FABAD J. Pharm. Sci., 48, 2, 219-230, 2023
Doi: 10.55262/fabadeczacilik.1268029
RESEARCH ARTICLE

Evaluation of Mechanical and Mucoadhesive Properties of Polyvinyl Alcohol Nanofibers As Vaginal Drug Delivery System

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SUMMARY

Electrospinning is a versatile and inexpensive technique to produce nanofibers. Nanofibers can be an excellent alternative to classical dosage forms in vaginal applications due to high surface area/volume ratio, high encapsulation efficiency, and mucoadhesive properties. Polyvinyl alcohol (PVA) is a biocompatible, easily degradable, and flexible polymer with mucoadhesive properties used in industrial, commercial, and medical applications. The scope of this study is to characterize electrospun nanofibers produced with different PVA types for vaginal use. PVA nanofibers were produced using the electrospinning method. Nanofiber formulations were prepared by dissolving PVA in dimethylformamide (DMF): distilled water (1:1) solvent system. Nanofibers were produced with three different types of PVA at 5%, 7.5%, and 10% concentrations. The surface tension, viscosity, and conductivity properties of the polymer mixtures were measured for the electrospinning process and these parameters were found suitable for nanofiber production. While the viscosity increased with increasing polymer concentration, the surface tension values were found to be close to each other since the solvent system was the same. The mechanical and mucoadhesive properties of nanofibers were examined and compared. Mucoadhesive and mechanical properties of nanofiber formulations differed depending on molecular weight and electrospinning process. The nanofiber formulations produced with Polyviol 13/140 were found suitable for vaginal applications in terms of their mechanical and mucoadhesive properties. PVA nanofibers can be a good alternative as a drug delivery system in vaginal applications.

Key Words: *Nanofiber, Polyvinyl alcohol, Vaginal application, Electrospinning.*

Vajinal İlaç Taşıyıcı Sistem Olarak Polivinil Alkol Nanoliflerinin Mekanik ve Mukoadezif Özelliklerinin Değerlendirilmesi

ÖZ

Elektroçekim, nanoliflerin üretilmesi için çok yönlü ve ucuz bir tekniktir. Nanolifler, yüksek yüzey alanı/hacim oranı, yüksek kapsülleme etkinliği ve mukoadezif özellikleri nedeniyle vajinal uygulamalarda klasik dozaj formlarına iyi bir alternatif olabilir. Polivinil alkol (PVA), endüstriyel, ticari ve tıbbi uygulamalarda kullanılan, biyouyumlu, kolay parçalanabilen ve mukoadezif özelliklere sahip esnek bir polimerdir. Bu çalışmanın kapsamı vajinal kullanım için farklı PVA tipleri ile üretilen elektropsun nanoliflerini karakterize etmektir. PVA nanolifleri, elektroeğirme yöntemi kullanılarak üretildi. Nanolif formülasyonları, PVA'nın dimetilformamid (DMF):saf su (1:1) solvent sisteminde çözülmesiyle hazırlandı. Nanolifler, %5, %7,5 ve %10 konsantrasyonlarında üç farklı tipte PVA ile üretilmiştir. Elektroçekim işlemi için polimer karışımlarının yüzey gerilimi, viskozite ve iletkenlik özellikleri ölçülmüş ve bu parametreler nanolif üretimi için uygun bulunmuştur. Artan polimer konsantrasyonu ile viskozite artarken, solvent sistemi aynı olduğu için yüzey gerilimi değerlerinin birbirine yakın olduğu görülmüştür. Nanoliflerin mekanik ve mukoadezif özellikleri incelenmiş ve karşılaştırılmıştır. Nanolif formülasyonlarının mukoadezif ve mekanik özellikleri moleküler ağırlık ve elektroeğirme işlemine bağlı olarak farklılık göstermiştir. Polyviol 13/140 ile üretilen nanolif formülasyonları, mekanik ve mukoadezif özellikleri açısından vajinal uygulamalar için uygun bulunmuştur. PVA nanolifleri, vajinal uygulamalarda ilaç taşıyıcı sistem olarak iyi bir alternatif olabilir.

Anahtar Kelimeler: *Nanolif, Polivinil alkol, Vajinal uygulama, Electroçekim.*

Received: 20.03.2023
Revised: 05.04.2023
Accepted: 05.04.2023

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INTRODUCTION

Vaginal drug administration provides unique properties for women in local or systemic administration of drugs to achieve the desired therapeutic effects (Vermani, 2000). Several drugs, such as antibacterial, antifungal, antiprotozoal, antiviral, spermicidal agents, and hormones have been administered through the vaginal route (Das Neves, 2006). Various drug delivery systems are used vaginally, including tablets, capsules, gels, suppositories, creams, ointments, rings, films, and foam (Bachhav, 2009; Dobaria, 2009; Ya, 2010; Woodsong & Holt, 2015; Tunpanich, 2019). Conventional drug delivery systems such as creams, foams, and gels stay in the vaginal cavity for a relatively short time due to the self-cleaning effect and leakage in vaginal use (Acartürk, 2009). Nanofibers provide potential advantages for vaginal drug delivery with their large surface area, increased solubility, stability, good mechanical properties, high flexibility, soft, non-abrasive, and rapid or controlled drug release (Stojanov, 2022).

Electrospinning is an easy, versatile, economical, productive, and applicable in different areas method used to manufacture fibers sizes from nanometers to micrometers (Kanjwal, 2022). The electrospinning method is a system consisting of a nozzle, collector, and high-voltage source. A cone called a Taylor cone is formed by the effect of the electric field applied to the polymer solution at the nozzle tip. After a critical voltage value, the first jet is formed, and the solvent of the solution evaporates and collects on the collector (Nematpour, 2020). Electrospinning technique is preferred for biomedical applications such as filtration and shielding materials, electrical and optical applications, and sensors (Agarwal, 2008).

Different bioadhesive systems have been developed using mucoadhesive polymers to prolong the residence time in the vaginal application. For this purpose, different mucoadhesive polymers such as alginate, gum arabic, chitosan, polyvinyl pyrrolidone, poly (ethylene oxide), pullulan, hydroxypropyl cellulose, and pectin have been studied (Tuğcu-Demiröz, 2020; Cazorla-Luna, 2021; Martín-Illana, 2021). Products developed for vaginal application must be safe, effective, and acceptable enough for women to use for a long time. Polyvinyl alcohol (PVA) is hydrophilic, non-toxic, biocompatible, and water-soluble. Excellent chemical resistance, physical properties, biodegradability, high swelling in water, and biological fluids and elastic structure enable PVA to be used in different areas such as contact lenses, skin, artificial cartilage, and drug release systems (Ekrem, 2017). PVA nanofibers can be produced by electrospinning, but their applications are limited due to their high hydrophilicity (Park, 2010). Many studies have investigated the effects of PVA fibers on manufacturing conditions, molecular weight, and solution composition, but they have not been evaluated for vaginal use (Park, 2010; Ekrem, 2017). In this study, we aimed to produce different types and concentrations of PVA fibers and to determine the appropriate nanofiber formulation for vaginal application. Contact angles, mucoadhesive and mechanical properties of PVA nanofibers produced with electrospinning were evaluated for vaginal use.

MATERIALS AND METHODS

Materials

Different molecular weight PVA were used for the preparation of nanofibers. Polyviol 13/140 (49.000 Da), Polyviol 26/140 (80.000 Da), and Polyviol 40/140 (100.000 Da) were purchased from Wacker Chemie AG. N, N-Dimethylformamide (DMF) was purchased from Sigma Aldrich (St. Louis, MO). All chemicals were of analytical grade. Distilled water was used for all studies.

Preparation of the polymer mixture for electrospinning

PVA was dissolved in hot water at 90 °C stirring at 500 rpm. Afterward, DMF was added and mixed until a homogeneous mixture was obtained. Before electrospinning, solutions were kept in an ultrasonic bath to remove air bubbles. Different types of PVA and different concentrations were used for formulation production. Polyviol 13/140, Polyviol 26/140 and Polyviol 40/140 were coded as A, B, and C, respectively. The content and codes of formulations are shown in Table 1.

Table 1. Concentration and codes of PVA nanofiber formulations

Concentration	Polyviol 13/14	Polyviol 26/140	Polyviol 40/140
5%	A1	B1	C1
7.5%	A2	B2	C2
10%	A3	B3	C3

Characterization of electrospinning solutions

Viscosity, conductivity, and surface tension of the polymer mixtures were characterized to determine the electrospinnability of the polymer mixtures. The viscosities of the polymer solutions were measured using a cone-plate viscometer (Brookfield, DV-III Rheometer with spindle type CPE-41, USA). Rheological experiments were performed with 0.5 mL of polymer with spindle 52 at room temperature. The viscosities of polymer mixtures obtained at 20 rpm were compared. The conductivity meter was used for conductivity measurements by immersing the probe in the polymer solution at room temperature (Seven2Go Cond meter S3, Mettler Toledo, UK). The conductivity values of the solutions were measured as μs/cm. Measurements of surface tension of polymer solutions were measured using an optical tensiometer (Attension-Theta Lite, Biolin Scientific, Finland). Surface tension was calculated by the device software using the Young-Laplace equation.

Production of nanofibers by electrospinning method

Nanofiber formulations were prepared using a single nozzle equipment electrospinning process (Inovenso Ltd, NE300, Turkey). Polymer solutions were drawn into a syringe. Fibers were collected on a rotary cylinder at 500 rpm rotation speed. The process parameters such as feed rate, voltage, and distance tip to the collector were adjusted for each polymer solution and these are given in Table 2. All processes were performed at room temperature.

Table 2. Process parameters of nanofibers produced via the electrospinning method

Formulation Code	Voltage (kV)	Feed Rate (mL/h)	Distance (mm)	Rotating Speed (rpm)
A1	18.2	1	90	500
A2	18.5	0.9	95	500
A3	17.7	0.5	95	500
B1	14.5	1.5	115	500
B2	15.9	1.2	132	500
B3	14	0.5	145	500
C1	16.5	1	125	500
C2	15	0.7	148	500
C3	18	1	130	500

Morphological studies

Fiber morphology and average fiber diameter of the PVA fibers were characterized using scanning electron microscopy (FEI Company, Quanta 400 F, USA). The average diameter of PVA nanofiber was calculated with the ImageJ (National Institute of Health) program. SEM images of nanofibers were taken at 20000X and 40000X magnifications.

Thermal analysis of nanofibers

Thermal analysis of pure PVA and PVA nanofiber was performed using a differential scanning calorimeter (Shimadzu, DSC-60, Japan). For DSC analysis, 2 mg samples were weighed and heated 300 °C at the heating rate of 10 °C/min under a nitrogen atmosphere. The flow rate of nitrogen gas was 20 mL/min.

Fourier transform infrared (FT-IR) spectroscopy studies

Fourier Transform Infrared Spectroscopy (FT-IR) was performed to examine the chemical changes and interactions occurring in PVA nanofiber formulations. FT-IR analyses were conducted from 350 to 4400 cm^{-1} at room temperature with an ATR probe (Perkin Elmer, Spectrum 400, USA).

Mechanical properties of nanofibers

Tensile strength and elongation at break values of the formulations were investigated using a texture analyzer (TA.XTPlus Texture Analyzer, Stable Micro Systems, UK). Mechanical properties were analyzed using a tensile grip apparatus. Nanofibers were cut into rectangles of 3 cm x 1 cm and attached to the apparatus. Elongation at break (%) and tensile strength (MPa) values were determined using stress-strain graphs. Elongation at break and tensile strength are calculated according to the values on the stress-strain graphs at the point where the elongation is maximum and the rupture does not occur (Figure 1.). The value on the x-axis is elongation at break, and the value on the y-axis is tensile strength.

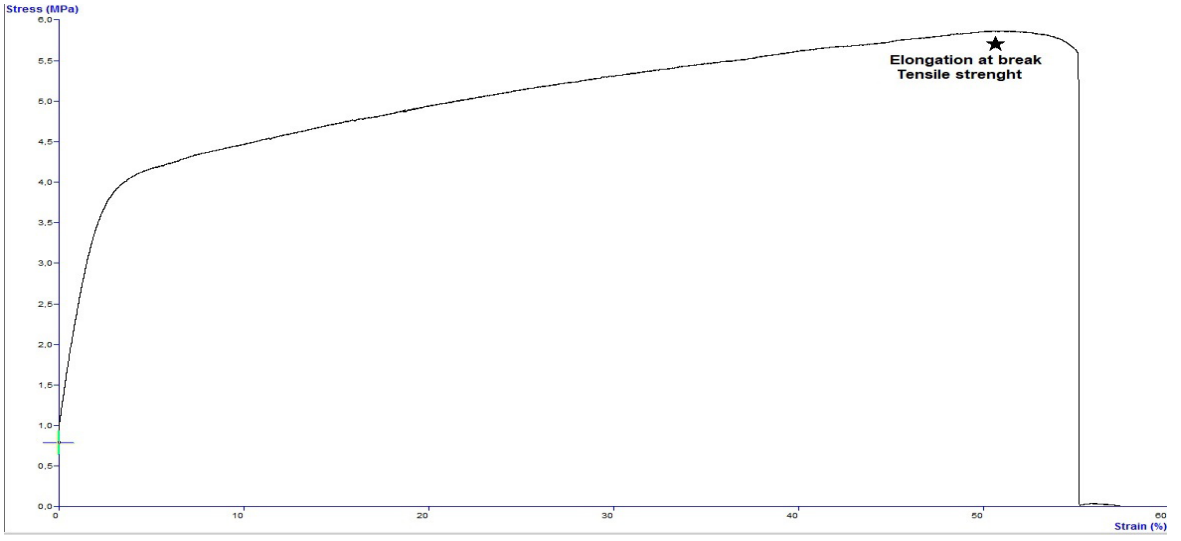

Figure 1. Stress-strain graphs of the tensile strength and elongation at break values obtained from texture analysis

Contact angle measurements

The Wettability of nanofiber was determined through contact angle measurements. Contact angles were measured by optical tensiometer using distilled water (Attension, Theta Lite, Finland). The nanofibers were stretched on a convex sample holder, and distilled water was dripped onto the nanofiber. The shape of the drop formed on the nanofiber and the contact angles were calculated using the software of the device.

Ex-Vivo Mucoadhesion studies

Mucoadhesion properties were determined by using the TA-XT Plus Texture Analyzer. The cow vagina was used as a model tissue for mucoadhesion studies. Nanofibers were attached to the upper probe of the device with double-sided tape, and vaginal tissue was placed on the platform at the bottom. In the mucoadhesion study, a probe speed of 1 mms^{-1}, a probe force of 0,2 N, and a contact time of 150 s with the mucosa were studied (Tuğcu-Demiröz, 2013; Tuğcu-Demiröz, 2015). The work of mucoadhesion was calculated from the area under the force-distance curve, and mucoadhesive properties were compared. Work of mucoadhesion values per cm^2 was calculated according to the formula (mJ/cm^2) = AUC/(πr^2) (Cevher, 2008).

Statistical analysis

GraphPad Prism version 7.0 (GraphPad Software Inc., San Diego, CA, USA) was used for all statistical analysis. $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Characterization of polymer solutions

The concentration of the polymer solution affects parameters such as viscosity, surface tension, and conductivity (İlbasmıs-Tamer, 2022). The physicochemical properties of the solutions directly affect the electrospinning process. As polymer concentration increased, the viscosity values of the solutions increased in all solutions. Selection of the appropriate solution concentration is one of the critical parameters to obtain nanofibers. High viscosity makes it extremely difficult for solutions to flow through the syringe needle to form nanofibers under an electrostatic force (Ding, 2010). Surface tension is influenced by the solvent system, and this parameter affects fiber formation and properties (Birer and Acartürk, 2022). Surface tension values of all polymer solutes were found to be similar, as seen in Table 3. The conductivity value was sufficient for the electrospinning process in all formulations. The highest conductivity value was found in A1, A2, and A3 formulations. Differences vin conductivity may have been observed due to the different molecular weights of the polymer used. Viscosity, conductivity, and surface tension measurements are important for the preliminary evaluation of polymer solutions in electrospinning method, but these parameters are not sufficient to decide on the optimum polymer solution properties. Many different parameters such as the evaporation of solvents and solvent systems affect the electrospinning process (Birer and Acartürk, 2022).

Table 3. The characterization results of viscosity, surface tension, and conductivity measurements (n = 3, mean ± SD).

Formulation Code	Viscosity (cP.s)	Conductivity (µS/cm)	Surface Tension (mN/m)
A1	41.42±2.87	174.73±4.97	43.74±0.20
A2	349.67±2.89	152.27±0.51	43.00±0.03
A3	542±0	129.47±2.26	42.11±0.08
B1	183.89±4.97	18.46±0.11	46.01±0.10
B2	699.11±2.87	32.31±0.30	43.01±0.02
B3	1740.16±93.91	36.49±0.23	44.78±0.07
C1	805.14±24.85	67.51±0.14	44.92±0.04
C2	1960±5.739	97.82±0.35	42.72±0.13
C3	2909±80.29	95.61±0.74	45.67±0.03

Morphological studies

SEM images demonstrated the successful production of nanofibers in formulations A3 and B3 using the electrospinning method (Figure 2.). Because the high molecular weight and concentration of PVA increase the viscosity, the solutions do not flow well from the injector and continuous fiber production becomes difficult. This situation was observed in group C formulations, and it was observed that high concentration nanofiber production did not occur. It was observed that the fibers were obtained wet due to the high-concentration and production parameters of the C3 formulation. No fiber structure was observed in C3. The average fiber diameter was found to be 159.416±65.015 nm for A3 and 167.171±39.016 nm for B3 formulation.

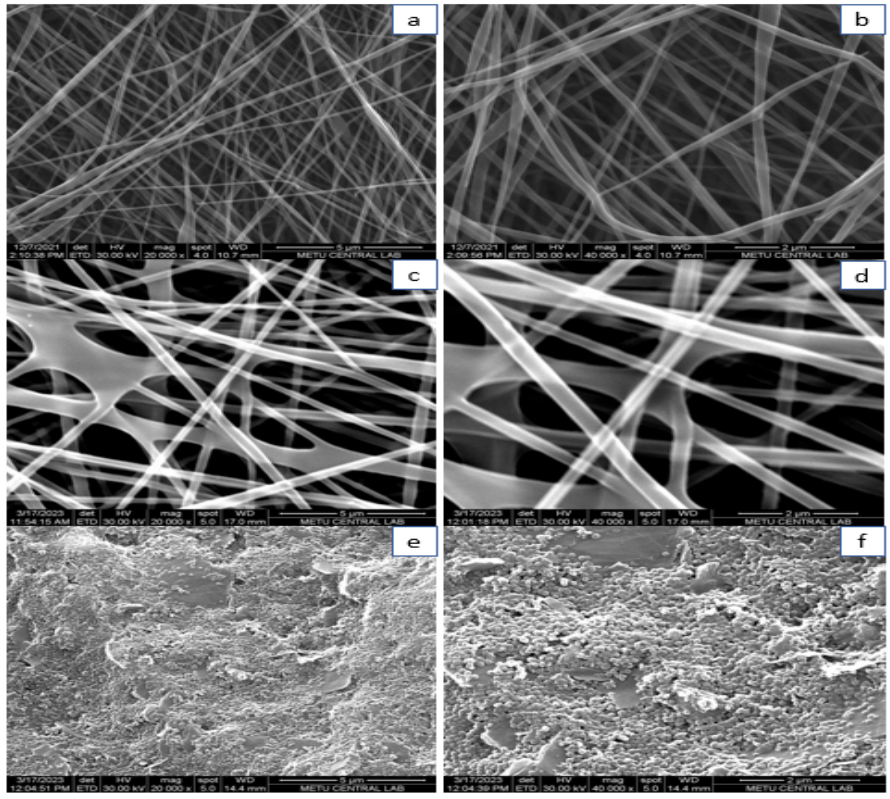

Figure 2. SEM images of A3 (magnifications a: 20000X, b: 40000X) and B3 formulation (magnifications c: 20000X, d: 40000X) and C3 formulation (magnifications e: 20000X, f: 40000X)

DSC analyses

We performed DSC measurements to investigate thermal behaviors such as melting, crystallization, and crystal structure formation. DSC thermograms of polymers and nanofibers are shown in Figure 3. DSC analysis showed an endothermic peak at 193,8 °C due to the melting point of PVA. Sudhamani et al. found the melting point of pure PVA as 202 °C in DSC analysis. In a study with PVA nanofibers, it was observed that the melting temperature in electrospun PVA fibers was almost unchanged compared to that of pure PVA (Kim, 2010).

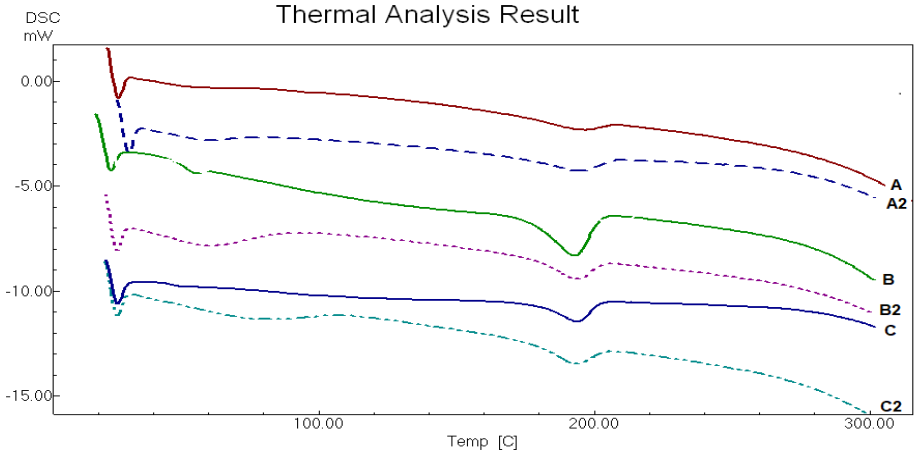

Figure 3. DSC thermogram of different pure PVA polymers (Polyviol 13/140 (A), Polyviol 26/140 (B) and Polyviol 40/140 (C)), and PVA nanofiber formulation

FT-IR analyses

Characteristic peaks of PVA were observed in the FT-IR spectra of the nanofibers. In addition, PVA absorption bands according to the literature (Ding, 2010). The spectrum of PVA and formulations showed a large band at 3268 cm^{-1} attributed to inter- and intramolecular hydrogen bonds in PVA (Reguieg, 2020). The peak at 2940 cm^{-1} is related to antisymmetric CH_2 stretching in the PVA sample, and two peaks at 1377 and 1432 cm^{-1} were CH-OH and CH_2 symmetric bending mode vibrations of PVA (Koosha, 2015). In the FT-IR analysis, specific PVA peaks were observed near 3320, 2940, 1420, and 840 cm^{-1}, consistent with the literature (Figure 4.). The observation of these FTIR bands in the spectra of the pure polymer and PVA nanofibers indicated that the electrospinning technique did not interfere with the chemical integrity of this polymer.

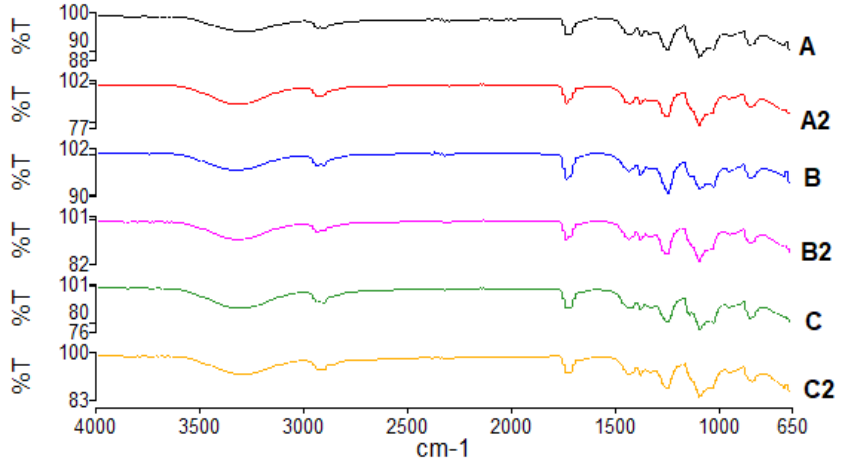

Figure 4. FT-IR spectrum of different pure PVA polymers (Polyviol 13/140 (A), Polyviol 26/140 (B) and Polyviol 40/140 (C)), and PVA nanofiber formulation

Mechanical properties of nanofibers

More flexible and elastic formulations are preferred for comfortable application in vaginal use (Szymańska, 2022). The mechanical properties of nanofibers are affected by different variables such as the rotation speed of the collector, the concentration of the polymers, the addition of crosslinking agents, and the change of production parameters (Sofi, 2020). The increase in the polymer concentration caused a change in the mechanical properties of the nanofibers. There is no increase in tensile strength was observed in formulations B1 and B2 with increasing PVA concentration. This difference was found to be statistically insignificant ($p>0.05$). Similarly, the difference between tensile strength value in A2 and A3 formulations was statistically insignificant ($p>0.05$). The tensile strength between A3 and B3 produced at high PVA concentration was found to be statistically insignificant ($p>0.05$). A3 was more suitable for electrospinning because it showed continuous production in the electrospinning method. The highest elongation at break values was found in the B3 formulation, whereas the highest tensile strength was found in the C3 formulation (Figure 5). Differences in the mechanical properties of nanofibers may be due to differences in the molecular weight of the polymer or changes in the structure of nanofibers by the electrospinning process. In the study of Koski et al., as the solution concentration increases, the fiber diameter and the distance between the fibers increase, and there is a gradual transition from circular to flat fibers (Koski, 2003). In addition, in low molecular weight samples, this transition from circular to straight fibers occurs at a higher concentration value than in high molecular weight polymers. As a result of such a change with the increase of polymer concentration and molecular weight in the fibers, changes in their mechanical properties may have been

observed. Ngadiman et al., in their study with PVA nanofibers, stated that although higher mechanical properties were obtained from high molecular weight PVA at high concentrations, the difficulty during the spinning process also increased (Ngadiman, 2015). It was stated that the high molecular weight and concentration of PVA would increase the viscosity. In this case, the solutions did not flow well, and extra force was required to extrude the solution from the syringe. Similar to these results, flow from the syrınge ıs dıffıcult at high PVA concentrations.

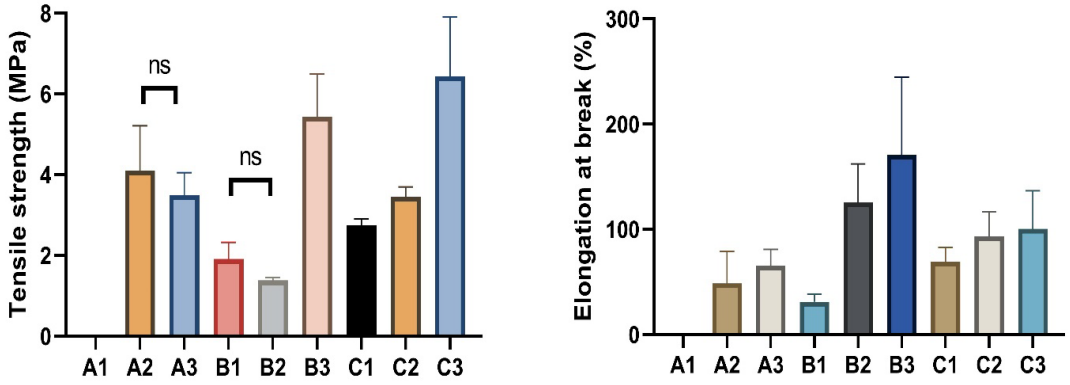

Figure 5. Tensile strength and elongation at break values of formulations (n=3, mean ± standard deviation, A1: Not detected, ns: not significant)

Contact angle measurements of nanofibers

The contact angle of all formulations was found to be less than 90 (Table 4.). These results show that the PVA nanofibers are hydrophilic structure. Among the fibers with high PVA concentration, the lowest contact angle was obtained in the A3 formulation. The difference in the contact angle in the fibers may be due to the changes in the fiber structure. Krogstad et al stated that PVA and PVP nanofibers for vaginal application should be wetted rapidly in order to provide nanoparticle diffusion into the vaginal tissue (Krogstad, 2017). Tissue regeneration and the biodegradation rate of the formulations will increase with their hydrophilic structure. Tuğcu-Demiröz et al. stated that the fibers for vaginal application could provide proper adhesion to the vagina due to their hydrophilic structure (Tuğcu-Demiröz, 2020). In another study shows that the complete wetting of the nanofibers releases the drug with a rapid effect, and this wettability increases the contact of the fibers with the environment (Tuğcu-Demiröz, 2021). The difference in molecular weight and solution concentration does not affect the hydrophilicity of PVA (Ngadiman, 2015).

Table 4. Characterization result of nanofiber formulations (n=3, *Not detected)

Formulation Code	Contact Angle (°)
A1	ND*
A2	17.29±2.69
A3	0±0
B1	0±0
B2	11.07±5.52
B3	19.62±5.21
C1	15.73±1.27
C2	24.78±0.71
C3	38.98±10.62

Mucoadhesion Studies

Work of mucoadhesion values of the nanofiber formulations are shown in Figure 6. Work of mucoadhesion increased by increase polymer concentration in C series formulations. The change in mucoadhesion in the A and B series may have resulted from the change in the structure of the fibers due to the electrospinning method. The highest work of mucoadhesion was found in B3 formulation. The difference between the mucoadhesion values of the A2 and A3 formulations and the B3 formulations was statistically insignificant ($p>0.05$). Mucoadhesion is an important factor for vaginal drug delivery and the use of mucoadhesive polymers such as carbopol, polyvinyl alcohol, hydroxyethyl cellulose, and chitosan provides various advantages such as prolonged residence time, improved location on the vagina, and controlled drug release rate (Zong, 2015). The fibers showed mucoadhesive properties for vaginal application.

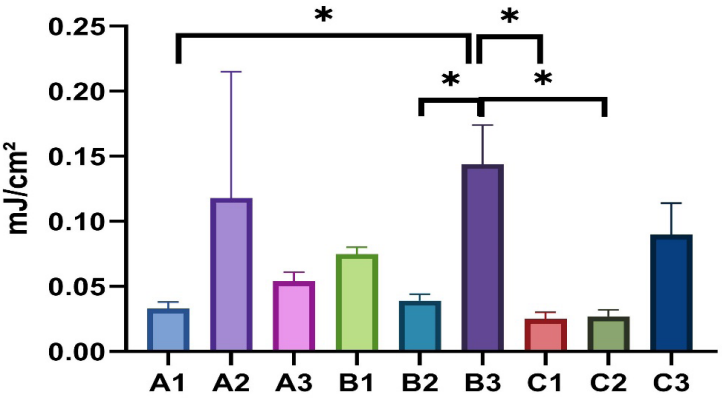

Figure 6. Work of mucoadhesion values of PVA nanofiber with cow vaginal tissue (n=3, mean ± standard deviation, *=$p<0.05$)

CONCLUSION

In this study, we evaluated PVA electrospun fibers as a vaginal delivery system for vaginal use according to their different concentration and different molecular weights. Structural properties, contact angles, mechanical and mucoadhesive properties of PVA nanofibers for vaginal application were investigated. PVA fibers have been shown to have different mechanical and mucoadhesive properties. We showed that the mucoadhesion and mechanical properties of PVA fibers differ depending on the concentration and molecular weight. The nanofiber prepared with PVA moleculer weight with 49.000 Da (Polyviol 13/140) showed continuous fiber production and showed sufficient mechanical and mucoadhesive properties for vaginal application. It was found that the A3 formulation among the vaginal nanofiber formulations prepared with PVA (Polyviol 13/140) has superior properties compared to the others. It was concluded that the A3 nanofiber formulation prepared using PVA (Polyviol 13/140) could be a promising alternative dosage form for the vaginal delivery of different drugs.

ACKNOWLEDGEMENTS

This study was supported by the Scientific Research Fund of Gazi University (BAP Project No: 02/2020-17).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

SS: Conceptualization, Methodology, Writing, Original draft preparation. **FTD:** Corresponding Author, Supervision, Writing, Reviewing and Editing.

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