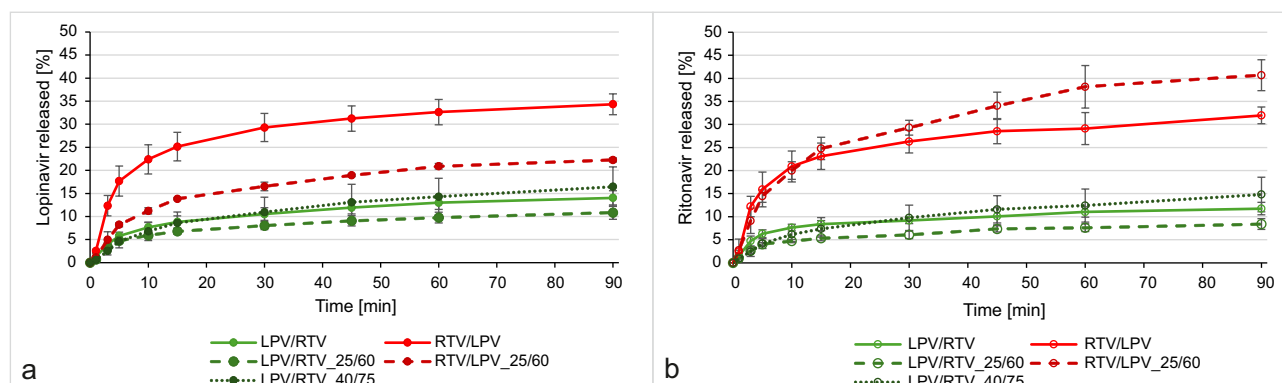


Figure 7 Release profiles of (a) lopinavir and (b) ritonavir from LPV/RTV and RTV/LPV films after six months of storage under long-term (25°C and 60% RH) and accelerated (40°C and 75% RH) stability conditions.

Analysis of Release Kinetics

The results of the release kinetics analysis are presented in Table 2. The dominating model was found to be Hopfenberg model originally developed for description of the release from the well-established geometries: slab, sphere and cylinder. The relevant geometry is established with pre-determined value of $n = 1, 2$ or 3 . In our analysis, the “ n ” parameter was also a subject of fitting, therefore its values were found to be different from any physics-related established values for the original model. The conclusion is that these results suggest a strong contribution of the erosion process to the drug

Table 2 Kinetic Parameters of Drug Release Profiles (RMSE – Root Mean Squared Error; R2 Adjusted – Adjusted Coefficient of Determination)

	Formulation	Model	Model Parameters	Goodness of Fit
LPV release	LPV/RTV	Hopfenberg	$K = -0.66$ $n = -0.0378$	RMSE = 0.34 R2 adjusted = 0.9941
	LPV/RTV_25/60	Hopfenberg	$k = -0.77$ $n = -0.0273$	RMSE = 0.37 R2 adjusted = 0.9878
	LPV/RTV_40/75	Hopfenberg	$k = -0.21$ $n = -0.0597$	RMSE = 0.30 R2 adjusted = 0.9967
	RTV/LPV	Hopfenberg	$k = -0.89$ $n = -0.10$	RMSE = 1.61 R2 adjusted = 0.9786
	RTV/LPV_25/60	Hopfenberg	$k = -0.4137$ $n = -0.0709$	RMSE = 0.57 R2 adjusted = 0.9937
RTV release	LPV/RTV	Hopfenberg	$k = -1.88$ $n = -0.0249$	RMSE = 0.47 R2 adjusted = 0.9821
	LPV/RTV_25/60	Peppas-Sahlin	$k_1 = -18.84$ $k_2 = 20.23$ $n = 0.0530$	RMSE = 0.29 R2 adjusted = 0.9857
	LPV/RTV_40/75	Hopfenberg	$k = -0.22$ $n = -0.0516$	RMSE = 0.254949 R2 adjusted = 0.9970415
	RTV/LPV	Hopfenberg	$k = -0.10$ $n = -0.0897$	RMSE = 1.53 R2 adjusted = 0.9767
	RTV/LPV_25/60	Hopfenberg	$k = -0.30$ $n = -0.16$	RMSE = 0.7992 R2 adjusted = 0.9962

release process and yet due to the negative values of parameter “n”, it is necessary to investigate further the fact that complex fibers geometry might be a subject of different manner of mathematical description under the umbrella of Hopfenberg model.

Limitations

This study demonstrated the possibility of preparation core-shell electrospun films containing LPV and RTV manufactured by coaxial electrospinning; however, several limitations should be acknowledged. First, the long-term stability of the films remains a challenge. After six months of storage, particularly under accelerated conditions, significant structural degradation was observed, including fiber fusion, fragmentation, and loss of integrity, likely due to moisture-induced plasticization and recrystallization. These changes may affect mechanical properties and handling, even though dissolution profiles remained largely comparable. Second, complete drug release was not achieved in any formulation, as film residues and undissolved crystalline LPV and RTV persisted after dissolution testing, which may limit bioavailability. Finally, the packaging barrier properties were not optimized, and additional strategies such as improved laminates or desiccant systems should be considered to ensure stability under humid conditions.

Conclusion

This study highlights the composition of core-shell electrospun orodispersible films that influences their performance and stability. The films with lopinavir in the core and ritonavir in the shell (LPV/RTV) exhibited superior fiber morphology, mechanical integrity, faster disintegration, consistent drug content, and stable APIs release profiles, even after prolonged storage. In contrast, the RTV/LPV configuration showed a higher initial drug release but suffered from poor disintegration and structural degradation over time. After storage, for both types of films, partial recrystallization of API was observed, however the influence of temperature and humidity of storing was higher for the RTV/LPV films. Overall, coaxial electrospinning with LPV in the core and RTV in the shell presents a promising strategy for developing pediatric-friendly antiretroviral formulations.

Prospective research should focus on enhancing the palatability of orodispersible films through effective taste-masking strategies to improve patient adherence, particularly in pediatric populations. In vivo pharmacokinetic studies are essential to confirm the therapeutic efficacy and bioavailability of the developed formulations. In addition, investigations are needed into the scalability and manufacturing feasibility of co-axial electrospinning to support clinical and commercial applications. Furthermore, selecting packaging with improved barrier properties will be critical to enhance the stability of the films during storage and distribution.

Abbreviations

AIDS, acquired immunodeficiency syndrome; API, active pharmaceutical ingredient; ART, antiretroviral therapy; AUC, area under the curve; DSC, differential scanning calorimetry; E100, Eudragit E100; HIV, human immunodeficiency virus; HPLC, High-performance liquid chromatography; KVA64, Kollidon VA64; LPV, lopinavir; ODF, orodispersible films; ODT, orodispersible tablets; RMSE, Root Mean Squared Error; RTV, ritonavir; RSD, relative standard deviation; SEM, scanning electron microscope; SD, standard deviation; WHO, World Health Organization; XRD, X-ray diffraction.

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Disclosure

The authors report no conflicts of interest in this work.

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