Research Article

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Development of Mucoadhesive Nanofiber Platform Using Eudragit® E100 for Vaginal Application

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ABSTRACT

Electrospun nanofibers represent an excellent alternative to classical vaginal dosage forms due to flexibility, high loading capacity, high mucoadhesive strength and enhanced patient compliance. Eudragit®s, which are synthetic polymers composed of methacrylate monomers, are preferred for vaginal drug delivery. The aim of this study was to develop and evaluate Eudragit®-based nanofiber formulations for vaginal drug delivery. Nanofibers were fabricated using electrospinning. Eudragit® E100 was dissolved at varying concentrations in an an ethanol:dimethylformamide mixture (7:3, v/v). The polymer solutions were characterized in terms of surface tension, viscosity, and electrical conductivity. Thermal behavior of both the raw polymer and the nanofiber formulations was analyzed using Differential Scanning Calorimetry. Surface wettability was assessed by contact angle measurements using an optical tensiometer. The mechanical strength and mucoadhesive properties of the nanofibers were evaluated using a texture analyzer. The viscosity, surface tension and conductivity of the polymer solutions were found to be suitable for electrospinning. All formulations exhibited hydrophobic properties. Mechanical properties of formulations were increased with increasing polymer concentration. No statistically significant differences in mucoadhesion were observed among formulations. Based on comprehensive characterization, the E5 formulation exhibited the most favorable properties for vaginal use. It was seen that Eudragit-based formulations may be a potential platform for vaginal application.

Keywords: Electrospinning, Nanofiber, Eudragit, Vaginal drug delivery

1. Introduction

Vaginal drug delivery systems hold promising potential due to their ability to exert both local and systemic effects [1]. Traditional vaginal dosage forms such as gel, film, and tablet provide easy administration but have disadvantages such as spreading in the vagina, short retention time, leakage, and poor patient [2]. Advanced drug delivery platforms, including nanoparticles, liposomes, and dendrimers, have been widely investigated for vaginal administration [3]. Among different drug delivery systems, nanofibers are useful due to their high surface area/porosity, tunable release, mucoadhesive behavior, and local delivery in the vagina. [4-6]. Electrospun nanofibers have been used in vaginal applications for HIV prophylaxis, treatment of vulvovaginal candidiasis and bacterial vaginosis, and contraception [7-9].

Nanofibers are widely used in various biomedical applications such as drug delivery, wound healing, diagnostic applications, cell therapy, cancer treatment, tissue engineering and regenerative medicine [10, 11]. Nanofibers can be fabricated by multiple techniques, including solution blowing, centrifugal spinning, pressurized gyration, phase separation, and electrospinning [12]. Electrospinning is a highly adaptable and rapidly developing technique that has attracted great attention for its ability to produce nanofibers [13]. The electrospinning method consists of a high-voltage source, a spinning head, and a grounded collector [14, 15]. Nanofibers can be easily produced by electrospinning with synthetic or natural polymers [16]. Nanofibers are commonly produced from natural polymers such as zein, gelatin, chitosan and alginate, and also from synthetic polymers such as polyvinyl alcohol, polycaprolactone, polylactic acid, polylactic-co-glycolic acid, polyethylene oxide and polyvinylpyrrolidone [17-20]. Natural polymers offer high biodegradability, good interactions with mucosal surfaces, and easy chemical modification via functional groups, but these polymers also carry drawbacks such as impurity risk, potential immunogenicity, and limited mechanical strength [21-23]. On the other hand, synthetic polymers offer high purity and batch consistency, tunable mechanical and degradation profiles, excellent electrospinnability, and storage stability [24-26].

Eudragit® methacrylate copolymers are widely used in pharmaceutical formulations, offering pH-dependent solubility, strong film-forming properties, tunable release, and residence in mucosal drug delivery [27-29]. Cationic methacrylate polymer Eudragit® E100 (EE100) was selected as the polymer carrier for the preparation of electrospun nanofiber [30]. EE100 is a pH-dependent polymer and acts by swelling when the pH is lower than 5, aligning with the vaginal pH [31]. EE100 promotes electrostatic interactions with negatively charged mucin supporting electrostatic interactions and thereby enhancing mucoadhesion [32]. Enteric anionic Eudragit® polymers (L/S/FS) dissolve only above pH 5.5–7.0 and RL/RS types are insoluble and exhibit swelling. The hydrophobicity of EE100 provides an advantage for vaginal drug delivery applications, as it limits the rapid dissolution of the dosage form in vaginal fluids and thereby supports prolonged residence time. Małolepsza-Jarmołowska et al. developed a methylcellulose-based tablet formulation combining E100 with lactic acid. These formulations were showed gel at appropriate rates and possess an acid reserve capable of neutralizing the excess alkalinity present in the vaginal environment during severe vaginal infections [33]. Vedha Hari and colleagues used EE100 in nanoparticle production and converted it into an aerosol foam formulation for vaginal administration of efavirenz for pre-exposure prophylaxis of HIV infections [34].

The aim of this study was to develop and characterize electrospun nanofiber formulations containing different concentrations of EE100 for vaginal drug delivery. The effects of polymer concentration on the processability and functional performance of the nanofibers were investigated. Contact angles, mucoadhesive and mechanical properties of nanofibers were evaluated for vaginal use. The results of this work are expected to contribute to the design of mucoadhesive nanofiber systems for future drug-loaded vaginal formulations.

2. Material and Methods

2.1. Materials

EE100 was a generous gift from Evonik (Germany). N, N-Dimethylformamide (DMF) and ethanol were purchased from Sigma Aldrich (St. Louis, MO). All chemicals were of analytical grade.

2.2. Preparation of nanofiber formulations

Nanofiber formulations were prepared using the electrospinning method. Solvent system consisting

of a mixture of ethanol and DMF (7:3) was chosen to dissolve the polymers. To obtain electrospinning solutions, the polymer was dissolved to different concentrations at room temperature. Each formulation was coded according to polymer concentration. The codes, compositions, and processing parameters of the nanofiber formulations are presented in Table 1.

2.3. Characterization studies of polymer solutions

Prior to electrospinning, the polymer solutions were characterized for viscosity, conductivity, and surface tension. These factors affect the electrospinning process [35]. The viscosity of the polymer mixtures was measured using a Brookfield viscometer, a stress-controlled cone and plate rheometer [36]. All samples were measured at room temperature. A 0.5 mL sample was used for each measurement. Each rheological measurement of polymer solutions was repeated at least three times. The results are given as mean and standard deviation. Conductivity measurements of the polymer mixtures were carried out using a conductometer (Mettler Toledo Seven2Go S3). The conductivity probe was immersed in the sample, ensuring complete immersion [37]. Measurements were performed at room temperature. Each measurement was repeated three times for accuracy. Average conductivity values were recorded and expressed in microsiemens per centimeter (μS/cm). Surface tension was determined via the pendant-drop method (Attension, Theta Lite, Finland). The surface tension of the polymer solution was calculated using the Young-Laplace equation [38, 39].

2.4. Electrospinning process

Nanofiber formulations were prepared using a single nozzle equipment electrospinning (Inovenso Ltd, NE300, Turkiye). Electrospinning device allows precise control over processing parameters such as voltage, feed rate, and tip-to-collector distance. Polymer mixtures were loaded into the pump using 10 mL syringes. Different voltage values were tested to produce each formulation, and production was carried out at voltages where continuous jet formation occurred. During electrospinning, nanofibers were collected on a rotating drum collector, operated at a constant speed of 100 rpm to ensure uniform fiber deposition and orientation. Production parameters for each formulation are given in Table 1.

2.5. Thermal analysis of formulations

Differential Scanning Calorimetry (DSC, Shimadzu, DSC-60, Japan) studies were performed on polymer and nanofiber formulations. EE100 and E5 were heated to 300 °C at a rate of 10 °C/min under a nitrogen atmosphere [40].

2.6. Fourier-transform infrared (FT-IR) spectroscopy studies

FT-IR spectroscopy analyses of the EE100 and E5 fiber formulation were performed using FT-IR spectrometer with an ATR probe (Perkin Elmer, Spectrum 400, USA) [41].

2.7. Mechanical characterization

Mechanical characterization of the nanofiber formulations was performed using a Texture Analyzer (TA. XT Plus Texture Analyzer, Stable Micro Systems, UK) equipped with a mini tensile grip. Nanofibers were cut into 1 cm × 3 cm samples and attached to the apparatus. Stress–strain curves were used to determine tensile strength (MPa) and elongation at break (%). Each measurement was repeated three times [14]. The obtained values were used to study the effect of polymer concentration on the mechanical integrity of the nanofibers, a critical parameter for vaginal applications where flexibility and durability are required.

Table 1. The codes, compositions, and processing parameters of the nanofiber formulations

Codes	Polymer Concentration (%)	Voltage (kV)	Feed rate (mL/h)	Distance (mm)	Rotating Speed (rpm)
E3	30	19	1	125	100
E4	40	21.5	1.7	130	100
E5	50	20	1.7	135	100

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2.8. Contact angle measurements

Formulation contact angles were measured with Attension Theta Lite optical tensiometer. The nanofiber formulations were placed on a convex sample holder. A droplet of 5 µL distilled water was gently placed on the nanofiber surface, and the contact angle was calculated using device software [42].

2.9. Ex-vivo mucoadhesion studies

Mucoadhesion is a critical parameter for achieving prolonged residence time and therapeutic efficacy in vaginal drug delivery applications [4]. It provides a reliable and reproducible quantitative assessment of the bioadhesive performance of nanofiber systems. The work of mucoadhesion of formulations was measured using the TA.XT. Plus Texture Analyzer (Stable Micro Systems, UK) with cow vaginal tissue. Nanofibers were attached to the upper probe of the device and vaginal tissue was placed on the bottom. The mucoadhesion study was assessed using a cylindrical probe (12,5 mm diameter) under a probe force of 0,20 N, with a contact time of 150 s and a probe speed of 1,0 $mm.s^{-1}$ [43]. The work of mucoadhesion was calculated from the area under the force-distance curve [14]. The work of mucoadhesion was calculated from the obtained data using the formula $(mJ/cm^2) = AUC/(\pi r^2)$, after which the mucoadhesive characteristics were evaluated [44].

2.10. Statistical analyses

Experimental studies were repeated three times for each formulation, and the results are presented as mean ± SD. Statistical analysis was performed using Analysis of Variance (ANOVA). Graphpad Prism 8 (GraphPad Software, San Diego, CA) software was used for all statistical analyses.

3. Results and Discussion

Polymer solutions were evaluated for viscosity, conductivity, and surface tension to determine their suitability for electrospinning. Characterization results of polymer solutions were presented in Figure 1. It was observed that conductivity, viscosity, and surface tension values differed due to the polymer concentration.

A significant increase in viscosity values was observed as the EE100 polymer concentration increased. While the E3 (30% EE100) formulation had a low viscosity, the highest viscosity was observed in the E5 formulation ($p<0.05$). This indicates that the polymer chains interact more intensely in solutions. While high viscosity promotes thicker and smoother fiber formation during electrospinning, jet formation can be difficult at very high viscosities [45]. Conductivity values of the solutions decreased in parallel with increasing polymer concentration. While the highest conductivity (12.65 µS/cm) was measured in formulation E3, this value decreased to 10.24 µS/cm in formulation E5. High conductivity during electrospinning allows for more stable jet formation, while very low conductivity can hinder fiber formation.

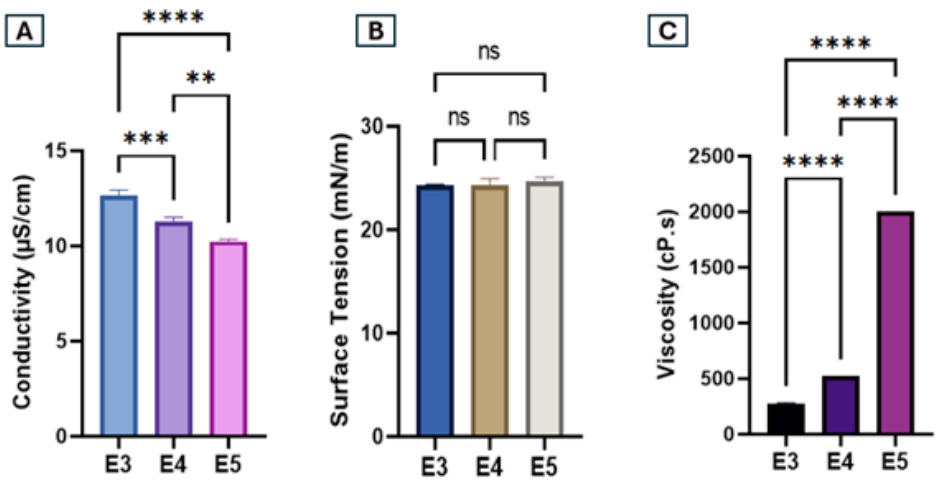

Figure 1. Characterization of polymer solutions: (A) Conductivity, (B) Surface tension and (C) Viscosity values of formulations (Mean ± SD, n=3, ns: not significant, **:p<0,01, ***:p<0,001, ****:p<0,0001).

Pablo Sánchez-Cid and colleagues observed that conductivity values generally decreased as polymer concentrations increased [46]. They suggested that, although the number of electrical charges in solution increases with higher polymer concentrations, the overall conductivity decreases due to restricted ionic mobility. In our study, this decrease may be attributed to limited ionic mobility caused by the increased polymer density. Surface tension is critical for droplet formation and jet initiation in electrospinning [47]. The surface tension values exhibited minimal variation across formulations, indicating that polymer concentration has a negligible effect on interfacial tension within the tested range. This stability is advantageous for maintaining consistent jet formation during electrospinning, regardless of formulation strength.

The thermogram of EE100 and E5 nanofiber is shown in Figure 2. Pure EE100 thermograms display a glass transition endotherm around 55–62 °C and a degradation-related peak near 237 °C, confirming its characteristic thermal behavior [48]. A shift or suppression of the peak was observed in the DSC thermogram of EE100 nanofibers.

The observed shift or suppression of peak in DSC studies of EE100 nanofibers may be attributed to physical modifications such as chain alignment resulting from the electrospinning process. Electrospinning can induce polymer chain stretching and orientation, which may reduce the mobility of amorphous regions and thus influence thermal transitions. This thermal behavior indicates that electrospun Eudragit® E100 nanofibers maintain structural integrity under physiological temperatures, supporting their potential as stable carriers in vaginal drug delivery applications.

FTIR spectroscopy is a widely used technique for assessment of intermolecular compatibility and investigating of degradation processes [49]. The FTIR spectrums of pure EE100 and as well as the nanofiber formulation are shown in Figure 3. The FTIR spectra of E100 shows the characteristic ester C=O stretch at 1730 cm^{-1} and aliphatic C–H stretches near 2950–2760 cm^{-1} [50]. Weak bands around 2820 and 2770 cm^{-1} attributed to the dimethylamino groups of the polymer are also visible, consistent with the structure of Eudragit E [51]. The E5 nanofiber formulation exhibits the same bands with EE100. Retention of the characteristic peaks in the formulation confirms that electrospinning did not change polymers' functional groups.

The mechanical properties of electrospun nanofibers are influenced by polymer molecular weight and concentration, processing parameters, and ambient conditions [52]. The mechanical characterization results of the formulations are presented in Figure 4.

For vaginal applications, an optimal mechanical profile is essential to ensure the dosage form maintains its structural integrity during insertion and remains throughout the residence in the vaginal cavity. The tensile strength and elongation at break values of nanofiber formulations increased depending on the EE100 concentration. The increase in the polymer concentration caused a change in the mechanical

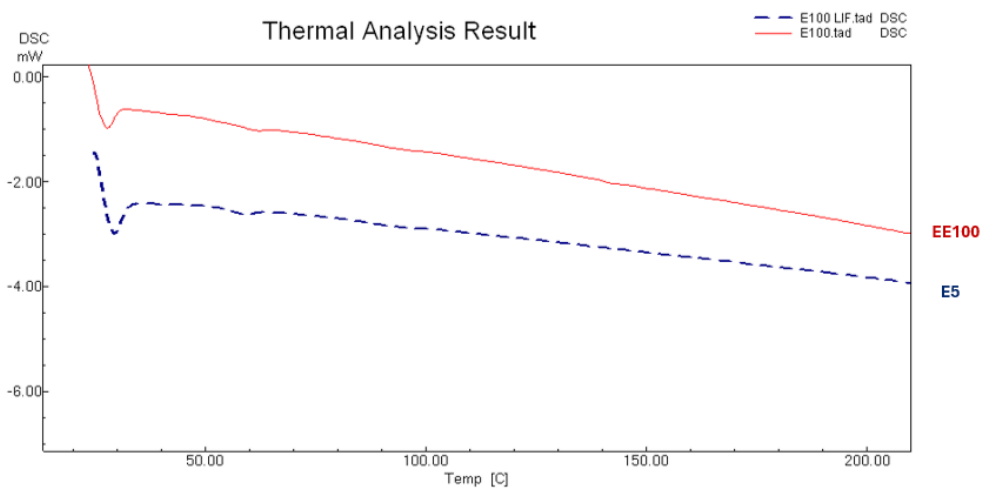

Figure 2. DSC thermograms of pure EE100 and E5 nanofiber formulation

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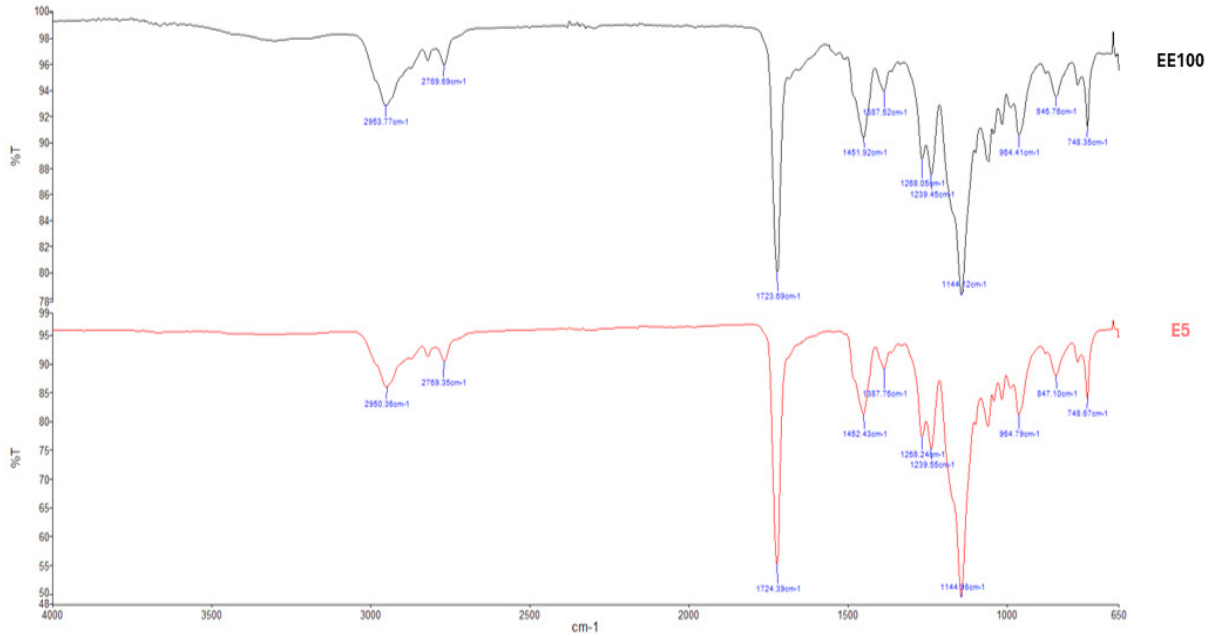

Figure 3. FTIR spectrum of pure EE100 and E5 nanofiber formulation

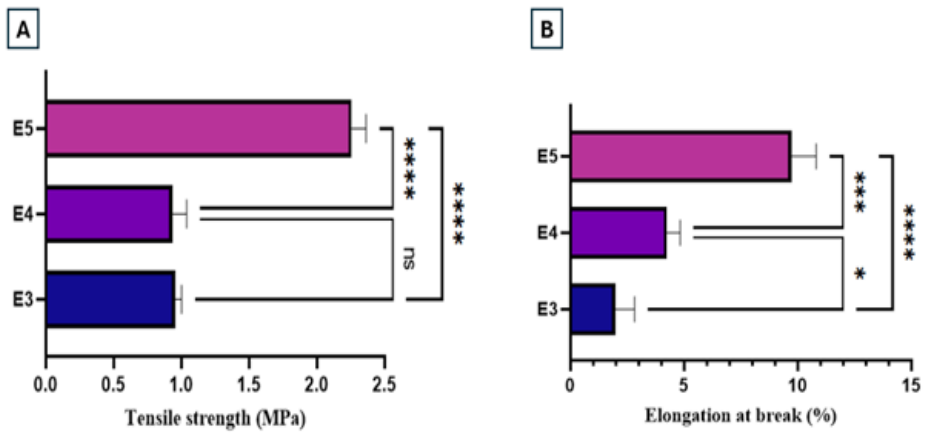

Figure 4. Mechanical characterization result of nanofiber formulations representing (A) tensile strength and (B) elongation at break of EE100-based nanofiber formulations. (Mean ± SD, n = 3, ns: not significant, *: p<0,05, ***: p<0,001, ****: p<0,0001).

properties of the nanofibers. Increasing polymer concentration resulted in enhanced mechanical strength and extensibility, particularly in formulation E5. Eudragit® polymers have been used in core-shell nanofibers that demonstrate superior mechanical performance in colon-targeted systems [53]. While literature specifically on EE100 in vaginal nanofibers is limited, these findings demonstrate that higher E100 concentrations in formulations enhance mechanical properties. As polymer concentration increases, fiber morphology becomes more uniform, bead defects are reduced, and tensile strength is achieved, which is important for maintaining structural integrity in vaginal applications.

The contact angle value indicates the wettability of the nanofiber. The contact angles of the formulations were found to be 118,58 ± 0,86°, 95.21 ± 3,84°, and 111,15 ± 3,45° for E3, E4, and E5, respectively. The contact angle value higher than 90° is indicative of the hydrophobicity of the polymer.

In their study, Abdel-Rahman et al. determined the contact angle of the nanofiber formulation produced with EE100 as 136° [54]. Formulation E3 exhibits a lower contact angle value. Among the formulations, the surface properties of E5 are at a moderate level in terms of hydrophobicity. Changing the polymer concentration affected parameters such as fiber diameter and surface roughness, thus altering the contact angle. The lower contact angle of the formulation suggests that it may have better interaction potential with the mucosal surface. Hydrophobic fibers exhibit slower initial wetting and delayed disintegration upon contact with vaginal fluids, preventing rapid drug release and preserving the structural integrity of the dosage form. This prolonged interaction with the mucosal surface provides longer residence time, ensuring effective formulation adhesion and prolonged drug release. Furthermore, the combination of hydrophobicity and mucoadhesive properties enhances adhesion, providing advantages for controlled and targeted vaginal use.

The work of mucoadhesion values of the nanofiber formulations are shown in Figure 5. The E4 formulation demonstrated the highest mucoadhesion that enhanced interaction with the vaginal mucosa. The work of mucoadhesion values obtained for E3, E4, and E5 formulations did not show a statistically significant difference ($p > 0.05$).

Mucoadhesion of formulations may not dependent on polymer concentration, but influenced by multiple factors including morphology, porosity and surface roughness [55]. The positive charge present in EE100 nanofibers may explain the stronger contact with the mucosa and provide the necessary residence time for the drug in the vagina [32]. Interactions with mucosa are critical for prolonging the residence time of the dosage form at the site of administration and thereby potentially enhancing local therapeutic efficacy. Cazorla-Luna et al. developed mucoadhesive bilayer films for vaginal administration of tenofovir [56]. The films exhibited a work of mucoadhesion effect of approximately 0.2–1.0 N.mm on bovine vaginal mucosa. Tuğcu-Demiröz's study on vaginal administration of benzydamine, liposomes displayed low mucoadhesion relative to liposomes dispersed in a mucoadhesive gel (lipogel) [57]. In our work, E3–E5 nanofibers exhibited higher mucoadhesion than liposomes but lower than mucoadhesive gels. Probe geometry, tissue source, and assay parameters can significantly influence the absolute values [58, 59].

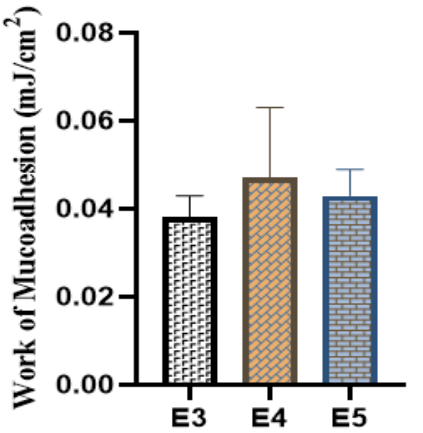

Figure 5. Work of mucoadhesion values of E3, E4, and E5 nanofiber formulations measured using cow vaginal tissue (Mean ± SD, n = 3).

Overall, the results demonstrate that polymer concentration is a key determinant of nanofiber performance, particularly in achieving optimal mechanical and mucoadhesive properties for vaginal applications. These findings provide a basis for future development of drug-loaded nanofiber platforms with targeted therapeutic potential.

4. Conclusion

In this study, EE100-based nanofiber formulations were successfully developed using the electrospinning technique for potential application in vaginal drug delivery. EE100 fibers with varying polymer concentrations were evaluated to assess their suitability as vaginal delivery systems. Characterization of the polymer solutions revealed that increasing the EE100 concentration led to higher viscosity and lower conductivity, while surface tension remained relatively constant. All formulations exhibited hydrophobic surface characteristics, as evidenced by contact angle values exceeding 90°. The mucoadhesive and mechanical properties of the nanofibers were also found to be dependent on EE100 concentration. Considering mechanical strength and the overall characterization studies, E5 formulation exhibited the most favorable characteristics among the tested formulations. These findings suggest that EE100-based nanofibers possess favorable characteristics for vaginal administration, and the E5 formulation holds promise as a potential carrier for the vaginal delivery of various therapeutic agents.

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Conflict of Interest

No potential conflict of interest was reported by the author(s).

Statement of Contribution of Researchers

Concept – S.S., F.N.T., F.A.; Design – S.S., F.N.T., F.A.; Supervision – F.N.T., F.A.; Resources – S.S., F.N.T., F.A.; Materials – S.S., F.N.T., F.A.; Data Collection and/or Processing – S.S., F.N.T., F.A.; Analysis and/or Interpretation – S.S., F.N.T., F.A.; Literature Search – S.S., F.N.T., F.A.; Writing – S.S., F.N.T., F.A.; Critical Reviews – S.S., F.N.T., F.A.

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