

ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE
ENGINEERING AND TECHNOLOGY

**DONEPEZIL HCl LOADED ELECTROSPUN FIBERS FOR TRANSDERMAL
DRUG DELIVERY SYSTEMS**

Ph.D. THESIS

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Department of Polymer Science and Technology

Polymer Science and Technology Programme

MARCH 2019

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**TRANSDERMAL SALIM SİSTEMLERİ İÇİN DONEPEZİL HCl YÜKLÜ
ELEKTRO-EĞİRİLMİŞ FİBERLER**

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To my family,



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ABBREVIATIONS

DP	: Donepezil HCl
DMF	: Dimethylformamide
DSC	: Differential Scanning Calorimetry
DL	: Detection Limit
EIS	: Electrochemical Impedance Spectroscopy
EMA	: European Medicines Agency
EP	: European Pharmacopoeia
EC	: Ethylcellulose
FDA	: Food and Drug Administration (US)
ATR-FTIR	: Fourier Transform Infrared Spectroscopy
HPC	: Hydroxypropylcellulose
NMR	: Nuclear Magnetic Resonance Spectroscopy
PU	: Polyurethane
PVP	: Polyvinylpyrrolidone
TDDS	: Transdermal Drug Delivery System
SEM	: Scanning Electron Microscopy
Tg	: Glass Transition Temperature
UV-Vis	: Ultraviolet Visible Spectrophotometer



SYMBOLS

C	: Capacitance
R_s	: Solution Resistance
C_{DL}	: Double Layer Capacitance
R_{ct}	: Charge-Transfer Resistance
R_{et}	: Electron-Transfer Resistance
W	: Warburg Impedance
CPE	: Constant Phase Element
t	: Time
f	: Frequency
Z_{IM}	: Imaginary Impedance
Z_{RE}	: Real Impedance
φ	: Phase Angle



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DONEPEZIL HCl LOADED ELECTROSPUN FIBERS FOR TRANSDERMAL DRUG DELIVERY SYSTEMS

SUMMARY

Nanofibers are solid state linear nanomaterials known as flexible and having an aspect ratio greater than 1000:1. Fibers have high porosity, high specific surface area, and superior directional strength and they are adopted over a broad range of sizes and shapes. These properties make fiber preferred materials in many applications and there is an increasing interest in nanofiber technology due to these advantages. Fiber producing technologies are the template method, vapor grown, phase separation and electrospinning method. Electrospinning can be used for producing long, three-dimensional (3D), ultrafine fibers. The general working principle of electrospinning can be described as follows: an electrical potential is applied between a grounded object and a droplet of a polymer solution that is held at the end of a spinning tip. This voltage enhances the formation of an ultra-thin jet from the spinning tip, resulting in the instant evaporation of the solvent. The solid polymer then builds up on the grounded collector. Physical properties of electrospun fibers depend on process parameters, such as applied voltage, rpm of collector, distance between jet and collector, flow rate and various formulation parameters such as selection of polymer, its viscosity, and surface tension (Reneker and Chun 1996). Materials in fiber form have a great practical and fundamental importance for many applications such as filtration, military protective clothing, energy-related applications, cosmetic mask, nano-sensor, tissue engineering scaffolds, enzyme immobilization, wound dressings and drug delivery.

Combined usage of electrospinning with pharmaceutical polymers and active materials provides novel strategies for developing novel drug delivery system (DDS). There are many biodegradable and biocompatible polymers that can be used to produce electrospinning fibers in pharmaceutical fields such as natural, synthetic polymers and blends of them (Huang, Nagapudi et al. 2001). These polymers and combinations are used to develop electrospun fibers with different functions based on the requirement.

Polyurethane (PU) is a hydrophobic and thermoplastic polymer. They can be designed in any desired form and can be satisfactorily decomposed and eliminated in the human body. Therefore, they have found many applications including biomedical devices, protective clothes, filtration equipments, biosensors, tissue engineering materials, and wound dressings, due to their good chemical resistance, wear resistance, good mechanical strength, elasticity, proper hardness, biocompatibility and biodegradability (Erdem et al. 2015). One promising approach is the use of polyurethane materials for the development of new controlled drug release preparations (Reddy, Hadano et al. 2006) and patches (Mannheim et al. 2005). In these studies, polyurethane was evaluated as a main drug carrier material to modulate the drug release profile.

Hydroxypropyl cellulose (HPC), which is a non-ionic and hydrophilic cellulose derivate, can be used in the field of biomedical engineering for drug delivery applications as coating, emulsifying, stabiliser, suspending, thickener, film-coating agent, tablet binder, and extended release-matrix former in various dosage forms (Abdelbary, Elshafeey et al. 2009, Bai, Zhang et al. 2012). Ethyl cellulose (EC) which is an inert, non-toxic and insoluble in the water due to its hydrophobic nature, has a relatively small swelling degree, therefore it is suitable for sustained release of pharmaceutical products (Huang, Branford-White et al. 2012). Polyvinylpyrrolidone (PVP) is a hydrophilic, non-ionic, biodegradable and biocompatible polymer made from the monomer N-vinylpyrrolidone. PVP has been broadly used as a template for preparing functional nanofibers for applications such as drug-containing nanofibers (Xu, Jiao et al. 2011).

The aim of this thesis is to design a drug loaded new electrospun fibers prepared by electrospinning method, characterize and describe the release profile of designated drug loaded electrospun fibers, determine the drug release kinetics, investigate their use as transdermal drug delivery system (TDDS) and also study the drug release profile by electrical impedance spectroscopy (EIS). For this purpose, biologically acceptable hydrophobic polyurethane was used as main carrier polymer. The properties of the polyurethane matrices were modified by adding another hydrophobic or hydrophilic polymers to the matrices in varying ratios such as hydrophobic ethylcellulose, hydrophilic hydroxypropylcellulose and polyvinylpyrrolidone.

Donepezil HCl is a new class of acetylcholinesterase inhibitor and the second drug approved by FDA for the treatment of mild to moderate Alzheimer's diseases (Asiri and Mostafa 2010, Liew, Tan et al. 2012). Alzheimer's disease, which is a chronic neurodegenerative disease, is particularly observed in elderly patients. These patients may not remember to take the medication or carry pills for administration later in the day. It is commercially available as oral formulations such as orodispersible tablet (ODT), film coated tablet. But, Donepezil HCl has adverse effect to the gastrointestinal system when it is administered orally. A new administration such as the transdermal administration is an alternative route for Donepezil HCl depending on patient compliance and tolerability. Because of these reasons, Donepezil HCl was chosen as a model drug to design a drug loaded electrospun fibers.

First of all, various parameters such as concentration of polymer solution and processing parameters (flow rate, voltage, distance between the tip and collector) were tried to be optimized for electrospinning method. N,N-dimethylformamide (DMF) which dissolves all polymers in this study was used to get drug-polymer solution. Also it has high dielectric property and the high dielectric constant of a solvent has a significant influence on electrospinning method by improving the fiber morphology. After deciding polymer concentrations and optimizing processing parameters, drug loaded electrospun fibers was achieved. Viscosity and conductivity analysis were performed for the electrospinning polymer solutions. Because a required parameter in electrospinning is using an electrospinning solution that has sufficient viscosity and conductivity. Some physical and chemical characterizations were performed for drug loaded electrospun fibers.

Morphology and porosity of fibers are important to provide a suitable environment to load drug to the polymer matrices. Scanning electron microscopy (SEM) and porosity analysis were taken in the study to examine of the geometric properties of

electrospun fibers such as fiber morphology/orientation and pore size of fibers. Average diameter of fibers was analysed from the SEM images of the electrospun fibers.

Fourier transform infrared spectroscopy (ATR-FTIR) was used to identify the interactions between the polymers and drug. Differential scanning calorimetry has been used to study transition temperatures of polymers and DSC studies were carried out to research the possible drug and distinct polymers interactions. Also, nuclear magnetic resonance (NMR) was used to characterize the drug loaded&unloaded electrospun fibers

Drug-loaded polyurethane (PU), polyurethane-hydroxypropyl cellulose (PU/HPC), polyurethane-ethyl cellulose (PU/EC) and polyurethane-polyvinylpyrrolidone (PU/PVP) electrospun fibers systems in various ratio were achieved by using electrospinning method. In vitro dissolution profile of the drug-loaded electrospun fibers systems were investigated in phosphate-buffered saline by using UV-Visible Spectroscopy. The in vitro release results of fibers were fitted to Zero Order, First Order, Hixson-Crowell, Higuchi Square Root and Korsmeyer-Peppas Kinetics Models to investigate the drug release kinetics of electrospun fibers.

The obtained data from the in vitro dissolution results showed that the all formulations of drug loaded PU/PVP electrospun fibers systems provide the immediate release of drug. According to the in vitro dissolution results, only drug loaded PU/HPC (10/2), PU/EC (10/8) fiber systems were chosen for the in vitro skin permeation studies and were performed via Franz diffusion cell and synthetic skin resembling membrane. Results show that the drug loaded PU/HPC (10/2) fiber system provided release of more drug than the drug loaded PU/EC (10/8) fiber system during skin permeation studies. Based on the observations, it can be reasonably concluded that drug loaded PU/HPC (10/2) suits better than drug loaded PU/EC (10/8/1) for the development of transdermal drug delivery of Donepezil. In vitro cytotoxicity studies were performed for drug loaded and unloaded PU/HPC 10/2 electrospun fibers through MTT viability assay on the mouse fibroblast cell line. It is found that this system is nontoxic and can be used in skin applications.

Electrochemical impedance spectroscopy (EIS) is one of the most effective method to get information about electrochemical characteristics of the electrochemical system, including double-layer capacitance, diffusion impedance, determination of the rate of charge transfer and charge transport processes, and solution resistance (Macdonald et al., 2005). Further, the impedance spectroscopy technique is sensitive to variations in the substructural layers of the microporous membranes and the measurements allow a determination of the porosity of these layers of membrane if their thickness and dielectric constant are known (Coster et al., 1997). The EIS results are usually presented as the complex impedance Z versus frequency f . Experimental values from the EIS were simulated with the ZSimpWin software to find the optimum electrical circuit model. Electrochemical impedance spectroscopy technique was studied to investigate the changes of interfacial properties at drug loaded electrospun fibers on the electrode surface and also changes in buffer solution after the drug release from the fiber. EIS was used as an alternative way to follow the drug release from the electrospun fibers and to investigate morphology changes on the electrospun fiber.



TRANSDERMAL SALIM İÇİN DONEPEZİL HCl YÜKLÜ ELEKTRO-EĞRİLMİŞ FİBERLER

ÖZET

Nanofiberler esnek, katı durumda doğrusal nanomalzemelerdir ve uzunluk oranı 1000:1'den daha büyüktür. Fiberler yüksek porozite, yüksek spesifik yüzey alanı, geniş boyut ve şekillere uyum sağlayan esneklik ve üstün bir doğrultu mukavemeti kombinasyonuna sahip oldukları için tercih edilen malzemelerdir. Bu avantajlarından dolayı nanofiber üretim teknolojilerine artan bir ilgi vardır. Lif üretme teknolojileri kalıp yöntemi, buhar yoğunlaşması, faz ayrılması ve elektro lif çekim yöntemidir.

Elektro lif çekim teknolojisi uzun, üç boyutlu, yüksek derecede ince fiber eğirme teknolojisidir. Elektro lif çekim yönteminin genel çalışma prensibi şu şekildedir: bir elektrik potansiyeli kılcal bir boru sonundaki damlacık halindeki polimer çözeltisi ile topraklanmış kollektör arasında uygulanır. Bu gerilim eğirme ucundan bir ultra-ince jet oluşumuna neden olur, anında çözücünün buharlaştırılmasıyla sonuçlanır. Sonra katı polimer (fiber) topraklı kollektör üzerinde birikir. Elektro eğrilmiş fiberlerin fiziksel özellikleri, gerilim, kollektörün dönüş hızı, jet ve kollektör arasındaki mesafe, akış hızı ve polimerin seçimi, viskozite ve yüzey gerilimi gibi çeşitli formülasyon parametrelerine bağlıdır (Reneker and Chun 1996).

Fiber formundaki malzemeler, filtrasyon, askeri koruyucu giysi, enerji ile ilgili uygulamalar, kozmetik maske, nano sensör, doku mühendisliği, enzim immobilizasyonu, yara sargıları ve ilaç salım sistemleri gibi birçok uygulama için büyük ve pratik bir öneme sahiptir.

Farmasötik polimerler, aktif maddeler ve elektro lif çekim yöntemi kombinasyonu yeni ilaç salım sistemlerinin gelişimi için yeni stratejiler sağlar. Elektro lif çekim yönteminde kullanılan doğal, sentetik ve bunların karışımları gibi farmasötik alanda kullanılan pekçok biyobozunur ve biyouyumlu polimer vardır (Huang, Nagapudi et al. 2001). Bu polimerler gereksinime göre farklı fonksiyonlarda elektropsun fiber gelişimi için kullanılır.

Poliüretan (PU), hidrofobik ve termoplastik bir polimerdir. İstenilen herhangi bir formda tasarlanabilirler ve insan vücudunda tatmin edici bir şekilde ayrıştırılabilir ve elimine edilebilirler. Bu nedenle, iyi kimyasal dayanımları, aşınma direnci, iyi mekanik dayanım, elastikiyet, uygun sertlik, biyolojik uyumluluk, biyolojik bozunabilirlik nedeniyle biyomedikal cihazlar, koruyucu giysiler, filtrasyon ekipmanları, biyosensörler, doku mühendisliği malzemeleri ve yara örtüleri gibi birçok uygulama bulmuşlardır (Erdem et al. 2015). Umut verici bir yaklaşımlardan biri de yeni kontrollü ilaç salım preparatlarının (Reddy, Hadano et al. 2006) ve yamaların (Mannheim et al. 2005) geliştirilmesi için poliüretan malzemelerin kullanılmasıdır. Bu çalışmada poliüretan, ilaç salım profilini geliştirmek için ana ilaç taşıyıcı madde olarak kullanılmıştır.

İyonik olmayan ve hidrofilik bir selüloz türevi olan hidroksipropil selüloz (HPC), ilaç verme uygulamaları için pekçok dozaj formunda kaplama, emülsifiye edici, stabilizör, süspanse edici, kalınlaştırıcı, film kaplama maddesi, tablet bağlayıcı ve uzatılmış salım için matris yapıcı olarak biomedikal alanda kullanılabilir (Abdelbary, Elshafeey et al. 2009, Bai, Zhang et al. 2012). Hidrofobik yapısı nedeniyle suda etkisiz, toksik olmayan ve suda çözünmeyen Etil selüloz (EC) nispeten küçük bir şişme derecesine sahiptir, bu nedenle sürekli salımlı farmasötik ürünler için uygundur. Etkisiz, toksik olmayan, doğal ve kararlı hidrofobik polimer olan etil selüloz uzatılmış salımlı farmasötik ürünler için uygun ve kullanılabilir bir polimerdir (Huang, Branford-White et al. 2012). Polivinilpirolidon (PVP), monomer N-vinilpirolidondan yapılan, hidrofilik, iyonik olmayan, biyolojik olarak parçalanabilir ve biyo-uyumlu bir polimerdir. PVP ilaç içeren nano elyaflar gibi uygulamalar için fonksiyonel nano elyafların hazırlanmasında geniş bir şablon olarak kullanılmıştır (Xu, Jiao et al. 2011).

Bu tezin amacı, elektrospinning yöntemi ile hazırlanan ilaç yüklü yeni elektrospun elyafları üretmek, karakterize etmek, bu ilaç yüklü sistemlerin salım profilini incelemek, ilaç salım kinetiklerini hesaplamak ve transdermal ilaç salım sistemi olarak kullanılıp kullanılamayacağını değerlendirmek ve salım profilini elektriksel empedans spektroskopisi ile incelemektir. Bu amaçla biyolojik olarak kabul edilen hidrofobik poliüretan temel taşıyıcı olarak kullanılmıştır. Poliüretan matrislerin özellikleri, matrislere değişen oranlarda bir hidrofobik veya hidrofilik polimer örneğin hidrofobik etil selüloz, hidrofilik hidroksipropil selüloz ve polivinilpirolidon gibi başka polimerler ilave edilerek modifiye edilmiştir.

Donepezil HCl, yeni bir asetilkolinesteraz inhibitör sınıfındadır ve hafiften orta derecede Alzheimer hastalıklarının tedavisi için FDA tarafından onaylanan ikinci ilaçtır (Asiri and Mostafa 2010, Liew, Tan et al. 2012). Kronik bir nörodejenaratif hastalık olan Alzheimer hastalığı, özellikle yaşlı hastalarla ilişkilidir. Bu hastalar günün ilerleyen saatlerinde ilacı almayı veya uygulama için ilaç taşımayı hatırlamayabilirler. Donepezil HCl ticari olarak orodispersible tablet (ODT), film kaplı tablet gibi oral formülasyonlar halinde piyasada bulunmaktadır. Ayrıca, Donepezil HCl, oral yoldan uygulandığında gastrointestinal sistem üzerinde olumsuz etkiye sahiptir. Transdermal uygulama Donepezil HCl için hasta uyumu ve tolere edilebilirliği açısından alternatif bir yoldur. Bu nedenlerden dolayı Donepezil HCl, ilaç yüklü bir elektrospun elyafı tasarlamak için model bir ilaç olarak seçildi.

İlk olarak, polimer çözeltisinin konsantrasyonu ve proses parametreleri (akış hızı, gerilim, tip ve kollektör arasındaki mesafe) gibi çeşitli parametreler, elektrospinning yöntemi için optimize edilmeye çalışılmıştır. İlaç polimeri çözeltisi elde etmek için çalışmadaki tüm polimerleri çözen N, N-dimetilformamid (DMF) kullanılmıştır. Ayrıca, DMF yüksek dielektrik özelliğe sahiptir ve bir çözücünün dielektrik sabiti, fiber morfolojisini geliştirmek için elektroegirme yöntemi üzerinde önemli bir etkiye sahiptir. Polimer konsantrasyonlarına ve optimize edilmiş parametrelere karar verildikten sonra, elektroegirme yöntemi ile ilaç yüklü elektrospun elyafları elde edildi. Elektroegirme yöntemi için hazırlanan polimer çözeltileri için viskozite ve iletkenlik analizi yapıldı. Çünkü elektroegirme yönteminde polimer çözeltisinin yeterli viskoziteye ve iletkenliğe sahip olması gerekmektedir. Elde edilen ilaç yüklü elektrospun fiberler bazı karakterizasyon yöntemleri ile karakterize edilmiştir.

Polimer matrislerine ilacı yüklemek için liflerin morfolojisi ve gözenekliliği uygun bir ortamı sağlamak için önemlidir. Fiber morfoloji/oryantasyon gibi elektrospun

liflerinin geometrik özelliklerini ve liflerin gözenek boyutlarını incelemek için taramalı elektron mikroskopisi (SEM) ve porozite analizi yapılmıştır. Ayrıca, elektrospun fiberlerin SEM görüntülerinden ortalama fiber çapı analiz edilmiştir.

Polimerlerle ilaç arasındaki etkileşimi belirlemek için Fourier Transform Infrared (Kızılötesi) Spektroskopisi kullanılmıştır. Diferansiyel Tarama Kalorimetrisi polimerlerin camsı geçiş sıcaklığı (T_g) ve erime sıcaklığını (T_m) incelemek için yapılmıştır ve olası ilaç ve farklı polimer etkileşimleri araştırılmıştır. Ayrıca, ilaç yüklü ve yüksüz elektrospun elyaflarını karakterize etmek için nükleer manyetik rezonans (NMR) kullanılmıştır.

Farklı oranlarda ilaç yüklü poliüretan (PU), poliüretan-hidroksipropil selüloz (PU/HPC), poliüretan-etilselüloz (PU/EC) ve poliüretan-polivinilpirolidon (PU/PVP) elektrospun fiber sistemleri elektro eğirme yöntemi kullanılarak elde edilmiştir. İlaç yüklü elektrospun fiber sistemlerinin fosfat buffer ortamda in-vitro çözünme profili UV-Görünür Spektroskopisi kullanılarak test edilmiştir. İlaç yüklü liflerin in vitro salım sonuçları kullanılarak Sıfır Derece Kinetiği, Birinci Derece Kinetiği, Hixson-Crowell Kinetiği, Higuchi Kinetiği ve Korsmeyer-Peppas Kinetiği parametreleri hesaplanmıştır.

In vitro çözünme testinden elde edilen sonuçlar göstermektedir ki tüm ilaç yüklü PU/PVP fiber sistemleri ilacın hemen salımını sağlamaktadır. In vitro çözünme sonuçlarına göre, in vitro deri nüfuz etme çalışmaları için sadece ilaç yüklü PU/HPC (10/2), PU/EC (10/8) fiber sistemleri seçilmiştir. In vitro deri nüfuz etme çalışmaları Franz difüzyon hücresi ve deriye benzeyen sentetik zar ile yapılmıştır. Sonuçlar, ilaç yüklü PU/HPC (10/2) fiber sisteminin, deriye nüfuz etme çalışmaları sırasında ilaç yüklü PU/EC (10/8) fiber sisteminden daha fazla ilaç salımı sağladığını göstermektedir. Gözlemlere dayanarak, Donepezil'in transdermal ilaç dağıtım sisteminin geliştirilmesi için ilaç yüklü PU/HPC'nin (10/2/1) ilaç yüklü PU/EC'den (10/8/1) daha uygun olduğu sonucuna varılabilir. İlaç yüklü ve yüksüz PU/HPC 10/2 elektrospun elyafları için fare fibroblast hücre hattındaki MTT canlılığı analizi ile in vitro sitotoksosite çalışmaları yapılmıştır. Bu sistemin toksik olmadığı ve cilt uygulamalarında kullanılabileceği bulunmuştur.

Elektriksel empedans spektroskopisi (EIS), çift katmanlı kapasitans, difüzyon empedansı, yük transfer hızının belirlenmesi ve yük taşıma prosesi ve çözelti direnci de dahil olmak üzere elektrokimyasal sistemin elektrokimyasal özellikleri hakkında bilgi edinmenin en etkili yöntemlerinden biridir (Macdonald et al., 2005). Ayrıca, empedans spektroskopisi tekniği, mikro gözenekli zarların alt tabakalarındaki değişikliklere karşı hassastır ve kalınlıkları ve dielektrik sabitleri biliniyorsa, ölçümler bu zar katmanlarının gözenekliliğinin tespitine izin verir (Coster et al., 1997). EIS sonuçları genellikle, frekansa karşı empedans (Z) olarak sunulmaktadır. EIS'den elde edilen deneysel değerler, optimum elektrik devresi modelini bulmak için ZSimpWin programıyla simüle edilmiştir. Elektrot yüzeyinde ilaç yüklü elektrospun liflerinin arayüzey özelliklerinin değişimini ve ayrıca liften ilaç salınmasından sonra tampon çözeltideki değişiklikleri araştırmak için elektrokimyasal empedans spektroskopisi tekniği incelenmiştir. EIS, elektrospun liflerden ilaç salımını takip etmek ve elektrospun elyafı üzerindeki morfoloji değişikliklerini araştırmak için alternatif bir yol olarak kullanılmıştır.



1. INTRODUCTION

The main aim of designing transdermal drug delivery system is to deliver drugs through the skin into the systemic circulation at predetermined rate. Transdermal drug delivery system is the one of the most promising method as it provides reduced side effects accompanied by oral therapy and sustain drug release. A discrete, self-contained dosage form of transdermal drugs contribute to patient compliance and maintenance of plasma level longer than regular oral drugs.

The most common and succesful form of transdermal drug delivery system is known as Transdermal patches. Transdermal patches have many advantages over oral medication, providing direct-to-bloodstream delivery which prevent overload effect on gastrointestinal tract, being easy treatment and easy use (Keith 1983) (Chien 1987).

Reservoir, matrix, microreservoir, adhesive, membrane–matrix type transdermal patches are designed for transdermal systems. Among the other transdermal systems, matrix type transdermal patches method is the most popular since the drug can be released in controlled manner.

Electrospinning method is a convenient process to design a suitable matrix type transdermal drug delivery system (TDDS). This method is a effective method to manufacture ultrafine, three-dimensional and high porous long fibers with a good pore size distrubition in cost effective way (Sun and Li 2011). Electrospun fibers not just only have great surface area and mechanical performance but also their surface can be modified to form desired functions. The general principle of electrospinning process can be defined as follows: the polymer-drug solution is subjected to high voltage, through a spinning tip to a grounded object. A charged ultrathin jet of the solution is ejected from the tip and the solvent evaporates as the jet travels in air. The drug-containing polymer is collected on the grounded material. It has been reported that the physical properties of fibers can be affected by several factors that include

distance between collector and jet, flow rate, voltage, surface tension and viscosity of the polymer (Reneker and Chun 1996).

Among the other factors, determining the polymer which will be used in a specific area, is the crucial criteria in the electrospinning process. Also, solubility and compatibility of the drugs in the drug/polymer/solvent system were the remarkable factors for the preparation of electrospun fiber systems. There are many biodegradable and biocompatible polymers which can be used to produce electrospun fibers in pharmaceutical fields such as natural, synthetic, and blends of synthetic and natural polymer (Huang, Nagapudi et al. 2001). These polymers were used to develop electrospun fibers with different functions based on the requirement. Depending on the polymer carrier fiber type, release of pharmaceutical dosage can be adjusted as in rapid, delayed, modified or immediate forms. In order to develop new controlled releasing preparations, Polyurethanes are one of the most frequently used among the other synthetic polymers. Because they can be designed in any desired forms, degraded and eliminated easily in the human body (Reddy, Hadano et al. 2006). Polyurethane has been chosen to modify the release profile drug carrier polymer. In many studies, polyurethane has been incorporated with other materials such as cellulose, PVP etc. to modify viscosity and dissolution rate, hydrophilicity and pore size of polymer system.

Cellulose has become the subject of interest in the transdermal formulation. This polymer has important roles in the coated dosage forms, compression tablets, osmotic drug delivery systems, extended and controlled release matrix, mucoadhesives, bioadhesives, preparations as stabilizers, gelling agents, thickening agents, binders and also many other applications. Additionally, it has been used as free flowing agents, filler, pressure sensitive adhesives, taste masker in the transdermal patches to modulate and provide the good release feature. Cellulose and its derivatives with variable amounts can be used to adjust the release rate of controlled release system (Shokri and Adibkia 2013).

Hydroxypropyl cellulose (HPC) is known as a non-ionic cellulose derivative and can be used in the area of drug delivery applications of biomedical engineering (Abdelbary, Elshafeey et al. 2009, Bai, Zhang et al. 2012). Vlad et al. explained that specific biodegradability and biostability are important features of polyurethane-hydroxypropyl cellulose (Mandru, Vlad et al. 2013). There is not enough information

about electrospinning of HPC polymer in the literature (Periasamy, Devarayan et al. 2012). Electrospun of HPC fibers have been produced by using solvent mixture of ethanol and 2-propanol (Shukla, Brinley et al. 2005). Moreover, HPC fibers have been produced in polyethyleneoxide aqueous solution as carrier matrix (Francis, Balakrishnan et al. 2010). Ethyl cellulose (EC) is a suitable polymer which has natural, hydrophobic, inert and non-toxic characteristic that can be used in sustained release matrix (Huang, Branford-White et al. 2012). PVP is a popular template to prepare functional drug-containing fibers (Xu, Jiao et al. 2011). The electrospun biphasic drug release from the polyvinylpyrrolidone/ethyl cellulose core/sheath fibers were studied Yu et al. (Yu, Wang et al. 2013).

The aim of this thesis is to design a new drug loaded electrospun fibers and investigate their drug release kinetics to determine their function as transdermal drug delivery system (TDDS). In order to achieve this, polyurethane and its blends with other polymers such as hydroxypropylcellulose (HPC), ethylcellulose (EC) and polyvinylpyrrolidone (PVP) were used.

Donepezil HCl (DP) was chosen as a model drug to develop drug delivery system administration by transdermal way. Donepezil belongs to the class of acetylcholinesterase inhibitor and it is the second drug approved by FDA to treat Alzheimer's diseases from mild to moderate (Asiri and Mostafa 2010, Liew, Tan et al. 2012). 5 or 10 mg immediate release (Reichman 2003), 23 mg sustained release, and 5 or 10 mg orally disintegrating Donepezil tablets are available formulations, and all of products are related with oral administration for Donepezil. Adverse events in the gastrointestinal system and in plasma fluctuations are occurred at the oral Donepezil administration. The transdermal administration is an alternative route for Donepezil. The benefits of transdermal application such as reducing any side effects, providing steady drug blood levels over a long period of time may be particularly relevant over oral delivery forms to improve patient and treatment compliance and tolerability. For example, Alzheimer's and Parkinson's disease patient may not be remember to take or carry the medication for administration later in the day, and it is particularly applicable in elderly patients with chronic conditions.

In the first part of the study, drug–excipient interaction between the used polymers and drug was studied. It has been seen that drug and polymer do not interact each other. Then, various parameters like concentration of polymers and Donepezil HCl,

viscosity or processing parameters (flow rate, voltage, distance between the tip and collector) were tried to be optimized for the optimum parameters of electrospinning. For the electrospinning polymer solutions, viscosity and conductivity analysis were performed to determine the as-prepared fiber size and morphology. After all, combination ratio between the chosen polymers and the drug loaded electrospun fibers has been prepared. Physical and chemical characterizations were performed to characterize polymer fiber systems.

Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR) is considered as basic method to study drugs and their interactions with carrier matrix. Further analysis has been carried out by Differential Scanning Calorimetry (DSC) method which provide significant data about polymorphism, crystallinity, stability, interactions/compatibility and glass transition temperature features of the fibers. Moreover, Nuclear magnetic resonance (NMR) have been used for structural characterization of the drug loaded and unloaded electrospun fibers, Scanning electron microscopy (SEM) and porosity analysis have been studied to evaluate the geometric features including average diameter of fibers, fiber morphology/orientation and pore size of fibers.

In vitro dissolution studies of the Donepezil HCl loaded polyurethane (PU), polyurethane-hydroxypropyl cellulose (PU/HPC), polyurethane-ethyl cellulose (PU/EC) and polyurethane-polyvinylpyrrolidone (PU/PVP) systems (in PU/Second Polymer/Donepezil HCl ratio of 10/1/1, 10/2/1, 10/4/1, 10/8/1) were performed to detect the drug released from the drug loaded electrospun fibers by using UV-vis spectroscopy. The in vitro release results of formulations were fitted to Zero Order, First Order, Hixson-Crowell, Higuchi Square Root and Korsmeyer-Peppas Kinetics Models to investigate the drug release kinetics of electrospun fibers. The best regression value (r^2) was found for PU/HPC/DP at ratio of 10/2/1 and PU/EC/DP (10/8/1) when Korsmeyer-Peppas model was fitted the data. The obtained data from the in vitro dissolution results indicated that the all formulations of drug loaded PU/PVP display an immediate release of drug.

According to best results of in vitro dissolution studies drug loaded PU/HPC/DP (10/2/1) and PU/EC/DP (10/8/1) fiber systems were chosen for studying in vitro skin permeation. In vitro skin permeation studies were conducted in a modified Franz's diffusion cell and permeation drug concentration from electrospun fibers were

determined by validated high pressure liquid chromatography (HPLC) method. The results show $1000 \mu\text{g}/\text{cm}^2$ Donepezil HCl permeates through pig skin in 72 hours from the formulation of PU/HPC/DP (10/2/1) while $75 \mu\text{g}/\text{cm}^2$ Donepezil HCl permeates through pig skin in 72 hours from the formulation of PU/EC/DP (10/8/1). From results, it can be concluded that PU/HPC/DP (10/2/1) well fitted in Donepezil than PU/EC/DP (10/8/1) for the TDDS. In vitro cytotoxicity studies were performed for drug loaded and unloaded polyurethane-hydroxypropyl cellulose of 10/2/1 (w/w/w) ratio to test of chemical's cytotoxicity.

In general, drug release kinetic profile is evaluated by different methods such as UV-Visible Spectroscopy, Fluorescence Spectrometry and HPLC method. On the other hand Electrochemical Impedance Spectroscopy (EIS) is an alternative and effective method applied in the study of drug release. Since, EIS is a sensitive method to analyze the interfacial interactions between electrode and electrolyte including solvent resistance and capacitance of the system. Therefore the release of drug from the drug loaded materials can be monitored by EIS to identify polymer-drug system clearly. Furthermore, the analysis of alternating current (AC) response of the systems using equivalent circuit model allows the evaluations of charge transfer resistance, bulk resistance, double electric layer parameters and others.

In order to obtain EIS results, drug loaded electrospun fibers were collected onto commercial ITO/PET electrode. The drug release from the coated ITO/PET electrode has been monitored and attempted several times to obtain reproducible electrochemical measurements. Equivalent circuit models were fitted to the experimental EIS studies and was found the best fitted model between calculated and experimental results. The results revealed that the electrochemical impedance spectroscopy is an additional good method to determine the release of drug from the electrospun fibers although it still needs to be carried out more detailed studies about reaction kinetics of fibers. The results of EIS alone is not sufficient to determine the drug release now. But combining the EIS results with other electrochemical techniques (such as cyclic voltammetry (CV), differential pulse voltammetry (DPV) etc) and also UV-visible spectroscopy and high performance liquid chromatography (HPLC) could better support to understand the release of drug.



2. THEORETICAL BACKGROUND

2.1 Nanofiber Technology

Nanofibers are defined as having flexible structure and fibers with diameters less than one micron, surface area of fibers are high specific and directional strength properties is high-quality. Nanofiber materials are practical and these properties make fiber a preferred material and they find application many important areas from clothing to reinforcements for aerospace construction. Nanofibers can be produced by vapor grown, phase separation, template method and electrospinning method (Ko 2004).

2.1.1 Electrospinning Method

Electrospinning method is an electrostatic fiber producing technique and has potential for applications in several fields. The distinguishable applications are;

1. Filtration and protective apparel

- Liquid/gas molecule filtration
- Anti biochemical gases apparel
- Efficiency in trapping particles

2. Sensor Applications

- Thermal sensor
- Biochemical sensor
- Optical chemical sensor
- Piezoelectric sensor

3. Nanocomposite and others

- Conductive nano materials
- Nano-solar cell
- Functional catalysis

-Nanoelectronic device

4. Applications by medically and Tissue Engineering

- Drug delivery carrier

- Wound dressing

-Porous membrane for skin

-Three dimensional scaffolds for bone and cartilage regenerations

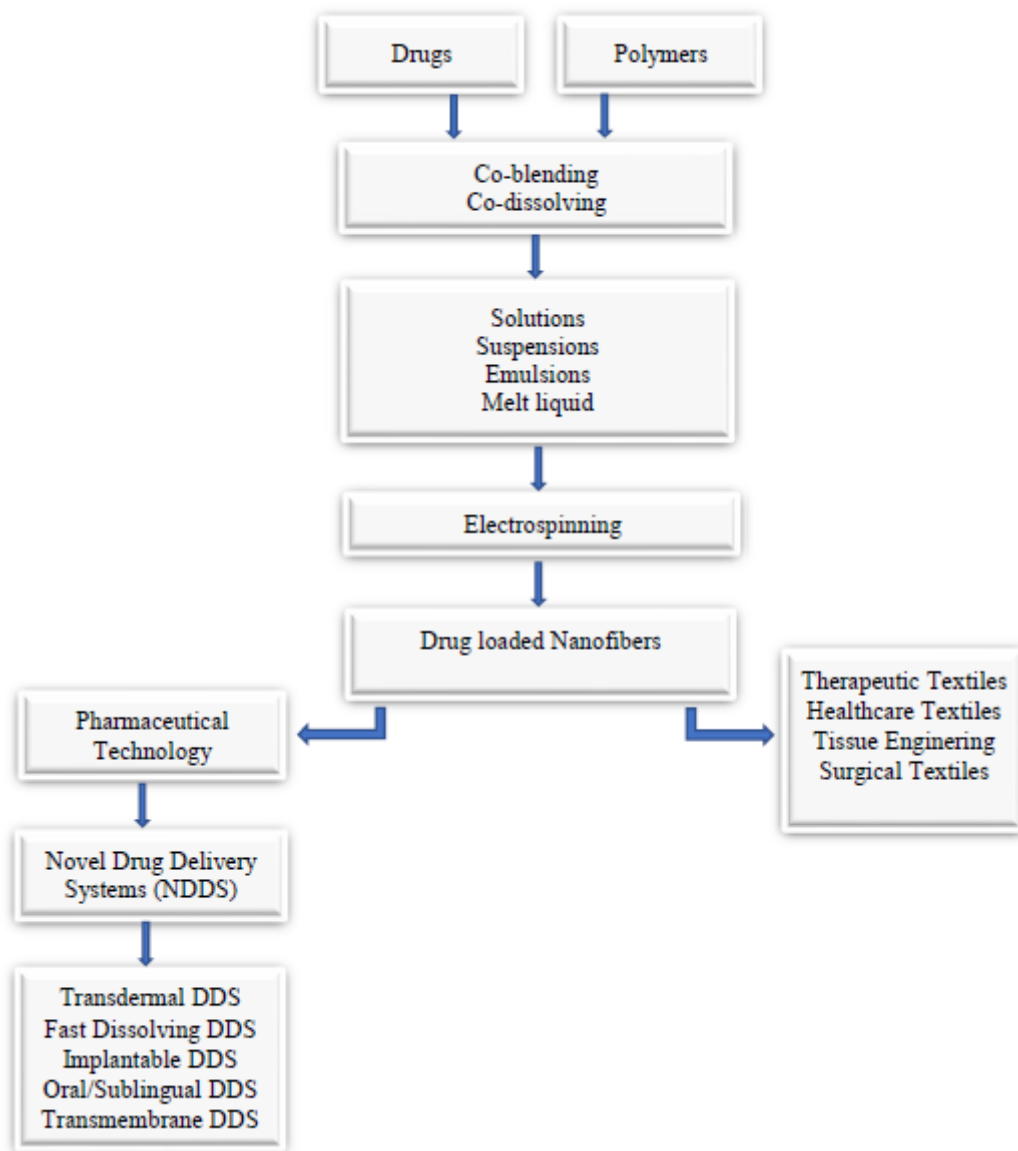


Figure 2.1 : Preparations and applications of electrospun drug-loaded nanofibers.

Polymer melts or solutions can produce high quality fibers by electrostatic forces at suitable atmosphere condition at room temperature using electrospinning technique. Vertical or horizontal electrospinning setups can be used at the electrospinning

technique. High surface area to volume ratio, the ability to operate fiber composition, sizeable porosity can be counted among the advantages of the fibers produced by electrospinning technique which is a good technique to get desired functionalities and properties (Bhardwaj and Kundu 2010).

Fundamentally, an electrospinning system comprises a high voltage supplier, a spinning tip (like a needle) and a grounded collector (like a metal plaque, screen or a rotating disk) shown in Figure 2.2 (Liang, Hsiao et al. 2007). Before electrospinning process starts, polymer fluid is formed accurately using convenient solvents from a great deal of the polymers then the polymer fluid is filled into the spinning tip to obtain fiber by electrospinning (Nagapudi, Brinkman, 2002). Its surface tension keeps the polymer fluid and an electric field applies at the end of a capillary tube in the electrospinning process. Electric field is created on the fluid surface. With the electric field reaching the important value, the propulsive electric forces exceed the forces of surface tension.

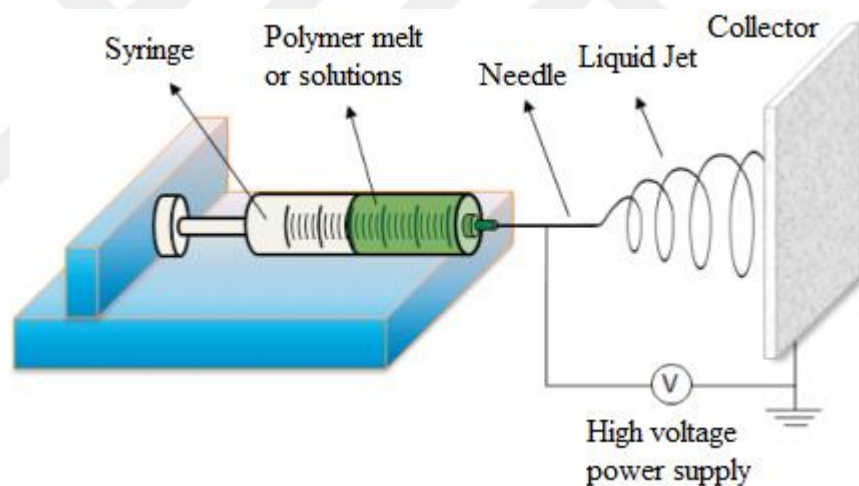


Figure 2.2 : Schematic representaion of the electrospinning method.

At last, jet of the solution having charged is thrown from the tip of the Taylor cone, an inconsistent and a fast whipping of the jet takes place between the capillary tip and collector plate area, solvent evaporates and solid polymer collects at the plaque (Taylor 1969, Sun, Zussman et al. 2003). Only, jet of the solution having charged is stable at the tip of the spinneret, after that it starts being unstable.

In the electrospinning method, the morphology of the fibers can be affected by several factor. These factors can be splitted into three major category: 1) polymer solution (surface tension, concentration, conductivity and viscosity of the polymer

solution), 2) polymer processing (such as feed rate, collection distance and electrostatic potential which is applied during the process) and 3) surrounding factors (for example temperature, relative velocity and humidity of the surrounding air in the spinning chamber) (Huang, Zhang et al. 2003). The desired morphology and diameter fibers can be obtained by using suitable factors, which affect the morphology of the fibers (Li and Xia 2004, Chong, Phan et al. 2007).

Properties of solution (e.g. surface tension, viscosity and electrical property of the solution) are very efficient factor in electrospinning method and on the morphology of the fibers. Polymer processing parameters (such as the diameter of needle, the feed rate, the solution's temperature, the voltage supplied and the needle tip and collector distance, which collector should be used) also can control the fiber morphology. But they are less important according to the solution factor. These factors are described in detail by A. Sezai Sarac (Sarac, A.S., 2016). The factors are summarized below:

Surface tension (σ) is the force used to the surface per unit length. For the starting of electrospinning, the charged polymer solution needs to rise above the surface tension of the solution. During the electrospinning process, the high surface tension values lead to the beaded fiber products within the polymer jet. By altering the polymer/solvent ratio in the electrospinning solution or adding surface active material to the solution, the surface tension can be adjusted. For example Hydroxypropylcellulose (HPC) is known a surface-active material in which combines surface activity, thermoplasticity and organic solvent solubility with the aqueous thickener and stabilizer features of other water soluble cellulose polymers.

Solution conductivity, the solution must obtain adequate charges because the propellant forces in the solution can be exceed the surface tension of the polymer solution for the initiating of electrospinning process. The capacity of the charge carrier of the solution is also affected by the drawing and subsequent stretching of the electrospinning jet. Conductivity of the solvent might be increased by addition salts, dissolved carbon dioxide, bases and acids.

Dielectric effect, dielectric constant of a solvent affects also significantly the electrospinning method. A higher dielectric constant solution lowering the diameter and bead generation of the electrospun fiber. The dielectric property of N,N-

dimethylformamide (DMF) may also increase the property of the electrolytic solution to improve fiber morphology.

Solution viscosity, molecular weight, concentration, temperature and also polymer chain entanglement are the main factors, these affect the viscosity of solution. The electrospinning solution should comprise adequately molecular weight of polymer and it should have sufficient viscosity. Viscosity of the solution is relevant to the molecular weight of a polymer directly. Fewer chain impediment amounts are found in the polymer solution at low viscosity. The forces of surface tension predominant, bead formation can be seen along the fibers. At high viscosity, jets can be stretched completely and non-beaded fibers may be achieved. Dividing of jets into little fibers may occur, and transmitting of the polymer solution can be hard, and getting dry of the solution on the tip of the needle can be seen in high viscosity values.

Volatility of the solvent, in the electrospinning method solvent evaporation takes place while the jet transmit from the tip of the syringe to the plaque of collector. The fiber might be formed and accumulated on the collector by evaporation of the entire solvent on the way. On the other hand, non-dry fibers or slim films can be seen with remaining some solvent on the polymer. The generation of pores on the fibers related also solvent volatility.

Applied voltage, the morphology and the obtained fibers can be affected by the applied voltage also. It defines the amount of charge of the polymer jet. The high voltage generates the required charges of the polymer solution and in parallel line with the external electric field which initiates the electrospinning method if the electrostatic force in the polymer solution exceed the surface tension of the solution.

Flow rate defines the amount of solution used for electrospinning. For an accurate voltage, a convenient flow rate should become to maintain the stability of Taylor cone. If the flow rate rises, bead size or fiber diameter increase correspondingly.

The tip of the needle and collector distance is important on the strength of the electric field and residence time of the jet in the air along the electrospinning way. If the distance is short, solvents might not have enough time to completely evaporate before the jet attains to the collector. Instead of dry fibers, the wet fibers may be resulted. Fibers may be stick together by the residual solvents and the residual solvents can result in joining of the fibers. The size and shape of the fibers were not

affected with reducing the distance, but inhomogeneously dispensed beads can be noticed, it can be related to increasing in the electric field strength. If there is a longer distance, the solution jet can find more time to evaporate the solvent and before collecting on the collector the jet can be stretched suitable.

Effect of the collector is also important parameter affect the electrospinning parameters. Usually a conductive collector is used in the electrospinning process. Conductive collectors may result in a further amount of fiber deposition because more jets can be attracted by the surface of the conductive collectors. Less fiber may deposite on the nonconductive collectors, because polymer jet charges flow onto the collector owing to the fact that quick accumulation of the charges. The production of aligned fibers can be achived by rotating cylinders which is a dynamic collector. Solvents have more time to evaporate using a rotating collector.

The inner diameter of the needle is important factor also at the electrospinning process. Decreasing the inner diameter effects the number of beads, and the final fiber diameter and observed an visible decrease in clogging. Decreasing the inner diameter creates an increase in the surface tension of the drops on the tip of the needle It means that starting the formation of a jet needs a greater amount of electrostatic force.

2.2 Material and Electrospinning Method

2.2.1 Biomaterials in electrospinning system for drug delivery

Synthetic and natural polymers which is used in electrospinning process exhibit a variety of alternative properties to reach needed biological and mechanical requirements. Classes and examples of polymers can be classified as given in Table 2.1 (Brannon-Peppas 1997, Pillai and Panchagnula 2001).

Table 2.1 : Natural and synthetic polymers.

NATURAL POLYMER			
Lipids	Aminopolysaccharide	Proteins	Carbohydrate
Wax Emulsion Low Density Lipoprotein (LDL)	Chitosan	Fibrinogen Albumin DNA Gelatin Collagen Erythrocyte	Alginate Dextrose Agar Starch Cellulose
SYNTHETIC POLYMER			
Biologically Degradable		Biologically Nondegradable	
Polyaminoacid Polylactic acid Polyglycolic acid Polyhydroxyvalerate Polyhydroxybutyrate Polycaprolactone Polyamide Polyanhydride Polyalicyanoacrylate Polyorthoesters Psödopolyaminoacid		Polyurethane Polyisobuthylene Cellulose acetate Silicon Polyethylene vinylacetate Polyethylene Copoly(ethylene vinyl acetate) Polynuclear polystyrene Polybutyl metacrylate	

Polyurethanes have many applications as biomaterials within synthetic polymers, because of their excellent physical properties which was designed in any desired forms, being degraded satisfactorily and being eliminated from the human body (Shalaby 1994) and considerably good biocompatibility such as used in catheters, implants, drug delivery systems, mammary prostheses and vascular grafts. The highest polymer concentration of the polyurethane was 12.8 % (wt) for electrospinning of fibers at room temperature, whereas at the high temperature, the polymer concentration was 21.2 % (wt). In the literature it was indicated that at the same concentration, the polyurethane solution viscosity was higher at room temperature than at some higher temperature (Huang, Zhang et al. 2003).

Table 2.2 : Chemical structures of selective inactive ingredients.

Ingredient	Chemical structures
Polyurethane (PU)	
Hydroxypropyl cellulose (HPC)	R=H or CH₂CH(OH)CH₃
Ethylcellulose (EC)	R=H or CH₂CH₃
Polyvinyl pyrrolidone (PVP)	

The type of polymer matrix, drug-polymer interaction and also drug solubility in these matrix are important factors for development of therapeutic research at the drug release from polyurethane matrix (Ruggeri, Francolini, 2007, Mandru, Ciobanu, 2011, Macocinschi, 2012). The use of polyurethane as drug delivery systems is a hopeful approach to develop a new controlled releasing preparations (Reddy, Hadano et al. 2006). The microporous or non-porous membrane which controls the drug release are qualified by structural and mechanical strength for long-time use, good tissue/blood compatibility and endurance (such as patches, orthopaedic and dental implants) (Cardea, Sessa et al. 2010). For example, an adhesive skin-containing polyurethanes layer is designed to administer nicotine to help people stop smoking (Baker, Kochinke et al. 1989). Polyurethane membranes were designed as controlled sustained release systems for antifungal agents. Materials with PU-based with

increasing disarrangement and many functions have been improved for drug release control, like pH-stimuli and temperature sensitive (Zhou, Yu et al. 2011, Chen, Wang et al. 2011). Polyurethanes such as various materials have been suggested for use as the drug reservoir of the transdermal drug delivery devices.

Cellulose and its derivatives which are attractive natural polymers because of abundance in nature. Most of cellulose derivatives are soluble in water; but, like hydroxypropyl cellulose, ethyl cellulose, methyl cellulose, cellulose acetate some of them are soluble in organic solvents (Granström, 2009). Increasing the hydrophilicity can be done by increasing the amount of hydrophilic functional groups on the membrane surface (Zhang, 2015). Hydrophilic materials which means that all or part of their chemical structure must be polar can also participate in hydrogen bonding. Cellulosic fibers comprise most oxygen molecules and -OH group which can interact with water. Water will interact conveniently with polar groups, such as -OH, R-Halogen, =O, and -NH₂, this is the key point. The cellulose based polymers such as hydroxypropyl methylcellulose (HPMC), hydroxypropyl cellulose (HPC) (Uchegbu and Schatzlein 2006), carboxymethyl cellulose (CMC) are many times used in medical devices or drug release. They have a balance of hydrophilic and hydrophobic groups on the cellulosic backbone which determines the eventual swelling and gelling or dissolution rates. These cellulosic system must be produced to the ultimate desired rate of drug release such as nystatin (Quinones and Ghaly 2008) for enhancing solubility and its feasibility for local fungal infections. But, there are less studies relating with electrospinning of HPC in the literature. Shukla et al. fabricated HPC electrospun fibers using ethanol and isopropanol dual solvent system (Shukla, Brinley et al. 2005). In another study, two types of enzymes were immobilized to HPC electrospun fibers by Periasamy et al. (Periasamy, Devarayan et al. 2012). Drug delivering biomaterials can be obtained by introduction of cellulose and its derivatives into synthetic polyurethane matrix (Rivera-Armenta, Heinze et al. 2004, Macocinschi, Filip et al. 2009). The polyurethane-hydroxypropyl cellulose has improved properties, such as specific biodegradability or biostability Vlad et al. explained that specific biodegradability or biostability is the improved properties of polyurethane-hydroxypropyl cellulose (Mandru, Vlad et al. 2013, Alvarez-Lorenzo, Gomez-Amoza et al. 2000, Macocinschi, Filip et al. 2012). Raschip et al. found that studied biocompatibility and compatibility properties of HPC/PU blends (various

ratios) could be used in pharmaceutical and medical applications because good surface properties and haemocompatibility was exhibited and they are not cytotoxic (Raschip et al. 2009).

Table 2.3 : Properties of used polymers.

Polymer	Properties
PU	*Hydrophobic *Thermoplastic polymer *Synthetic polymer
HPC	*Bioadhesives *Increase the permeability *Hydrophilic polymer *Water soluble cellulose ethers *Exposing with water, the polymers form to hydrogel and gradually dissolve in water until disappear *Good swelling properties *These dosage forms generally called dissolution-controlled drug delivery *Natural polymer
EC	*Bioadhesives *Poor water permeability *Hydrophobic polymer *Water insoluble cellulose ethers *Exposing with water, remain as a viscous gel which doesn't allow to drug to freely release from dosage form *good in mechanical properties *These dosage forms generally called diffusion-controlled drug delivery systems *Natural polymer
PVP	*Hydrophilic and hygroscopic *Synthetic polymer

Various types of release profiles may be obtained by introducing the selected polymer into the main polymer matrix. For example, Huang et al. was studied the sustained drug release by preparing fibers from polymers insoluble in water or biodegradable macromolecules (chitosan, ethyl cellulose, cellulose acetate and polycaprolactone) (Huang, Branford-White et al. 2012), on the other hand Yu et al. was achieved the immediate drug release by fabricated fibers from hydrophilic polymers (poly(vinyl pyrrolidone) (PVP)) and polyethylene oxide (Yu, Branford-White et al. 2010). The basis of a fast dissolving drug delivery systems composed PVP may be developed for drugs which it is poorly water-soluble for possible oral delivery system. Yu DG et.al. have been studied the release behaviour of drug loaded

PVP and EC or PVP/EC electrospun nanofibers (Yu, Shen et al. 2009, Yu, White et al. 2012).

The drug release depends on how the drug is embedded into the polymer and also by the properties of polymers: chemical and morphological structures, the degree of order, permeability, glass transition temperature, degree of crystallinity, miscibility, biocompatibility (Mandru, Ciobanu et al. 2011). Drugs move from their first status in a polymer composition to the release environment if the "drugs release" occurs in the polymeric systems. An other choice, drug which firstly are hold on to a surface material may be delivered once brought in contact with an external environment. Drug releasing is driven mainly by diffusion in the membrane which does not degraded or matrix systems. The release rate is related by the permeability and thickness of polymer matrix, also the solubility of the drug in the polymer matrix (Fu and Kao 2010).

2.3 Transdermal Delivery

Human skin is an ideal application to study release the active agents for both systemic and local administration because of being well-engineered and the largest organ and it protects organism against environmental factors. Mainly, the epidermis, the dermis, and the hypodermis layers are constructed the skin. Stratum corneum (SC), formed from keratinized and dead cells is the outmost layer of epidermis. Passage of drugs via the skin is hindranced by SC. The drug molecules comes from transdermal or dermal systems have to access via the SC layer to enter systemic cycle and/or to arrive lower layers of the skin (Okyar, Güngör, 2012). "Transdermal system" provides to enter the drugs to systemic cycle by controlled drug release rather than topical application of some drugs to the skin, especially for local effects (Chandrasekaran, Michaels et al. 1976).

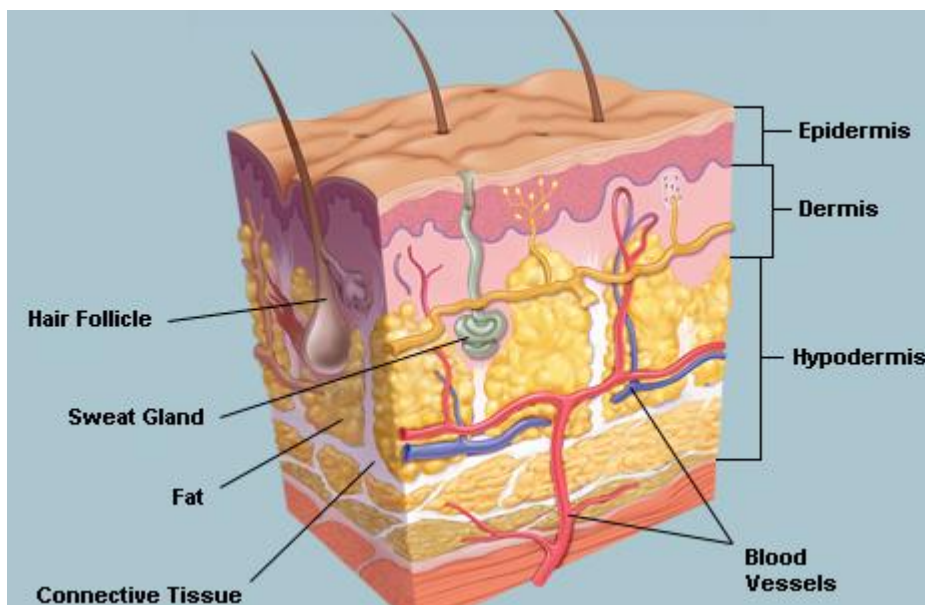


Figure 2.3 : Cross section of the human skin.

Transdermal drug delivery systems (TDDS), tailored to release the drug therapeutically effective amount through to the skin in a prearranged time by controlled rate, and in this way drug plasma concentration can be continued constant over a long period (Wokovich, Prodduturi, Doub, Hussain, & Buhse, 2006). TDD systems superiority to the oral drug delivery, hypodermic injections, gastrointestinal drug degradation (Brown & Langer, 1988), poor absorption, and local irritation (Bariya, Gohel, Mehta, & Sharma, 2012). Transdermal drug delivery systems can be removed immediately when patient compliance or irritation on the skin is occurred (Güngör, Erdal, & Özsoy, 2012).

The followings are the advantages of TDD systems to other traditional release systems (Deshwal and Verma 2012):

1. Bioavailability and efficacy of drugs increase by leapping the first pass metabolism, salivary metabolism and intestinal metabolism.
2. Possible self sufficient administration.
3. Removal of the patch from the skin at any time during application, at an emergency action.
4. Disposal of gastrointestinal incompatibility, the fluctuation in plasma level of drug and it is easy application for patient.
5. Steady, consistent and optimum blood concentration.

6. By the only single application release of drug for prolonged time
7. Easy of use the drugs with short biological half-lives
8. If oral administration causes vomit, diarrhoea, intolerable side effects this way is an alternative way to take drug.

The followings are the disadvantage of TDD systems (Deshwal and Verma 2012)

1. Only potent drugs are convenient, ionic drugs are not available candidate for TDD
2. Skin application area can be irritation in some patient
3. High blood levels drugs is not available
4. Uneconomic system
5. It can be used only for chronic conditions not acute, because it is required that drug therapy for a long period of time.

2.4 Drug Release Kinetic Model

Drug release process consists of the steps absorption, distribution, metabolism, and excretion (ADME) when a drug leaves product. In vitro dissolution is an important parameter in development of drug. It is related to function of time (t) and the amount of drug released from the therapeutic formulation. Zero and first order release kinetics, Higuchi, Hixson Crowell and Korsmeyer Peppas models are just used in our study (Bruschi, 2015).

2.4.1 Zero-order release kinetics

“Zero order kinetics” is an ideal delivery of drugs, if blood levels of drugs would keep constant throughout the delivery time, the dissolution of a drug molecule is only a function of time in the zero order kinetics. This model is only valid in a condition of very slow drug release.

In a general way Zero Order Release Kinetics may resume the following expression:

$$Rate = k[W]^n$$

$$Rate = -\frac{d[W]^0}{dt} = k \quad (n = 0)$$

$$d[W] = -kdt$$

$$\int_{[W_o]}^{[W]} d[W] = - \int_0^t k dt$$

$$[W] = [W_o] - kt \quad (2.1)$$

W_o and W : the initial amount of drug and the amount of drug at time t in the pharmaceutical dosage form,

k : the release constant of Zero-Order with units of concentration per time.

Plot of the drug dissolved fraction versus “ t ” shall be linear if the specified situation were implemented before this.

Describing the dissolution of drug in modified release dosage forms (like in osmotic systems, matrix tablets with low soluble drugs in coated forms, some transdermal systems etc) can be related for this equation.

2.4.2 First order release kinetics

The first-order model identified the drug release from dosage form, in this way release rate is concentration dependent.

In a general way First Order Release Kinetics may resume the following expression:

$$Rate = \frac{d[W]}{dt} = -k_1[W]$$

$$\log[W_1] = \log[W_o] + \frac{k_1 t}{2.303} \text{ or}$$

$$W = W_o - e^{k_1 t} \quad (2.2)$$

W_o and W : the initial drug concentration and the amount of drug released in time (t),
 k_1 : constant of first order.

Obtained data are plotted as $\ln$ cumulative percentage of drug remaining versus “ t ” which would yield a straight line with a slope of $-k$.

This relation can be related for describing the dissolution of drug in pharmaceutical forms (like, comprising water-soluble drugs in porous matrix)

2.4.3 Higuchi model

The Higuchi model was improved by Higuchi to examine the release of low-soluble and water-soluble drugs inserted in solid and semi-solid matrix. In a general way, the mathematical equation of Higuchi model derived for drug particles dispersed in a uniform system acting as diffusion medium may refer to following expression:

$$W = k_H t^{1/2} \quad (2.3)$$

W: the amount of drug released in t (time) per unit area A,

k_H : constant of Higuchi dissolution.

t: time in hours

Higuchi explains drug release square root time dependent as a diffusion process on the basis of Fick's law. A plot of W versus square root of time is linear for diffusion controlled system used to explain the drug dissolution from various types of modified release pharmaceutical systems, matrix tablets with water soluble drugs and some transdermal systems

2.4.4 Hixson Crowell model

The Hixson-Crowell cube root law describes the release from systems where there is a change in surface area and diameter of particles or tablets. Hence, particles of regular area are proportional to the cube root of its volume.

In a general way Hixson Crowell model may resume the following expression:

$$W_0^{1/3} - W_t^{1/3} = k_{HC} t \quad (2.4)$$

W_0 and W_t : the amount of drug in the first time and the remaining amount of drug in the pharmaceutical system at "t",

k_{HC} : the rate constant for Hixson-Crowell rate equation incorporating the surface volume relation.

A plot is cube root of drug percentage remaining in matrix versus time. The statement applicates to pharmaceutical system (like tablets, where the dissolution achives in planes that the first geometrical form keeps constant all the time).

2.4.5 Korsmeyer-Peppas Model

The Korsmeyer-Peppas Model was improved for specific system that the release of a drug from a polymeric matrix (like a hydrogel). The kinetics of this model are characterized by diffusivity.

Korsmeyer-Peppas model may resume the following expression:

$$\frac{W_t}{W_\infty} = k t^n$$
$$\log\left(\frac{W_t}{W_\infty}\right) = \log k + n \log t \quad (2.5)$$

wherein ;

W_t : the concentration of the drug in the release solution at “t”

W_∞ : the equilibrium concentration of drug in the release solution

W_t / W_∞ : the fraction of drug in the release solution at “t”.

k : rate constant of drug release

n : the diffusion or release exponent.

2.5 Donepezil

Development of Donepezil research began in 1983 by Eisai. FDA (The Food and Drug Administration) approved the Donepezil which helps to prevent the acetylcholine breakdown in the brain and treats the symptoms of Alzheimer’s disease (Shintani and Uchida 1997). In November 1996, 5 or 10 mg (recommended dosage is 5 mg/day for the first 4 weeks and 10 mg/day thereafter) immediate release (IR) of Donepezil tablets were approved in the US to treat the dementia which is the Alzheimer’s type (Black, Doody et al. 2007, Di Stefano, Iannitelli et al. 2011).

23 mg sustained-release (SR) Donepezil tablets were approved by FDA in 2010, to provide a higher once daily dose. Also, 5 or 10 mg orally disintegrating Donepezil tablet (ODT) which is practical for patients who swallows tablets difficultly, were approved in the US in 2004. Furthermore, they allow for administration once daily.

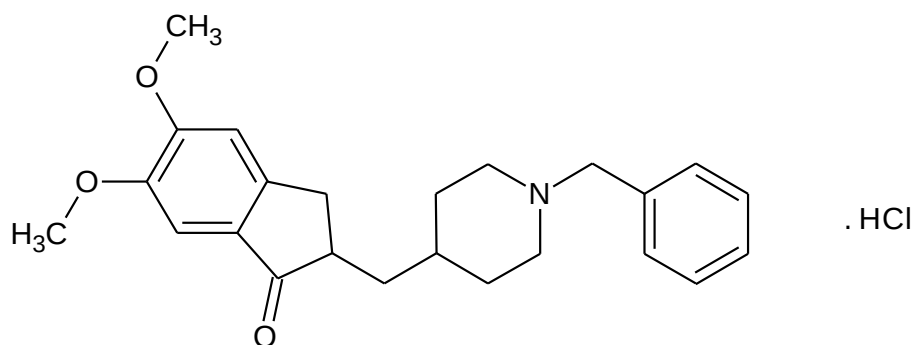


Figure 2.4 : Structure of Donepezil HCl.

Donepezil was chosen a model drug in this study because of it's adverse events proportional to the gastrointestinal system (diarrhea, nausea and vomiting), sleep disorders, the severity and frequency of adverse events according as selected dose (Farlow, Veloso et al. 2011). The incidence of adverse events increases with taking of drug by oral way, the alternative route to the oral administration, transdermal delivery systems of donepezil was attempted by Valia et al (Valia and Ramaraju 2016) in order to decrease the adverse events. The molecular structure is given in Figure 2.4.

2.6 Determination Method of Active Material

Several analytical methods (like ultraviolet visible spectroscopy, mass detection and liquid chromatography) (Nakashima, Itoh et al. 2006) have been used in the literature for drug determination. However, these methods require sample pretreatment because of not all of them are suitable for the routine detection. Electrochemical methods which do not need pre-treatment eventually lowers the analysis expense (Sayın and Kır 2001) it can be useful for sensitive and selective determination in pharmaceutical compounds. Gotti et al. detected the Donepezil by using a capillary electrophoretic method based on the rapid migration of the analyte gained under acidic situations (Gotti, Cavrini et al. 2001). Wang et al. studied that the electrochemical oxidation of pioglitazone, they have been investigated by impedance and cyclic voltammetry (CV) using a gold and silver electrode (Wang, 2014).

Electrochemical impedance spectroscopy (EIS) is a powerful method for investigating the changes of interface properties at electrode surfaces, the changes of many physical and chemical processes in solutions among the electrochemical

methods. The current response is detected in the impedance analysis by applied small sinusoidal AC voltage probe (typically 2–10 mV). The results of an impedance measurement can be illustrated in two different ways: imaginary impedance, $Z''(\omega)$ plotted against real impedance $Z'(\omega)$ in Nyquist plot, and phase angle or absolute impedance values (Z) plotted against the frequency in Bode plot (Bode Z and Bode phase) (Figure 2.5).

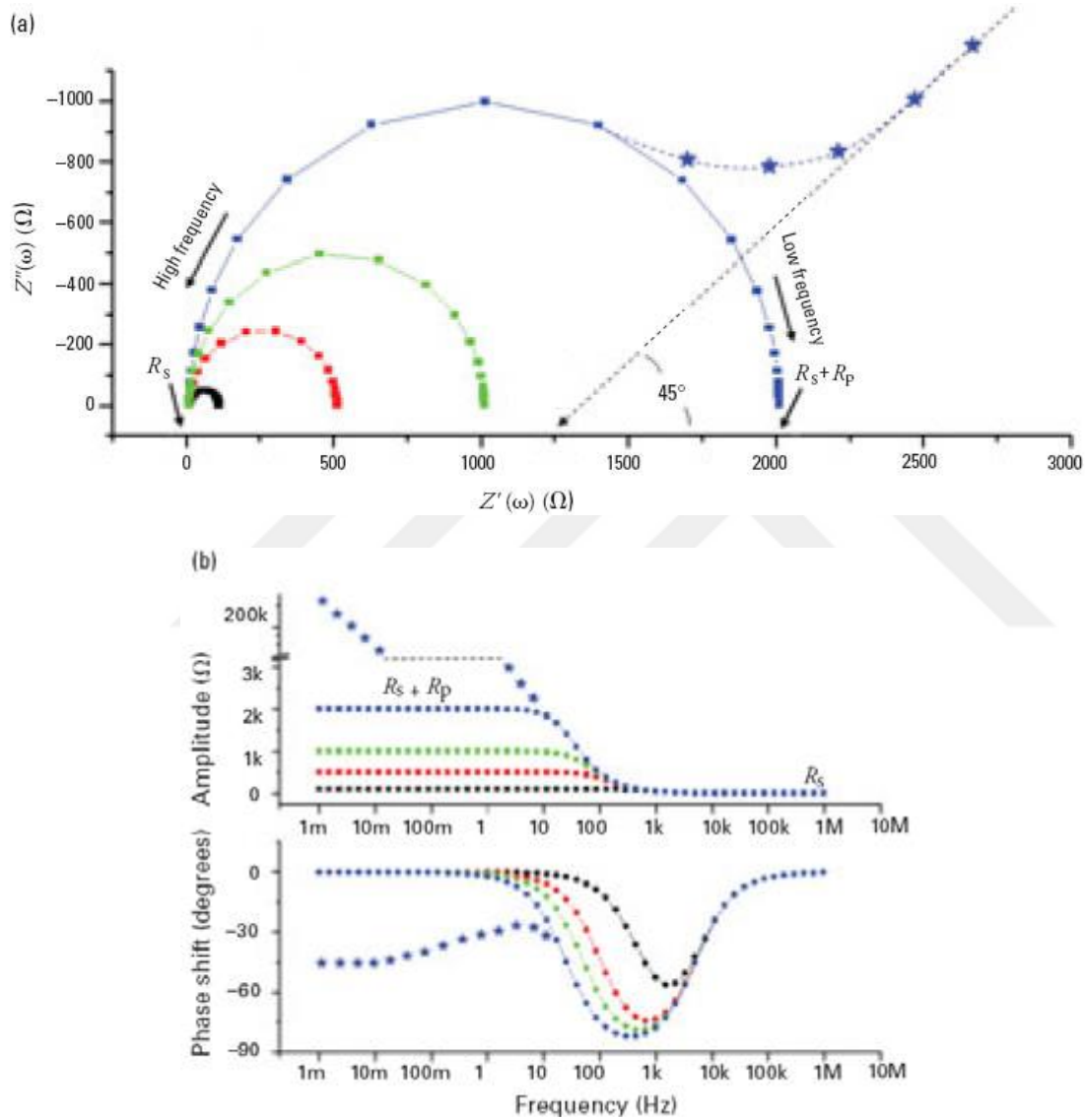


Figure 2.5 : (a) Nyquist plot (b) Magnitude of Z and Bode phase angle.

The reaction information and the interface of electrolyte and electrode can be obtained from the Nyquist plot and the Bode diagram. The impedance comprised two components: one is impedance of electrolyte or medium, and the other is electrode and the interface of electrode and electrolyte, called the interface or electrode impedance which was reported in literature (Hause, Komorowski et al. 1981).

The aim of the studies from EIS results are clarifying the process of electrode and its characteristic parameters derivation, it is a sensitive method although it does not supply a direct measure of the physical phenomenon. The experimental impedances in the way of mathematical functions was clarified by electrical impedance models for the purpose of obtaining good fit between the calculated and experimental impedances.

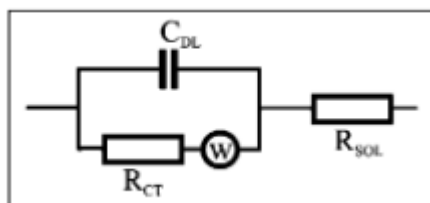


Figure 2.6 : Randles equivalent circuit model.

Randles circuit model which is simple electrochemical system was seen in Figure 2.6. Mostly, a solution or electrolyte resistance (R_{sol} or R_s) is an important element in the impedance of an electrochemical cell. Any solution resistance should be taken into account between the reference and the working electrode. The ionic solution resistance is related to the type and concentration of the ions, the geometry and temperature of the medium through which the current is transported. R_s is mainly due to the resistance of the electrolyte and is analytically helpful for conductivity sensors. R_{ct} is the resistance of electron-transfer or resistance of charge-transfer, it is related inversely to the rate of electron transfer. Nyquist plots (Z_{re} and Z_{im}) is a good way to establish R_{ct} to Bode Plot diagram (Bard, Faulkner et al. 1980).

Double layer capacitance (C_{dl}), appears between the electrode and the surrounding electrolyte interface. C_{dl} is generated as ions from the solution "stick on" the electrode surface. Electrode charges are separated from the charges of these ions. Electrode potential, type of ions, temperature, ionic concentrations, properties of the electrode surface (such as electrode roughness, adsorptions) are dependent on the double layer capacitance value.

Warburg impedance (Z_w) is known as diffusional impedance. At high frequencies, Warburg impedance is small and at low frequencies, Warburg impedance becomes large. Because at high frequencies, reactants do not diffuse very far away. But at low frequencies, the reactants of diffusing species have to move farther. Z_w is helpful

infrequently in the analytical applications for measuring the effective diffusion coefficients.

R_{et} and C_{dl} related with insulating and the dielectric properties between the electrolyte/electrode interface, though Z_w and R_s exhibit electrolyte solution features and diffusion of the redox probe. The properties change appeared at the electrode interface effects the parameters. Electrical impedance methods and electrical impedance models can be used for analyte determination if one or more of parameters of equivalent circuit are affected from the analyte and if interfering species do not affect the parameters between the elements of equivalent circuit.



3. MATERIALS AND METHODS

3.1 Materials

Thermoplastic polyurethane (PU, Mw is 93,000 g/mol) was supplied from Flokser Corporation, Istanbul, Turkey. Molecular weight (Mw) of Hydroxypropylcellulose was 140,000 g/mol (Nippon Soda Corporation Ltd) apparent viscosity was 8.6 mPa.s (2% aqueous solution at 20°C), assay for hydroxypropoxy group 71.4%. Polyvinylpyrrolidone (PVP) (Mw=360,000 g/mol) was purchased from Hangzhou Sunflower Technology and Ethylcellulose (EC) was obtained from Dow. N,N-dimethylformamide (DMF) has been taken from Merck. analytical grade compounds were used in the experiments. Sodium hydroxide (NaOH) has taken from Sigma–Aldrich. Potassium dihydrogen phosphate (KH_2PO_4) was purchased from Riedel-de Haën. Donepezil hydrochloride (DP) was kindly donated by Santa Farma Pharmaceutical Company (Turkey). The de-ionized water was supplied from Millipore Milli-Q ultrapure water system. All the chemical materials were used as received without any purification process.

For electrochemical impedance measurements, gold based screen-printed electrodes (SPEs) were belongs to Dropsens. SPEs is comprised gold working electrode which the diameter is 0.2 cm resulting in a geometric area of 0.126 cm^2 and pseudo-reference which is silver and an auxiliary electrodes which is platin. Indium Tin Oxide coated PET Electrodes (ITO-PET) were bought from Aldrich in which the surface resistivity of the ITO-PET is $60 \text{ } \Omega/\text{sq}$. The geometric area of the working electrode in ITO-PET is 3 cm^2 .

3.2 Methods

Studies mainly separated several sections. The first section is the assessment of electrospinning parameters to achieve the efficiently drug loaded electrospun fibers,

and the second section is comprised the drug loaded electrospun fibers characterization with different technique such as ATR-FTIR, SEM, porosity analysis and DSC. The third section consists of evaluation of drug release profile from electrospun fibers loaded drug, and the last section consists of electrochemical properties of the solution which comprise drug molecules and the drug loaded electrospun fibers. Studies of electrical properties were achieved by Electrochemical Impedance Spectroscopy

3.2.1 Electrospinning Method

The electrospinning method was used to fabricate drug loaded electrospun fibers for the use as transdermal patches. Preparation of polymers solutions, preliminary studies and electrospinning parameters were described in detail as given below. The electrospinning device comprises a syringe pump, a grounded collector and a high voltage direct current power supplier which produce positive direct current voltage up to 50 kV direct current power supply (ES 30 Model, Gamma High Voltage Inc., USA). Syringe pump is NE-500 Model, New Era Pump Systems, Inc. USA and its rate of feed from 5.5 $\mu\text{L/h}$ to 400 mL/h. Grounded collector is an aluminum plate.

3.2.2 Preliminary studies of the electrospun fibers

The solvent selection is one of the important factor for preparing drug-loaded fibers using electrospinning technique. Three conditions should be met: (i) should have good electro-spinnability of the polymer after dissolving in the solvent, (ii) amount of enough and therapeutically useful drug should dissolve in the solvent, (iii) the obtained polymer and drug solution should be responsible for electrospinning method (Li, Wang et al. 2014). N,N-Dimethylformamide (DMF) has a high boiling point of 155 °C and dipole moment (at 25°C, $\mu=3.86$ D) and dielectric constant (at 25°C, $\epsilon=37.51$) and it is an aprotic solvent. It should be noted that DMF increases the dielectric property, makes contribution to the solution and improves the fiber morphology (Lee, Kim et al. 2003). In the light of this information DMF is common solvent for all materials at the thesis was chosen as a solvent for dissolving all the ingredients (PU, HPC, EC, PVP and Donepezil). Various concentrations (10-20%, w/v) of PU in DMF was prepared and tested to determine the required polymer

conditions for efficient electrospinning parameters. Besides, the electrospinning process parameters including the flow rates (0.5, 1, 2 ml/h), applied voltages (10, 12.5, 15 kV), distance between tip and collector (10, 12.5, 15 cm) were tested to find the optimum electrospinning process parameters. The preliminary results showed that the bead-free electrospun fibers could be only obtained at 12.5% (w/v) concentration of PU solution. The polymer solution viscosity should be suitable enough for producing fibers consistently and non-bead fibers. The same optimization parameters practiced to the HPC, but no fiber formation has been resulted at the electrospinning of HPC alone in DMF.

3.2.3 Preparation of electrospinning solutions

HPC, EC or PVP at different ratio was added as a second polymers to optimize the properties of PU solution. Second polymer was added in order to determine the effect on PU electrospun fibers and release properties at different blend ratios of polymers. DP as a model drug was dissolved molecularly or in particulate form in polymeric network to prepare electrospinning solutions for getting drug loaded PU/HPC, PU/EC, PU/PVP electrospun fiber mats. Finally PU/Second Polymer/DP ratio of 10/1/1, 10/2/1, 10/4/1, 10/8/1 (w/w/w) polymer solutions were prepared.

3.2.3.1 Characterization of the electrospinning solution

Viscosity and conductivity analysis were done to characterize the electrospinning solutions of PU/HPC and PU/HPC/DP.

Viscosity of electrospinning solution

The viscosity of PU/HPC solutions at different ratio of PU and HPC (between 10:0 and 10:8) were determined by viscometer (DV II+RV, Brookfield Digital Viscometer, Toronto, Canada) fitted with Spindle 21 at 10 rpm, 25°C.

Conductivity of electrospinning solutions

The solutions conductivity were analysed by conductivity meter (Eutech Instruments, PC2700, Singapore) at ambient temperature. Each of measurements was performed in triplicate.

3.2.4 Preparation of the electrospun fibers

Polymer solutions (described in 3.2.3) were loaded to the syringe needle having 0.7 mm outer diameter. Positive electrode (+) was attached the syringe needle. Negative electrode (-) was attached to the metal spinneret and high-voltage power supply was employed to generate the electric field. The feeding rate in the electrospinning method was checked by a syringe pumping apparatus. Syringe pumping apparatus and the collector placed by horizontally.

Table 3.1 : Electrospinning parameters.

Electrospinning parameters	
Applied voltage (kV)	15
Flow rate (mL/h)	1
Tip to collector distance (cm)	15

The question what the effective factor is on the morphology in electrospinning under various experimental requirement were investigated and the optimized electrospinning factors were evaluated to obtain having best morphology for the fibers. After determining the optimized parameters for PU solution, fibers were prepared according to optimized parameters indicated in Table 3.2 using the electrospinning method. At room temperature, 15 kV driving voltages was applied to the polymers solutions to obtain electrospun fibers. The polymer solution feed rate was 1ml/h and the distance was fixed to 15 cm between the capillary tip and the collector. The amount of PU and Donepezil were fixed at 0.5 mg and 0.05 mg and the amount of second polymer was varied as given in Table 3.2. Table 3.1 and 3.2 show electrospinning parameters and ratio of ingredients in the electrospun fibers.

Table 3.2 : Ratio of the ingredients of the fibers.

PU/HPC/DP	PU/EC/DP	PU/PVP/DP
(w/w/w)	(w/w/w)	(w/w/w)
0:10:0	-	-
10:0:0	-	-
10:1:0	-	-
10:2:0	-	-
10:4:0	-	-
10:8:0	-	-
10:10:0	-	-
10:0:1	-	-
10:1:1	10:1:1	10:1:1
10:2:1	10:2:1	10:2:1
10:4:1	10:4:1	10:4:1
10:8:1	10:8:1	10:8:1

3.2.4.1 Characterization of the electrospun fibers

Different methods were employed to characterize the PU/HPC/DP electrospun fibers and determine the thermal, spectroscopic and morphologic properties and the porosity sizes of the samples. These are;

Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR)

ATR-FTIR spectroscopy was used to monitor the incorporation of the second polymer and drug in the polyurethane matrix. Pure powders and electrospun fibers were analysed by ATR-FTIR reflectance spectrometer (Perkin Elmer, spectrum One; with a diamond and C70951 ZnSe crystal with a universal ATR accessory).

Differential scanning calorimetry

Differential scanning calorimetry (DSC) (DSC Q1000, TA Instruments, New Castle, USA) was used to investigate the thermal properties of electrospun fibers. The DSC analysis were performed at a heating rate of 10 °C/min under N₂ atmosphere by heating from 25 °C to 350 °C.

Nuclear magnetic resonance spectroscopy

The structural changes of electrospun fibers were examined by proton nuclear magnetic resonance (¹H NMR) (Agilent VNMRs 500 MHz, Agilent Technologies, California, USA) system. The experiments were performed in CDCl₃ at 25±5°C. The ¹H chemical shift values were informed on the δ scale in ppm.

Scanning electron microscope

Electrospun fibers diameters and morphology of the surface were evaluated with scanning electron microscope (SEM) (Philips-XL30 SFEG, Tokyo, Japan). Thin layer of gold/palladium (Au/Pd) alloy were used for coating the each surfaces sample, it sputtered to the surface for obtaining a conductive surface before SEM imaging. The SEM images were obtained with maximal resolution up to 150 eV at an accelerating voltage of 20 kV and magnification up to 6000-8000x. Image J program was used to calculate their average fiber diameters (AFD). Image J was measured the diameters of 50 individual fibers at random for each sample.

Porosity analysis

Porosity of electrospun nanofibers was analyzed at 200 mPa by Pascal mercury porosimeter (ThermoFisher Scientific, Inc., Massachusetts, USA).

3.3 In-vitro Release Studies of Active Material by UV Detection

In vitro release researching in PBS solution was done to determine the drug release from electrospun fibers with drug loaded. In vitro release researching was used the "dissolution test for transdermal patch" of United State Pharmacopeia (USP) 38 method. Dissolution rate of model drug (DP) was determined from all ratios of PU/HPC/DP, PU/EC/DP and PU/PVP/DP electrospun fiber mats.

Electrospun fiber mat section with 2.5 cm diameter was fixed to a stainless steel sieve which having a pore diameter of 125 μm . The prepared sample was put into a glass vessel containing the release medium, which was used 500 mL of pre-tempered phosphate buffer (PBS, pH:6.5, which is the most soluble for DP) in the experiments. The analysis was done at 50 rpm stirring rate in $32.0 \pm 0.5^\circ\text{C}$.

At the predetermined intervals (from 30th minute up to 6th hour), 2 mL of dissolution sample was withdrawn periodically from release medium and the fresh phosphate buffer at equivalent volume was replaced immediately in order to maintain sink condition. UV-visible spectrophotometer (Shimadzu, UV-1601, Tokyo, Japan) monitored the drug release. DP concentration in the samples was analyzed by UV-

visible spectrophotometer using calibration curve prepared at the wavelength of 229 nm. Each experiment was performed in triplicate.

The method was validated for accuracy, precision, linearity and selectivity. It was linear over the 2.5-20 $\mu\text{g/mL}$ concentration range having high accurate (recovery>98%) and correlation coefficient ($r^2>0.999$). Interfering absorbance with DP confirming the selectivity of the method was not seen.

In vitro release results were fitted to Kinetic Models such as Zero Order, First Order, Hixson-Crowell, Higuchi Square Root and Korsmeyer-Peppas to investigate the drug release kinetics of electrospun fibers.

3.4 In-vitro Permeation Studies of Active Material

In vitro permeation studies were done for PU/HPC/DP (10/2/1) and PU/EC/DP (10/4/1) electrospun fibers which gave the best results at the in vitro release researches. In vitro permeation researches were conducted in modified Franz diffusion cell (Hellertown, PA, USA, Permeagear, V6A Stirrer,) the diffusional surface area was 1.77 cm^2 and the receptor phase volume was 12 mL. The PBS (pH 6.5) was used for the receptor phase to maintain sink condition. Strat-MTM membrane (Millipore®, Massachusetts, USA), which is considered as an alternative skin resembling synthetic membrane to human and animal skins for permeation studies (Uchida, Kadhum et al. 2015) were placed onto the surface of diffusion cells, PU/HPC/DP and PU/EC/DP electrospun fibers mat was fixed between artificial membrane and donor compartment. Electrospun fiber mat was wetted with 200 μL of PBS (pH 6.5) to provide adhesion onto the membrane. The cells surfaces were covered by ParaFilm® to avoid the electrospun fibers drying. The temperature was sustained at $37\pm0.5^\circ\text{C}$ via a circulating water bath through a jacket surrounding the cell, and magnetically stirred at 300 rpm. 0.5 mL of samples were withdrawn from receptor phase in the prearranged intervals (from 3th hour up to 72nd hour) and at the equivalent volume and temperature of PBS (pH 6.5) was replaced into the receptor phase. DP concentration in the electrospun fibers were determined by validated high pressure liquid chromatography (HPLC) method.

The permeation rate of DP through the artificial membrane and permeation parameters were calculated. Each experiment was made in triplicates. The cumulative amount of permeated DP through the artificial membrane against time was drawn as a graph. The steady state flux (J) was estimated from the slope of straight line portion of the cumulative amount of permeated DP versus time plot. The lag time was calculated extrapolating the linear part of the cumulative drug amount against time plot.

3.4.1 Analysis method

Analysis of the Donepezil at permeation studies was done by HPLC Method (Galipoğlu, Erdal et al. 2015). The quantification of DP concentrations in the electrospun fibers were done by the high pressure liquid chromatography (HPLC) which was equipped with a PDA detector (Kyoto, Japan, LC 20AT Shimadzu Model). LiChrospher 100-RP18, CN, 125 mm x 4.0 mm, 5 μ m column (Waters Symmetry, Massachusetts, USA) was used as the stationary phase. The 14 mL of triethanolamine was mixed in 1 L ultrapure water and 200 mL of this solution was diluted to 1 L with ultrapure water. Then, pH value of the solution was arranged with ortho-phosphoric acid (10 %, v/v) to 2.5 and filtered through with 0.45 μ m of pore size membrane filter (Millipore®, Massachusetts, USA). Mobile phase was a mixture of this solution and acetonitrile in ratio of 60:40. The detection wavelength and flow rate were specified at 270 nm and 1.0 mL/min, respectively. The temperature was adjusted to 25°C. The method was validated for precision, accuracy, linearity and selectivity. The calibration curve was established between 0.1-150 μ g.mL⁻¹ ($r^2 > 0.999$). The precision of method was less than 2% as a relative standard deviation (RSD). Accuracy was ranged from 95% to 105% in which declared as a percentage of the mean recovery.

3.5 In-vitro Cytotoxicity Studies of Drug Loaded Electrospun Fibers

Cytotoxicity studies of PU/HPC/DP electrospun fibers were also performed. Cytotoxicity assays and cell viability was used to screen cytotoxicity of electrospun fibers. Institutionally, counting viable cells after staining with a vital dye was done to

establish of growth of cell. WST methods, MTT, Tritium-Labeled Thymidine Uptake method, Crystal Violet method and Colony Formation method approaches have been used for counting the number of live cells. MTT is a colorimetric assays using the tetrazolium salt thiazolyl blue, after methyl-thiazolyl-tetrazolium (Mosmann 1983) are widely used for evaluation of cell viability, cytotoxicity and proliferation researches in cell biology (Horobin and Kiernan 2002).

The relative cytotoxicity of PU/HPC/DP electrospun fiber (10:2:1, w/w) mat selected based on the release behaviour, which follows Korsmeyer-Peppas kinetic model and, the PU/HPC (10:2, w/w) electrospun fiber samples without drug loaded were evaluated in the mouse fibroblast cell culture (BALB/3T3) which was provided from American Type Culture Collection (ATCC® CLL-163, USA). The mouse fibroblast cells were taken into each well at concentration of 1×10^4 cell/well and the aqueous solutions of NFs in the range of 0.125% and 2.0% (v/v) were waited in the cell culture for 8 and 16 hours. The cells were seeded into Dulbecco's Modified Eagle's (DMEM) containing 1% penicillin-streptomycine and 10% inactivated fetal bovine serum (FBS) and incubated at 37°C in 5% CO₂ atmosphere. In all experiments, isotonic PBS at concentration of 1% and sodium lauryl sulphate solution (SLS, 10% w/v) which is an irritan molecule for skin was used as negative and positive control, respectively (Horobin and Kiernan 2002). The cell viability was assayed by MTT test. The viable cells were identified by precipitating insoluble purple formazan (van Meerloo, Kaspers et al. 2011). As proposed by Alley et al. (Alley, Scudiero et al. 1988), the cells were washed in PBS (pH 7.4) after treating with NF mats. 20 µL of MTT solution and 200 µL of cell culture medium in PBS (5 mg.mL⁻¹) were poured into each well. The plates were incubated at 37°C in 5% CO₂ atmosphere for 2 hours. MTT were taken out, then dimethyl sulphoxide (DMSO) (200 µL) was poured into the wells and the cell culture medium to solve the purple formazan crystals. The plates were then shaken for 10 minutes and the absorbance of content in each well was measured at 590 nm with a reference wavelenght of 670 nm by a microplate reader (Epoch, BioTek Instruments, Vermont, USA). The each experiment was performed using the cells from 5 to 25 passages at least three times. The cytotoxicity activity of each specimen was expressed as the percentage of the cell viability.

3.6 Statistical Analysis

The determination were exhibited as mean value of at least three experiments $\pm$ standart deviation (SD). GraphPad Prism Software version 6.05® (La Jolla, CA, USA) was used for the statistical analysis data. The data were compared with unpaired Student t-test. Statistical significance degree was expressed as $p < 0.05$.

3.7 Electrochemical Impedance Spectroscopic Measurements

3.7.1 In-vitro release studies of active material by electrochemical impedance

The electrical properties of the solution was researched also by Electrochemical Impedance Spectroscopy (EIS) during the drug release, addition of in-vitro release researches of drug by UV-vis spectroscopic determination. Evaluation of in vitro release studies have been done according to above section 3.3 at the predetermined intervals. 2 mL of dissolution sample was withdrawn periodically (from 30th minute up to 6th hour) from release medium and pour to the electrochemical cell immediately to determine the electrical properties of the buffer solution containing drug molecules.

EIS measurements were studied at room temperature (25°C) by using the screen printed electrodes (SPEs) which are miniaturized devices. SPEs has been improved for several progressive practices in analytical chemistry (Wang 1994), particularly at the analytical analyses improvement and the biosensors production (Tertis, Florea et al. 2013). On the other hand, if they compare with the current electrodes, SPEs have extra features such as simplicity use.

The electrochemical cell was connected to a PARSTAT 2263 Potentiostat by three electrodes. Impedance measurements was measured by PowerSine (an software of electrochemical impedance) between 10 mHz and 100 kHz. The AC amplitude voltage used for the experiments was 10 mV as DC potential. The Electrochemical Impedance Spectroscopic results were studied ZSimpWin V3.10 program and were fitted to best equivalent circuit model to investigate the circuit elements.

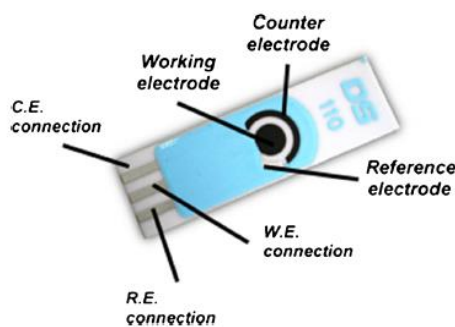


Figure 3.1 : Screen printed electrode.

3.7.2 Electrochemical impedance spectroscopic measurements of drug loaded electrospun fibers

Effect of the released drug on solution electrical properties was investigated by EIS as described in above. In these studies, drug loaded PU, PU/HPC and PU/EC electrospun fibers were collected onto commercial ITOPET with geometric area of 3 cm² as illustrated in Figure 3.2 for reproducible electrochemical measurements.

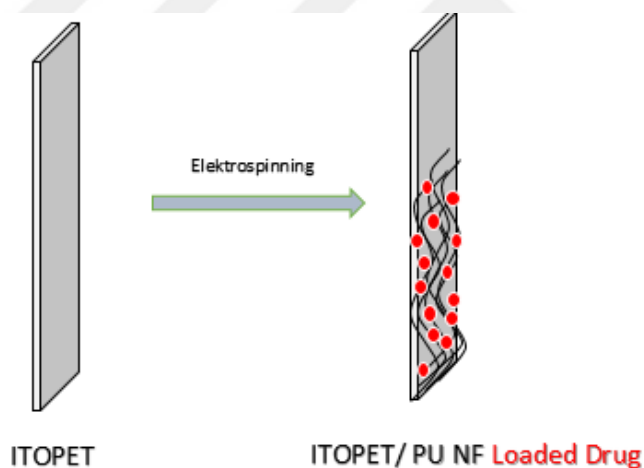


Figure 3.2 : Schematic representation of ITOPET/PU NFs drug loaded electrode.

Analysis were done in PBS medium using between 10 mHz and 100 kHz frequency range and AC voltage of 10 mV at the predetermined intervals (between 15 and 60 minutes (every 15 minutes) and 90th, 120th and 180th minutes). Three-electrode system was used, working electrode was coated ITOPET with drug loaded electrospun fiber (ITOPET/PU NFs drug loaded), pseudo reference electrode was silver wire and counter electrode was platinum wire. Equivalent circuit fitting was done according to the results of these measurements.



4. RESULTS AND DISCUSSIONS

Electrospinning solutions of PU, PU/HPC, PU/EC and PU/PVP (in various ratio) with drug or without drug were prepared with respect to method described in “Material and Method” part. Preparation details are given in below.

4.1 Preparation of Electrospinning Polymer Solutions

Optimum parameters for electrospinning solution of PU were found after several tries of PU concentrations. 0.5 g PU dissolved in 4 mL DMF was determined as optimized amount, and was stirred continually for 4 hours until the solution became transparent. Then HPC (or EC, PVP) with different contents were added to the homogenous electrospinning solution of PU separately, they were stirred magnetically for 30 minutes. Blended electrospinning solutions of PU/HPC, PU/EC and PU/PVP were prepared. Then, Donepezil HCl (DP) as a model drug (0.05 g) which the maximum amount of soluble drug in the polymer matrix (containing at least polymer) was dispersed within these blended solutions. Preliminary studies have shown that after adding more than 10 % (w/w) DP, it could not be dispersed in the solutions. Finally, electrospinning polymer solutions described in Table 3.2 were prepared. Conductivity and viscosity analysis of the electrospinning solutions for drug loaded and unloaded PU and PU/HPC were done. Also, conductivity measurements of the electrospinning solutions were done for drug loaded PU/EC and PU/PVP. Electrospinning solutions were then placed into the syringe and fed to the tip of the spinneret capillary.

4.2 Characterization of the Electrospinning Polymer Solutions

Drug loaded and unloaded PU and PU/HPC electrospinning solutions were characterized by viscosity and conductivity methods. Results of the measurements are represented in Table 4.1 and 4.2. Viscosity study was performed only for PU and PU/HPC electrospinning solutions, studies were not done for PU/EC and PU/PVP, because significantly visible changes have not seen between in various ratios of

PU/EC and PU/PVP electrospinning solutions. Conductivity measurements of the electrospinning solutions were also performed for drug loaded PU/EC and PU/PVP.

4.2.1 Viscosity and conductivity of the electrospinning polymer solutions

The viscosity and conductivity results of unloded drug PU and PU/HPC electrospinning solutions were given in Table 4.1 and Figure 4.1. The viscosity was found between the range of 740 and 6290 mPas. As expected, the viscosity of the electrospinning solutions increased dramatically with increasing of HPC in the polymer solutions. Polymer chains interactions happens according as HPC spreaded into the solution. (Ma'alania A., 2018). HPC can solubilize in organic compounds and also water and it is known as a natural polymer, secondary -OH groups of HPC can be oxidized to ketones, their cross-linking properties can be improved. Viscosity of polymer solution was not detected at 10/10 (PU/HPC) ratio. It is worth noting that generally higher HPC values gives to the solutions gel-viscous properties. It is determined that the viscosities of the PU/HPC solutions increased by increasing concentration of HPC in PU solutions due to the being gelling agent of HPC. It was seen in Figure 4.1, there is a linear increase in conductivity with increasing HPC content. 10/8 (PU/HPC) sample solution has slightly higher conductivity and much higher viscosity than 10/0 solution.

Table 4.1 : Viscosity, conductivity of PU/HPC solutions and the average fiber diameter of fibers.

Ratios of PU/HPC (w/w)	Viscosity (mPas)	Conductivity ($\mu\text{S.cm}^{-1}$)	Average fiber diameter (nm)
10:0	740	7.58 ± 0.24	1172 ± 10
10:1	890	4.52 ± 0.10	962 ± 15
10:2	1410	6.84 ± 0.10	791 ± 17
10:4	3170	12.03 ± 0.09	679 ± 23
10:8	6290	14.12 ± 0.54	687 ± 25

Diameters of average fiber obtained from the morphological images shown in Section 4.4.4 were also added to the Table 4.1. Average diameters of fibers of 10/8 (PU/HPC) which SEM images are shown in the section 4.4.4 were thinner than 10/0 possibly it is related with high conductivity and high viscosity of the solution comprising more HPC molecule. In the binary electrospinning polymer system average fiber diameter decreases from 962 nm to 687 nm with increasing conductivity from $4.52 \mu\text{S.cm}^{-1}$ to $14.12 \mu\text{S.cm}^{-1}$. The electrical conductivity

increase as the electrospun fiber diameter decreased (Park J.Y, 2007). Because salt, type of the polymer and solvent are important factors in conductive properties of resultant material. In nature, natural polymers generally show polyelectrolyte properties. Unlike the synthetic polymers, fiber formation of the natural fibers is poor. Because, the charge capacity of the polymer jet increases with the ions. Under the electric field, polymer jet are expose to high stress (Zhang C., 2005).

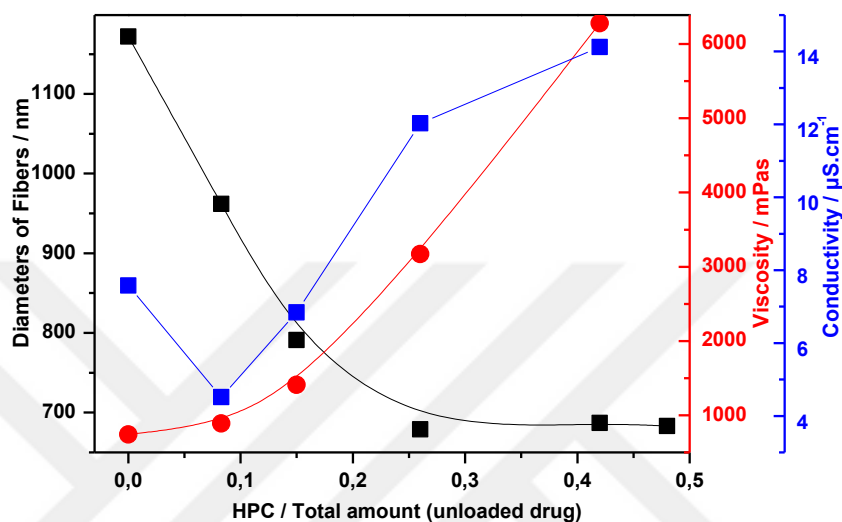


Figure 4.1 : Correlation of conductivity, viscosity of PU/HPC solutions and the average fiber diameter of fiber with HPC ratio.

Also, jet continuity is affected by viscosity which one of the important factors during electrospinning process. The literatures indicates that thicker electrospun fibers can be obtained by increased viscosity due to the increasing the concentration of polymer, and low viscosity leads to lower diameter electrospun fibers (Ramakrishna S., 2005). On the contrary, in the literature there are reports considering that not only the viscosity of the solutions do not affect the average fibers diameter of electrospun fibers. On the other hand, it was found that the addition of salt had greater effects on the fiber diameter than other parameters. In our findings increase in the solution conductivity favors the formation of thinner fibers.

Figure 4.1 shows that the average fiber diameters did not change considerable degree by adding HPC between 40 to 100 % to the PU. Because of that model drug, DP, was added to PU/HPC solutions which comprised between 0-80 % HPC.

Table 4.2 : The conductivity of PU/HPC/DP electrospinning solutions and the diameters of average fiber.

PU/HPC/DP (w/w/w)	DP/Total Amount	HPC/Total Amount	Conductivity ($\mu\text{S}.\text{cm}^{-1}$)	Average fiber diameter (nm)
10:0:1	0.090	0	149.70 ± 5.36	775 ± 16
10:1:1	0.083	0.083	131.67 ± 6.49	464 ± 24
10:2:1	0.077	0.150	117.27 ± 3.96	995 ± 14
10:4:1	0.067	0.260	147.40 ± 2.10	775 ± 20
10:8:1	0.053	0.420	116.43 ± 4.62	707 ± 28

The conductivity results of PU/HPC and PU/HPC/DP electrospinning solutions are given in Table 4.1, 4.2 and Figure 4.1, 4.2. Moreover, the conductivity of the solutions increased about 10-fold when the model drug (DP) was added into the blends of PU/HPC solutions because of the hydrochloride salt of DP. Conductivity of the PU/HPC blend solutions decrease with decreasing of the drug concentration in total solutions by adding different ratio of second polymer. Exception of the 0.260 “HPC/Total Amount” the conductivity of the electrospinning solutions decreased with decreasing drug and increasing HPC ratio. There are two reasons for this, one of them is the decreasing the concentration of the drug molecule (ions from salt form of the drug) and the other one is limitation of the ions motion area by gelation in the existence of HPC with increasing viscosity. Also, HPC support the polymer chains interaction, limit travelling of the ions in electropinning solutions and speed up the connection of polymer chains (Ma’alinia A. et al, 2018).

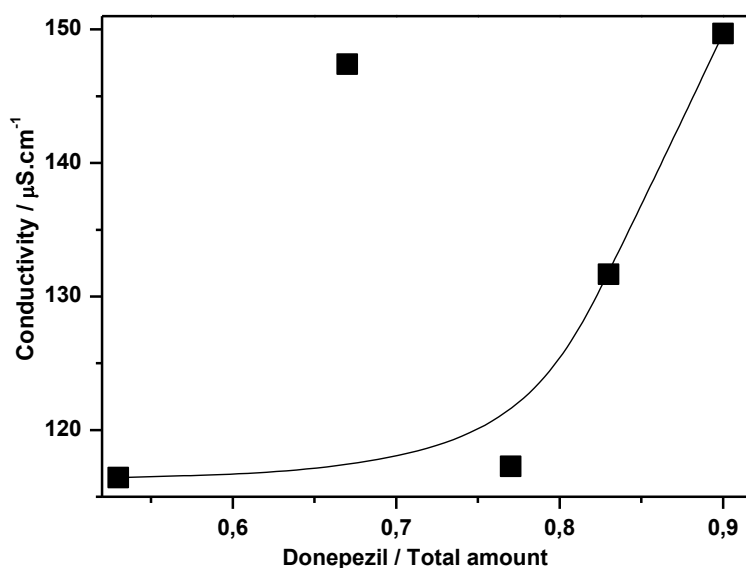


Figure 4.2 : Correlation between the solution conductivity and Donepezil HCl/ Total amount ratio.

Figure 4.2 shows, the conductivity of the drug loaded PU/HPC solutions decreases with the decreasing DP concentration in total polymer network. There are linear relationship between the conductivity and DP ratio in total polymer amount. These results show that conductivity is more dependent to the drug content in this study. However, there are no linear relationship between the conductivity measurements and average fiber diameters obtained from the SEM images which will be explained in detail the section 4.4.4. Because there are two variable parameters in these blends. One of them is the amount of Donepezil HCl and the other one is the amount of PU and HPC in the total mixture. While amount of Donepezil HCl and PU decreases, amount of the HPC increases in the total mixture.

Table 4.3 : The conductivity of PU/EC/DP solutions and the average fiber diameter of fibers.

PU/EC/DP (w/w/w)	DP/Total Amount	EC/Total Amount	Conductivity ($\mu\text{S.cm}^{-1}$)	Average fiber diameter (nm)
10:0:1	0.090	0	149.70 ± 5.36	775 ± 16
10:1:1	0.083	0.083	109.33 ± 4.15	410 ± 47
10:2:1	0.077	0.150	115.70 ± 6.65	556 ± 46
10:4:1	0.067	0.260	116.66 ± 1.65	518 ± 39
10:8:1	0.053	0.420	123.60 ± 2.20	603 ± 42

During this study, another trend was the increase in conductivity with the increasing concentration of EC in the PU/EC/DP solutions despite the drop in DP concentration observed in Table 4.3. Also in Table 4.3 with the exception of 0.077 of “DP/Total amount” a linear relationship is presented if the conductivity measurements and average fiber diameters obtained from SEM images for PU/EC/DP electrospun fibers for comparison.

Table 4.4 : The conductivity of PU/PVP/DP solutions and the average fiber diameter of fibers.

PU/PVP/DP (w/w/w)	DP/Total Amount	PVP/Total Amount	Conductivity ($\mu\text{S.cm}^{-1}$)	Average fiber diameter (nm)
10:0:1	0.090	0	149.70 ± 5.36	775 ± 16
10:1:1	0.083	0.083	134.03 ± 13.85	340 ± 56
10:2:1	0.077	0.150	110.20 ± 2.70	294 ± 39
10:4:1	0.067	0.260	109.57 ± 2.55	279 ± 57
10:8:1	0.053	0.420	131.40 ± 1.50	328 ± 66

There is a trend of decrease in the fiber diameter of PU/PVP/DP system by the decrease of conductivity with the exception of 0.053 of “DP/Total amount” which is

given in Table 4.4. There are two variable parameters in these blends. One of them is the amount of Donepezil HCl and the other one is the amount of PU and second polymer (EC or PVP) in the total mixture. While the amount of Donepezil HCl decreases, amount of the EC increases in the total mixture.

4.3 Preparation of Electrospun Fibers

Electrospun fibers loaded with drug or not were prepared by electrospinning technique. The effects of various electrospinning factors on fiber morphology were researched using many experimental parameters to obtain having the best morphology fibers. Optimum parameters for PU polymer solution were found as 1 mL/h flow rate, 15 kV applied voltage and 15 cm for, and distance between the collector and syringe respectively. To study on the impact of second polymers (HPC, EC and PVP) on drug loaded PU fiber formation, these parameters were kept constant throughout the study. The amount of PU and Donepezil HCl were fixed at 0.5 mg and 0.05 mg and the amount of second polymer was varied. During electrospinning process to fabricate of electrospun fibers, the collection of fibers have become harder with increasing of HPC ratios due to the increased viscosity in the blend solution. Conversely the collection of fibers have not become harder with increasing of EC and PVP ratios in the blend solutions.

4.4 Characterization of the Electrospun Fibers

ATR-FTIR Spectroscopy, NMR, DSC, SEM and porosity measurements were used to characterize drug loaded and unloaded electrospun fibers of PU/HPC in various ratio. ATR-FTIR Spectroscopy and SEM measurements were done also for drug loaded PU/EC and PU/PVP electrospun fibers.

4.4.1 ATR-FTIR of electrospun fibers

It is well known that the FTIR is a powerful technique to investigate of the bonding behavior between PU and celluloses or PVP. ATR-FTIR measurements were performed to characterize the electrospun fibers of the drug loaded PU, PU/HPC, PU/EC and PU/PVP and the effects of HPC, EC and PVP on the PU fibers. ATR-FTIR spectra of pure DP, pure PU, solution and fibers of PU, PU/HPC and also drug

loaded PU/HPC, PU/EC and PU/PVP (at various content) were noted in the wavenumber between 4000 and 800 cm^{-1} .

Figure 4.3 represents the ATR-FTIR of the (raw, first state before electrospinning), solution of PU in DMF and electrospun fibers of PU, it was measured to examine the difference between PU (raw), PU solution and electrospun fibers of PU where PU structure includes a benzene ring, an ether bond and urethane linkage. Characteristic absorption bands of PU can be observed like: the 1727 cm^{-1} band, owing to the C=O groups in the urethane segments; the 1529 cm^{-1} band, generally attributed to RCONHR' (secondary amide group); the 1170 cm^{-1} band due to C-O stretching and C-N stretching; the absorbance at 3350 cm^{-1} is consequent with the N-H stretching, N-H bond is characteristic of urea and urethane groups. The other band at 2941 cm^{-1} is in connection with the alkane C-H stretching vibration also characteristic band. The absorption band at 655 cm^{-1} in PU spectrum in DMF can attribute to O=C-N-C torsion mode. A band at 655 cm^{-1} also has a contribution from motion of O=C-N bending. The band at 1093 cm^{-1} and 1384 cm^{-1} can be assigned to the deformation mode of N-Cmethyl-H and C-N stretching mode in the spectrum of PU in DMF (Y. A. Fadeeva, 2003). The 1727 cm^{-1} band shifted to 1664 cm^{-1} when DMF is present in PU. However, there is no change can be observed between the PU (first state before electrospin) and PU fibers from the Figure 4.3 and there is not any DMF residual at the end of the electrospinning process.

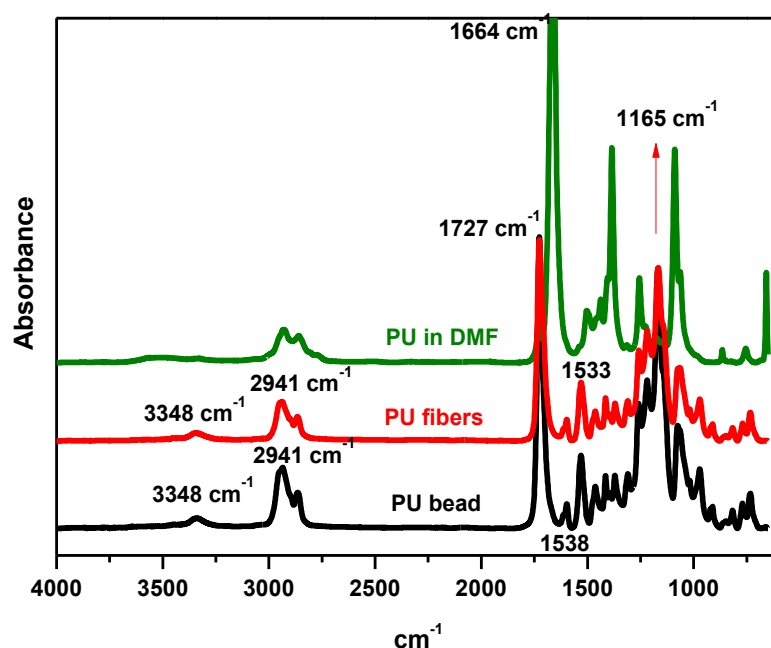


Figure 4.3 : ATR-FTIR spectra of PU pure, fibers of PU, PU in DMF solutions.

In pure DP spectra, aromatic phenone and parasubstituted aromatic hydrocarbon that the fundamental groups of the compound were observed in Figure 4.4. The most intensive absorption band in the spectra around 1680 cm^{-1} and 1591 cm^{-1} was the stretching vibrations of C=O group and aromatic C=C stretching in the structure of DP (Park, Ko et al. 2009). At 1315 cm^{-1} which is a sharp absorption band, is implied the C–N bond existence in the structure (Reddy, Babu et al. 2004). Also absorption bands at $3359\text{--}3579\text{ cm}^{-1}$ and 2926 cm^{-1} were referred the presence of C–N bond, OH stretching and aromatic C–H stretching bond in the structure. 748 and 701 cm^{-1} were attributed aromatic C–H bending existence in the structure.

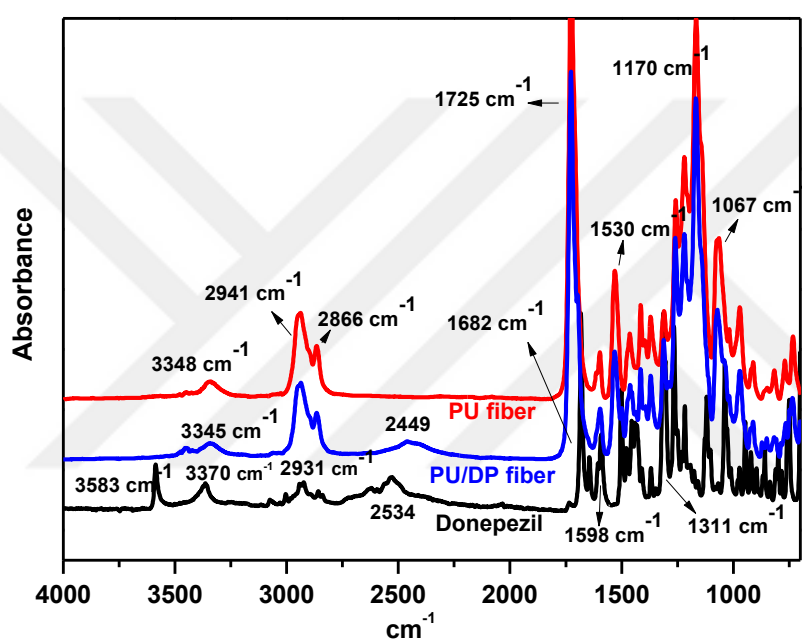


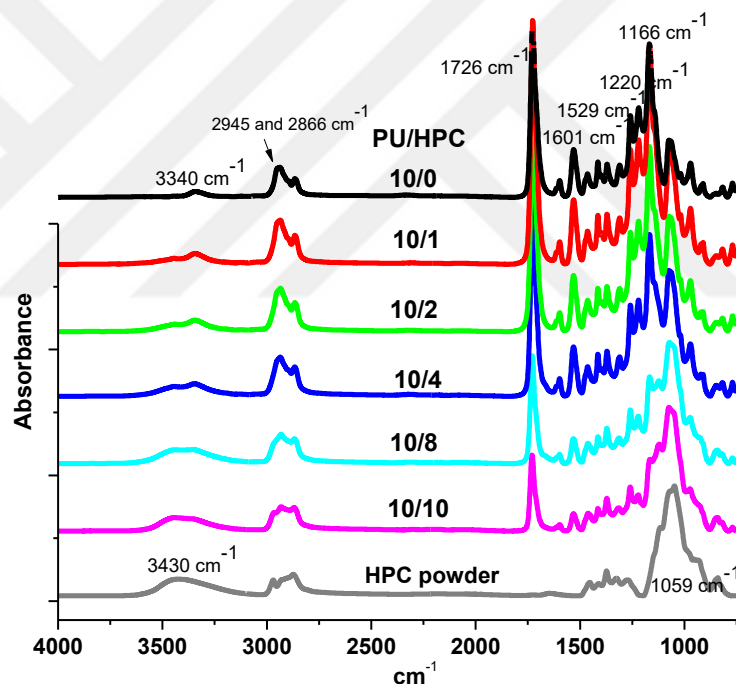
Figure 4.4 : ATR-FTIR spectrum of Donepezil HCl, PU and PU/DP fibers.

Figure 4.4 shows the ATR-FTIR spectra of Donepezil HCl loaded and unloaded PU fibers. It helps to compare the spectra differences. Since, N–H donor groups and C=O acceptor groups in polyurethane structure can form intermolecular hydrogen bond (Coleman, Skrovanek et al. 1988, Mattia and Painter 2007), while original intermolecular H bond may be broken down by hydrogen bond acceptors in the Donepezil structure to form new H bond with the groups N–H donor in polyurethane structure. Based on the data (Huheey J.E. 1965), it could be said that the C=O hydrogen bond acceptor in polyurethane had stronger electronegativity than H bond acceptors in DP, by this way it provides inducing the reregulation of hydrogen atom in the N–H group and it provides blueshift of the N–H group stretching absorption (Xi, Cun et al. 2012).

Table 4.5 : Characteristic peaks of Donepezil HCl.

Wavelength cm^{-1}	Assignment
2926	aromatic C-H stretching
1682	C=O stretching
1598	aromatic C=C stretching
1500 and 1457	aliphatic C-H bending
1311-1265	C-N stretching
1034	C-O stretching
748 and 700	aromatic C-H bending

From the spectrum in Figure 4.4, as for PU, the absorbance bands were not changed after DP loaded and also it was observed that all the characteristic peaks in DP were not seen in the spectrum due to the amount of DP is low in the fiber or these results might indicate that there is weak interaction between DP and the PU polymer, therefore, DP can not damage the original intermolecular hydrogen bond of PU.

**Figure 4.5 :** ATR-FTIR spectra of HPC and PU/HPC fibers with various HPC ratio.

ATR-FTIR spectra of drug unloaded PU and PU/HPC electrospun fibers with different ratio were described in Figure 4.5. HPC is polymer soluble in water and it displays many -OH functional groups which has active role in H bonding. In the pure HPC spectrum at $3600\text{--}3100\text{ cm}^{-1}$ which is the broadband with a maximum at nearly 3430 cm^{-1} , was attributed to stretching vibrations of hydroxyl groups. The stretching vibrations of $\nu(\text{C-O-C})$ was positioned at $1060\text{--}1080\text{ cm}^{-1}$ and aliphatic groups bands were positioned at 2873 cm^{-1} and positioned close to 2922 cm^{-1} that represent a C-H

stretching, and positioned at about 1059 cm^{-1} that identify C-O stretching, and at 1458 cm^{-1} is relevant to the $-\text{CH}_2$ bending, 1375 cm^{-1} is related to C-H bending and 945 cm^{-1} is related to CH_2 stretching in Figure 4.5. The basic structure of cellulose is consistent with these functional groups (Qu., 2010).

The polyurethane is comprehensively hydrogen bonded, the donor group being the NH of the urethane segment. Furthermore, the C=O group of the urethane/ester carbonyl/ether oxygen may be hydrogen bond acceptor (Seymour, 1973). The analysis of the carbonyl absorption region can provide information about H bonds formation in PU spectra. In the ATR-FTIR of the PU, HPC and PU/HPC fibers in various ratio, the stretching vibration (N-H) peak from PU group moved from 3406 cm^{-1} to 3340 cm^{-1} and narrow peak convert into broad peaks because of the hydrogen bonded from HPC and interaction between PU and HPC increases with the increasing HPC content. When PU are mixed with HPC, the intensity of the peak at 1726 cm^{-1} , 1601 cm^{-1} and 1529 cm^{-1} decrease, this refers that the carbonyl fraction, which is hydrogen-bonded with OH from the HPC, is increased. Also, the C=O stretching vibration peak shifts from 1726 cm^{-1} to 1731 cm^{-1} at the higher HPC ratio. Shifting to high wavenumber propound the attendance of HPC to the matrix and PU and HPC generate urethane cellulose interactions by the hydrogen bond. (Cao, Dong et al. 2007, Mandru, Vlad et al. 2013). While the peak at 1071 cm^{-1} lessens, the intensity of the peak at 1166 cm^{-1} rises and also 1071 cm^{-1} combines with the 1100 cm^{-1} (C–O–C) from the HPC. It is relating to the C–O–C stretching which demonstrate that the ether group is also involved in H-bonding. So that, between the urethane carbonyl groups and the hydroxyl groups of the HPC, and also between NH group and ether linkages of the polyurethane chains are formed hydrogen bonds. These changes are observed in consequence of the interconnection of the polyurethane chains with HPC molecules (Stefan, 2013). ATR-FTIR spectra of novel segmented PU based on HPC and a cellulose-waterborne PU nanocomposite films have also been reported (Cao, Dong et al. 2007, Mandru, Vlad et al. 2013).

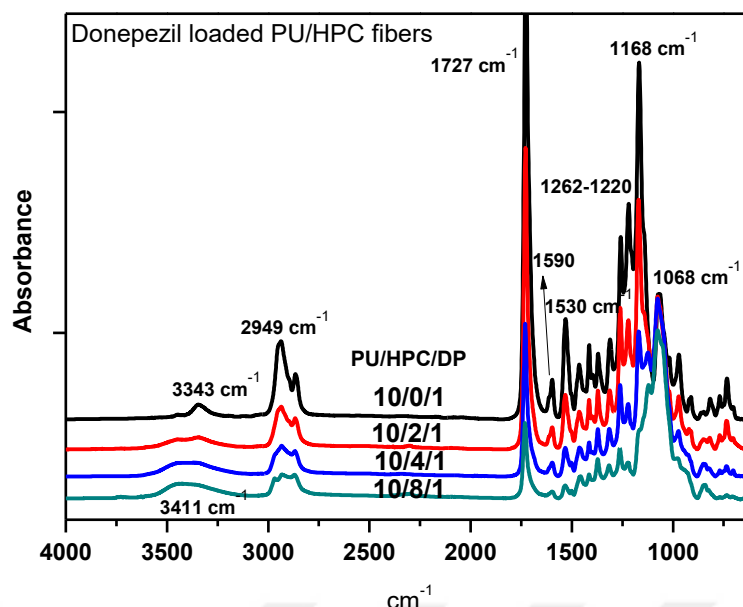


Figure 4.6 : ATR-FTIR spectra of drug loaded PU/HPC fibers in various ratio.

In Figure 4.6 represented the spectra of drug loaded electrospun fibers of PU/HPC, results showed that the increase the broad band at nearly 3411 cm^{-1} was attributed to stretching vibrations of hydroxyl groups belongs to HPC. The broad band at nearly 3411 cm^{-1} was shifted to the narrow band at 3343 cm^{-1} by decreasing HPC in the total volume. The absorbance peaks at 1727 cm^{-1} which shows C=O group was shifted to the 1732 cm^{-1} with increasing HPC content. The intensity of absorbance peaks at 1168 cm^{-1} which shows C-O group, $1220\text{--}1262\text{ cm}^{-1}$ which shows O-C=O group and 1590 cm^{-1} which represents the absorbance of CO-NH functional group decrease with increasing HPC content. C-O stretch gives two bands between $1300\text{--}1100\text{ cm}^{-1}$ in different intensity where one of the peak disappears by the increase of HPC content due to interaction. This can be explained by cellulosic material has -OH groups and these groups could spare as interaction region with different functional groups in PU (Sharma, 2012). This is probably with the interaction of hydroxyl groups of HPC with -NCO groups of PU. From ATR-FTIR studies it can be resulted that the inclusion of hydroxypropylcellulose in the polurethane structure occurs (Munajad, 2018 and Armenta, 2004).

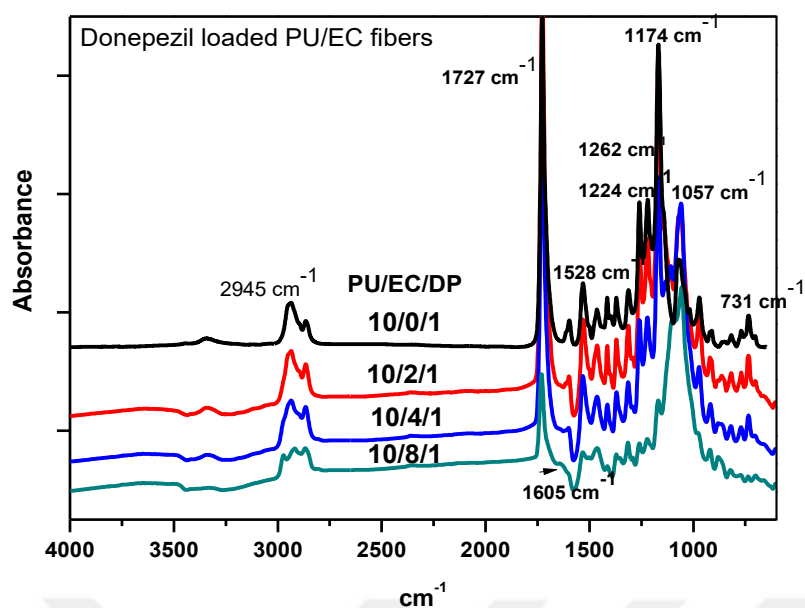


Figure 4.7 : ATR-FTIR spectrum of PU/EC fibers loaded drug in various ratio.

ATR-FTIR spectra of drug loaded electrospun fibers of PU/EC are seen in Figure 4.7. By increasing cellulose unit in the PU matrix, the intensity of characteristic absorption bands of PU at 1727 cm^{-1} decreases also 1057 cm^{-1} , 2945 cm^{-1} and 1000-1300 cm^{-1} have shown changes, these variation clarify that EC incorporate to the PU matrix. But characteristic absorption bands of Donepezil HCl can not be observed due to the small amount of Donepezil HCl presence in PU/HPC and PU/EC mixture.

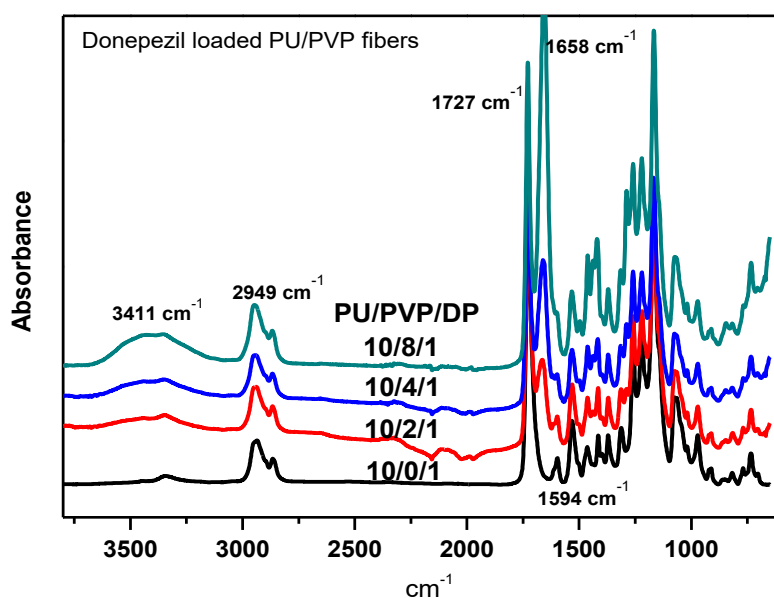


Figure 4.8 : ATR-FTIR spectrum of PU/PVP fibers loaded drug in various ratio.

ATR-FTIR spectrum of electrospun fibers of PU/PVP loaded with drug are seen in Figure 4.8. Strong peak around 1727 cm^{-1} remarks the carbonyl bond of PU. The pyrrolidone ring in PVP contains a proton-accepting carbonyl group (Bernal, 2011). With the addition of PVP into PU matrix, a new broad peak located at 3411 cm^{-1} because of the stretching vibration of the hydroxyl group (OH) and a new strong peak at 1658 cm^{-1} that indicates the stretching mode of C=O bond of PVP are observed in Figure 4.8. Also 1594 cm^{-1} peak is disappeared and $1000\text{--}1300\text{ cm}^{-1}$ bands which correspond to the C-N vibrations variate with addition of the PVP to the drug loaded PU matrix. The peak at 1430 cm^{-1} indicates CH_2 bending (Bhavsar, 2017). The C-H asymmetric stretching of the CH_2 absorption band is seen to be located at 2949 cm^{-1} from PVP and PU. stretching vibration of C=O peak shifts from 1726 cm^{-1} to 1732 cm^{-1} at the higher HPC, EC or PVP ratio as shown in Figure 4.6, 4.7 and 4.8.

4.4.2 Differential scanning calorimetry of electrospun fibers

Differential scanning calorimetry has been done to study transition temperatures of polymers and DSC studies were carried out so as to research the possible drug and distinct polymers interactions. The samples of PU, Donepezil HCl, HPC, and 10/8/1, 10/4/1, 10/2/1, 10/1/1 and 10/0/1 ratio of PU/HPC/DP were chosen for DSC analysis.

In our study it demonstrated an endothermic transition round $+46\text{ }^{\circ}\text{C}$ referred to T_g belongs to hard segments or melting belongs to soft segments of PU. A sharp endothermic peak can be observed related to the melting of soft segment distinguishable at around $+46\text{ }^{\circ}\text{C}$ for having high PU ratio samples.

The melting temperature values of the soft segment PU changes from $45.53\text{ }^{\circ}\text{C}$ to $46.82\text{ }^{\circ}\text{C}$ and ΔH changes from 51.98 J/g to 16.17 J/g in the drug loaded PU/HPC fibers. The heat of melting (ΔH) decreases by decreasing PU ratio in total fiber blend.

Table 4.6 : Characteristic Tg and Tm temperature of the fibers.

Samples	Tg of HPC ($\Delta H/J/g$)	Tg of DP ($\Delta H/J/g$)	Tm of HPC ($\Delta H/J/g$)	Tm of DP ($\Delta H/J/g$)
Donepezil HCl		133.14 $^{\circ}C$ (81.66 J/g)		227.61 $^{\circ}C$ (136.20 J/g)
HPC	92.45 $^{\circ}C$ (70.93 J/g)		198.03 $^{\circ}C$ (16.27 J/g)	
PU/ HPC/DP	Tm of PU ($\Delta H/J/g$)	Tg of HPC ($\Delta H/J/g$)	Tm of HPC ($\Delta H/J/g$)	Tm of DP ($\Delta H/J/g$)
10/0/0	46.09 $^{\circ}C$ (51.98 J/g)			
10/0/1	45.53 $^{\circ}C$ (28.54 J/g)			206.13 $^{\circ}C$ (3.02 J/g)
10/1/1	44.90 $^{\circ}C$ (28.83 J/g)			261.91 $^{\circ}C$ (1.486 J/g)
10/2/1	46,24 $^{\circ}C$ (17.20 J/g)		105,9 $^{\circ}C$ (5.84 J/g)	217,95 $^{\circ}C$ (8.51 J/g)
10/4/1	46.60 $^{\circ}C$ (3.63 J/g)		194.14 $^{\circ}C$ (0.28 J/g)	283.97 $^{\circ}C$ (1.56 J/g)
10/8/1	46.82 $^{\circ}C$ (16.17 J/g)	88.31 $^{\circ}C$ (48.49 J/g)		

The melting of the soft segments observed at about 45 $^{\circ}C$ in all PUs (Vega-Baudrit, Sibaja-Ballesteros et al. 2007) and at about 49 $^{\circ}C$ (Raschip et al. 2009). There is a good agreement between data with the results of PU's thermal curve displays a single sharp peak with an onset of 36,44 $^{\circ}C$ and maximum occurrence at 46.09 $^{\circ}C$ (enthalpy of fusion equal to 51.98 J/g), corresponding to melting temperature (Tm) value of PU soft segments. Pure HPC shows the large shallow broad peak over the temperature at 92.45 $^{\circ}C$ (enthalpy of fusion equal to 70.93 J/g), For the Tg value of HPC the second shallow broad peak temperature at 198.03 $^{\circ}C$ (enthalpy of fusion equal to 16.27 J/g) is observed for the melting of HPC. Essentially, an endothermic effect of the thermal analysis of cellulose was exhibited above 100 $^{\circ}C$, reported in the literature (Ford 1999, McPhillips, Craig et al. 1999, Guyot and Fawaz 2000).

The DP has been characterized by DSC, which shows small exotherm peak at 133.14 $^{\circ}C$ and a single sharp melting endotherm peak at 227.61 $^{\circ}C$ (enthalpy of fusion equal to 136.2 J/g) (Appendices, Figure B1). The exotherm at 133.14 $^{\circ}C$ could be due to crystallization of dehydrated phase, and this crystallized material melts at 227.61 $^{\circ}C$ as evidenced by a sharp endotherm.

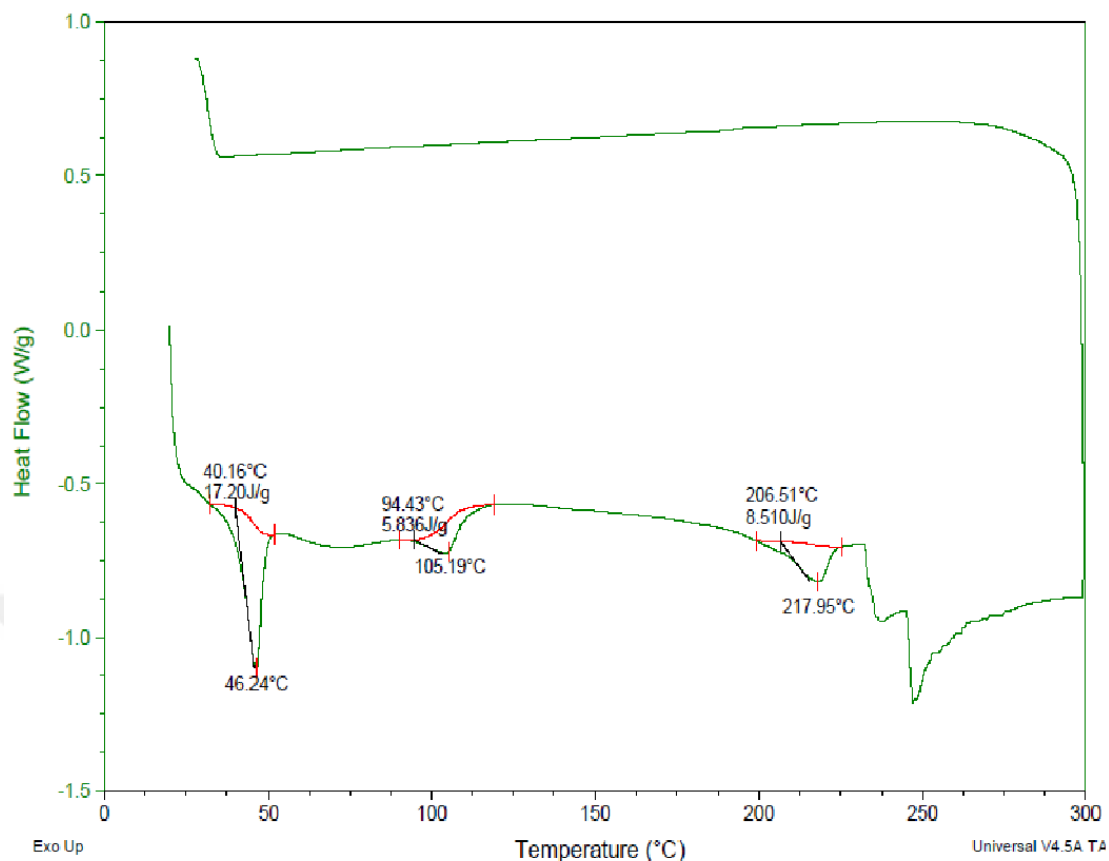


Figure 4.9 : DSC thermogram of PU/HPC/DP (10/2/1) fibers.

Figure 4.9 shows the DSC thermograms of the PU/HPC/DP (10/2/1) electrospun fibers. Three peaks are seen from the Figure 4.9. The temperature at 46.24°C (enthalpy of fusion equal to 17.20 J/g) is the T_m value of PU soft segment and over the temperature at 105.19°C (enthalpy of fusion equal to 5.84 J/g) the second large shallow broad endothermic effect are observed for the polymer HPC. The values of the glass transition temperature of HPC increased by the incorporation of the hydroxypropyl cellulose into the polyurethane. Also there is an upward shift of the PU signal with decreasing PU and increasing HPC content in the total mixture of fibers, in which is related with interaction of polymer:polymer:drug.

Regarding the PU/HPC/DP studied, PU/HPC/DP (10/8/1) electrospun fibers showed no peak temperature of Donepezil HCl, which might be because of the effect of the melted PU and HPC. So, Donepezil HCl were removed from 205°C to 230°C step by step from solid form (Suzuki, Kasahara et al. 2002).

4.4.3 Nuclear magnetic resonance spectroscopy of electrospun fibers

The Donepezil HCl, PU, HPC, PU/HPC and PU/HPC/DP samples were chosen for NMR analysis. The characteristic peaks and their assignments of the structures for Donepezil HCl are as follows:

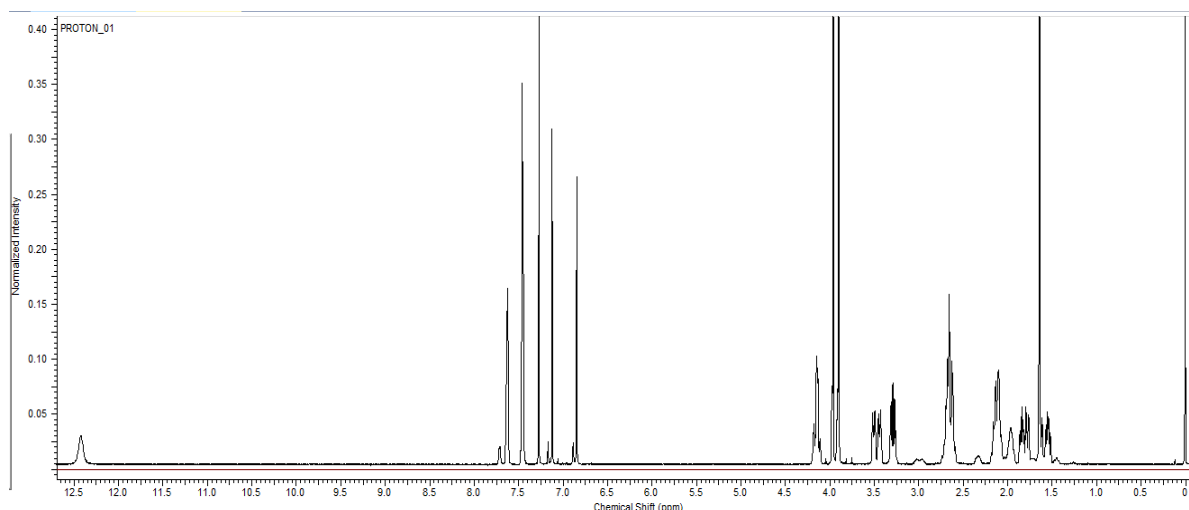


Figure 4.10 : ^1H NMR spectrum of Donepezil HCl in CDCl_3 .

Table 4.7 : Assignment of Donepezil HCl and polyurethane.

Donepezil HCl			Polyurethane		
Chemical Shift (δ) ^1H (ppm)	Assignment		Chemical Shift (δ) ^1H (ppm)	Assignment	
1.50-1.75	1H	-CH-	1.30-1.75	4H	-CH ₂ - -CH ₂ -
1.77-1.90	2H	-CH ₂ -	2.30	2H	CONH CONH
2.0-2.25	4H	-CH ₂ - -CH ₂ -	4.00-4.15	2H	-CH ₂ -
2.60-2.75	4H	-CH ₂ - -CH ₂ -	7.00-7.30	8H	Ar-H Ar-H
3.25-3.35	1H	-CH-			
3.40-3.60	2H	-CH ₂ -			
3.80	3H	-O-CH ₃			
3.90	3H	-O-CH ₃			
4.12	2H	N-CH ₂			
6.75-7.75	7H	Ar-H			

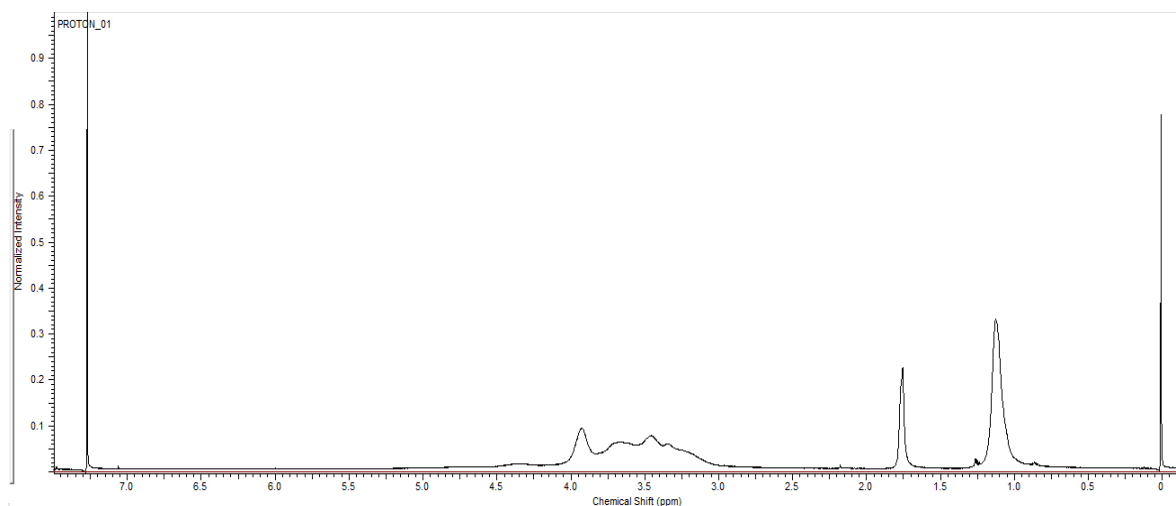


Figure 4.11 : ^1H NMR spectrum of HPC in CDCl_3 .

Figure 4.10, 4.12 and Table 4.7 show the NMR spectra and assignments of Donepezil HCl and PU. Figure 4.11 shows the NMR spectra and assignments of HPC. Characteristic HPC peaks were found at 1.1 ppm which was attributed to the hydrogens linked to the CH_3 and 3-4.5 ppm was attributed to the cellulose backbone $[(\text{CH}_2\text{CHCH}_3)\text{OH}]$ (Ohlendorf and Greiner 2015).

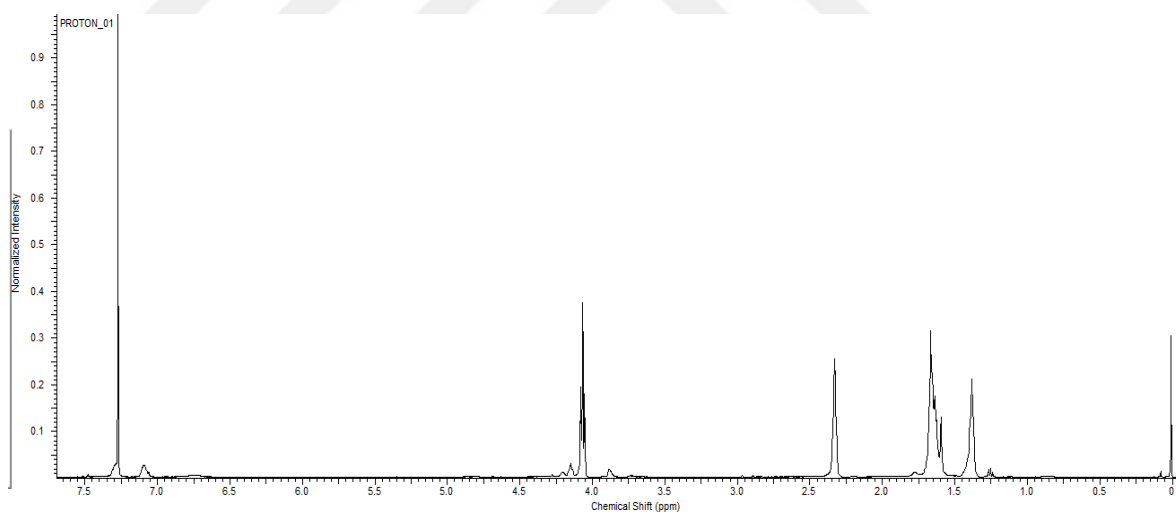


Figure 4.12 : ^1H NMR spectrum of Polyurethane in CDCl_3 .

The relative increase in the number of protons in the region between $\delta=3.6\text{-}4.0$ and $\delta=6.4\text{-}7.7$ indicates that the Donepezil HCl has been loaded to the electrospun fibers in Figure 4.13. The structure of the produced derivatives are confirmed by ^1H -NMR

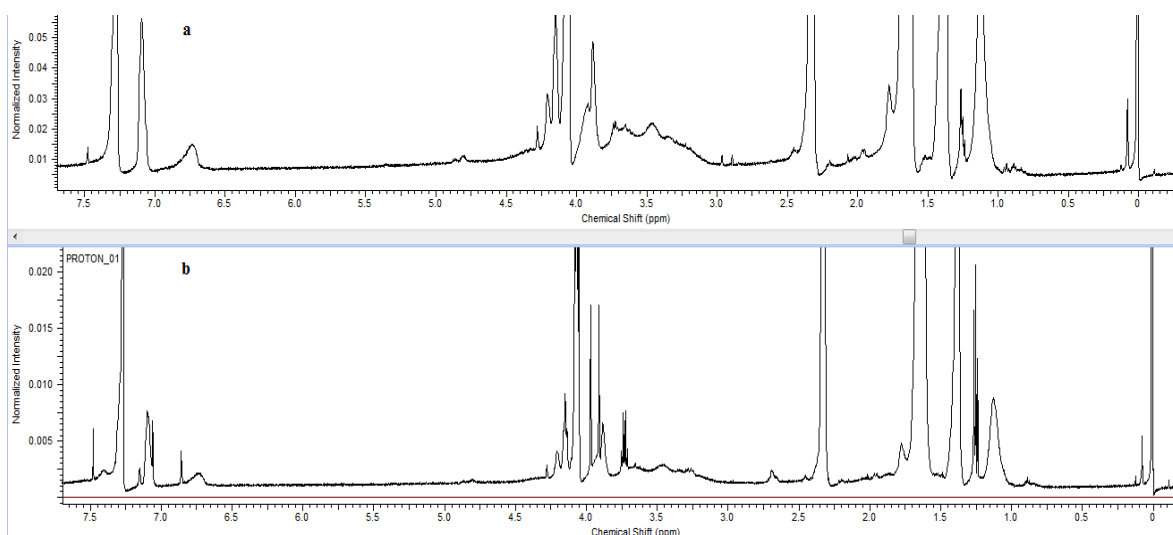


Figure 4.13 : ¹H NMR spectrum of PU/HPC fibers a) unloaded and b) loaded Donepezil HCl, in CDCl₃.

4.4.4 Morphology studies of electrospun fibers

Morphology of fibers such as fiber size, pore size, the density of pores and nonbeaded fibers is an important factor for drug delivery applications, so it is critical to supply an available environment to load the drug (Sill&Von Recum, 2008). Thus, it was aimed that the appropriate morphologic structures with suitable porosity between high surface and volume ratio to become electrospun fibers for loading and releasing of drug. Morphology of the fibers were obtained by SEM images of electrospun fibers. In this part, morphologic effect of the HPC, EC and PVP with different ratio on the electrospun fibers of PU were investigated using SEM. Porosity analysis were performed also for drug loaded PU/HPC fibers. The fiber morphology obtained under optimized parameters from three different blends depicted below

4.4.4.1 Effect of HPC on morphology of the PU/HPC electrospun fibers

The effect of HPC on morphology and PU/HPC electrospun fibers's average diameters were investigated. The morphologies of electrospun fibers of PU/HPC (10/0, 10/1, 10/2, 10/4, 10/8) are presented in Figure 4.14. According to the SEM images, all types of fibers have uniform design and smooth surfaces. They are found with no sign of beads -on-a-string morphology. Electrospinning of the PU/HPC is resulted in an uniform fibers structures in the lower range diameters. The average diameters of the electrospun fibers are dependent on HPC content at the PU/HPC electrospun fibers.

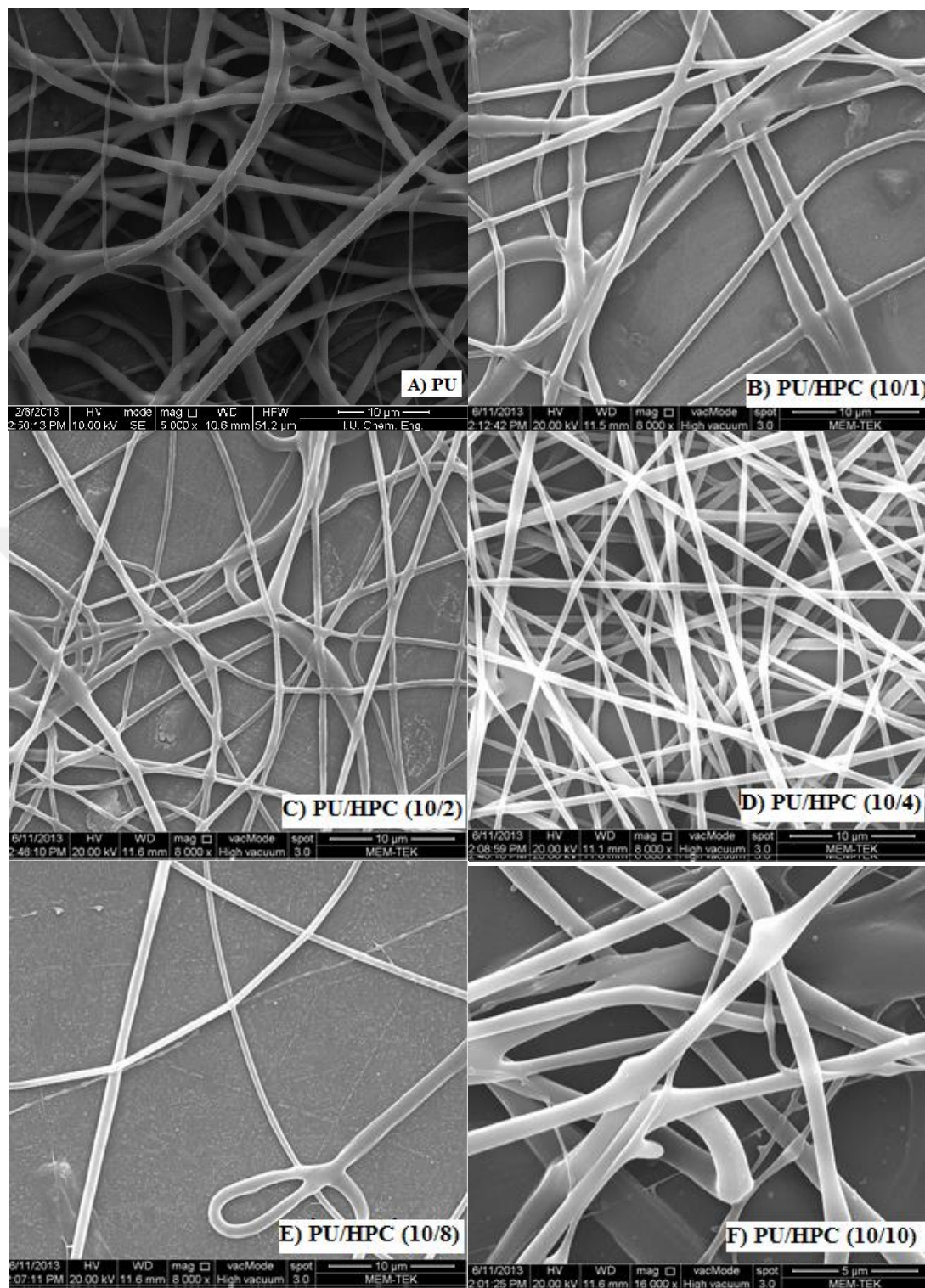


Figure 4.14 : SEM images of PU/HPC electrospun fibers with various compositions (The scale bars exhibit 10 μ m for A, B, C, D, E and 5 μ m for F).

The average diameters of PU/HPC electrospun fibers were smaller than PU fibers. The average fiber diameter decreased with increasing HPC content from 1200 nm to 600 nm for samples of PU/HPC (10/0, 10/1, 10/2, 10/4, 10/8, 10/10) (Table 4.8 and Figure 4.14) in the blends. When HPC concentration is approximately 50 % or more

at the PU/HPC electrospun fibers, average diameters of the fibers are kept constant such as 687 nm for 10/8 (PU/HPC) and 683 nm for 10/10 (PU/HPC) and do not change sharply. Results shows that PU and 10/4 ratio of PU/HPC fibers more uniform structure than the other ratios. Moreover, 10/4, 10/8 and 10/10 ratio of PU/HPC fibers have similar values.

Owing to increasing the viscosity of the electrospinning solution by adding HPC, viscosity was increased large and the viscosity of polymer solution of 10/10 (PU/HPC) ratio was not detected.

Table 4.8 : Average diameter of drug unloaded PU and PU/HPC electrospun fibers (nearly 50 fibers).

PU/HPC (w/w)	Average fiber diameter (nm)
0:10	Could not be processed
10:0	1172 $\pm$ 10
10:1	962 $\pm$ 15
10:2	791 $\pm$ 17
10:4	679 $\pm$ 23
10:8	687 $\pm$ 25
10:10	683 $\pm$ 24

4.4.4.2 Effect of HPC on morphology of PU/HPC loaded drug electrospun fibers

Effect of HPC and Donepezil HCl incorporation on morphology and average diameters of fibers were investigated in this study. SEM images of the electrospun fibers of PU/HPC/DP in a ratio of 10/0/1, 10/1/1, 10/2/1, 10/4/1 and 10/8/1 w/w/w are represented in Figure 4.15. Incorporation of HPC in PU electrospun fibers was deteriorated the shape and uniformity of the fibers. Neither aggregates nor drug crystals were monitored on the surfaces of electrospun fibers. Drug particles does not observed on fibers surface by demonstrating that polymers and drug have a good coordination. But the electrospun fibers of 10/8/1 were formed along with some beads. The fibers with beads at high concentration of HPC was presumably due to the high viscosity of the drug loaded PU solution. SEM images are shown that PU/HPC/DP electrospun fibers morphology are changed with the ratio of HPC in the blend, the average diameters of the PU/HPC/DP fibers depend on added HPC quantity.

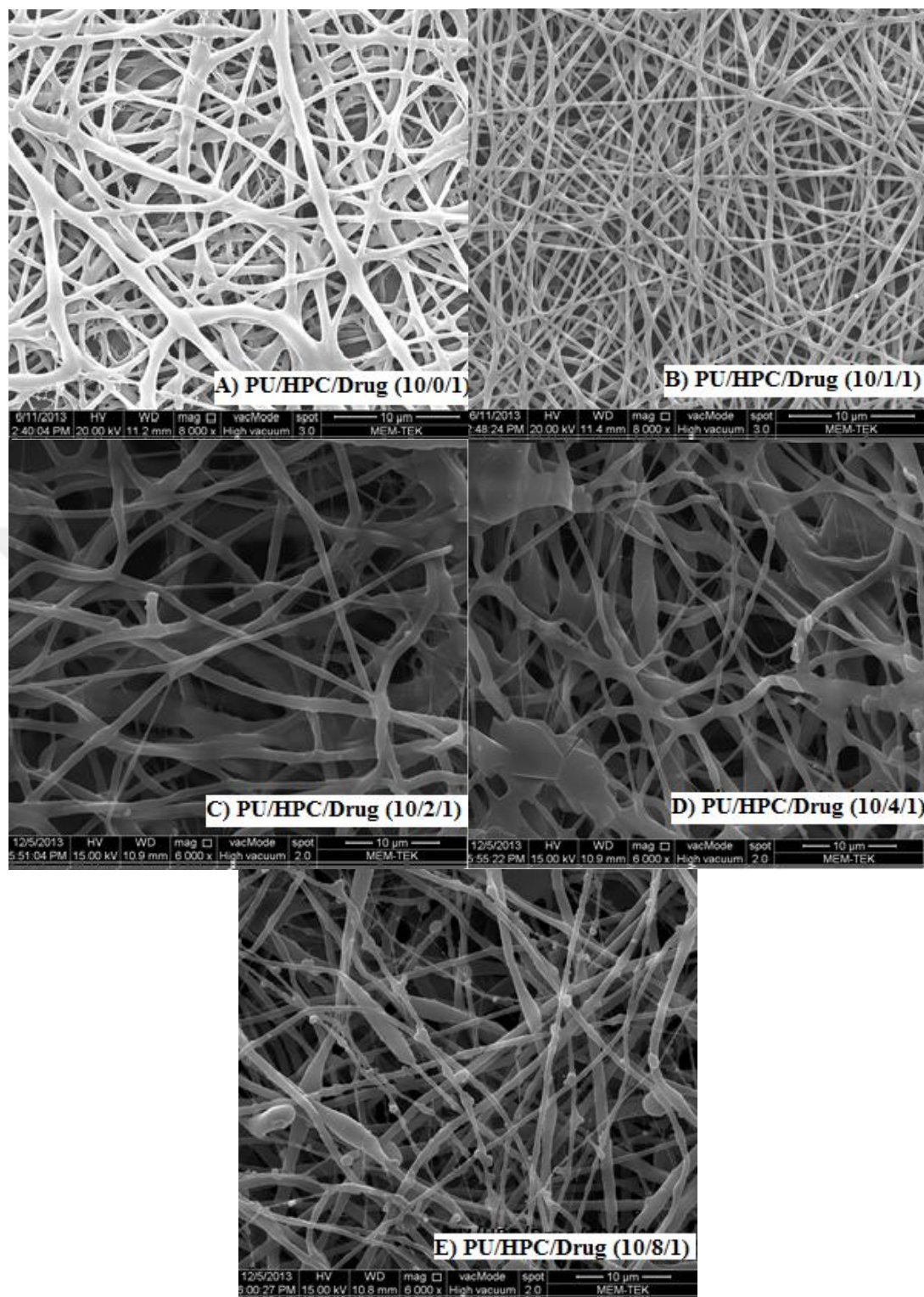


Figure 4.15 : SEM images of PU/HPC/DP fibers with various compositions (The scale bars exhibit 10 µm for A, B, C, D, E).

Furthermore, nonbeaded structure and uniformity of the fibers lower with decreasing conductivity of the electrospun fibers solution from $149 \mu\text{S}.\text{cm}^{-1}$ to $116 \mu\text{S}.\text{cm}^{-1}$ because of the decreasing ratio of drug molecule in total mixture from 0.09 to 0.05. But at the same time, ratio of HPC in total mixture varies from 0 to 0.42. There are

different variables in this study. In this case, uniformity and average fiber diameters depends on ratio of drug and HPC and consequently conductivity and viscosity.

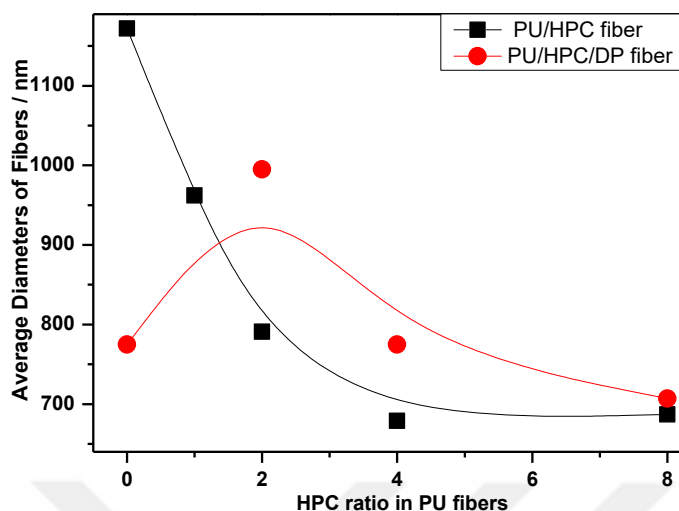


Figure 4.16 : Correlation between HPC ratio and average fiber diameters.

Figure 4.16 shows that average diameters of fibers decreases proportionally when the quantity of HPC increases in the PU/HPC electrospun fibers. It is seen from the Figure 4.17 that drug loaded fibers of PU have less average diameter size then unloaded fibers of PU. It can be explained by the solution conductivity that playing lesser role, while conductivity of PU solution is $7.58 \mu\text{S.cm}^{-1}$, PU solution conductivity comprising drug is $149.7 \mu\text{S.cm}^{-1}$.

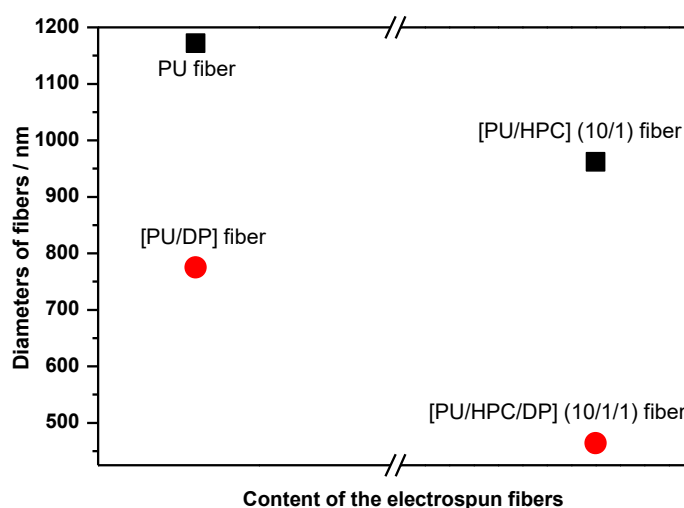


Figure 4.17 : Correlation of the Donepezil and the diameters of fibers.

Also, conductivity of PU/HPC solution is $4.57 \mu\text{S.cm}^{-1}$, conductivity of Donepezil HCl loaded PU/HPC solution is $131.7 \mu\text{S.cm}^{-1}$. Polymer solutions with high charge

carrying capacity will be highly influential in jet formation. The fiber jet having low conductive solutions will not be exposed to a great tensile force than a high conductive solution in the presence of an electric field.

4.4.5 Porosity analysis of PU/HPC loaded drug electrospun fibers

The diameter of average pore, total pore volume and total pore surface area of the DP loaded PU/HPC electrospun fibers were analyzed at 200 mPa by Pascal mercury porosimeter (ThermoFisher Scientific, Inc., Massachusetts, USA) and the results of DP loaded PU/HPC electrospun fibers are given in Table 4.9. The porosity, total pore volume and total pore surface areas of electrospun fibers decreased significantly with the increase of the amount of HPC in PU solutions while average pore diameter enhanced with the increase of pore diameter of electrospun fibers, except the ones consisting of the highest ratios of HPC (10/8/1) and lacking of HPC (10/0/1).

Table 4.9 : Porosity values of DP loaded PU/HPC fibers.

	PU/HPC/DP (w/ww)			
	10/0/1	10/2/1	10/4/1	10/8/1
Porosity by Hg intrusion (%)	80.91	73.40	52.52	63.48
Total pore volume (cm³/g)	2.72	2.98	1.74	2.80
Total pore surface area (m²/g)	10.79	9.42	1.56	3.68
Average pore diameter (μm)	1.01	1.26	4.48	3.04

The increased viscosity with the presence of HPC may affected the electrospun fiber diameter and average pore diameter of the fiber. The high porosity might be an advantage for the drug loading capacity when optimum ratio of HPC is ensured for release study (Yu, Zhu et al. 2009). Moreover, the average pore diameter enhanced with the increasing of amount of HPC.

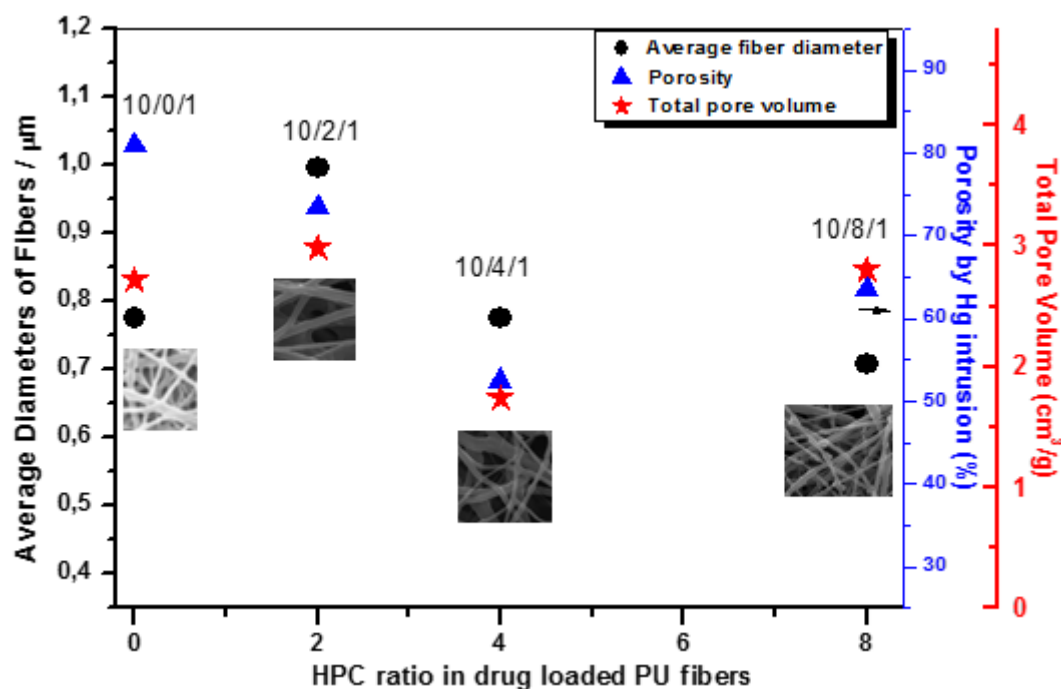


Figure 4.18 : Effect of of the HPC content on the diameters and porosity by Hg intrusion of fibers.

4.4.5.1 Effect of EC on morphology of the PU/EC loaded drug electrospun fibers

Effect of EC incorporation on the formation of drug loaded PU/EC electrospun fibers was investigated using SEM analysis. Figure 4.19 presents the morphological features of PU/EC/DP electrospun fibers containing different ratios of EC (10/1/1, 10/2/1, 10/4/1 and 10/8/1). SEM images verified the fabrication of the electrospun fibers with uniform, smooth surface, non-beads on-a-string morphology. Drug particules or aggregates of drug were not observed on electrospun fibers surfaces, the polymers and drug show good compatibility each other. Also, the shape and uniformity of PU/EC/DP electrospun fibers were kept even when increasing of the EC content in the fibers.

The average diameters of the PU/EC/DP electrospun fibers depends on the presence of EC in the electrospun fibers compositon. PU/DP (10:1) electrospun fibers had average diameter of 775 ± 16 nm. Average diameter of the electrospun fibers with addition of EC decreased in the range of 410 ± 47 and 518 ± 39 nm, without exhibiting a linear change in fiber diameters (Figure 4.19). This case can be explained by the fact that the increment in the electric conductivity of the electrospinning solutions after addition of EC (Ahmad, Stoyanov et al. 2013) and, then, it was resulted with decreasing of average diameter of the electrospun fibers. A meaningful decrease in

electrospun fibers diameters with the rising of electrical conductivity of the solutions was previously reported by Fong et al. (Fong, Chun et al. 1999)

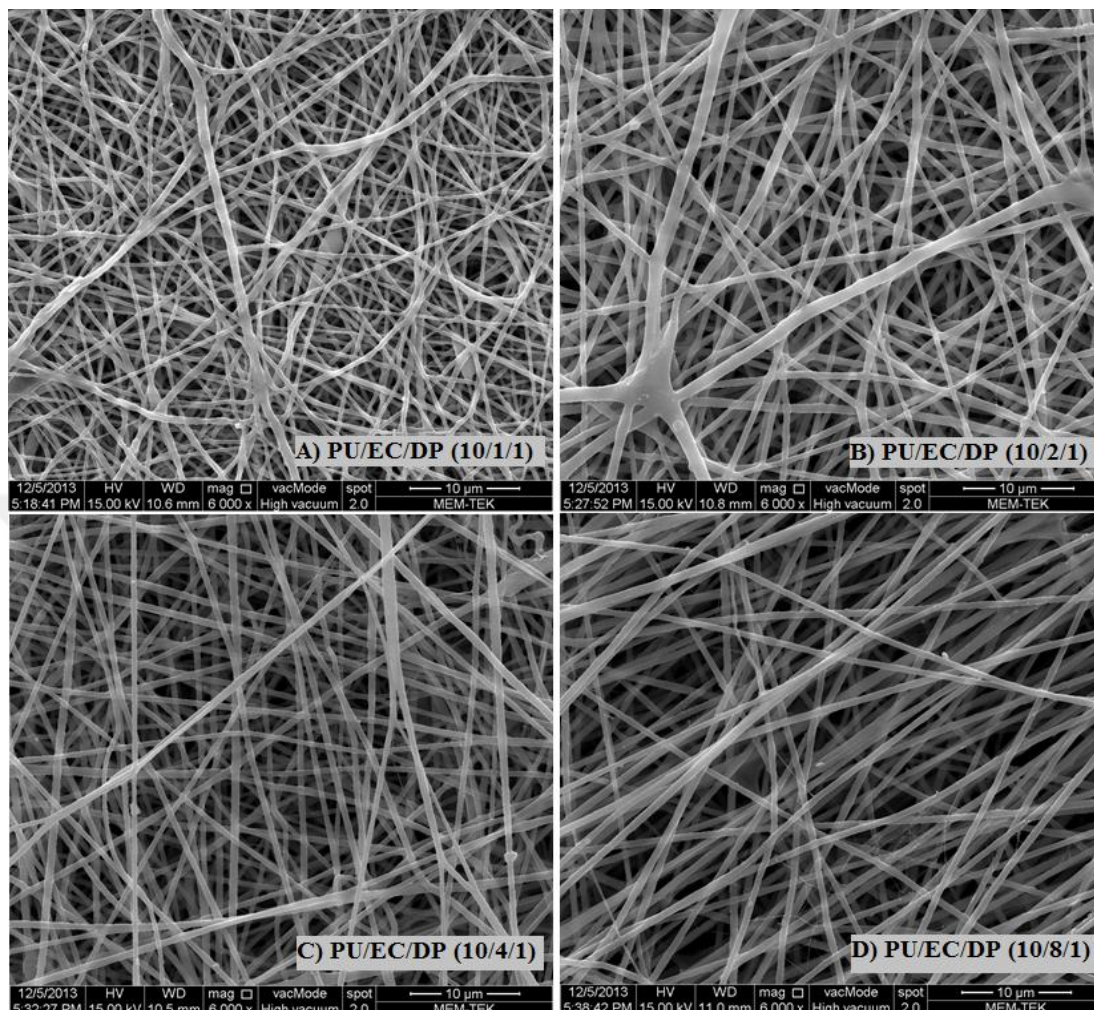


Figure 4.19 : SEM images of PU/EC/DP fibers with various compositions (A, B, C, D have 10 µm scale bars).

4.4.5.2 Effect of PVP on morphology of the drug loaded PU/PVP fibers

The effect of PVP incorporation on the formation of PU/PVP/DP electrospun fibers was investigated using SEM analysis. Figure 4.20 presents the morphological features of PU/PVP/DP electrospun fibers containing different ratios of PVP (10/1/1, 10/2/1, 10/4/1 and 10/8/1). SEM images verified the fabrication of the all four types of electrospun fibers with uniform, smooth surface, non-beads on-a-string morphology. Drug crystals did not show any aggregates on the surfaces of electrospun fibers the polymers and drug show a good compatibility in between. The average diameter of fibers significantly decreased with addition to PVP to electrospun fiber formulations, such as observed by PU/HPC/DP electrospun fibers.

The PU/PVP/DP electrospun fibers had mean diameters from 340 ± 56 nm to 328 ± 66 nm (Table 4.10 and Figure 4.20). However, some clumps were observed in PU/PVP/DP electrospun fibers at ratio of 10/8/1, due to low solubility of PVP in electrospinning solution, as reported earlier (Yu, Shen et al. 2009). Overall, these results verified that PU/PVP/DP and PU/EC/DP electrospun fibers with smooth surface, non-beads in nanoscale were fabricated almost in all of ratios.

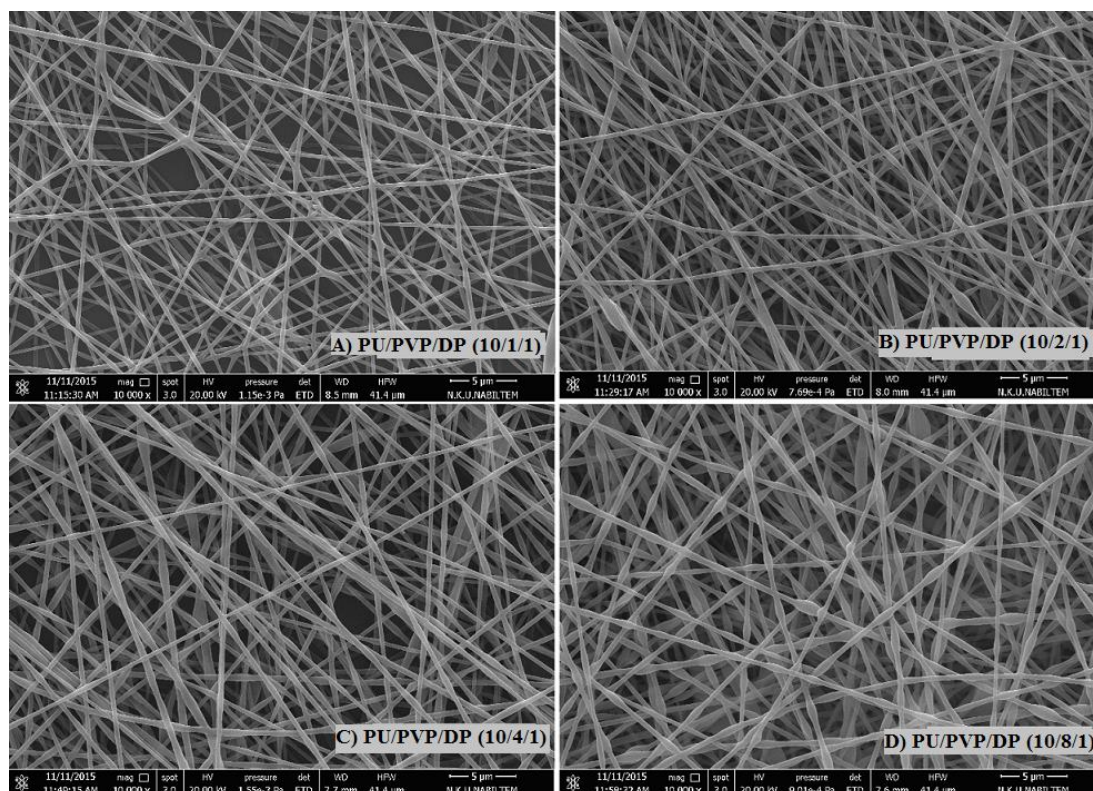


Figure 4.20 : SEM images of PU/PVP/DP fibers with various compositions (A, B, C, D have 5 μm scale bars).

These results (Figure 4.19 and 4.20) verified that PU/PVP/DP and PU/EC/DP blends had good compatibility in selected solvent and content ratio to electrospun the fibers. The PU/PVP/DP electrospun fibers had the lowest fiber diameters, while PU/HPC/DP fibers had the highest fiber diameter in 10/2/1 ratio among the fibers obtained from different polymer blends in different ratios. PU/HPC/DP fibers also exhibited beaded morphology while PU/EC/DP and PU/PVP/DP fibers exhibited nonbeaded, smooth surface in all ratio.

Table 4.10 : Average diameter of Donepezil HCl loaded fibers (nearly 50 fibers).

PU/HPC (or EC, PVP)/DP (w/w/w)	DP/Total Amount	HPC/Total (EC/Total, PVP/ Total Amount)	Average fiber diameter (nm)		
			PU/HPC/DP	PU/EC/DP	PU/PVP/DP
10:0:1	0.090	0	775± 16		
10:1:1	0.083	0.083	464 ± 24	410±47	340±56
10:2:1	0.077	0.15	995 ± 14	556±46	294±39
10:4:1	0.067	0.26	775 ± 20	518±39	279±57
10:8:1	0.053	0.42	707 ± 28	603±42	328±66

From previous FTIR and other results indicate that HPC is more interactive with other components in the fiber compared to the EC and PVP, this is reflected to the fiber diameter distributions, where in the presence of EC and PVP standard deviation is high and it can be considered not much effect is expected from the interaction of EC and PVP. Since the amount of HPC is effective on the fiber diameter and also the porosity values of DP loaded PU/HPC electrospun fibers is observed as a parallel changes according to the Figure 4.18. This fact can be explained that the OH group of HPC is more interactive with the polyurethane C=O groups by forming hydrogen bond, on the other hand Donepezil HCl carbonyl groups can also form a hydrogen bond with the polyurethane N-H bonds. HPC has more OH bond (wherein R is H or CH₂CH(OH)CH₃ in HPC) according to EC (wherein R is H or CH₂CH₃ in the EC) due to the R functional group. Due to this properties, HPC can make more interaction with PU functional group. FTIR of Figure 4.6 and Figure 4.7 show that 3400-3500 cm⁻¹ area is different while Figure 4.6 has OH peak at about 3346 cm⁻¹ such peak does not seen from Figure 4.7.

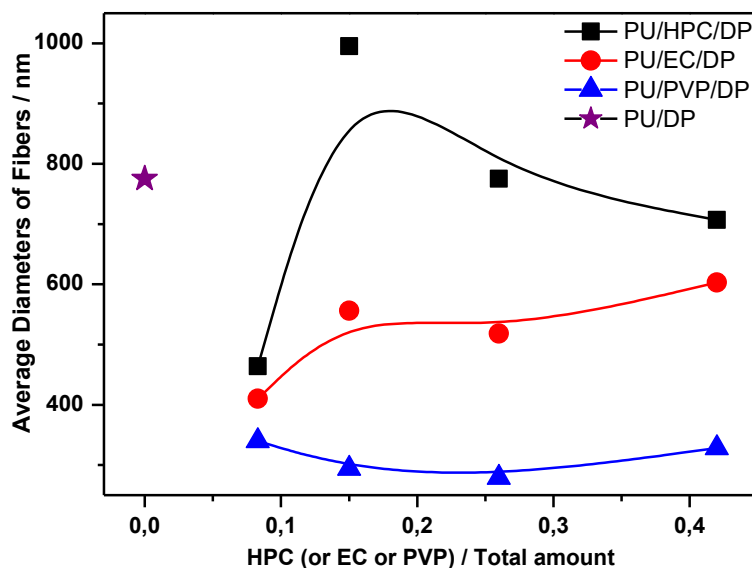


Figure 4.21 : The correlation between diameters of fibers and amount of polymer in PU fibers.

Compared to EC and PVP hydrogen bond interaction is expected to be more strong in PU/HPC. Similar interaction was reported for the DP with hydroxypropyl- β -cyclodextrin (Tingting, 2018).

Figure 4.21 shows that when EC content increases from 10/1/1 to 10/8/1, the average diameters of fibers increase in the electrospun fibers. When the morphology of the fibers comprising EC or HPC were compared, despite the increasing EC ratio in the PU/EC/DP electrospun fibers show fine fibers with bead free unlike fiber comprising HPC. When the amount of HPC increased in the electrospun fibers, beads increase in the fibers because viscosity of the solution also increases. Second polymer addition to PU effects as well as the morphology and average diameters of fiber.

Morphology and porosity of fibers are important to provide a suitable environment to load the drug on them. The most suitable fibrous structures with appropriate porosity with high surface to volume ratio were aimed to be on the electrospun. Although the average pore diameter of 10/8/1 (4.48 μm) and 10/4/1 (3.04 μm) (PU/HPC/DP) is higher, % porosity and surface to volume ratio are higher for the electrospun fibers of 10/0/1 and 10/2/1 than 10/8/1 and 10/4/1. Surface to volume ratios are 3.97, 3.16, 0.89 and 1.31 cm^3/m^2 for 10/0/1, 10/2/1, 10/4/1 and 10/8/1 respectively. Beads are known as defect structures because they disturb the unique property of electrospun fibers and decrease the surface area to volume ratio. From the results of average fiber

diameters, porosity and release of drug shows that the high average pore diameter shows more drug release in first 30th min. But it should be noted that these electrospun fibers have beads and beads disturb the porosity and morphology. 10/0/1 and 10/2/1 having high surface area to volume ratio and average fiber diameters release drug slower and controlled. It is known that, the presence of the pores does not only increase the mobility of the drug molecules, but alters the underlying drug release mechanisms (Klose et al, 2006).

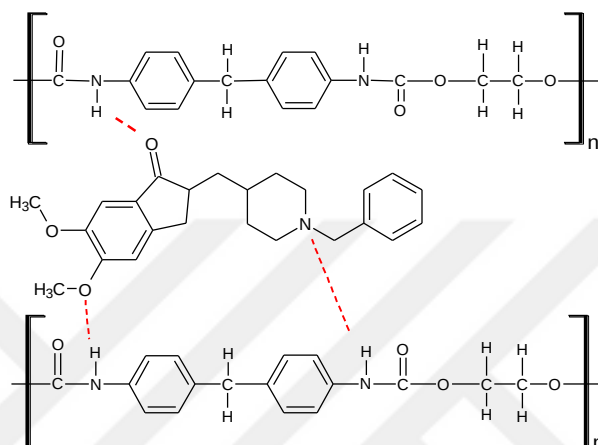


Figure 4.22 : Schematic representation of bonds between PU and drug.

4.5 In-vitro Release Studies of Drug Loaded Fibers by UV-vis Spectroscopic Detection and Kinetic Model

In vitro release studies of the drug were performed in 500 mL isotonic pH 6.5 phosphate buffer at 32⁰C before in vitro release studies electrospun fibers was weighed and dried. After different time intervals (at 30. min, 1th, 2nd, 3rd, 4th, 5th and 6th hour), 2 ml solution has taken from flask. λ max value was determined by scanning the solutions between 500 and 200 nm spectrum mode. Donepezil HCl release from PU/HPC, PU/EC and PU/PVP electrospun fibers are measured with ultra violet visible spectroscopy. The UV absorption spectrum of DP showed at 229 nm maximum peak and two small maximum at 270 nm and 314 nm wavelengths (Figure 4.23). 229 nm was used as detection wavelength and the absorbances of resulting solutions were measured at 229 nm. It is based on the fact that the content of Donepezil HCl in all the electrospun fibers is equivalent to the calculation of theoretical values and indicates that there is no drug loss along the process of electrospinning and homogeneous distribution in all area of the fibers.

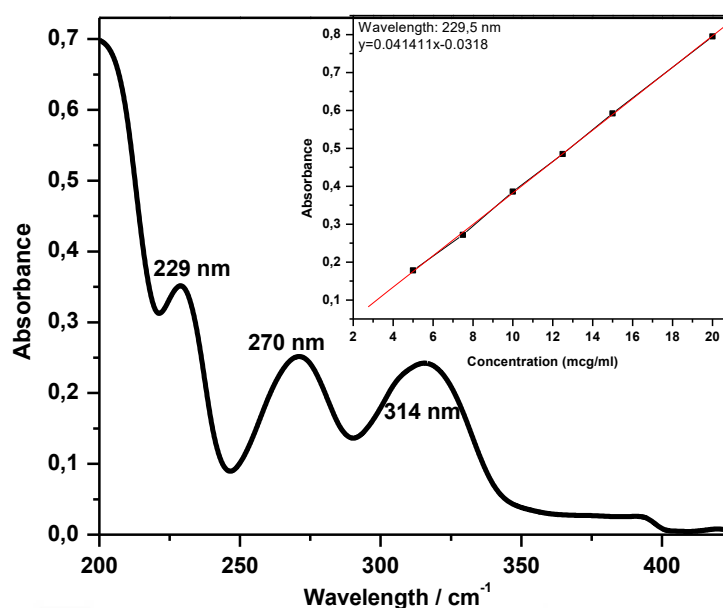


Figure 4.23 : The UV spectra of Donepezil HCl and calibration curve.

The release profiles of DP from PU/HPC electrospun fibers are shown in Figure 4.24. At the end of the first 30 minutes, the electrospun fibers composed of PU/HPC/DP at ratios of 10/0/1, 10/1/1, 10/2/1 and 10/4/1 exhibited an initial burst release of approximately 66%, 66%, 61% and 71%, respectively. Then, the about 85-90% of the drug in the electrospun fibers was released at slightly slower rates within six hours. As pointed out in literature by other researcher an initial fast release of drug from electrospun fiber at the first phase may be related to the drug molecules which dissolved on the surface of the electrospun fibers, which are quickly dissolved into buffer solution in 30 minutes, on the other hand at the second stage, the drug molecules positioned in the center of fibers was released slowly in a sustained manner (Wang, Geng et al. 2009, Huang, Yu et al. 2012, Xu, Shi et al. 2015). Significant change did not seen in the release rate of DP from the electrospun fibers when the ratio of HPC in PU fibers was increased from 10/0 to 10/4 (PU/HPC). However, it has been reported by (Mandru, Vlad et al. 2013) that the addition of HPC into PU to form electrospun fibers has enhanced the release rate of drug. That diverse finding could be explained by the fact that the decreased amount of DP in electrospun fibers while the ratio of HPC was gradually increased in the electrospun fibers.

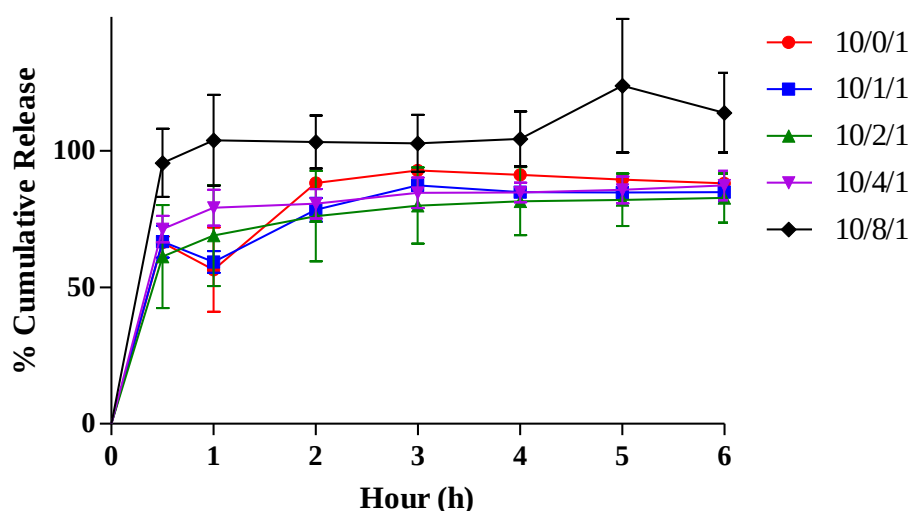


Figure 4.24 : In-vitro DP release profiles from drug loaded PU/HPC fibers.

The PU/HPC/DP electrospun fibers at ratio of 10/8/1 showed immediate drug release as presented in Figure 4.24. That burst drug release behaviour can be due to the existence of the highest ratio of HPC in the composition of electrospun fibers and, the observed some beads on surface of the electrospun fibers, which are likely drug molecules at the outer layer of electrospun fibers (Figure 4.24). Because HPC has the properties which tend to form hydrogel and dissolves slowly in water until it disappears by exposing with water.

Table 4.11 : Coefficient of correlation (r^2) values of DP loaded PU/HPC fibers.

	Ratio of PU/HPC/DP				
	10:0:1	10:1:1	10:2:1	10:4:1	10:8:1
Zero Order Kinetic Model	0.516	0.633	0.787	0.791	0.637
First Order Kinetic Model	0.510	0.619	0.762	0.768	0.655
Hixson-Crowell Kinetic Model	0.513	0.624	0.770	0.776	0.649
Higuchi Square Root Kinetic Model	0.613	0.713	0.896	0.881	0.612
Korsmeyer-Peppas Kinetic Model	0.649	0.721	0.964	0.938	0.591

The in vitro release results of formulations were fitted to above mentioned kinetic models to investigate the drug release kinetics of electrospun fibers. The data obtained from the analysis were evaluated based on the determination coefficients (r^2 values), which signifies the goodness of the fit. The best regression value (r^2) was found for PU/HPC/DP at ratio of 10/2/1 when Korsmeyer-Peppas model was fitted the data, as shown in Table 4.11. The value of exponential factor 'n' for this model

was calculated as 0.12, showing Fickian diffusion controlled release system. The similar release kinetic model for electrospun fibers has also been described by Taepaiboon and Kumar. (Taepaiboon, Rungsardthong et al. 2007, Kumar and Gopinath 2015).

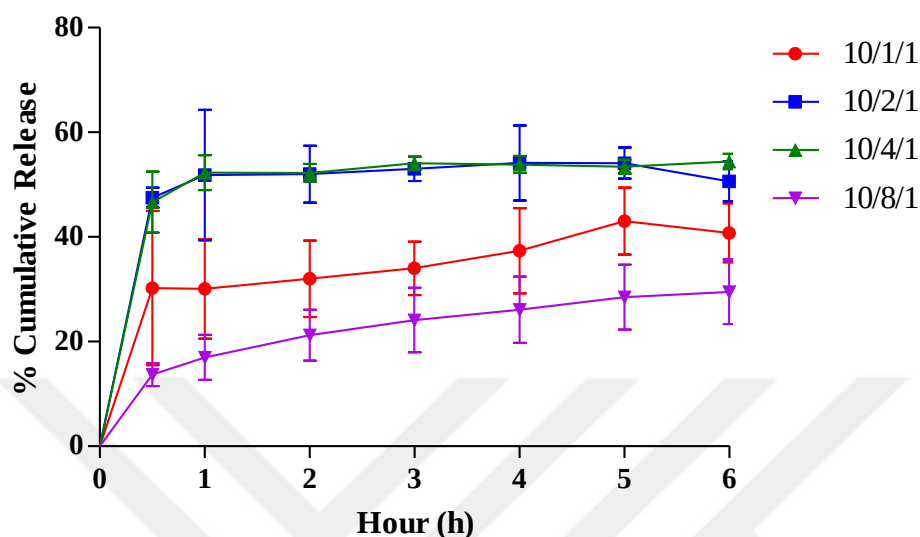


Figure 4.25 : In-vitro DP release profiles from drug loaded PU/EC fibers.

EC or PVP was added for researching the EC and PVP effect to PU solutions on the drug release; PU/EC/DP and PU/PVP/DP (at ratios of 10/1/1, 10/2/1 10/4/1 and 10/8/1, respectively) electrospun fibers were utilized. The release profiles of DP from these PU/EC and PU/PVP electrospun fibers are shown in Figure 4.25 and 4.26, respectively.

Table 4.12 : Coefficient of correlation (r^2) values of DP loaded PU/EC fibers.

	Ratio of PU/EC/DP				
	10:0:1	10:1:1	10:2:1	10:4:1	10:8:1
Zero Order Kinetic Model	0.516	0.905	0.240	0.542	0.949
First Order Kinetic Model	0.510	0.922	0.241	0.530	0.896
Hixson-Crowell Kinetic Model	0.513	0.917	0.241	0.534	0.915
Higuchi Square Root Kinetic Model	0.613	0.863	0.358	0.658	0.994
Korsmeyer-Peppas Kinetic Model	0.649	0.797	0.493	0.767	0.999

As can be seen from these figures, all of the electrospun fibers fabricated showed biphasic drug release profiles with relatively fast release in 30 min, they later released the remaining of the drug slowly. Significantly higher amount of drug release was observed by PU/PVP/DP (10/8/1) electrospun fibers consisted of the

highest ratio of PVP than those of other electrospun fibers comprised of PVP (Figure 4.26). However, the increment of the EC amount added to PU solution was resulted in decrease of drug release (Figure 4.25). While about 60% of DP from that PU/PVP/DP (10/8/1) electrospun fibers was released in an hour (Figure 4.26), the concentration of released DP from electrospun fibers PU/EC/DP (10/8/1) was about 17.0% at the end of an hour as shown in Figure 4.25. This case was attributed to the hydrophilic character of PVP, which might accelerate the release rate of drug from PU electrospun fibers when its ratio was increased. The observed slow drug release effect of EC may be explained by its hydrophobic property when its ratio was increased in the composition of PU electrospun fibers.

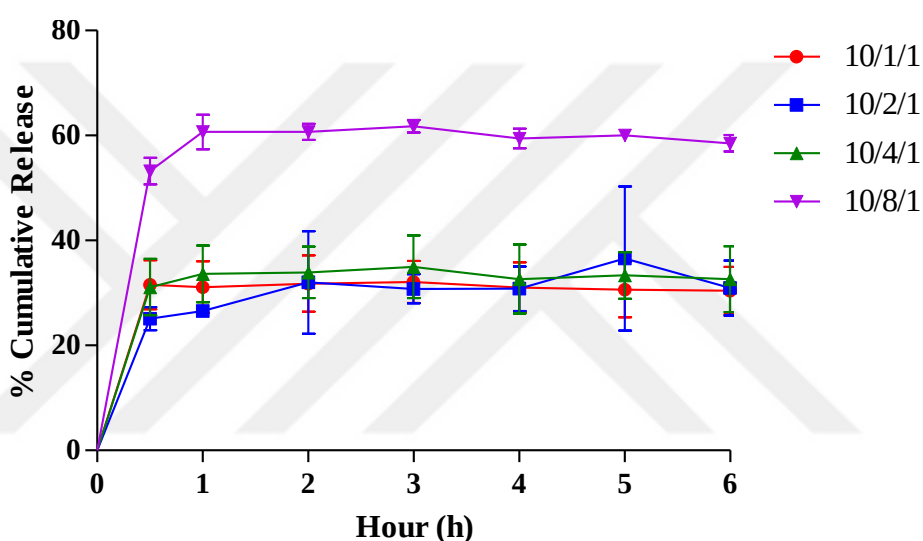


Figure 4.26 : In-vitro DP release from PU/PVP fibers loaded drug.

Besides, the clumps on the electrospun fibers consisted of PVP in Figure 4.20 might contribute to that higher immediate drug release. Interestingly, among the other PU/DP electrospun fibers composed of either PVP or EC, no linear correlation between the released amount of drug and their ratios in the electrospun fibers composition were observed. The results revealed that in the polymer EC may lead to retention of the hydrophilic drug, which was resulted in inhibiting drug release and presenting a sustained drug release, as reported earlier (Rowe R.C., 2009). Furthermore, drug does not release freely from dosage form with exposing with water which is as a viscose gel due to the properties of EC.

On the other hand, PVP could also accelerate the release of a hydrophilic drug, as also reported for poorly water-soluble drug (ibuprofen) (Yu, Shen et al. 2009). Moreover, it is well known that the hydrophilic drugs are released fast, this case are a

problem for the controlled release of these types of drugs (Enayatifard, Saeedi et al. 2009). Based on that data, the sustained release behaviour of a hydrophilic drug from PU/EC electrospun fibers can be possible when the ratio of EC was increased as shown in Figure 4.25, that would be an advantage for providing a sustained release manner for hydrophilic compounds.

Table 4.13 : Coefficient of correlation (r^2) values of DP loaded PU/PVP fibers.

	Ratio of PU/PVP/DP				
	10:0:1	10:1:1	10:2:1	10:4:1	10:8:1
Zero Order Kinetic Model	0.516	0.449	0.537	0.011	0.088
First Order Kinetic Model	0.510	0.455	0.558	0.013	0.097
Hixson-Crowell Kinetic Model	0.513	0.453	0.551	0.013	0.094
Higuchi Square Root Kinetic Model	0.613	0.345	0.618	0.052	0.174
Korsmeyer-Peppas Kinetic Model	0.649	0.247	0.713	0.141	0.316

The in vitro release data of electrospun fibers were analyzed using the kinetic models mentioned in method section to understand the release mechanism and kinetics of model drug from electrospun PU/EC and PU/PVP fibers. The mechanism of drug release and kinetics were identified based on the coefficient of correlation (r^2) values, which signifies the goodness of the fit. Table 4.12 and Table 4.13 present coefficient of correlation (r^2) values estimated for linear curves of PU/EC/DP and PU/PVP/DP electrospun fibers, respectively. The regressed results for PU/EC/DP electrospun fibers at ratio of 10/8/1 exhibited the highest coefficient of correlation ($r^2=0.999$) (Table 4.12) for Korsmeyer-Peppas model. This result indicated that the release of drug from PU/EC/DP electrospun fibers in ratio of 10/8/1 was controlled by mechanism of Fickian diffusion with the release exponent result (n) 0.31 (<0.5). Namely, the drug was released by molecular diffusion based on the chemical potential gradient. The similar release kinetic for PVP/EC electrospun fibers loaded with ketoprofen, which is a poorly soluble drug, has also been reported by Yu et.al. (Yu D.G. 2013). However, the other electrospun fibers consisting of EC (at ratios of 10/1/1 10/2/1 and 10/4/1) also did not exhibit Korsmeyer-Peppas model (Table 4.12). In this case, an increase of EC ratio in the composition of PU based electrospun fibers can affect release kinetics and mechanisms of a hydrophilic drug, which was resulted in a sustained-drug release fashion. On the other hand, PU/PVP/DP

electrospun fibers exhibited very low coefficient of correlation (r^2) values for kinetic models (Table 4.13).

4.6 In-vitro Permeation Studies of Drug Loaded PU/HPC and PU/EC Fibers

The electrospun fibers composed of PU/HPC/DP blends (10:2:1, w/w/w) and PU/EC/DP blends (10:8:1, w/w/w) presented the best regression for Korsmeyer-Peppas kinetic model which are the model of diffusion controlled drug release were chosen during 72 h for in vitro permeation study. However, we paid no attention the cumulative DP amounts of 72th hour as it was reached to the saturation after 54th hour. Following 24, 48 and 54 h in vitro permeation, the cumulative amounts (Q₂₄, Q₄₈ and Q₅₄) of DP permeated from electrospun fibers across Strat-MTM membrane, which is skin resembling synthetic membrane to human and animal skin for permeation studies were $259.32 \pm 55.46 \mu\text{g} \cdot \text{cm}^{-2}$, $863.67 \pm 118.31 \mu\text{g} \cdot \text{cm}^{-2}$ and $956.00 \pm 107.95 \mu\text{g} \cdot \text{cm}^{-2}$, respectively given in Figure 4.27 for PU/HPC/DP blends (10:2:1, w/w/w) (Uchida, Kadhun et al. 2015). The flux of DP was also calculated as $20.33 \pm 3.62 \mu\text{g} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$ while lag time was predicted $6.70 \pm 3.10 \text{ h}$ for PU/HPC/DP blends (w/w/w, 10:2:1).

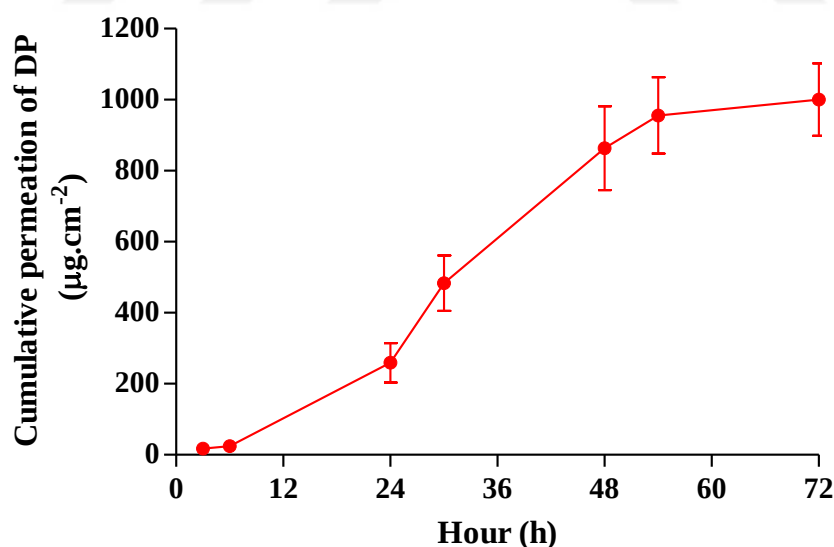


Figure 4.27 : The cumulative amount of permeated DP across M-Strate® membrane for sample of (PU/HPC/DP, 10:2:1, w/w/w).

Previously, the targeted transdermal flux of DP across skin has been estimated to be $16.5 \mu\text{g} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$ to attain its therapeutic plasma value with applying a patch on skin surface with size of 20 cm^2 (Choi, Choi et al. 2012, Galipoğlu, Erdal et al. 2015).

Table 4.14 : Amount of released Donepezil HCl through the pig skin from the electrospun fibers as a result of permeation study.

Time /Hour	PU/HPC/DP (10:2:1) Amount of drug release ($\mu\text{g}/\text{cm}^2$)	PU/ EC/DP (10:8:1) Amount of drug release ($\mu\text{g}/\text{cm}^2$)
3	17.55 $\pm$ 30.8	0.48 $\pm$ 0.24
6	24.07 $\pm$ 28.3	2.29 $\pm$ 0.19
24	259.32 $\pm$ 98.2	13.88 $\pm$ 3.19
30	483.57 $\pm$ 138.7	23.12 $\pm$ 3.94
48	863.67 $\pm$ 209.4	47.29 $\pm$ 33.8
54	956.00 $\pm$ 191.1	58.04 $\pm$ 49.45
72	1000.55 $\pm$ 180.2	75.15 $\pm$ 61.08

On the other hand, in vitro permeations for PU/EC/DP blends (10:8:1, w/w/w) were 13.88 $\pm$ 3.19 $\mu\text{g}.\text{cm}^{-2}$, 47.29 $\pm$ 33.8 $\mu\text{g}.\text{cm}^{-2}$ and 58.04 $\pm$ 49.45 $\mu\text{g}.\text{cm}^{-2}$, respectively as shown in Figure 4.28. The flux of DP was also calculated as 1.1772 $\mu\text{g}.\text{cm}^{-2}.\text{h}^{-1}$ while lag time was predicted 7.88 h.

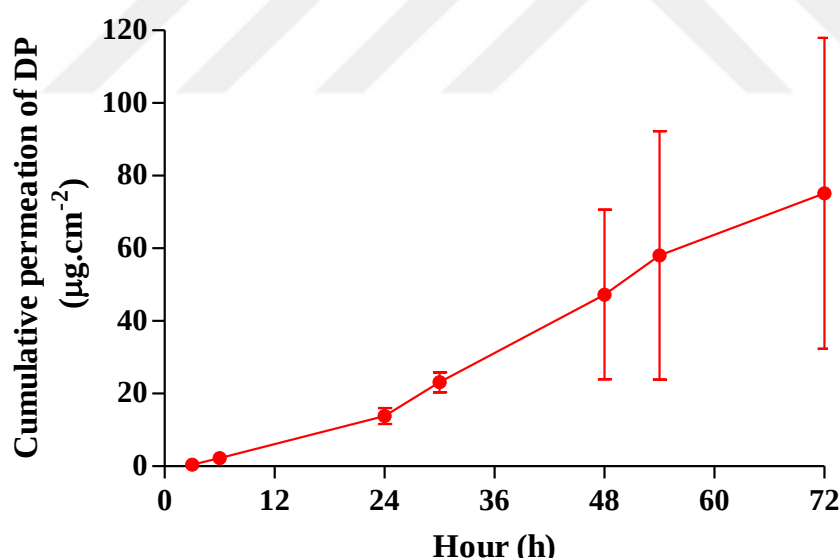


Figure 4.28 : The cumulative amount of permeated DP across M-Strate® membrane for sample of (PU/ EC/DP, 10:8:1, w/w/w).

This finding indicates that the targeted permeation rate (20.33 $\pm$ 3.62 $\mu\text{g}.\text{cm}^{-2}.\text{h}^{-1}$) of DP across synthetic skin resembling membrane was attained following the administration of the electrospun fibers composed of PU/HPC/DP blends (10:2:1, w/w/w). Thus, electrospun fibers optimized seems to be feasible to deliver DP across skin in its therapeutic range. 1000 $\mu\text{g}/\text{cm}^2$ Donepezil HCl permeates through pig skin

in 72 hours from transdermal formulation of PU/HPC/DP blends (10:2:1, w/w/w) containing approximately 1860 $\mu\text{g}/\text{cm}^2$ of Donepezil HCl.

4.7 In-vitro Cytotoxicity Studies of PU/HPC and PU/HPC/DP Fibers

To evaluate the relative reliability cytotoxicity of the optimized electrospun fibers formulation was evaluated with cell culture study in comparison with sodium lauryl sulfate (SLS) as a moderate irritant molecule. The relative cytotoxicity of PU/HPC/DP electrospun fibers (10:2:1, w/w) selected based on the release behaviours, (in vitro release behaviour which follows Korsmeyer–Peppas kinetic model and in vitro permeation behaviour) and the PU/HPC (10:2, w/w) electrospun fiber samples without drug loading were evaluated in the mouse fibroblast cell culture. The cells seeded into Dulbecco's Modified Eagle's medium (DMEM) incubated at 37°C temperature in 5% CO₂ atmosphere. DMEM comprises additive penicillin-streptomycin (1%) and 10 % inactivated fetal calf serum albumin (FBS).

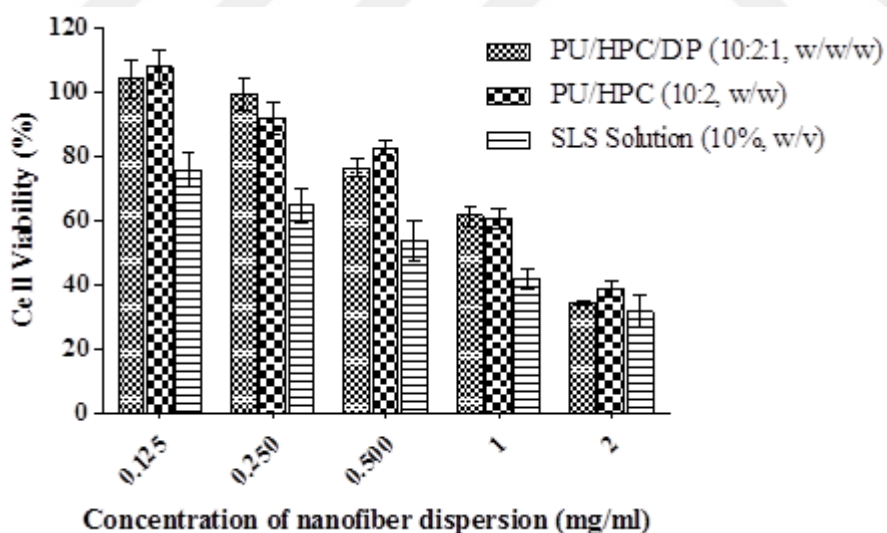
For evaluation; the cells were added to a well in a 96-well plate-surface tissue culture (104 cell/well) and aqueous solutions of electrospun fibers formulations loaded and unloaded Donepezil HCl were kept in the cell culture between 0.125 to 2% (v/v) concentration at 8 and 16 hour. Isotonic phosphate buffer (PBS) in 1% (v/v) concentration was used as a negative control at all tests. SLS (10% concentration) used as a positive control.

The cell viability proliferation was evaluated by MTT assay. The viable cells were identified by precipitating insoluble purple formazan. Test was performed according to the method proposed by Alley et al. (Alley, Scudiero et al. 1988). Briefly, the cells were washed with PBS (pH 7.4) after treating with electrospun fibers. Then, 20 μL MTT solution in PBS (5 mg/ml) and 200 μL the cell culture medium was supplemented to every well. At 37 °C for 2 h in humidified atmosphere of 5% CO₂ in air, the plates were incubated. Dimethyl sulfoxide (200 μL) was added in order to dissolve the purple formazan crystal into the wells after MTT and culture medium were removed. The plates were then shaken for 10 min, they were rinsed and the absorbance of content in each well was recorded at a 590 nm wavelength using an microplate reader (Epoch, BioTek Instruments, Vermont, USA) 670 nm wavelength was used as a reference wavelength.

Table 4.15 : The relative cytotoxicity data of PU/HPC (10:2, w/w) fibers.

Concentration of the electrospun fibers dispersion (mg/ml)	% Cell Viability		
	PU/HPC/DP (10:2:1, w/w/w)	PU/HPC (10:2, w/w)	SLS Solution (10 %, w/v)
0.125	104.0 ± 5.67	107.90 ± 5.25	75.75 ± 5.05
0.250	99.25 ± 4.89	91.91 ± 5.05	64.73 ± 5.38
0.500	76.37 ± 2.99	82.70 ± 2.18	53.70 ± 6.13
1	61.40 ± 3.11	60.45 ± 3.21	41.74 ± 3.41
2	34.22 ± 0.57	38.79 ± 2.57	31.64 ± 4.89
IC₅₀ value	1.247	1.300	0.631

The cytotoxicity activity of each specimen was expressed as an IC₅₀ value (percentage of the cell viability). The results are given by finding the mean value of $\pm$ standard deviation (SD) of at least three experiments. GraphPad Prism Software version 6.05® (La Jolla, CA) was used to calculate the statistical data analysis. The data comparison was done by Unpaired Student t-test. Statistical significance level was remarked as P<0.05.

**Figure 4.29 :** % Cell viability of PU/HPC (10:2, w/w) electrospun fibers in mouse fibroblast cell.

The relative cytotoxicity results are shown as the percentage (%) of cell viability and IC₅₀ value in Figure 4.29 and 4.30. As the data examined, it was determined that the cells viability was lowered with ascending concentration of the electrospun fibers. However, it was observed that % cell viability in all of concentrations for both of DP

loaded and unloaded PU/HPC (10:2, w/w) electrospun fibers was significantly higher than 10 % (w/v) SLS concentration ($p < 0.05$).

Otherwise, IC₅₀ value for either PU/HPC (10:2, w/w) fibers with DP (1.247 mg/ml) or without DP (1.300 mg/ml) was calculated as 2-fold higher than IC₅₀ value for SLS (0.631 mg/ml), which has stated in International Conference on Harmonisation Guideline [International Conference on Harmonisation Guideline]. According to these findings, it could be concluded that PU/HPC (10:2, w/w) electrospun fibers could be well tolerated by the skin and the components of electrospun fibers would not irritant for the skin. Thus, electrospun fibers optimized seems to be feasible to deliver DP across skin in its therapeutic range.

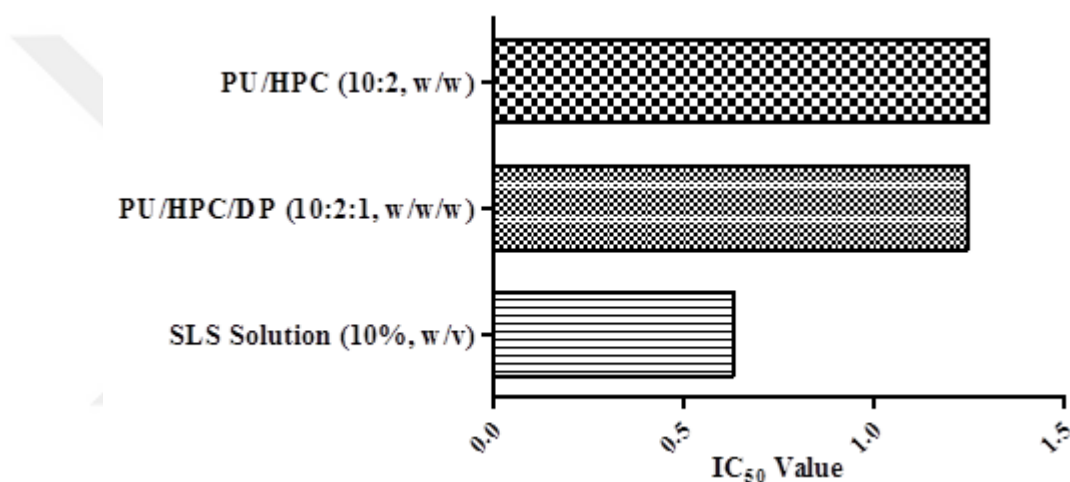


Figure 4.30 : IC₅₀ values of PU/HPC (10:2, w/w) fibers.

4.8 In-Vitro Release Studies of Drug Loaded Fibers with Electrochemical Impedance Spectroscopy and Model of Equivalent Circuit

This part of the thesis will focus on the electrochemical impedance studies and equivalent electrical circuit model. In these works, electrochemical impedance spectroscopy technique was used to investigate the changes of interfacial properties at electrospun fibers on the electrode surface and also changes in buffer solution after the drug release from the fiber. Electrochemical impedance studies were performed according to 3.7.1 experimental part, to monitor electrical properties of buffer solution after the releasing of drug from the drug loaded PU, PU/HPC and PU/EC electrospun fibers. In this part, commercial screen-printed electrode (SPEs) was used as electrode system at the electrochemical impedance studies. Working electrode is

gold, pseudo-reference electrode is silver and auxiliary electrode is platinum in this electrode system.

Bhargav et.al indicated that conductivity (σ) of the electrolyte depends on the actual concentration of conducting species and their mobility (Bhargav, Mohan et al. 2007). Resistivity and conductivity are reciprocals:

$$\rho = 1/\sigma \quad (4.6)$$

It can be observed that conductivity of electrolyte, increases by rising in salt concentration in the electrolyte. In other words, resistivity of electrolyte decreases with increase in salt content at the electrolyte. According to this point of view, we expect that resistivity of buffer solution decreases with increase in released drug in buffer solution.

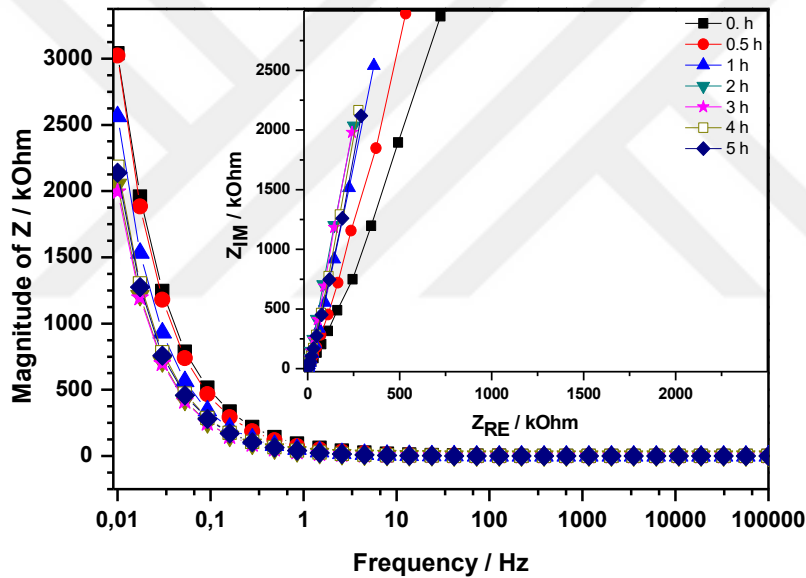


Figure 4.31 : Magnitude of Z (inset: Nyquist plot) of SPE in buffer solution containing released Donepezil HCl from PU/HPC/DP (10/2/1) fibers.

Figure 4.31 and 4.33 show Magnitude of Z and Nyquist plot of impedance (inset) of PU/HPC/DP (10/2/1) and PU/EC/DP (10/8/1) electrospun fibers. PU/HPC/DP (10/2/1) and PU/EC/DP (10/8/1) electrospun fibers were presented the best regression for Korsmeyer-Peppas kinetic model. Magnitude of Z and Nyquist plot of impedance were presented between 10 mHz and 100 kHz frequency range. Nyquist plots and Magnitude of Z give us information about the resistivity of the system/solution. When the drug release increases over time, electrode exhibits more capacitive behavior in buffer solution, such as electrode is more capacitive after 2

hours than first hours in buffer solution. As it can be observed from Figure 4.32, Magnitude of Z values of the electrode decreases from 3046 kOhm to 2042 kOhm with the increasing drug release in buffer solution between 0 and 2 hours. Magnitude of Z values between 2 and 5 hours shows parallel behavior with the in-vitro drug release studies for PU/HPC/DP (10/2/1) electrospun fibers, in this case not much changes are observed between 2 and 5 hours. On the other hand Magnitude of Z values of the electrode decreases from 2700 kOhm to 2400 kOhm for PU/EC/DP (10/8/1) electrospun fibers between 0 and 6 hours, it is more significantly controlled release than PU/HPC/DP (10/2/1) electrospun fibers.

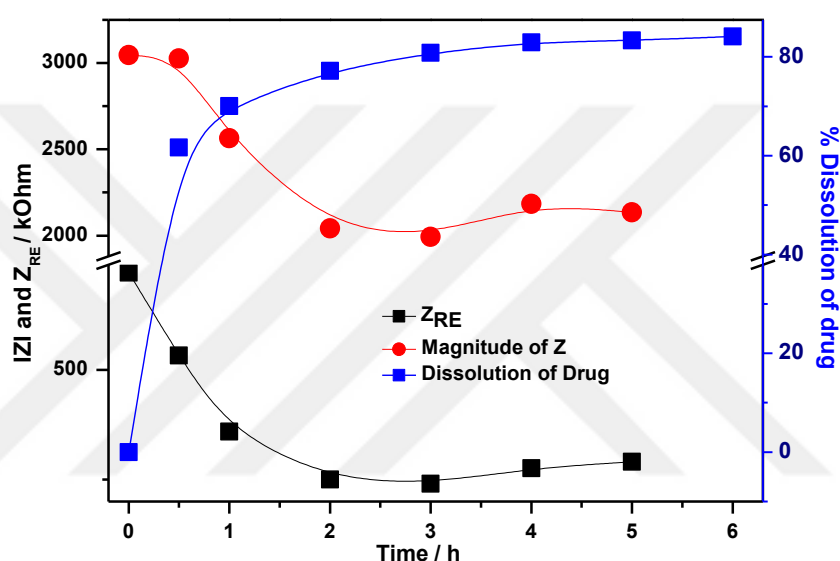


Figure 4.32 : The correlation between time and Magnitude of Z, Z_{RE} (at 10 mHz) and percent of drug release from PU/HPC/DP (10/2/1) fibers from UV analysis.

Figure 4.32 and 4.34 show the correlation between time and Magnitude of Z, Z_{RE} values at 10 mHz (from Nyquist plots) and % dissolution of DP from PU/HPC/DP (10/2/1) and PU/EC/DP (10/8/1) electrospun fibers. The expected effect is seen between resistance and dissolution of drug, the electrode in buffer solutions generated a decrease in impedance in progress of time since the electrical characteristics of the electrode/solution interface was changed.

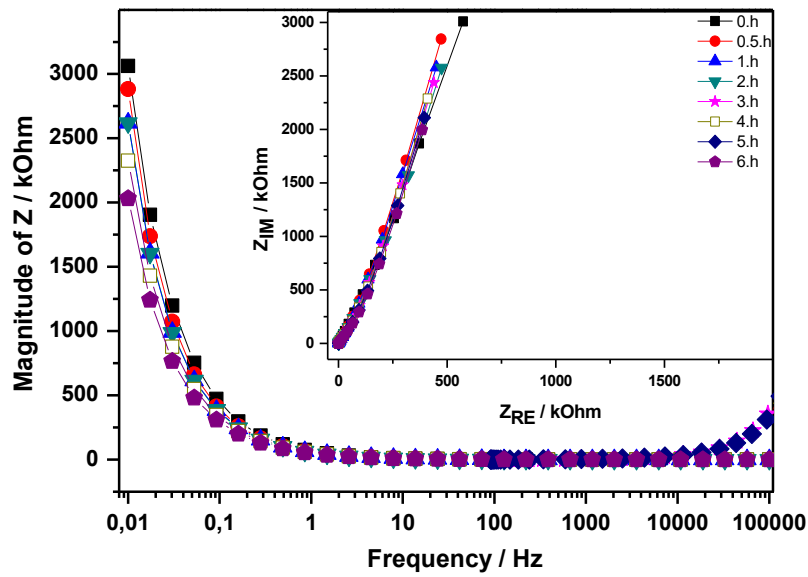


Figure 4.33 : Magnitude of Z (inset: Nyquist plot) of SPE in buffer solution containing released DP from PU/EC/DP (10/8/1) fibers.

Dissolution of drug increases with decreasing Z values. But for PU/HPC/DP fibers resistivity differences almost the same between hour 2th and 5th according to Magnitude of Z and Z_{RE} values.

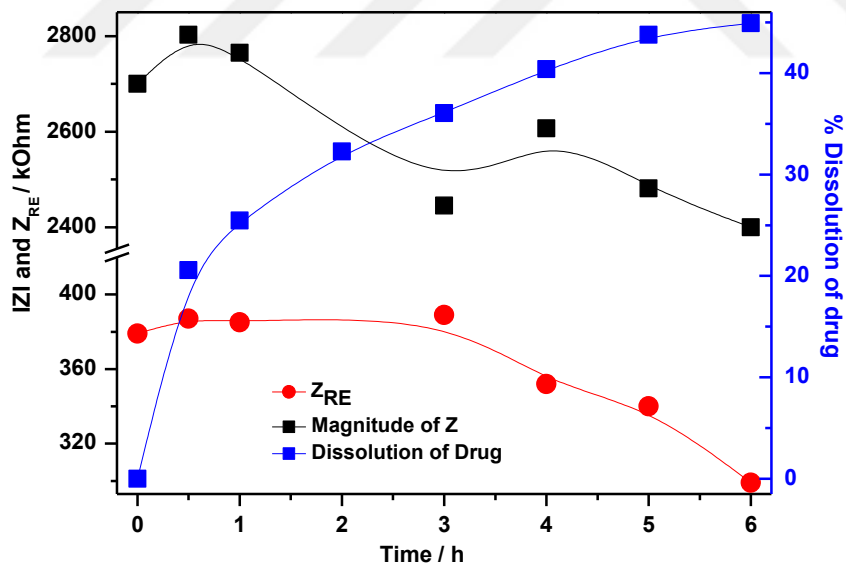


Figure 4.34 : The correlation between time and Magnitude of Z, Z_{RE} (at 10 mHz) and percent of drug release from PU/EC/DP (10/8/1) fibers.

The impedance results were analyzed by fitting into electrical equivalent circuit models. ZSimWin V.3.10 (Princeton Applied Research, Tennessee, USA) program was used for analysis. Electrical equivalent circuit models usually consists of a number o elements connected in the network either in series and/or parallel

combinations. $R(Q(RW))$, $RQRW$ and $R(Q(RW))QR$ equivalent circuit models were fitted models with experimental data. Figure 4.35 shows $RQRW$ circuit model.

At the proposed model of $RQRW$, the first unit is the solution or electrolyte resistance (R_s) of the buffer solution which is useful analytically in conductivity sensors mainly. The resistance offered by the ionic solution (electrolyte) is an important factor contributing to the overall impedance of the cell. The other units is constant phase element, (define as Q or CPE), the charge transfer resistance (R_{ct1}) and Warburg Impedance (W). CPE reflecting the double layer capacitance at the electrode/electrolyte interface. R_{ct1} is resistance amongst the buffer solution and the electrode interface, W is a diffusion of drug species at the interface. All components in this electrical circuit model are the series connection. The chi square values is a function defined as the sum of the squares of the residuals and were around 10^{-3} , 10^{-4} , 10^{-5} for chosen models.

In generally, constant phase element used in heterogeneous systems is related to capacitive behavior of non-ideal system which outcomes from non-homogeneous conductivity, diffusion or electrode roughness (Rubinson, J. F., 2009). CPE is also dependent on the electrospun fibers composition.



Figure 4.35 : The best fitting ($RQRW$) Model for SPE system.

Results of R_s which was achieved from the best fitting model, ranges from 59Ω to 0.13Ω at $RQRW$ model at 30th minutes for drug release of PU/HPC/DP (10/2/1) electrospun fibers (Table 4.16). 10/8/1 ratio of PU/HPC/DP showed immediate drug release in the first 30th minutes, as shown in Figure 4.24. The smallest and highest R_s value (0.13Ω and 81.3Ω) obtained from the circuit model belongs to the 10/8/1 and 10/2/1 ratio of electrospun fibers. In vitro results of PU/HPC/DP are compatible with R_s value of EIS. In addition to R_s values, R_{ct} values of 10/8/1 and 10/4/1 ratio of electrospun fibers have the highest value (about 88Ω). Charge transfer resistances of 10/8/1 and 10/4/1 ratios can be related to HPC amount in the matrixes. Since, high HPC amount in the matrix structure can be created gelation and can increased the charge transfer resistance.

Table 4.16 : Equivalent circuit model parameters [RQRW] for Donepezil HCl loaded PU/HPC electrospun fibers (released at 30th min).

Equivalent Circuit Model	PU/HPC/DP (w/w/w) electrospun fibers				
Parameters	(10/0/1)	(10/1/1)	(10/2/1)	(10/4/1)	(10/8/1)
Rs [Ω]	58.84	14.68	81.3	1.83	0.13
CPE [Y_0/Ss^{-n}]	4.86E-6	4.91E-6	3.21E-6	5.55E-6	4.94E-6
n	0.87	0.93	0.82	0.92	0.91
Rct [Ω]	26.23	81.15	28.3	88.78	88.31
W [Y_0/Ss^{-n}]	8.1E-3	7.23E5	1.57E5	1.23E4	4.53E-3
Chi Squared	3.13E-03	2.40E-03	5.06E-03	1.47E-03	5.66E-04

Rs values of PU/EC/Drug (10/8/1) electrospun fibers ranges from 11 Ω to 115 Ω at for drug release at 30th minutes. Rs and Rct values show the opposite effect. Whereas Rs shows the maximum value, Rct shows the minimum value. In other words, if the charge transfer resistance is high, the drug release is delayed. Warburg Impedance (W) values (at released 30th min) calculated from RQRW model are 8.1E-3, 7.23E5, 1.57E5, 1.23E4, 4.53E-03 for PU/HPC/DP (10/0/1-10/8/1) and 5.92E6, 1.30E9, 2.49E8 for PU/EC/DP (10/1/1, 10/2/1, 10/8/1) samples respectively shown in Table 4.16 and 4.17.

Table 4.17 : Equivalent circuit model parameters [RQRW] for Donepezil HCl loaded PU/EC electrospun fibers (released at 30th min).

Equivalent Circuit Model	PU/EC/DP (w/w/w) electrospun fibers		
Parameters	(10/1/1)	(10/2/1)	(10/8/1)
Rs [Ω]	11.26	115.40	79.77
CPE [Y_0/Ss^{-n}]	3.64E-6	4.35E-6	3.29E-6
n	0.86	0.90	0.85
R _{CT} [Ω]	81.18	2.40	37.25
W [Y_0/Ss^{-n}]	5.92E6	1.30E9	2.49E8
Chi Squared	4.81E-03	1.51E-03	6.12E-03

CPE values are 4.86E-6, 4.91E-6, 3.21E-6, 5.55E-6, 4.94E-6 for PU/HPC/DP (10/0/1-10/8/1) and 3.64E-6, 4.35E-6, 3.29E-6 for PU/EC/DP (10/1/1, 10/2/1 and 10/8/1) they exhibit almost similar results as given in Table 4.16 and 4.17. CPE has not been shown considerable change.

Table 4.18 : Some equivalent circuit model results of 10/2/1 ratio PU/HPC/DP.

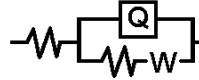
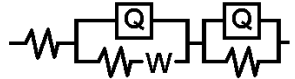
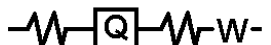
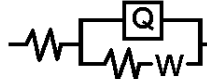
Rs [Ω]	CPE [Y₀/Ss⁻ⁿ] (10⁻⁵)	Rct [Ω]	W [Y₀/Ss⁻ⁿ]	Chi Squared	Equivalent Circuit Models
81	3.21E-1	28	1.57E5	5.06E-3	
110	3.21E-1	1.09E8	1.05E-23	5.06E-3	
114	3.40E-1	3.23E11	4.62E-18	1.54E-3	

Table 4.18 and Table 4.19 show some equivalent circuit models of electrospun fibers composed of PU/HPC/DP (10/2/1) and PU/EC/DP (10/8/1) presented the best results in vitro dissolution studies. Experimental and calculation results is best fitting with these three models. The results show that Rs and CPE parameters do not change depending on the circuit design which is given in the table. Against Rs and CPE results, Rct and W depends on the circuit design.

Table 4.19 : Some equivalent circuit results of ratio 10/8/1 PU/EC/DP.

Rs [Ω]	CPE [Y₀/Ss⁻ⁿ] (10⁻⁵)	Rct [Ω]	W [Y₀/Ss⁻ⁿ]	Chi Squared	Equivalent Circuit Model
80	3.29E-1	37.25	2.49E8	6.12E-3	
118	4.27E-1	1.52E6	8.56E-8	1.38E-3	
119	4.46E-1	2.93E6	4.87E-8	8.69E-4	

4.9 Evaluation of Drug Release by Using Substrate System

4.9.1 Evaluation of Drug Release Using Substrate System by Electrochemical Impedance Spectroscopy

A goal of this section was to demonstrate that the measurement of impedance would allow us to evaluate surface of the electrospun fibers coated onto the electrode system in terms of the electrical properties and releasing of drug from the electrospun fibers. For this purpose, ITOPET with dimensions of 1x3 cm was chosen as an electrode for reproducible electrochemical measurements. Drug loaded PU, PU/HPC

and PU/EC electrospun fibers (100 % PU, 75/25, 50/50, 25/75 % PU/HPC and 75/25, 50/50 % PU/EC respectively) were collected onto ITO/PET electrode for 5 minutes. Coated ITO/PET electrodes with fibers were immersed into the 5 ml isotonic phosphate buffer (di-sodium hydrogen phosphate and potassium dihydrogen phosphate) solutions at pH 6.5. Drug release was evaluated by EIS and equivalent electrical circuit models from this electrode system (Gencturk and Sarac 2016). Figure 4.36 (in Figure t_0 : the first time, the fiber is dipped in solution, t_1 : the time, solution begins to enter the space of the fiber, the drug release starts, t_2 : the time, solution penetrates into fiber, the drug release occurred and WE: drug loaded electrospun fibers based substrate electrode) shows the simulation of system. Electrospun fibers based The working electrode was an ITO/PET, the counter electrode was a platinum (wire) and the pseudo-reference electrode was a silver (wire) in EIS measurements.

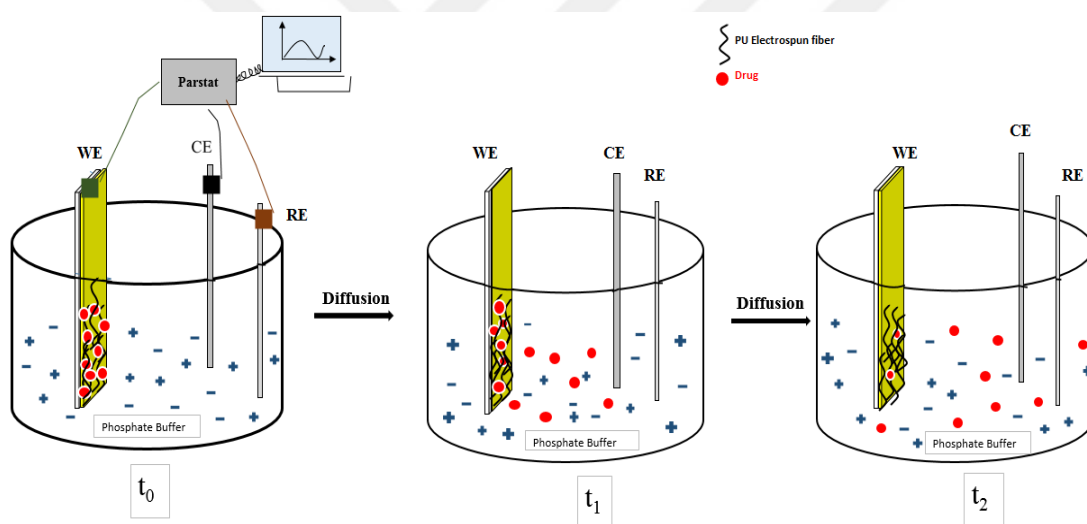


Figure 4.36 : Drug release representation from PU and PU/HPC drug loaded fibers in electrochemical cell.

In this stage, at room temperature time intervals were chosen such as 15., 30., 45., 60., 90., 120., 180., 240. and 270. minute for EIS measurements in buffer solution. Because in previous study, no significant changes were detected between 4-6 hours at in vitro dissolution release studies.

Equivalent electrical circuit models have studied the electrical properties of drug loaded PU, PU/HPC and PU/EC electrospun fibers. Best fitting between experimental data and electrical circuit models was succeeded by using the ZSimpWin software from the EIS measurements. The best fitting models were

determined as $(R(QRW)(QR))$ and $(R(Q(RW))(QR))$ as shown in Figure 4.37 for electrospun fibers collected on ITOPET. Figure 4.36 shows the simulation of the drug release from drug loaded PU, PU/HPC and PU/EC electrospun fibers in the electrochemical cell.

$R(Q(RW))(QR)$ electrical circuit model was presented in detail in our publication (Gencturk and Sarac 2016). In this section we will compare both electrical circuit models of $R(Q(RW))(QR)$ with $R(QRW)(QR)$. R_s value at 15th minutes differs from 254Ω to 224Ω at $R(Q(RW))(QR)$ and differs from 366Ω to 224Ω at $R(QRW)(QR)$ for Donepezil HCl loaded electrospun fibers samples comprised of 0, 25, 50, 75 % HPC in PU matrix (Table 4.20).

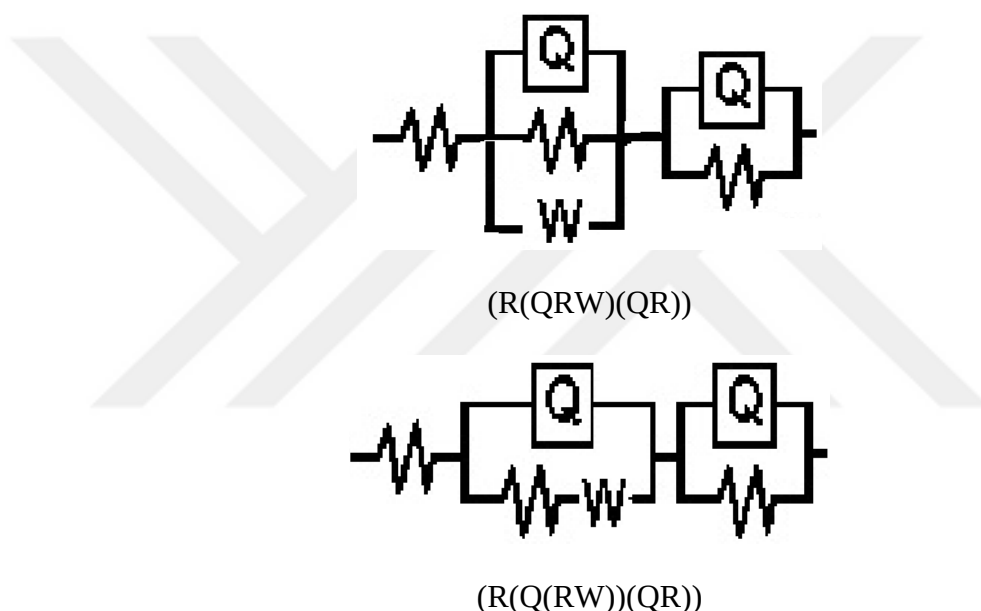


Figure 4.37 : The best fitting Equivalent Circuit Models for electrospun fibers coated on ITOPET.

The value of R_s decreases as the amount of HPC in matrix increases. It depends on the increasing Donepezil HCl in the buffer solution because released Donepezil HCl increased by increasing HPC content in the electrospun fiber matrix. CPE_1 values are close to each other with a value of $2.42E-5$, $2.01E-5$, $2.56E-5$ and $1.49E-5$ [Y_0/Ss^{-n}] in $R(Q(RW))(QR)$ model for Donepezil HCl loaded electrospun fibers samples comprised of 0, 25, 50, 75 % HPC in PU matrix respectively (Table is not given in the thesis, it was presented in detail in our publication). In Table 4.20, by applying $R(QRW)(QR)$ electrical circuit model results indicate that CPE_1 values increase by incorporation of HPC into the electrospun fiber matrix. CPE_2 values stay almost the same for Donepezil HCl loaded electrospun fibers comprised of 0, 25, 50 % HPC in

PU matrix, but if the fibers comprise of 75 % HPC in PU matrix CPE_2 value increases. n values were closer to 1 and gave better results in $R(QRW)(QR)$ model. R_{ct1} values increase by incorporation of HPC into the electrospun fiber matrix. Unlike R_{ct1} , R_{ct2} values decreases by incorporation of HPC into the electrospun fiber matrix. This can be explained as follows: R_1 (R_{CT1}) exhibits the charge transfer resistance in interface between fiber on ITOPET and the buffer solution. R_2 (R_{CT2}) represents the charge transfer resistance of the ions (drug) in fiber collected onto ITOPET. As the amount of HPC increases in the fiber matrix, more HPC accumulates at the interface in the 15th minute, and R_1 increases. However, the resistance of the fiber matrix on the ITOPET is reduced by the (dissolved) HPC separated from the fiber matrix. The reduction of R_s at 15th minutes by the increase in the amount of HPC in the fiber matrix confirms this explanation. Chi Squared values did not change and they are almost the same at the both applied electrical circuit models. Also, both electrical circuit models seems to be suitable and can be used for Donepezil HCl loaded electrospun fibers comprised 75 % HPC in PU matrix by giving similar results.

Table 4.20 : Parameters from $R(QRW)(QR)$ model best fitted with EIS data for Donepezil HCl loaded fibers (released at 15th min).

Equivalent Circuit Model Parameters	Electrospun Fibers Content			
	(0 % HPC)	(25 % HPC)	(50 % HPC)	(75 % HPC)
R_s [Ω]	366.1	251.4	241.0	224.5
CPE_1 (10^{-5}) [Y_0/Ss^{-n}]	$4.32E^{-4}$	45.90	9.28	1.49
n	0.99	0.81	0.90	0.94
R_{ct1} [Ω]	235.9	458.0	917.6	$1,754E^{14}$
W [Y_0/Ss^{-n}]	$3.77E^{-6}$	$4.50E^{-9}$	$1.55E^{-20}$	$5.65E^{-7}$
CPE_2 (10^{-5}) [Y_0/Ss^{-n}]	2.43	2.01	2.57	10.34
n	0.95	0.93	0.94	0.73
R_{ct2} [Ω]	$4.33E^8$	$7.77E^7$	$2.31E^7$	9.07
Chi Squared	$5.63 E^{-5}$	$1.41 E^{-4}$	$1.24 E^{-4}$	$1.42 E^{-4}$

Table 4.20 summarizes the data extracted from the models $R(QRW)(QR)$. Warburg Impedance, W value are $5,65E^{-7}$, $1,55E^{-20}$, $4,50E^{-9}$ and $3,77E^{-6}$ at $R(QRW)(QR)$ in Table 4.20 and $5.52E^{-7}$, $2.92E^{-20}$, $6.31E^{-13}$ and $3.57E^{-6}$ in $R(Q(RW))(QR)$ model for Donepezil HCl loaded electrospun fibers samples comprised of 75, 50, 25 % and

0 % HPC in PU matrix respectively. The tendency and value of the two electrical circuit models are almost the same.

Warburg Impedance (W) decreases due to increased HPC ratio. The release profile of PU/HPC electrospun fibers can be explained by a combination of some processes. Release profile of PU/HPC fibers occurs according to certain processes: Solution or water spreads into fiber, starts to swell out the Hydroxypropylcellulose. Hydroxypropylcellulose quickly forms a gel layer when contacts with solution or water. The release of drug is retarded the gel layer and diffusion or erosion help the releases of active materials. Diffusion of active materials at the interface decrease because it was seen that the resistance of charge transfer (R_{ct1}) increases (electrospun fibers comprised 0% HPC: 235.9Ω - 25%: 458.0Ω - 50%: 917.6Ω - 75%: $1.754E14\Omega$ for R(QRW)(QR) electrical circuit model) by the increased HPC ratio. In real systems, both regions may not be well defined depending on R_{ct} and its relation to Z. If R_{ct} is small in comparison to R_s and Z over nearly all system is so kinetically facile that mass transfer always plays a role. If system is kinetically slow, large R_{ct} and only limited frequency region where mass transfer significant (Chang and coworkers, 2006).

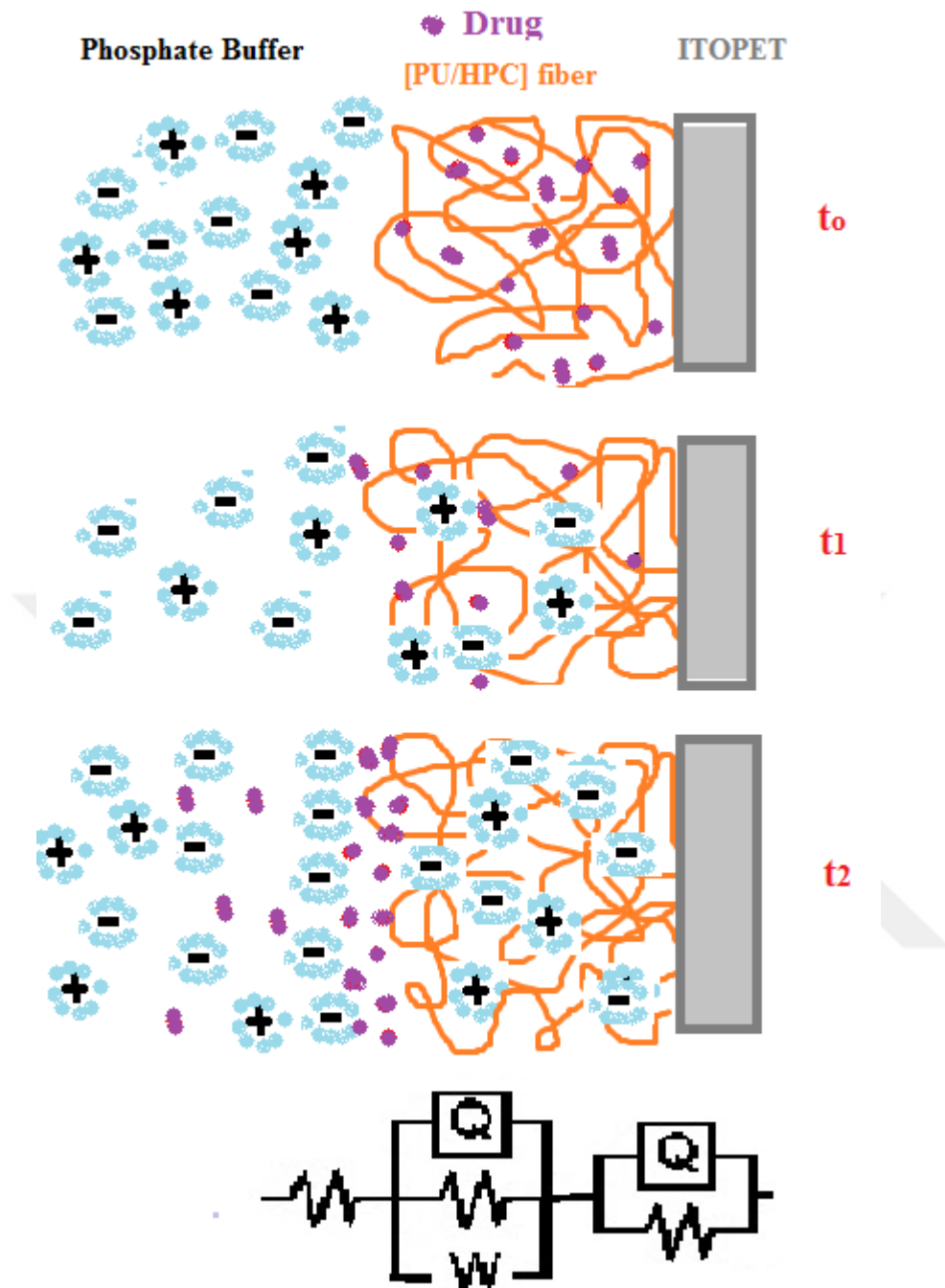


Figure 4.38 : Simulation of the drug release from drug loaded [PU or PU/HPC or PU/EC] fibers coated on ITOPET and equivalent electrical circuit model.

Figure 4.38 shows the simulation of drug release from drug loaded [PU or PU/HPC or PU/EC] electrospun fibers coated onto ITOPET (t_0 : the first time, the fiber is dipped in solution, t_1 : the time, solution begins to enter the space of the fiber, the drug release starts, t_2 : the time, solution penetrates into fiber, the drug release occurred) and equivalent electrical circuit model.

Table 4.21 : Z_{RE} , R_{CT1} and R_{CT2} results for electrospun fibers at 15th min for R(QRW)(QR) model.

Electrospun Fibers Content			(for 15 th minute)		
% PU	% HPC	Donepezil HCl (g)	Z_{RE} (kOhm) at 10 mHz	R_{CT1}	R_{CT2}
100	0	0.05	43.92	235.9	4.33E ⁸
75	25	0.05	73.77	458.0	7.77E ⁷
50	50	0.05	62.19	917.6	2.31E ⁷
25	75	0.05	124.00	1.75E ¹⁴	9.07

Table 4.21 shows the Z_{RE} (at 10 mHz) results from the electrochemical impedance data and R_{CT1} , R_{CT2} results obtained from the application of R(QRW)(QR) electrical circuit model to this data. Z_{RE} and R_{CT1} increases are coherent, but changes are conversely between Z_{RE} and R_{CT2} indicating inverse proportionality as Z_{RE} increases R_{CT2} decreases.

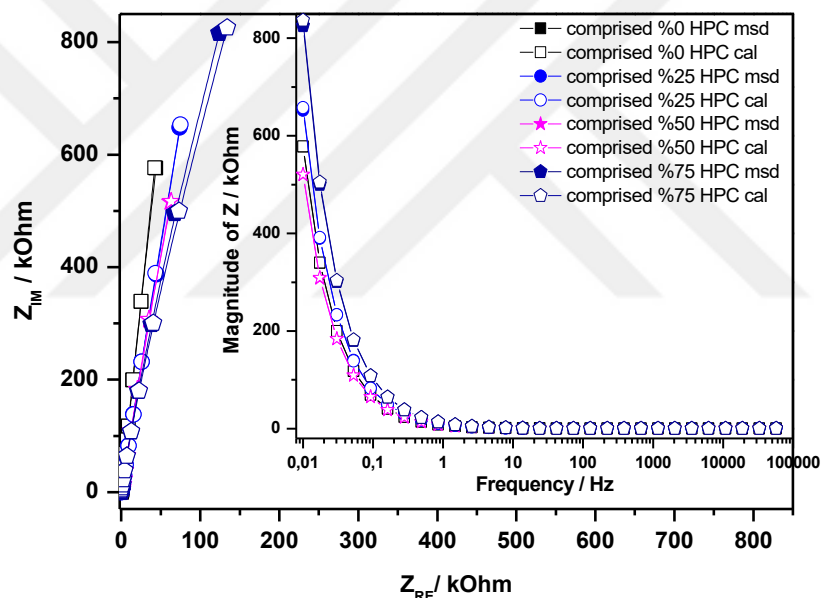


Figure 4.39 : Nyquist and Bode Magnitude (inset) plots for drug loaded PU/HPC fibers (at 15th min) from EIS and fitted with [R(QRW)(QR)] model.

Nyquist plot (Z_{RE} vs. Z_{IM}) and Bode Magnitude of measured data resulted from electrochemical analysis and calculated data from the best fitted with R(QRW)(QR) electrical circuit model (Figure 4.37) are shown in Figure 4.39.

Characterization of the electrochemical properties of ITOPET coated with drug-loaded electrospun fibers was performed by Electrochemical Impedance Spectroscopy. Electrochemical analysis was done by the aim of describing the electrochemical properties of ITOPET which was coated with drug-loaded fibers.

ITOPET coated with drug loaded electrospun fibers comprised of 0 % HPC is more capacitive than fibers comprised 25, 50 and 75 % HPC (Figure 4.39).

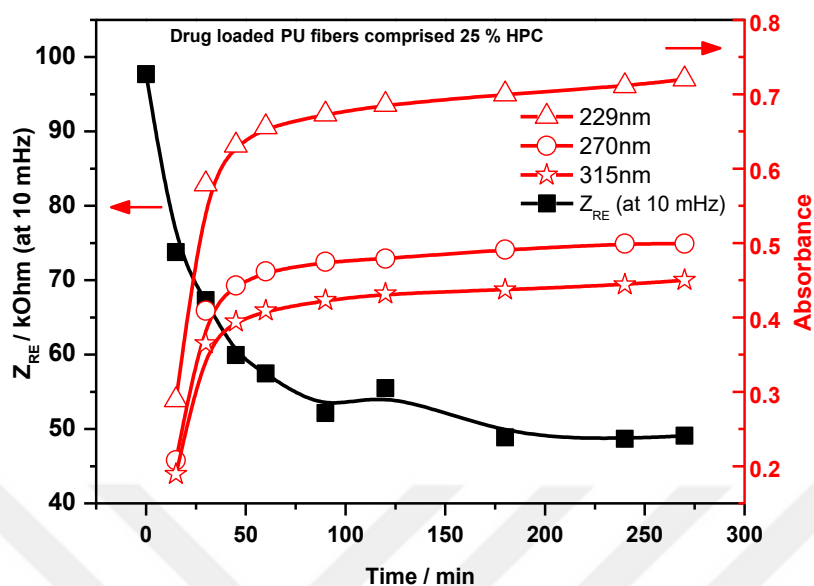


Figure 4.40 : The correlation between Z_{RE} (at 10 mHz) and absorbance with time.

The correlation between time (0 min-270 min) and Z_{RE} (from the EIS data at 10 mHz), time (0 min-270 min) and absorbance (from UV analysis) for ITOPET coated with drug loaded electrospun fibers comprised 25 % HPC is presented in Figure 4.40. It was found that the Z_{RE} decreases with the increasing of DP concentration in the buffer solution. As the time passes, the drug are released from fibers in buffer solution, while the absorbance values are increased and the Z_{RE} decreases. Because, increasing of quantity of drug in buffer solution results with increasing the solution conductivity and decreasing resistance.

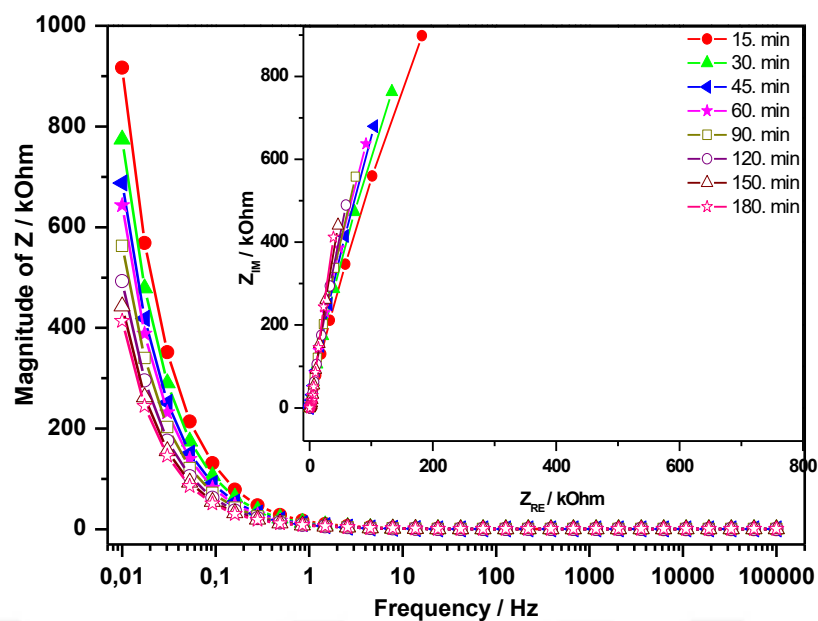


Figure 4.41 : Magnitude of Z and Nyquist plots of ITOPET electrode coated with drug loaded PU/EC/DP fibers (comprised 25 % EC).

Figure 4.41 shows Magnitude of Z and Nyquist plots (inset) of drug loaded PU/EC (comprised 25 % EC) based on ITOPET coated electrode. Resistivity of total decreases with the increasing of time, means that drug molecules release to the buffer solution.

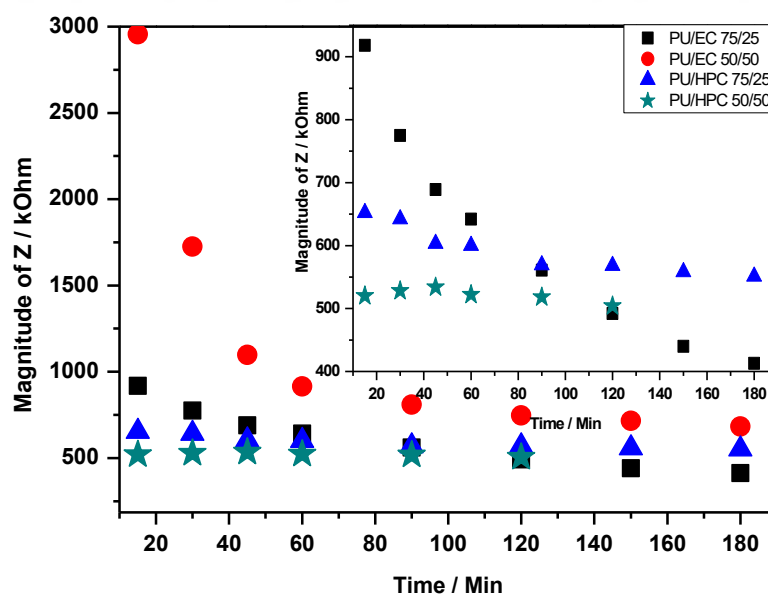


Figure 4.42 : Change of Magnitude of Z (at 10 mHz) versus time for electrospun fibers comprised 25 % and 50 % HPC or EC in fiber.

Figure 4.42 shows variation of Magnitude of Z over time for 25 % and 50 % HPC or EC in drug loaded PU electrospun fibers. Existence of EC in the PU electrospun fibers affect release of drug more than by the existence of HPC. Also drug release from electrospun fibers comprising 50 % EC in the PU matrix is providing more steady decrease trend and it provides an easier measurement for EIS than the other matrix. This result is compatible with the vitro release studies as given in section 4.5. Electrospun fibers containing about 50 % EC exhibit controlled release as given in the section 4.5. In addition to this, Magnitude of Z values of electrospun fibers containing about 50 % EC (Figure 4.42) decreases more controlled than the other fibers containing HPC. The electrochemical impedance measurements seems to be an alternative method compared to the UV-vis spectroscopy, especially for the PU/EC fibers (50 % EC) due to the characteristic properties of EC, non-swelling pH independent release retardancy and poor water permeability. It was viewed that drug release determination from electrospun fibers based ITOPET using hydrophobic and poor water permeability polymer combinations is found to be more suitable than using good water permeability polymers used in this study.

Table 4.22 : Parameters from the [R(QRW)(QR)] model best fitted with EIS data for Donepezil HCl loaded fibers (released at 15th min).

Equivalent Circuit Model Parameters	Electrospun Fibers Content		
	0 % EC 100 % PU	25 % EC 75 % PU	50 % EC 50 % PU
R_s [Ω]	366.1	351	316
CPE_1 (10^{-5}) [Y_0/Ss^{-n}]	$4.32E^{-4}$	1.22	0.74
n	0.99	0.92	0.92
R_{ct1} [Ω]	235.9	$3.21E^{14}$	$9.99E^{14}$
W [Y_0/Ss^{-n}]	$3.77E^{-6}$	$5.00E^{-7}$	$1.89E^{-6}$
CPE_2 (10^{-5}) [Y_0/Ss^{-n}]	2.43	0.01	0.69
n	0.95	0.19	0.99
R_{ct2} [Ω]	$4.33E^8$	$1.65E^{11}$	$1E^{20}$
Chi Squared	$5.63 E^{-5}$	$1.82E^{-4}$	$1.050E^{-3}$

R_s value at 15th minutes differs from 366 Ω to 316 Ω for Donepezil HCl loaded electrospun fibers comprised 0 %, 25 % and 50 % EC in PU matrix in Table 4.22, like tendency of PU/HPC matrix, but R_s is lower in the PU/HPC matrix. Because at the 15th minutes releasing of Donepezil HCl from PU/HPC is much faster than PU/EC matrix. R_{CT1} increases with the increasing of EC in the electrospun fibers. CPE_1 increases from $4.32E^{-4}$ to 0.74 by incorporation of EC into the electrospun fiber matrix and Warburg Impedance (W) does not display a stable trend by

increasing EC in the electrospun fibers. CPE_2 values are almost the same for Donepezil HCl loaded electrospun fibers comprised 0, 25, 50 % EC in PU matrix like drug loaded PU/HPC electrospun fibers. n values were closer to 1 in R(QRW)(QR) model except 25 % EC in the CPE_1 . R_{ct1} values increase from 235 to $9.99E14$ and also, R_{ct2} values increases from $4.33E8$ to $1E20$ by incorporation of EC into the electrospun fiber matrix. unlike PU/HPC matrix. This can be explained as follows: as mentioned in previous paragraphs R_{CT1} is charge transfer resistance at the interface between fiber on ITO/PET and the buffer solution. R_{CT2} is charge transfer resistance of fiber on ITO/PET. EC is chemically modified cellulose derivative. It represents plasticity, excellent hydrophobicity, and good solubility in organic solvents but hydrophobic polymer (Crowley, et al, 2004). Release of drug may be performed with the following steps: (i) the initial concentration of drug in fibers; (ii) the quality of being porous of fibers and tortuous of fibers; (iii) the fibers forming polymer system; (iv) the drug solubility. Release of drug from drug release system which has hydrophobic polymeric and porous properties takes place when the drug contacts with the medium of drug release, immediately afterwards drug molecules dissolve and diffuse from filled pores through medium. In accordance with Percolation Theory, if a drug release system is made up of water insoluble polymer and a water soluble drug, drug dissolves by way of capillaries, capillaries make up of connection of drug particle clusters and the pore meshwork (Holman, Leuenberger, 1988). As long as the drug release is in progress, the pore meshwork is increased by the connection drug particle clusters by the way which inner drug clusters can diffuse.

Michael M. Crowley and coworkers also explained that with more ethyl cellulose particles presence, they predict that less cluster of soluble drug are occurred. The obtained pore meshwork takes place fewer intensive and further tortuous, so the drug is released more slowly.

According to this information, with the presence of more EC in matrix, drug can be encapsulated within the matrix and contacting with the release medium of the drug can be delayed. At the 15th minutes of the drug releasing matrix can show resistance to release the drug and because of that R_{CT2} increases by the increasing EC content in the matrix at this minute unlike HPC. Shortly after about 30rd minutes R_{CT2} was reduced to $1.48E7$.

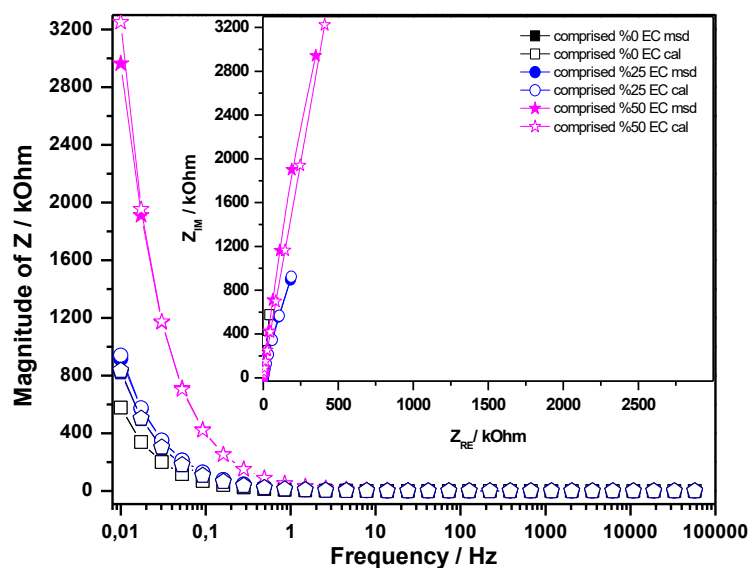


Figure 4.43 : Bode Magnitude (inset: Nyquist) plot of electrochemical analysis and fitting of [R(QRW)(QR)] model for drug loaded PU/EC fibers (at 15th min).

Nyquist plot (Z_{RE} vs. Z_{IM}) and Bode Magnitude of measured data resulted from electrochemical analysis and calculated data are shown in Figure 4.43, calculated data were best fitted with R(QRW)(QR) electrical circuit model. Magnitude of Z (at 10 mHz) values obtained from the electrochemical impedance data increases by the increasing EC content in the PU/EC/DP electrospun fiber. In addition to the electrical circuit models examined in detail above, also the experimental data is well fitted with the four different electrical circuit model such as RQRW(QR), R(Q(R(Q(RW))))), R(QR)(QR)W, R(QR(QRW)) shown in Table 23-Table 27. Electrical circuit model results for electrospun fibers comprised 100 % PU, 25 % HPC, 50 % HPC, 25 % EC, 50 % EC in PU matrixes. All drug loaded electrospun fiber formulations coated on ITOPET contain combination of polymers of different proportions. The polymer type and polymer ratios on the formulation change both the release of the drug and the properties of the fiber such as average fiber diameter, porosity, swelling, dispersion, adhesion, water retention, because there are many variable parameters obtained from ITOPET-coated electrode results. While evaluating the circuit model results, when the drug loaded fiber coated ITOPET electrode surface contact with the solvent, it is necessary to take into account the changes at the electrolyte, changes on the surface of fiber, changes between the surface of the fiber and electrolyte, changes between the surface of the fiber and ITOPET. Therefore, using a single and fixed electrical circuit model for each ITOPET electrode does not always present more accurate and more reliable results.

Table 4.23 : Parameters from the some models best fitted with EIS data 100 % PU electrospun fibers for (released at 15th min).

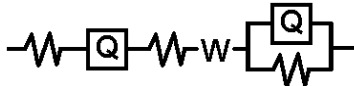
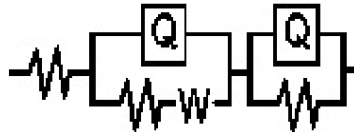
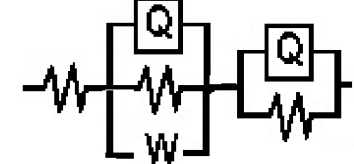
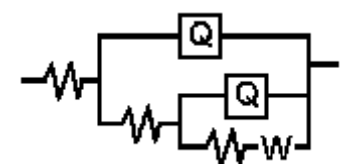
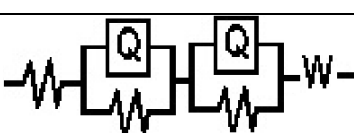
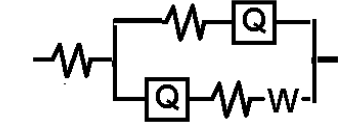
R_s [Ω]	CPE₁ [Y₀/Ss⁻ⁿ] (10⁻⁵)	R_{ct1} [Ω]	W [Y₀/Ss⁻ⁿ]	CPE₂ [Y₀/Ss⁻ⁿ] (10⁻⁵)	R_{ct2} [Ω]	Chi Squared	Equivalent Circuit Model
2.00E-6	2.43	289	1.50E-2	7.30E-2	309	5.68E-5	
254	2.42	57.48	3.57E-6	8.63E-2	346	5.92E-5	
366	4.32E-4	236	3.77E-6	2.43	4.33E8	5.63E-5	
372	4.09E-2	226	9.75E9	2.38	9.75E19	1.16E-4	
288	7.42E-2	310	1.50E-2	2.43	1.01E8	5.61E-5	
341	4.71E-2	2.25E18	3.88E-3	2.40	253	5.79E-5	

Table 4.24 : Parameters from the some models best fitted with EIS data 25 % HPC electrospun fibers for (released at 15th min).

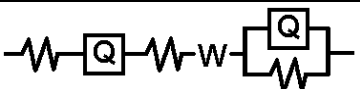
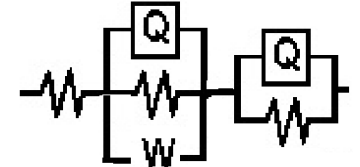
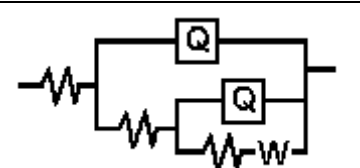
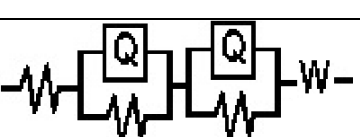
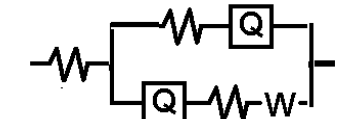
R_s [Ω]	CPE₁ [Y₀/Ss⁻ⁿ] (10⁻⁵)	R_{ct1} [Ω]	W [Y₀/Ss⁻ⁿ]	CPE₂ [Y₀/Ss⁻ⁿ] (10⁻⁵)	R_{ct2} [Ω]	Chi Squared	Equivalent Circuit Model
250	2.01	0.52	4.43E-3	33.4	252	9.80E-5	
252	2.01	3.00E14	6.31E-13	50.71	369	1.44E-4	
251	45.90	458	4.50E-9	2.01	7.77E7	1.41E-4	
252	1.93	1.31E5	5.66E-20	7.71E-2	6.92E15	1.40E-4	
250	30.17	270	4.45E-3	2.02	6.39E7	9.29E-5	
6.46E-3	4.71E-2	2.25E18	3.88E-3	2.40	253	5.79E-5	

Table 4.25 : Parameters from the some models best fitted with EIS data 50 % HPC electrospun fibers for (released at 15th min).

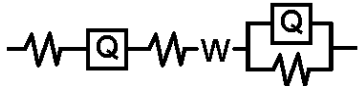
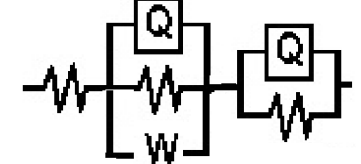
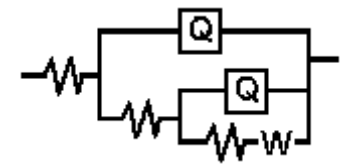
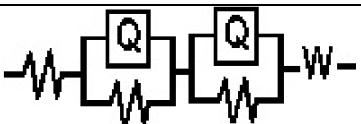
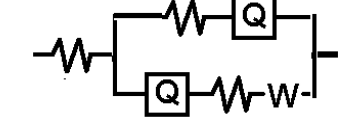
R_s [Ω]	CPE₁ [Y_0/Ss^{-n}] (10⁻⁵)	R_{ct1} [Ω]	W [Y_0/Ss^{-n}]	CPE₂ [Y_0/Ss^{-n}] (10⁻⁵)	R_{ct2} [Ω]	Chi Squared	Equivalent Circuit Model
1.02E-2	2.57	239	2.92E-3	7.76	636	9.16E-5	
241	2.56	26.86E-3	2.92E-20	9.40	815	1.47E-4	
241	9.28	918	1.55E-20	2.57	2.31E7	1.24E-4	
241	2.11	2.15E4	1.02E-3	4.42E-1	4.05E7	1.03E-4	
239	7.61	692	2.72E-3	2.58	2.44E7	6.96E-5	
241	2.11	4.06E7	6.68E10	4.44E-1	2.15E4	2.66E-4	

Table 4.26 : Parameters from the some models best fitted with EIS data 25 % EC electrospun fibers for (released at 15th min).

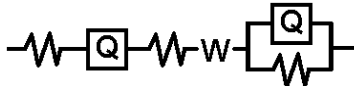
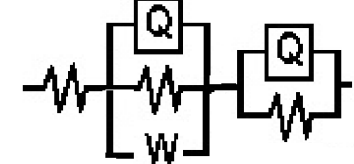
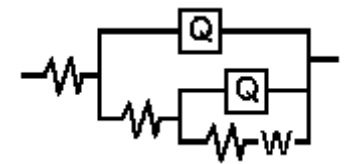
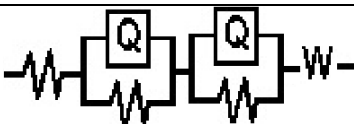
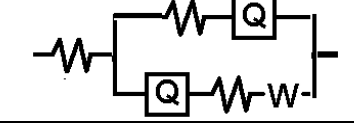
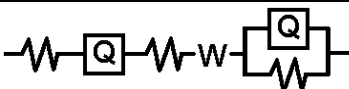
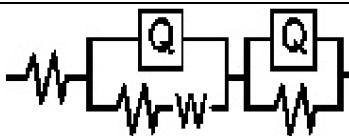
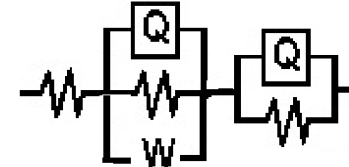
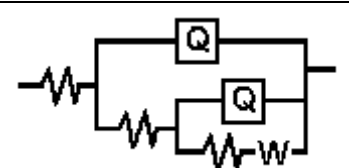
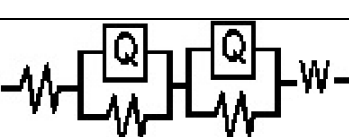
R_s [Ω]	CPE₁ [Y_0/Ss^{-n}] (10⁻⁵)	R_{ct1} [Ω]	W [Y_0/Ss^{-n}]	CPE₂ [Y_0/Ss^{-n}] (10⁻⁵)	R_{ct2} [Ω]	Chi Squared	Equivalent Circuit Model
360	1.49	3.50E-2	1.96E-3	7.81	1.16E5	1.67E-4	
358	1.25	2.70E6	4.38E-7	2.00E-4	16.71	1.73E-4	
351	1.22	3.21E14	5.00E-7	0.01	1.65E11	1.82E-4	
360	7.19E-1	87.28	4.28E-7	5.31E-1	3.07E6	1.73E-4	
360	1.49	3.54E17	1.96E-3	7.82	1.16E5	1.67E-4	
301	3.22E-2	2.78E13	1.18E-3	1.58	62.22	2.70E-4	

Table 4.27 : Parameters from the some models best fitted with EIS data 50 % EC electrospun fibers for (released at 15th min)

R_s [Ω]	CPE₁ [Y_0/Ss^{-n}] (10⁻⁵)	R_{ct1} [Ω]	W [Y_0/Ss^{-n}]	CPE₂ [Y_0/Ss^{-n}] (10⁻⁵)	R_{ct2} [Ω]	Chi Squared	Equivalent Circuit Model
15.74	5.18E-1	299	4.48E4	1.38	3.99E5	8.40E-4	
312	3.86E-1	2.02E6	1.30E-7	79.13	14.58	1.16E-3	
316	0.74	9.99E14	1.89E-6	0.69	1E20	1.05E-3	
317	3.87E-1	1.27E7	9.93E-20	5.54E-2	1.22E11	9.30E-4	
315	5.16E-1	1E20	1.60E11	1.38	3.99E5	8.40E-4	



5. CONCLUSIONS

Electrospinning is a simple and applicable method for loading drugs into polymer matrixes. Electrospinning process improves dissolution rates of drugs more effectively because large surface area formed, porosity can be adjusted and the fiber composition can be regulated in order to obtain fibers with desired properties. Parameters like scan rate, distance and applied voltage of electrospinning process have important effects on the morphological structure of electrospun fibers. The aim in this thesis is to produce a new transdermal drug delivery system using electrospinning method. Hydrophobic polymers such as Polyurethane (PU) and ethylcellulose (EC) and hydrophilic polymers such as hydroxypropylcellulose (HPC) and polyvinylpyrrolidone (PVP), which are used in pharmaceuticals to control the release rate of drugs, were used in various combination ratios. Donepezil HCl (DP) is a new class of acetylcholinesterase inhibitor and the second drug approved by FDA for treatment of mild to moderate Alzheimer's diseases. DP was chosen as a model drug for the drug loaded electrospun fibers. Since DP has side effects to the gastrointestinal system when it is administered orally, transdermal delivery could be an alternative route for Donepezil HCl.

Polyurethane electrospun fibers were fabricated successfully using electrospinning process parameters at 15 kV.cm⁻¹ applied voltage, 15 cm distance and 1 mL.h⁻¹ scan rate. In view of this respect, electrospinning process parameters were fixed to these known parameters to produce drug loaded electrospun fibers. DP loaded electrospun fiber systems were produced in various ratios of PU with its combinations such as 10/0/1, 10/2/1, 10/4/1, 10/8/1 (PU/HPC/DP, PU/EC/DP or PU/PVP/DP) by electrospinning using the fixed parameters.

ATR-FTIR spectroscopy was used to characterize the incorporation of DP to PU, PU/HPC, PU/EC and PU/PVP matrix. The intensity of the characteristic carbonyl stretching vibration bands of PU at 1727 cm⁻¹ decreases with the increasing ratio of HPC, EC or PVP in the fiber systems. Characteristic bands at 1057 cm⁻¹, 2945 cm⁻¹

and $1000\text{--}1300\text{ cm}^{-1}$ were changed with the increasing ratio of HPC, EC or PVP in the PU fiber systems. In addition, HPC has hydroxyl groups and these groups can present interaction region with functional groups of polyurethane. The possible interaction between HPC and PU is hydroxyl groups in HPC and --NCO groups in PU. So that, the broad peak located at 3411 cm^{-1} which is the stretching vibration of the hydroxyl group (OH) was formed with the addition of HPC and PVP to the PU matrix. Due to the interaction between hydroxyl group and carbonyl group, 1727 cm^{-1} was shifted to higher absorption band. But characteristic absorption bands of DP can not be seen in the spectra due to the small amount of DP inside of the PU/HPC, PU/EC and PU/PVP matrixes. ATR-FTIR analysis showed that physical blends of PU, HPC (or EC or PVP) and DP are suitable.

The homogeneity of DP loaded PU and PU/HPC, PU/EC and PU/PVP electrospun fibers were verified by SEM analysis and size distribution analysis with Image J, which is an image processing software. The information provided by SEM images show that all electrospun fibers except some ratio (10/8/1 and 10/4/1) of DP loaded PU/HPC fibers had uniform structures and smooth surfaces with no beads. These uniform structures clarify that HPC, EC and PVP incorporate to the PU matrix. 10/8/1 and 10/4/1 for PU/HPC/DP formulations have some beads and morphology is not in the uniform structure due to the high concentration of the HPC in the PU matrix. The average fiber diameter statistics showed that 10/2/1 ratio of PU/HPC/DP fibers has the highest average fiber diameter (995 nm) among all electrospun fibers. The sizes of PU/EC/DP and PU/PVP/DP electrospun fiber systems range between 400 nm to 600 nm and 300 nm to 350 nm, respectively. In addition to morphology analysis, porosity results of the PU/HPC/DP fiber systems showed that 10/0/1 and 10/2/1 fiber systems have high surface area to volume ratio.

The PU fiber system blended with appropriate amount of cellulose improved morphologic and releasing properties of the fiber system. In-vitro release profiles of DP loaded electrospun fibers were fitted to Zero Order, First Order, Hixson-Crowell, Higuchi Square Root and Korsmeyer-Peppas release kinetics models. The best regression value (r^2) was found at the ratio of 10/2/1 for PU/HPC/DP and at the ratio of 10/8/1 for PU/EC/DP, indicating Korsmeyer-Peppas drug release kinetic model which is often used to describe the drug release behaviour from polymeric systems.

In Korsmeyer-Peppas kinetic model, the rate of drug release is controlled by diffusion.

In vitro preliminary permeation analysis were done for optimized electrospun fibers in the ratio of 10/2/1 for PU/HPC/DP and in the ratio of 10/8/1 for PU/EC/DP. Based on in vitro preliminary permeation data, the optimized electrospun fibers could be feasible to deliver required amount of DP. The results show that $1000 \mu\text{g}/\text{cm}^2$ DP permeates through pig skin in 72 hours from the formulation of PU/HPC/DP (10/2/1) while $75 \mu\text{g}/\text{cm}^2$ DP permeates through pig skin in 72 hours from the formulation of PU/EC/DP (10/8/1). From these results, it can be concluded that PU/HPC/DP (10/2/1) well fitted as transdermal drug delivery system than PU/EC/DP (10/8/1). So, it can be said that the drug release from the matrix of PU and a hydrophobic polymer was less than the matrix of PU and a hydrophilic polymer.

Cytotoxic analysis done for optimized electrospun fibers in the ratio of 10/2/1 for PU/HPC/DP shows that PU/HPC/DP electrospun fibers are well-tolerated and are not irritant to the skin. Overall, all these findings suggest that the possible use of the 10/2/1 ratio of PU/HPC/DP electrospun fibers can be used as transdermal drug delivery system. However, it should be confirmed with further in vivo studies to conclude an utter judgement on the feasibility of optimized electrospun fibers as transdermal delivery carrier.

Moreover, in vitro study results confirm that DP loaded PU/PVP electrospun fibers exhibited immediate drug release. These electrospun fibers comprising PVP are not suitable drug carriers for release of a hydrophilic drug such as DP. Because PVP could accelerate the release of a hydrophilic drug. PVP can be considered as a matrix polymer for release of a hydrophobic drug. The results also suggest that the types and ratios of the polymer blends need to be judiciously adjusted so as to optimize the rate of drug release.

Electrochemical impedance spectroscopy (EIS) and equivalent electrical circuit model have been used to investigate the changes in buffer solution and changes of interfacial properties at electrospun fibers on the electrode surface after the drug release. The EIS results for both studies (one of them is in-vitro release studies by using SPE electrode and the other one is evaluation of drug release by using ITOPET substrate system) were analyzed in terms of electrical equivalent circuit models.

RQRW equivalent circuit model for the data of SPE electrode, $R(Q(RW))(QR)$ and $R(QRW)(QR)$ equivalent circuit models for the data of ITOPET substrate system were achieved with the good fitting between model and experimental data.

In the first electrochemical impedance spectroscopic studies, the correlation between time and Magnitude of Z , Z_{RE} values at 10 mHz (from Nyquist plots) and also the correlation between time and % dissolution of DP from PU/HPC/DP (10/2/1) and PU/EC/DP (10/8/1) electrospun fibers show that the electrode resistance and % dissolution of drug in buffer solutions were generated a decrease in impedance in progress of time since the electrical characteristics of the electrode/solution interface was changed.

In the second electrical impedance studies were achieved by coated ITOPET electrode with drug loaded fibers (such as PU, PU/HPC and PU/EC electrospun fibers (100 % PU, 75/25, 50/50, 25/75 % PU/HPC and 75/25, 50/50 % PU/EC respectively)). Z_{RE} values decreases with the increasing of DP concentration in the buffer solution. The EIS data is good fitted with the many electrical circuit model such as $R(QRW)(QR)$, $R(Q(RW)(QR))$, $RQRW(QR)$, $R(Q(R(Q(RW))))$, $R(QR)(QR)W$, $R(QR(QRW))$. All drug loaded electrospun fiber formulations coated on ITOPET contain combination of polymers of different proportions and have different properties. There are many variable parameters obtained from ITOPET-coated electrode results. While evaluating the circuit model results, when the drug loaded fiber coated ITOPET electrode surface contact with the solvent, it is necessary to take into account the changes at the electrolyte, changes on the surface of fiber, changes between the surface of the fiber and electrolyte, changes between the surface of the fiber and ITOPET. Therefore, using a single and fixed electrical circuit model for each ITOPET electrode does not present accurate and reliable results.

For future expectation, different polymers can be used instead of HPC, EC or PVP for preparation of different drug loaded PU combinations to release Donepezil HCl for longer time period. Also, PU/HPC or PU/EC fibers can be loaded with other hydrophilic drugs and PU/PVP fibers can be loaded hydrophobic drugs. It can be said that electrochemical impedance measurement can be an alternative method to detect the releasing of drug from the drug loaded materials.

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APPENDICES

APPENDIX A: DSC thermogram of Donepezil HCl powder.



APPENDIX A

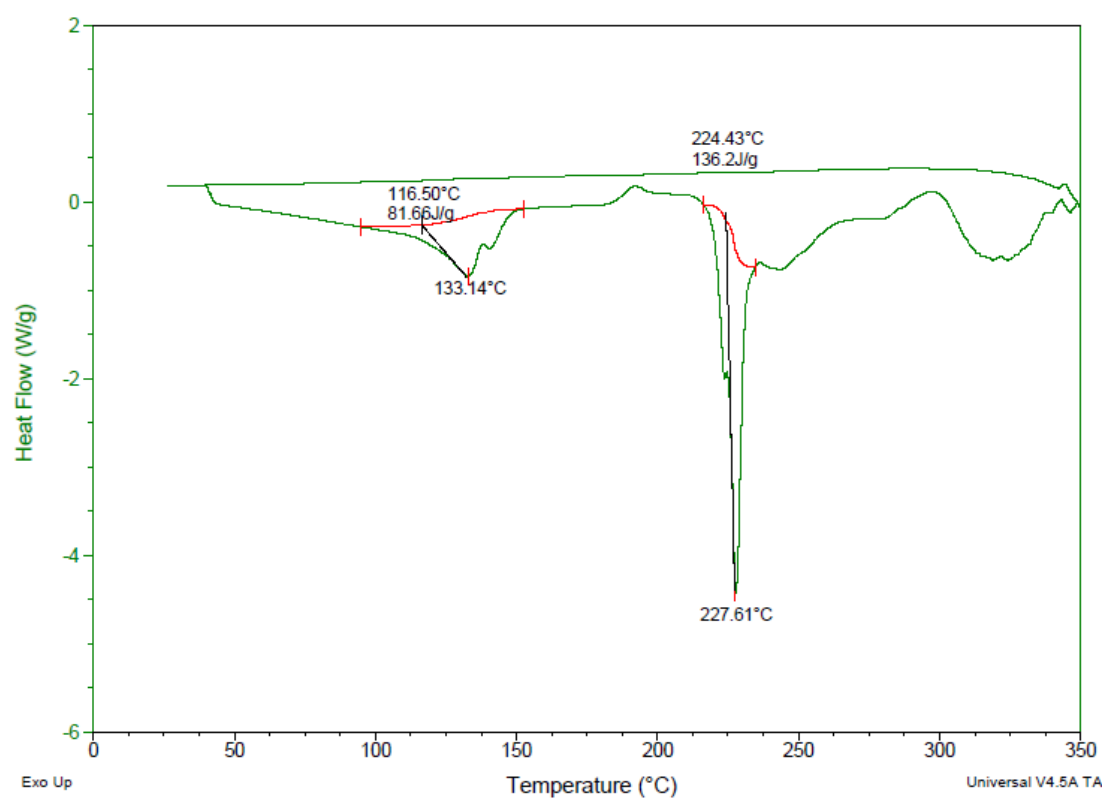
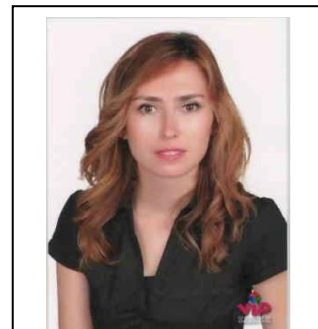


Figure A.1: DSC thermogram of Donepezil HCl powder.

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