ELECTROSPINNING OF POLY(ETHYLENE OXIDE)/ALGINATE NANOFIBERS CONTAINING ESTRADIOL/β-CYCLODEXTRIN COMPLEX

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การปั่นเส้นใยด้วยไฟฟ้าสถิตของเส้นใยนาโนพอลิเอทิลีนออกไซด์/แอลจิเนตที่มีสารเชิงซ้อนเอสทราไดออล/บีตาไซโคลเดกซ์ทริน

นางสาวกษมา ทังสุพานิช

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Thesis Title	ELECTROSPINNING OF POLY(ETHYLENE OXIDE)/ALGINATE NANOFIBERS CONTAINING ESTRADIOL/β-CYCLODEXTRIN COMPLEX
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Field of Study	Petrochemistry and Polymer Science
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กษมา ทังสุพานิช : การปั่นเส้นใยด้วยไฟฟ้าสถิตของเส้นใยนาโนพอลิเอทิลีนออกไซด์/แอลจิเนตที่มีสารเชิงซ้อนเอสทราไดออล/บีตาไซโคลเดกซ์ทริน (ELECTROSPINNING OF POLY (ETHYLENE OXIDE)/ALGINATE NANOFIBERS CONTAINING ESTRADIOL/β-CYCLODEXTRIN COMPLEX) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: รศ.ดร.นงนุช เหมืองสิน, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: ดร.กฤษณา ศิรเลิศมุกุล 70 หน้า.

การใช้แอลจิเนตเพื่อการนำส่งยา นิยมใช้ในรูปของอนุภาคขนาดไมโครและนาโนเมตร งานวิจัยนี้เสนอวิธีการเตรียมแอลจิเนตในรูปแบบเส้นใยขนาดนาโนเมตร สำหรับการนำส่งฮอร์โมนทางผิวหนัง ด้วยการใช้เอสทราไดออลเป็นยาต้นแบบ เส้นใยขนาดนาโนเมตรสามารถเตรียมได้จากการปั่นเส้นใยด้วยไฟฟ้าสถิตของสารละลายผสมระหว่างแอลจิเนต พอลิเอทิลีนออกไซด์ และบีตาไซโคลเดกซ์ทริน การใช้พอลิเอทิลีนออกไซด์จะช่วยลดแรงผลักประจุระหว่างโมเลกุลของแอลจิเนตลง และบีตาไซโคลเดกซ์ทรินช่วยเพิ่มความสามารถในการละลายน้ำของยาในสารละลายพอลิเมอร์ ปัจจัยที่มีผลต่อลักษณะของเส้นใยประกอบด้วย ความเข้มข้นของสารละลาย อัตราส่วนโดยมวลของพอลิเมอร์ ศักย์ไฟฟ้าที่ใช้ อัตราการไหลของพอลิเมอร์เมื่อออกจากเข็ม และระยะทางจากปลายเข็มถึงแผ่นรองรับ เส้นใยขนาดนาโนเมตรถูกวิเคราะห์ลักษณะสัณฐานด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด (SEM) รวมถึงวิเคราะห์ลักษณะทางกายและโครงสร้างผลึกด้วยเทคนิค FT-IR และ XRD ตามลำดับ เส้นใยที่เตรียมได้มีขนาดน้อยกว่า 200 นาโนเมตร โดยใช้อัตราส่วนโดยมวลของพอลิเอทิลีนออกไซด์ต่อแอลจิเนตต่อบีตาไซโคลเดกซ์ทรินเป็น 2:1:2 ใช้ศักย์ไฟฟ้า 20 กิโลโวลต์ ระยะทาง 18 เซนติเมตร และอัตราการไหลของพอลิเมอร์ 1.3 มิลลิลิตรต่อชั่วโมง สำหรับการประยุกต์ใช้เส้นใยที่ได้ในการนำส่งยา เอสทราไดออลจะถูกกักเก็บในโมเลกุลของบีตาไซโคลเดกซ์ทรินผ่านการเกิดสารเชิงซ้อนแบบอินคลูชันก่อนการปั่นเส้นใย และศึกษาการกักเก็บและการปลดปล่อยยาด้วยเทคนิค UV ผลการทดลองพบว่า เส้นใยที่มีสารเชิงซ้อนของเอสทราไดออลและบีตาไซโคลเดกซ์ทรินอัตราส่วน 1:1 (โดยโมล) มีประสิทธิภาพในการกักเก็บสูงถึง 93% และสามารถปลดปล่อยยาเอสทราไดออลในสารละลายฟอสเฟตบัฟเฟอร์ที่มีเอทานอล 25% โดยปริมาตร (พีเอส 7.4) ที่อุณหภูมิ 37 องศาเซลเซียส เป็นระยะเวลา 7 วัน และพบว่าการซึมผ่านสภาวะเลียนแบบผิวหนังของเอสทราไดออลโดยการใช้เมมเบรนเซลลูโลสแอซิเทตในระยะเวลา 7 วันด้วยฟลักซ์ 10.431 ไมโครกรัมต่อตารางเซนติเมตรต่อวัน

สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต.....

ปีการศึกษา 2553 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์.....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

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KEYWORDS : ESTRADIOL / β-CYCLODEXTRIN / PEO / ALGINATE / ELECTROSPINNING/ DRUG DELIVERY SYSTEM

GUSMAR THUNGSUPANICH: ELECTROSPINNING OF POLY (ETHYLENE OXIDE)/ALGINATE NANOFIBERS CONTAINING ESTRADIOL/β-CYCLODEXTRIN COMPLEX. ADVISOR: ASSOC. PROF. NONGNUJ MUANGSIN, Ph.D., CO-ADVISOR: KRISANA SIRALERTMUKUL, Ph.D., 70 pp.

Alginate is a polymer widely used in drug delivery field in the form of micro/nanoparticles. This work presents the new technique for preparation of alginate as nanofiber and its pharmaceutical application as a transdermal hormone drug delivery system using estradiol as a model drug. These can be archived by electrospinning the blend of alginate/poly (ethylene oxide) (PEO)/β-cyclodextrin (β-CD) by using PEO to decrease the repulsive force of alginate and using β-CD to increase the compatibility of hydrophobic drug with the hydrophilic polymer system. The electrospinning parameters for preparation the well-formed nanofibers including solution concentration, mass ratio of polymers, applied voltage, flow rate and working distance were investigated. The morphology and size of nanofiber were investigated by Scanning Electron Microscope (SEM) and the physical properties were investigated by Fourier transformed infrared spectroscopy (FT-IR) and X-rays Diffractometer The well-formed nanofibers PEO/Alg/β-CD with the diameter size less than 200 nm were successively prepared from 2:1:2 (w/w/w) of PEO/Alg/β-CD at 20 kV, 18 cm, 1.3 ml/hr. To investigate the possibility of PEO/Alg/β-CD nanofibers as transdermal estradiol delivery systems, the estradiol were formed an inclusion complex with β-CD before added to the polymer blend. The encapsulation efficiency and *in vitro* release behavior were investigated by UV spectophotometer. The high entrapment efficiency of 93% of estradiol was obtained from the nanofibers containing 1:1 mole ratio of estradiol/β-CD complex. The estradiol loaded nanofiber could be sustained released the drug within 7 days in 25% (v/v) ethanol/phosphate buffered saline (pH 7.4) at 37°C. The permeation study of estradiol through cellulose acetate membrane as a simulated skin (Franz's cell) provided controlled release for 7 days with flux of 10.431 μg/cm^2.day.

Field of Study: Petrochemistry and Polymer Science Student's Signature .....

Academic Year: 2010 Advisor's Signature .....

Co-advisor's Signature .....

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LIST OF ABBREVIATIONS

%	percentage
µg	microgram
µL	microliter
aq	aqueous
cm	centimeter
cm^{-1}	unit of wave number
conc.	concentration
Alg	alginate
β-CD	beta-cyclodextrin
^oC	degree Celsius (centrigrade)
D	day
EE	entrapment efficiency
EST	estradiol
F	flux
FT-IR	Fourier Transform Infrared Spectrophotometer
g	gram
h	hour
KBr	potassium bromide disk
kDa	kilodalton
kV	kilovolt
M	concentration in molar
mg	milligram
min	minute
mL	milliliter
mL/h	milliliter per hour
$\overline{M}_w$	molecular weight
$\overline{M}v$	viscosity average molecular weight
nm	nanometer
PEO	poly(ethylene oxide)

pH	power of hydrogen ion or the negative logarithm (base ten)
ppm	part per million
r^2	correlation coefficient
rpm	round per minute
S.D.	standard deviation
SEM	Scanning Electron Microscope
t	time
UV	ultraviolet
v/v	volume/volume
w/w	weight/weight
w/o	without
XRD	X-ray diffraction

CHAPTER I
INTRODUCTION

1.1 Introduction

Electrospinning has been widely used as an effective technique for generate micro/nano-scale synthetic and natural polymers fibers. The principle of electro-spinning is applying a high voltage to polymeric solution to forces the polymer to occur the jet and deposit on the collector. These unique characteristics are used in the biomedical area, membrane/filtration, drug delivery, wound dressing and tissue engineering scaffold, etc. Many natural macromolecule and synthetic polymer can be manufactured nanofibers by electrospinning such as polycaprolactam, poly polycaprolactam, (vinyl alcohol) (PVA), poly (ethylene oxide) (PEO), cellulose, chitin, chitosan and alginate.

Alginate is a natural polysaccide which obtained marine brown algae; consist of 1, 4-linked b-D-mannuronic (M) and a-L-guluronic acid (G) units arranged in a nonregular pattern [1]. It is widely used this natural polymers in the biomedical application such as drug delivery carrier, wound dressing and tissue engineering.

Poly (ethylene oxide) is a water soluble synthetic polymer with non-toxic, nonion, biocompatible and associating with natural polymers through hydrogen bonding. Hence, poly (ethylene oxide) has been widely used to improve fiber fabrication of natural polymer and avoid electrospun from toxic organic solvent for instance, β-cyclodextrin [2], chitosan [3], carboxymethyl cellulose [4] and alginate [1].

The mixture of alginate and poly (ethylene oxide) would be produced to nanofibers for controlled release application. Estradiol was used as a model drug. The drug is a steroid hormone and used as treatment menopause. However, it is poor solubility in water and unstable in the upper part of the gastrointestinal and undergoes inactive metabolites. This behavior made substantial first-pass hepatic metabolism which caused abdominal pain, nausea and vomiting [5].

Therefore, drug control release system plays an important role in the drug delivery which can reduce its side effects and improve efficiency of the drug. In the recently, drug delivary system based on polymers for example cyclodextrin hydrogels [6], poly-(lactide-co-glycolide-50/50)-microspheres [7] and chitosan particles [8] was used. The encapsulation of drug into cyclodextrin can reduce its side effect and a prolong residence time at the site of drug [9] including it can improve drug solubility in aqueous solution [10].

β-Cyclodextrin is cyclic oligosaccharide forms enzymatically from starch by *Bacillus macerans* which consist of 7 glucose units and forms a doughnut shape structure with hydrophobic cavity for incorporate hydrophobic molecules and hydrophilic outer surface which It can enhance the solubility of insoluble drug [11, 12]. It also nontoxic and mostly used a drug delivery carrier for complexation in pharmacuetical formulations [2, 13].

Inclusion complexation is one of the complexes which suitable small molecular called “guest molecule” incorporate into cavity of bigger molecule called “host” or called “host-guest inclusion complexation”. This complex can stabilize some properties of small molecules such as resistivity at atmosphere, control for evaporation and increasing solubility. Inclusion complexes can be prepared by grinding method, kneading method, freeze drying [14] or lyophilization, spray drying and co-precipitate method.

The preparation of estradiol/β-cyclodextrin complexes performed a co-precipitate method by the estradiol was dissolved in methanol and added in β-cyclodextrin solution. The aqueous solubility of estradiol/β-cyclodextrin complexes increased remarkably in comparison with the solubility of estradiol alone [11].

The purpose of the present work was to prepare a controlled release of poly (ethylene oxide)/alginate/β-cyclodextrin nanofibers containing estradiol by electro-spinning technique. Various process parameters that affect on the morphology and the size of the obtained nanofibers such as applied voltage, working distance, flow rate and solution concentration including drug ratio were optimized. The optimal conditions are appropriate for drug delivery; *in vitro* drug release behavior.While the physical properties were characted by scanning electron microscopy (SEM), Fourier Transform Infrared Spectroscopy (FT-IR), X-ray Diffractrometer (XRD).

1.2 Objectives

1.2.1. To prepare poly (ethylene oxide)/alginate nanofibers containing estradiol/β-cyclodextrin complex for estradiol delivery system by electrospinning technique.

1.2.2. To study the release behaviors of estradiol from the obtained nanofibers using Franz cell.

1.3 Scope and work

1.3.1 Literature review of related works.

1.3.2 Preparation of PEO/alginate/β-cyclodextrin nanofibers and estradiol-loaded PEO/alginate/β-cyclodextrin nanofibers by varying parameters including solution concentration, poly (ethylene oxide): alginate: β-cyclodextrin ratio, voltage, flow rate and working distance.

1.3.3 Characterization of obtained nanofibers in terms of morphology, size, chemical analysis, thermal behavior and crystallinity.

1.3.4 Evaluation of drug content and drug encapsulation efficiency as a function of preparation parameters; molar ratio of β-cyclodextrin to estradiol.

1.3.5 Study the *In vitro* release behavior of estradiol from poly (ethylene oxide)/alginate/β-cyclodextrin nanofibers at various molar ratios of β-cyclodextrin to estradiol in 25% EtOH/phosphate buffered saline.

1.3.6 Study the *In vitro* release behavior of estradiol under simulated skin permeation condition by using cellulose acetate membrane.

1.3.7 Report, Discussion and Writing up thesis.

CHAPTER II

BACKGROUND AND LITERATURE REVIEWS

2.1 Menopause and Treatment

Memopause is widely found in the most elder females (average age as 51 years). The menopause syndrome is characterised a various symptoms such as hot flushes, sleep disturbance and atrophic vaginitis [15]. These symptoms can be involved with imbalance of hormone level from the cessation of ovarian follicular. Estrogen replacement therapy (ERT) is often in decreasing menopause symptoms by counteract the decreased production of estrogens. This medication is various administrations for instance oral tables, implants or injection, topical [16], gel and transdermal delivery system [17].

2.2 Estradiol

17β-estradiol (also known as E2) is a sex hormone to use in the area of Estrogen replacement therapy (ERT) due to it is the most potent of the natural human estrogens [18]. Estradiol is derived from cholesterol. After side chain cleavage and utilizing the delta-5 pathway or the delta-4 pathway, androstenedione is the key intermediary. A fraction of the androstenedione is converted to testosterone, which in turn undergoes conversion to estradiol by an enzyme called aromatase. In an alternative pathway, androstenedione is aromatized to estrone, which is subsequently converted to estradiol [19].

2.2.1 Physicochemical properties

Estradiol is a white, crystalline solid, slightly. It is soluble in methanol, ethanol, dimethyl sulfoxide and slightly soluble in water. When heated to decompose, carbondioxide and carbon monoxide is by product.

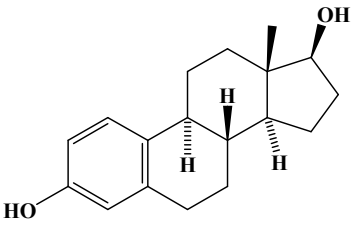


Figure 2.1 Chemical structure of estradiol

Chemical data [19]

Formula	:	$C_{18}H_{24}O_2$
Molecular weight	:	272.38
IUPAC name	:	(17β)-estra-1,3,5(10)-triene-3,17-diol
Synonyms	:	(8*R*,9*S*,13*S*,14*S*,17*S*)-13-methyl-6,7,8,9,11,12,14,15,16, 17-decahydrocyclopenta[*a*]phenanthrene-3,17-diol

Pharmacokinetic data [19]

Bioavailability	:	97-99% is bound
Metabolism	:	Liver
Half life	:	~13 h
Excretion	:	Urine
Route	:	Oral, transdermal

Adverse effect

Adverse effects of estradiol have been associated with estrogen and/or progestin therapy for example:

1. To Change in vaginal bleeding, dysmenorrhea, increase in size of uterine leiomyomata, vaginitis including vaginal candidiasis, changes in cervical secretion and cervical ectropion, ovarian cancer; endometrial hyperplasia; endometrial cancer, nipple discharge, galactorrhea; fibrocystic breast changes and breast cancer.

2. Cardiovascular effects include chest pain, deep and superficial venous thrombosis; pulmonary embolism; thrombophlebitis; myocardial infarction; stroke; increase in blood pressure.

3. Gastrointestinal effects include nausea and vomiting, abdominal cramps, bloating, diarrhea, dyspepsia, dysuria, gastritis, cholestatic jaundice, increased incidence of gallbladder disease, pancreatitis, enlargement of hepatic hemangiomas.

4. Skin adverse effects including chloasma or melasma that may continue despite discontinuation of the drug.

5. Other effects on the skin include erythema multiforme, erythema nodosum, otitis media, hemorrhagic eruption, loss of scalp hair, hirsutism, pruritus, rash.

Estrogen combined with medroxyprogesterone is associated with an increased risk of dementia. It is not known whether estradiol taken alone is associated with an increased risk of dementia. Estrogens should only be used for the shortest possible time and at the lowest effective dose due to these risks. Attempts to gradually reduce the medication via a dose taper should be made every 3 – 6 months.

2.6 Polymer in pharmaceutical field

Polymers are becoming increasingly important in the pharmaceutical industry as both drug encapsulants and vehicles of drug carriage in order to either protect an active agent during its passage through the body until its release, or control its release. Carrier technology obtained the drug delivery system by coupling the drug to the carrier polymers in various dosage forms such as beads, microspheres, nanoparticles, liposomes. Those formulations could delay the release of drug and also generate a response in a specific area or organ of the body requiring treatment. Moreover, a target drug, encapsulated in a polymer can be released sustainedly to improve drug therapeutic efficacy and decrease the dosing time and side effect [20].

Naturally occurring polymers are attractive as drug delivery system since they possess the biocompatibility, biodegradability and non-toxicity required for used in human [21].

2.6.1 Poly (ethylene oxide)

Poly (ethylene oxide) (PEO) is a water soluble synthetic polymer, obtained polymerization reaction of ethylene oxide monomer (Figure 2.2). It is non-toxicity, non-ionic, biocompatibility, biodegradability and can occurr hydrogen bonding with macromolecules. Thus, PEO were used to blend with other macromolecules which need to improve the fabrication and avoid toxic solvent such as chitsan, alginate, casien, cellulose and β-cyclodextrin.

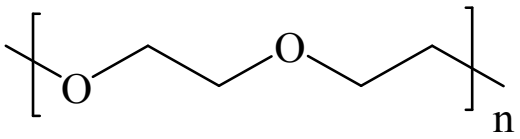


Figure 2.2 Structure of PEO

Application of poly (ethylene oxide)

Poly (ethylene oxide) (PEO) is a essential material in solid polymer electrolytes [22], biomaterial applications and pharmaceutical areas e.g. drug carriers, surface modifiers, and components of membranes, and hydrogels [23], and pH–sensitive sensors [24]. In addition, it was produced to nanofiber in electrospinning technique and was mixed with natural polymer for nanofibers fabrication.

2.6.2 Alginate

Alginate is a anionic polymer which obtained from marine brown algae which consist of 1,4 linked β-D-mannuromic (M) and α-L-guluronic (G) units [25]. It is non-toxicity, biodegradability, biocompatibility and hydrophilicity properties that make it widely used in medical applications.

Nanofibers of alginate can be produced by incorporating it with synthetic polymer such as PEO, PVA to reduce repulsive effect through formation hydrogen bond between polyanions and non-ionic polymer and allows successful electrospinning of the polymer blends [1, 25]. The advantage of using electrospinning technique in biomedical applications is controlled release of drugs which are slightly soluble in water or insoluble water can be

applied [26]. The soluble efficacy of drugs could be improved by incorporating the drug with drug carrier materials such as cyclodextrins

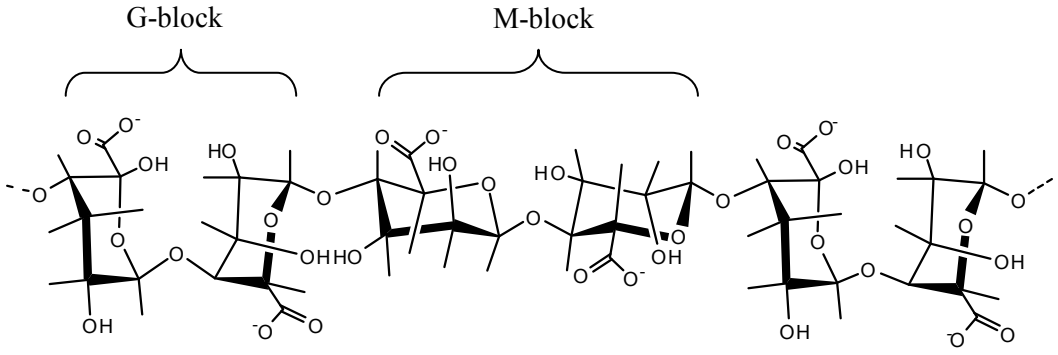


Figure 2.3 Copolymer of α-L-guluronic acid (G) and β-D-mannuromic acid (M) [27]

Application of alginate [27]

The uses of alginates are based on three main properties. The first is their ability to increase the viscosity of aqueous solutions. The second is their ability to form gels; gels form when a calcium salt is added to a solution of sodium alginate in water. The gel forms by chemical reaction, the calcium displaces the sodium from the alginate, holds the long alginate molecules together and a gel is the result. No heat is required and the gels do not melt when heated. The third property of alginates is the ability to form films of sodium or calcium alginate and fibres of calcium alginates. Upon these properties, alginates are widely used in various applications such as textile printing, food and pharmaceutical uses.

1. Textile printing

In textile printing, alginates are used as thickeners for the paste containing the dye. These pastes may be applied to the fabric by either screen or roller printing equipment. Alginates became important thickeners with the advent of reactive dyes. These combine chemically with cellulose in the fabric. Many of the usual thickeners, such as starch, react with the reactive dyes, and this leads to lower colour yields and sometimes by-products that are not easily washed out. Alginates do not react with the dyes, they easily wash out of the finished textile and are the best thickeners for reactive dyes. Textlie printing accounts for about 50 percent of the global alginate market.

2. Food

The thickening property of alginate is useful in food product such as sauces, syrups and toppings for ice cream. Addition of alginate can make icings non-sticky and allow the baked goods to be covered with plastic wrap. Alginate can be used as emulsifier in water-in-oil emulsions such as mayonnaise and salad dressings are less likely to separate into their original oil and water phases.

Alginates have some applications that are not related to either their viscosity or gel properties. They act as stabilizers in ice cream by reducing the formation of ice crystals during freezing, giving a smooth product. A variety of agents are used in the clarification of wine and removal of unwanted coloring, in some cases it has been found that the addition of sodium alginate can be effective.

The gelling properties of alginate were used in the production of artificial cherries since 1946. A flavored and colored solution of sodium alginate was allowed to drop into a solution of calcium salts. Moreover, calcium alginate films and coatings have been used to help to preserve frozen fish. If the fish is frozen in a calcium alginate jelly, the fish is protected from the air and rancidity from oxidation is very limited.

3. Pharmaceutical and medical uses

The fibers of calcium alginate are used in wound dressings. They have very good wound healing and haemostatic properties and can be absorbed by body fluids because the calcium in the fiber is exchanged for sodium from the body fluid to give a soluble sodium alginate. This also makes it easy to remove these dressings from the large open wounds or burns since they do not adhere to the wound. In addition, removal also can be assisted by rinsing the dressing with saline solution to ensure its conversion to soluble sodium alginate.

The good swelling properties of alginic acid power led to its use as a tablet disintegrant for some specialized applications. Alginic acid has also been used in some dietary foods; it swells in the stomach and gives a full feeling if sufficient amount is taken so the person is dissuaded from further eating.

Alginate is widely used in the controlled release of drugs and other chemicals. In some applications, the active ingredient is placed in a calcium alginate bead and slowly released when the bead is exposed in the appropriate environment.

2.6.3 β-cyclodextrin (β-CD) is cyclic oligosaccharides consisting of seven glucopyranose units and having a toroid- glucopyranose shaped molecular structure. The interior hydrophobic cavity make it able to form noncovalent host-guest inclusion complex with various drugs [2] and the exterior cavity is hydrophilic which cause β-CDs can improve the solubility of drugs [28]

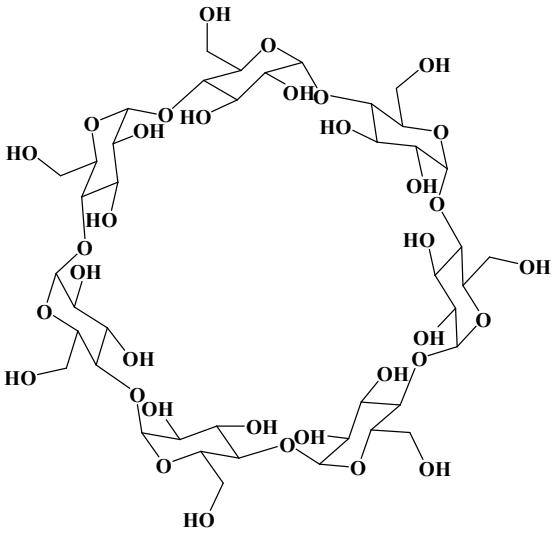


Figure 2.4 Structure of β-cyclodextrin.

Application of β-cyclodextrin

The cavities of CDs can fit various small molecules to form supramolecular inclusion complexes. Because of the unique capability of forming inclusion complexes in the inner cavities and many other favorable physicochemical and biological properties, natural CDs and their derivatives have been applied in drug delivery systems to enhance the solubilization, stabilization, and absorption. There have been a lot of developments of drug delivery systems based on CDs, which were used in nasal drug delivery, in peptide and protein delivery, in ophthalmic drug delivery, and in many other areas [9, 29].

Therefore, electrospinning technique in biomedical applications is controlled release of insoluble drugs can be applied by incorporating the drug with drug carrier materials such as cyclodextrins [30].

2.7 Electrospinning

Electrospinning is a process to produce ultra-fine fibers and nonwoven fabrics from applying high voltage between tip of needle and grounded collector. These unique characteristics are used in the biomedical area, Membrane/filtration [31], drug delivery [32], wound dressing and tissue engineering scaffold [33], etc. Many natural macro- sue macromolecule and synthetic polymer can be manufactured nanofibers by electrospinning such as polycaprolactam, poly polycaprolactam, (vinyl alcohol) (PVA), poly (ethylene oxide) (PEO), cellulose, chitin, chitosan and alginate [32-34]. In addition, the electrospinning process has some advantages such as this processing is simple, cost effective method and obtained nanofibers size is micro-nanometers, high surface area and porosity.

Venugopal et al [35] have been briefly reviewed about electrospinning technology that nanotechnology has the potential to revolutionize many sectors, including pharmaceuticals, information technology, medical devices, materials science, chemicals, and energy. Nanofibres provide a connection between the nanoscale world and the macroscale world, since their diameters are in the range of 1 to 100 nanometres and several metres in length.

The drug delivery application of electrospinning has been reviewed by Sill and von Recum that the electrospinning affords great flexibility in selecting materials for drug delivery applications. Either biodegradable or non-degradable materials can be used to control whether drug release occurs via diffusion alone or diffusion and scaffold degradation. Additionally, due to the flexibility in material selection a number of drugs can be delivered including: antibiotics, anticancer drugs, proteins, and DNA. Using the various electrospinning techniques a number of different drug loading methods can also be utilized: coatings, embedded drug, and encapsulated drug (coaxial and emulsion electrospinning). These techniques can be used to give finer control over drug release kinetics [36].

An electrospinning apparatus, include: (1) a syringe with a needle, (2) a high power voltage supply and (3) a collector (Fig. 2.7). The polymer solution in syringe is forced through the syringe pump to the needle by an advancement pump and is subjected to an electric field.

Initially, as a result of surface tension, pendant droplets of the solution are held in place. When a critical voltage is applied to the system, a conical protrusion known as a Taylor cone is formed and when the repulsive electrostatic force overcomes the surface tension, a charged jet of fluid is ejected from the tip of the Taylor cone. The jet is directed towards the collector, which has the opposite electrical charge. In the time it takes the jet to reach the collector, the solvent evaporates and dry polymer fibers are deposited [34, 37].

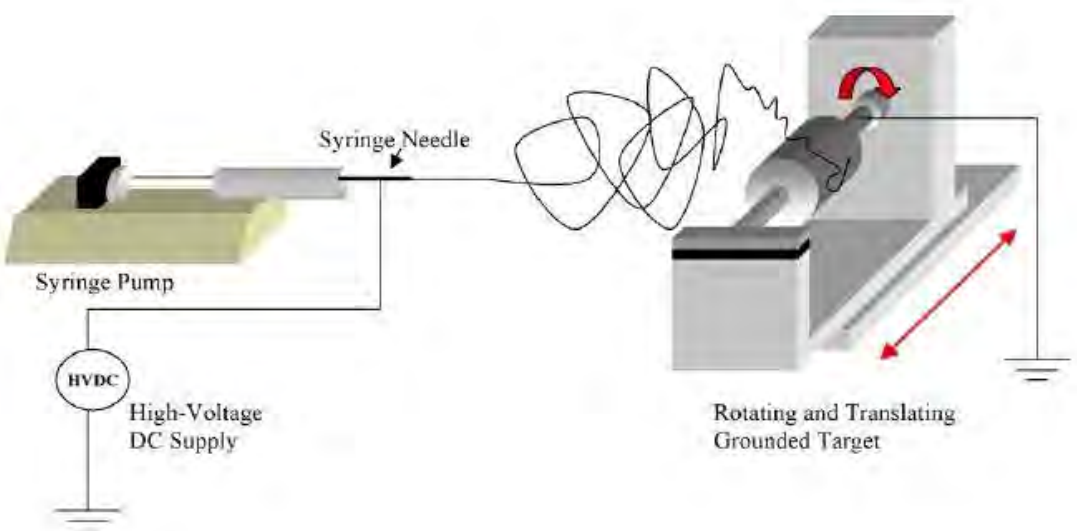


Figure 2.5 Schematic of a typical electrospinning apparatus. [34]

Controlled release system [38]

The means by which a drug is introduced into the body is almost as important as the drug itself. Drug concentration at the site of action must be maintained at a level that provides maximum therapeutic benefit and minimum toxicity. The pharmaceutical developer must also consider how to transport the drug to the appropriate part of the body and, once there, make it available for use [38].

Controlled drug delivery occurs when a polymer is combined with the drug or other active agents in such a way that the active agent is released from the material in a predesigned manner.

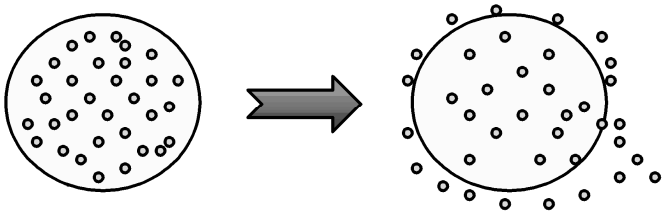


Figure 2.6 Presentation of controlled release system [38]

The drug can be released from the system by 3 mechanisms.

1) Diffusion Controlled Release

Diffusion occurs when drug molecules pass from the polymer matrix to the external environment. As the release continues, its rate normally decreases with this type of system, since drug has progressively longer distance to travel and therefore requires a longer diffusion time to release.

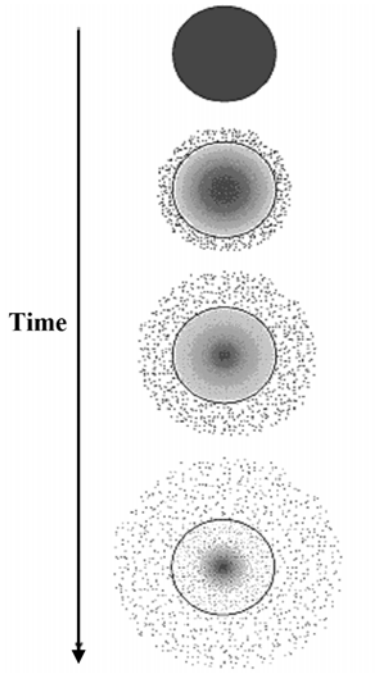


Figure 2.7 Presentation of diffusion controlled release [38]

2) Swelling Controlled Release

The swelling of the carrier increases the aqueous solvent content within the polymer matrix, enabling the drug to diffuse through the swollen network into the external environment. Most of materials used are based on hydrogel. The swelling can be triggered by a change in the environment surrounding such as pH, temperature, ionic strength, etc.

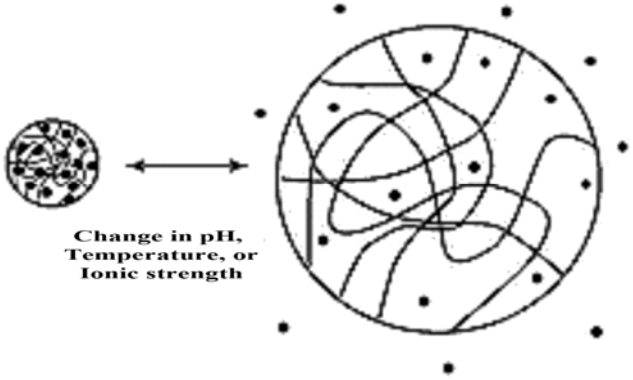


Figure 2.8 Presentation of swelling controlled release [38]

3) Erosion Controlled Release

The drug can be released from the matrix due to erosion of polymers, which can be classified into 2 types.

Bulk erosion: The polymer degrades in a fairly uniform manner throughout the polymer matrix.

Surface erosion: The degradation occurs only at the surface of the polymer device.

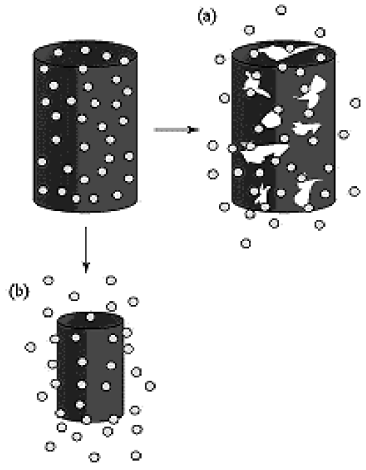


Figure 2.9 Presentation of erosion controlled release-(a) bulk erosion and (b) surface erosion [39]

2.9 Transdermal delivery systems [8]

The skin is an important barrier to controlled drug delivery. Approaches for delivering drugs throughout the skin as well as recent advances in iontophoresis, ultrasound, chemical enhancers, and chemical treatment of drugs for transdermal delivery are discussed [39].

Transdermal delivery system is the results of sophisticated procedures, where technology prevailed over a well-known pharmacological component, resulting in the development of the system in a short time. Such development progressed through three stages, or generations, aimed at improving delivery and absorption, reducing patch size and making it easier to use. Furthermore, the therapeutic benefits of transdermal drug delivery systems are an important issue in the development of any drug products.

The advantages transdermal drug delivery are:

- Adaptability to drugs with a short half-life.
- Avoidance of variation in gastrointestinal absorption.
- By pass of the hepatic first pass metabolism.
- Good patient compliance.
- Production of sustained and constant plasma concentrations of drugs
- Reduction in repeated dosing intervals.
- Reduction of potential adverse side effects.
- Removal of transdermal drug delivery systems provokes an immediate decrease of drug plasma levels.
- Substitute for oral or parenteral administration in certain clinical situation (pediatrics, geriatrics, nausea, etc.)
- Suitable for drugs which produce a therapeutic response at very low plasma concentrations.

2.10 Franz's cell apparatus [8]

Franz's cell (Figure 2.10) is used for *in vitro* study to quantify the release rate of drugs from topical preparation. In these systems, skin membranes or synthetic membranes may be used as barriers to the flow of drug and vehicle to simulate the biological system. The typical of Franz's cell has two chambers, one on each side of the test diffusion membrane. A temperature-controlled solution is placed in one chamber and a receptor solution in the others. Drug

permeation may be determined by periodic sampling and assay of the drug content in the receptor solution. Franz's cell is the most widely used apparatus to determine the drug release profile from the topical drug products because of the reliability and reproducibility. The test sample is placed in the donor phase, which was separated from the receptor phase by a semipermeable membrane. The suitable receptor medium is suggested to increase the drug solubility for detection of drug release by the ultraviolet spectroscopy or high-pressure liquid chromatography (HPLC).

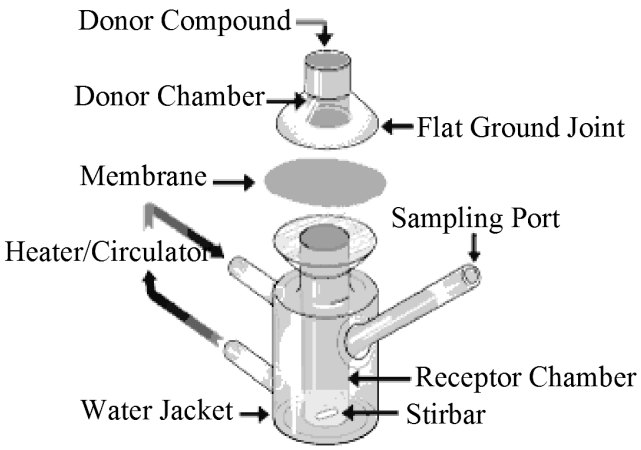


Figure 2.10 The components of Franz's cell [8]

2.11 Skin sources

The difference of skin sources give the different results in percutaneous absorption, these differences are due to the physiology of the skin. The types of skin sources in percutaneous absorption studies were human skin, animal skin and artificial skin and skin cultures.

CHAPTER III
MATERIALS AND METHODS

3.1 Materials

The following materials were obtained from commercial suppliers.

3.1.1 Model drugs

17β-Estradiol was purchased from Sigma.

3.1.2 Polymers and chemicals

- Poly (ethylene oxide), PEO, $\overline{Mv}$ of 900 kDa (Sigma-aldrich)
- Sodium alginate (Carlo Erba Reactifs SA)
- β-cyclodextrin (Sigma-aldrich)
- Calcium chloride (Carlo Erba Reactifs SA)
- Methanol, HPLC grade (Merck, Germany)
- Ethanol, analyze grade (Merck, Germany)
- Phosphate Buffered Saline, pH 7.4 (Sigma-aldrich)
- Cellulose dialysis membrane with $\overline{M}_w$ cut off at 3,500 Da (Spectrum Laboratories Inc.)
- Cellulose acetate membrane pore size 0.45 μm for Franz diffusion analysis

3.2 Instruments

The instruments used in this study are listed in Table 3.1

Table 3.1 Instruments

Instrument	Manufacture	Model
High voltage	Spellman	SL300
Syring pump	New Era pump systems	NE-1000
Analytical balance	Mettler	AT200
Ultracentrifuge	Refrigerated centrifuge	Sigma 30K
UV-spectrophotometer	SPECORD	S100
Fourier transform infrared spectrometer	Perkin Elmer	Spectrum One
Scanning electron microscope	Philips	XL30CP
X-ray diffractometer	JEOL	JDX-8030
pH-meter	Metrohm	744
Micropipette (100-1000 µl)	Mettler Toledo	Volumate
Magnetic stirrer	IKA®C-MAG	HS7
Freez dryer	Labconco	Freeze 6

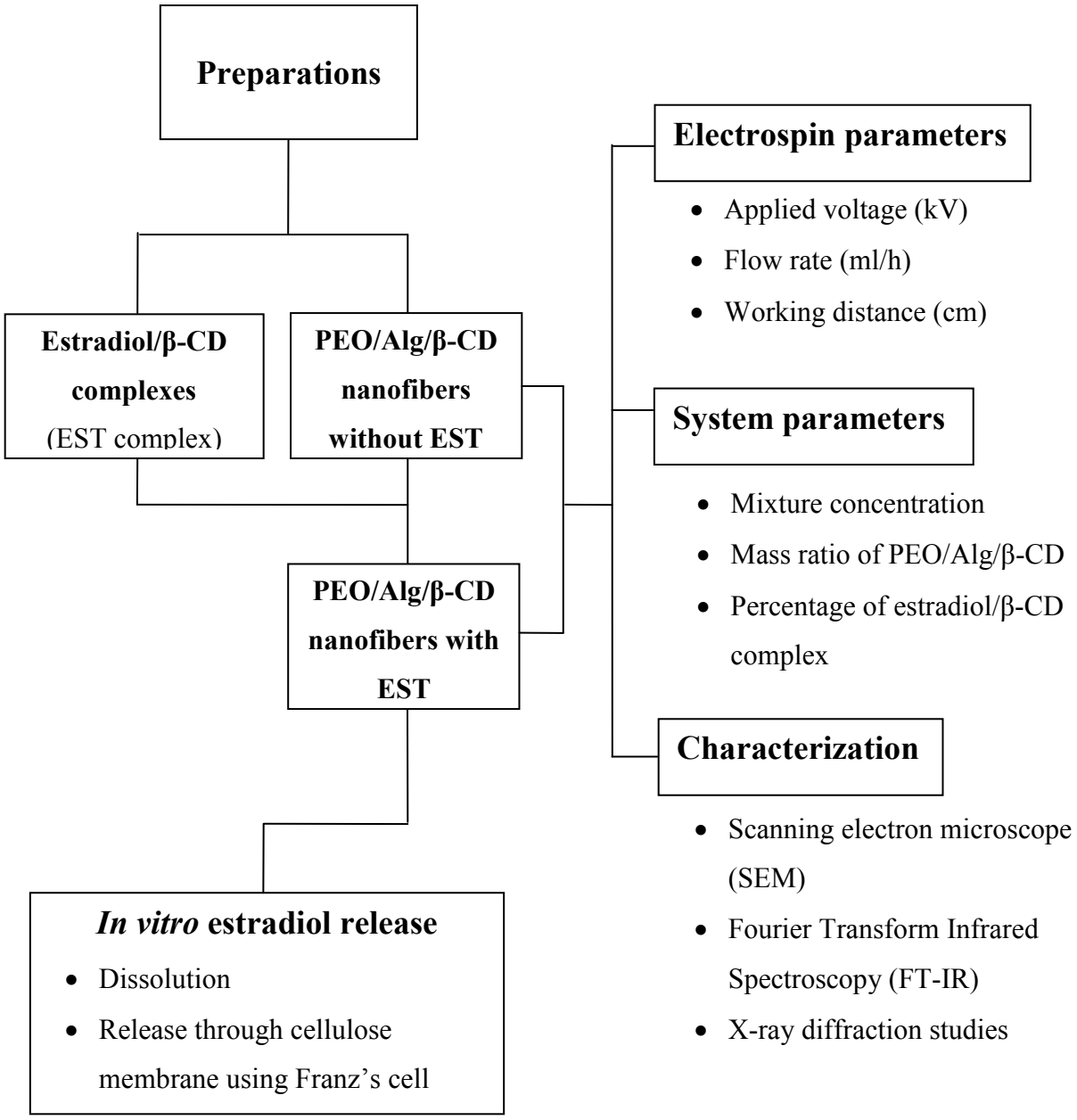


Figure 3.1 Flow chart of methodology

3.3 Preparation of estradiol/β-cyclodexrin complex

Each molar ratio of estradiol and β-cyclodextrin were prepared to be complex as 0.1:1, 0.3:1, 0.5:1, 0.7:1 and 1:1 of estradiol to β-cyclodextrin, respectively. The estradiol was dissolved in 5 ml methanol and was added in β-CD solution to produce 100 ml of the final mixture. The systems of estradiol/β-CD were shaken at 500 rpm for 24 h. The complexes suspension was centrifuged (3500 rpm) at 4°C. The free drug was determined in the supernatant. The solvent remained in the precipitate was removed by freeze-drying which samples were previously frozen at -20° C and then dried in vacuum condition.

3.4 Nanofibers Preparation

3.4.1 Poly (ethylene oxide)/alginate/β-cyclodextrin (PEO/Alg/β-CD) nanofibers

Poly (ethylene oxide)/alginate/β-cyclodextrin nanofibers were prepared by electrospinning technique. The PEO/Alg/β-CD solution is placed into a 5 mL syring with a capillary tip. A high electric field applied to the polymer solution in the syring. A syring pump was used to feed the polymer solution into the needle tip. Nanofiber falls into an aluminium foil as nonwoven. After that, the nanofibers were immersed into 30 ml of 0.33 mg/ml $CaCl_2$ solution overnight to stabilization and were eliminated sodium chloride salts by washing. The nanofibers were dried at 50 ºC overnight to remove the remaining solvent.

3.4.2 Poly (ethylene oxide)/alginate nanofibers containing estradiol/β-cyclodextrin complexes

Poly (ethylene oxide)/alginate solutions containing estradiol/β-cyclodextrin complex were prepared by adding a specified amount of estradiol/β-cyclodextrin complex into the poly (ethylene oxide)/alginate solution under magnetic stirring at 1000 rpm for 24 hour. The parameters studied for the nanofibers preparation by using an electrospining technique are shown in Table 3.2

Table 3.2 The parameters studied for the nanofibers preparation

Applied Voltage (kV)	16, 18, 20, 23
Flow rate (ml/h)	1.3, 1.5, 2
Working distance (cm)	16, 18, 20
Concentration of poly(ethylene oxide)/alginate/β-cyclodextrin solution	2, 3, 4, 4.5, 5
Mass ratio (w/w/w) of poly(ethylene oxide) : alginate : β-cyclodextrin based on the overall weight	1:1:3, 1:2:2, 1:3:1, 2:2:1, 2:1:2, 3:1:1
Percent (w/w) of estradiol/β-cyclodextrin complex based on the weight of β-CD part	0, 2, 4, 6, 8, 10

3.5 Characterization of nanofibers

3.5.1 Microscopic analysis

The morphology of the dried nanofibers was investigated with scanning electron microscope (SEM). Scanning was performed under high vacuum with electron voltage of 20 kV.

The mean fiber diameter of nanofibers was determined from the scanning electron micrographs which were measured by digital software.

3.5.2 Functional group analysis

The compositional and chemical characteristics of poly(ethylene oxide), alginate, β-cyclodextrin, estradiol, PEO/alg/β-cyclodextrin nanofiber and estradiol loaded nanofibers were examined by using the potassium bromide disk (KBr) method with a Fourier transform infrared spectrometry (FT-IR) in the range of 4000-400 cm^{-1}.

3.5.3 Power X-ray diffraction study

Estradiol distribution within β-cyclodextrin complexes and some cyclodextrin crystalline aggregates within the poly (ethylene oxide)/alginate matrix were investigated by X-rays diffractometry. The samples for X-ray diffraction studies were firmly packed into a cavity of a thin rectangular metal plate using two glass slides attached to the metal plate with adhesive tape. The first glass slide was then removed, and the prepared sample was taken to expose to the X-ray diffraction chamber. The X-ray diffraction patterns were recorded from 5º to 65º in terms of 2θ angle.

3.6 Evaluation of drug entrapment efficiency

3.6.1 Calibration curve of estradiol in methanol

Estradiol (10 mg) was accurately weighed into a 100 ml volumetric flask which was dissolved in methanol. The solution was adjusted to volume and used as stock solution. The stock solution was individually pipetted (0.5, 2.5, 5, 10 and 25 ml) into a 50 mL volumetric flask by micropipette and adjusted to volume with methanol. The final concentration of each solution was 1, 5, 10, 20 and 50 µg/mL accordingly.

The known of estradiol concentration was determined by UV/visible spectrophotometer in a 1 cm cell at a wavelength of 203 nm. The peak area and the calibration curves of estradiol in methanol are presented in Table 1A and Figure 1A (Appendix A), respectively.

3.6.2 Determination of drug encapsulation efficiency (EE)

Dry samples of various estradiol/β-cyclodextrin complexes, 10 mg, were dissolved in 50 ml methanol overnight and keep out evaporation of methanol. The free estradiol was dissolved in the solution. The amount of the free estradiol was determined by UV. All experiments were carried out in triplicate. The drug encapsulation efficiency was calculated from the following equation:

$$\%\ EE = \frac{\text{weight of the total estradiol - weight of free estradiol}}{\text{weight of the total estradiol}} \text{ x } 100$$

The drug entrapment efficiency was studies as a function of estradiol to β-cyclodextrin molar ratio (0.1:1, 0.3:1, 0.5:1, 0.7:1 and 1:1)

3.7 *In vitro* estradiol release from nanofibers

3.7.1 Preparation of buffur medium for drug release study

The phosphate buffered saline with pH 7.4 was used as medium for drug release study. The buffer solution was prepared by dissolved dry power phosphate buffered saline in 1 liters deionized water will yield 0.01M phosphate buffered saline.

3.7.2 Calibration curve of estradiol in 25% ethanol/pH 7.4 buffer solution

In order to make a standard curve, 10 mg of estradiol was accurately weighed into a 100 mL volumetric flask. The 25% (v/v) ethanol/phosphate buffered saline was added to dissolve the estradiol and adjusted to volume for used as stock solution. The stock solution was individually pipetted (1, 2, 3, 4 and 5 mL) into a 50 mL volumetric flask using micropipette and adjusted the volume with 25% (v/v) ethanol/phosphate buffered saline pH 7.4. The final concentrations of each solution were 2, 4, 6, 8, 10 μg/mL accordingly.

The known of estradiol concentration was determined by UV in a 1 cm cell at a wavelength of 203 nm. The peak area and the calibration curve of estradiol in 25% (v/v) ethanol/phosphate buffered saline (pH 7.4) are presented in Table 2A and Figure 2A, respectively, in appendix A.

3.7.3 *In vitro* drug release

The estradiol release from nanofibers was performed in 25% (v/v) ethanol/phosphate buffered saline pH 7.4 by dialysis bag diffusion technique [15].
The accuratedly weighted quantities of 5 mg nanofibers were suspended in 3 ml 25% (v/v) ethanol/phosphate buffered saline pH 7.4 which enclosed in a dialysis bag with molecular weight cut off of 3500 Da. The dialysis bag was immersed into 50 ml of 25% (v/v) ethanol/phosphate buffered saline pH 7.4 in a flask and the flask was placed in a shaken water bath at 100 rpm and incubated at 37±1 ºC. Samples of 3 ml were withdrawn at the time intervals of 1, 2, 3, 4, 5, 6, 7, 8, 12, 24, 30, 48, 72, 96, 120, 144, 168 h and an equal volume of fresh medium was compensated.

Each sampling solution was filtered through nylon filters (0.45 μm, Whatman, England) and the released estradiol amount was assayed by UV. The amount of estradiol was calculated by interpolation calibration curves containing increasing concentrations of estradiol. The percentages of cumulative estradiol release were calculated from the following equations:

$$\text{Amount of estradiol release (mg/mg of estradiol in nanofiber)} = \frac{\text{Concentration of estadiol (mg/mL)}}{5\ \text{mg}} \text{ x } 50\ \text{mL}$$

$$\%\ \text{Cumulative release} = \frac{\text{Amount of estradiol from releasing (mg/mg of estrdiol in nanofiber)}}{\text{Amount of estradiol before releasing (mg/mg of estrdio in nanofiberl)}} \text{ x } 100$$

The percentage of cumulative estradiol release is present in Table 1B-4B in Appendix B.

3.8 *In vitro* study of drug permeation through cellulose membrane

3.8.1 Pretreatment of cellulose membrane

The cellulose membranes were soaked in phosphate buffered saline pH 7.4 for 12 hours before used.

3.8.2 Drug permeation studies

The permeation of estradiol from nanofibers was determined using Franz diffusion cell [14]. The cellulose membrane with pore size of 0.45 μm was placed between the donor and receptor chambers. The upper donor chamber was filled with 5 mg of PEO/alg/estradio-β-CD nanofiers in 25% (v/v) ethano/ phosphate buffered saline pH 7.4. The diameter of the Franz cell was 1.5 cm corresponding to an effectively permeable area of 1.77 cm^2. The receptor chamber contained 12 mL of 25% (v/v) ethanol/ phosphate buffered saline pH 7.4 as the receptor fluid. The receptor phase was stirred at 500 rpm and maintanced the temperature at 37±1°C by circulating water through a jacket surrounding the cell body throughout the experiment. Two milliliters of receptor fluid was withdrawn at 0, 1, 2, 3, 4, 5, 6 and 7 day. while equal volume of fresh medium was compensated. The samples were filtered through 0.45 μm nylon filter and determined the released estradiol amount by HPLC with UV detection 203 nm.

The cumulative amount of drug permeated through a unit area of the membrane was calculated from the following equations:

$$Q_p = \frac{C_{LC} \times V_B}{A_M}$$

where Q_p is the cumulative amount of drug permeated through a unit area of cellulose membrane (μg/cm^2), C_{LC} is the concentration of estradiol permeated in receptor (g/mL), V_B is the volume of 25% (v/v) ethanol/phosphate buffered saline pH 7.4 in the receptor, A_M is the area of cellulose membrane.

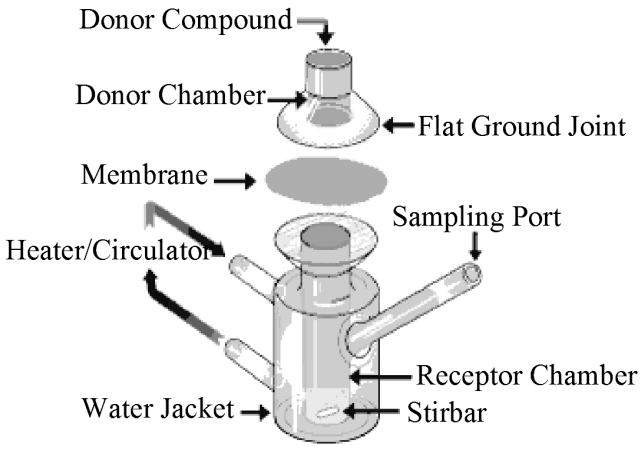


Figure 3.2 The component of Franz's cell and instrument set-up [8]

The cumulative amounts of drug permeated through the cellulose membrane are presented in Table 1E-4E, in Appendix E and Table 1F-4F, in Appendix F, respectively.

The flux of estradiol from PEO/alg/estradio-β-CD nanofiers in the receptor compartment obtained from plotting the slope of the linear correlation between cumulative amount of drug (Q_p) and time are presented in Figure 1C-4C, in Appendix C.

3.9 Statistical analysis

All measurements were performed in triplicate in each experiment. Results are presented as means ± SD. Statistical analysis was performed by one-way ANOVA using Microsoft Excel (Microsoft Corporation) with $P < 0.05$ considered to indicate statistical significance.

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Synthesis and optimization of fabrication parameter

The PEO/Alg/β-CD nanofibers were performed by electrospin technique. The morphology and size of the nanofibers were affected on various electrospinning system parameters and process parameters for example;

I. Effect of mixture solution concentration
II. Effect of mass ratio of PEO/alg/β-CD
III. Effect of applied voltage
IV. Effect of flow rate
V. Effect of working distance
VI. Effect of percentage of estradiol/β-CD complex

Each parameter was studied to obtain the optimization condition for nanofibers fabrication (while keeping other variables constant) and appropriate for drug delivery applications.

4.1.1 Effect of solution concentration

The effect of solution concentration was studied by varying a solution concentration from 2 to 5 % while all other conditions were kept at 20kV, 1.3 ml/hr, 18 cm, 26 g and 2:1:2 ratio of PEO/alg/β-CD. SEM analysis (Figure 4.1(a)-(d)) revealed that at low concentration (2%-4%) of the mixture solution, the beads were observed and the shape of beads changed from spherical to spindle-like when increasing concentration from 2% to 4%. The fibers morphology was archieved at 5% concentration with the diameter size of 120-190 nm (Table 4.1).

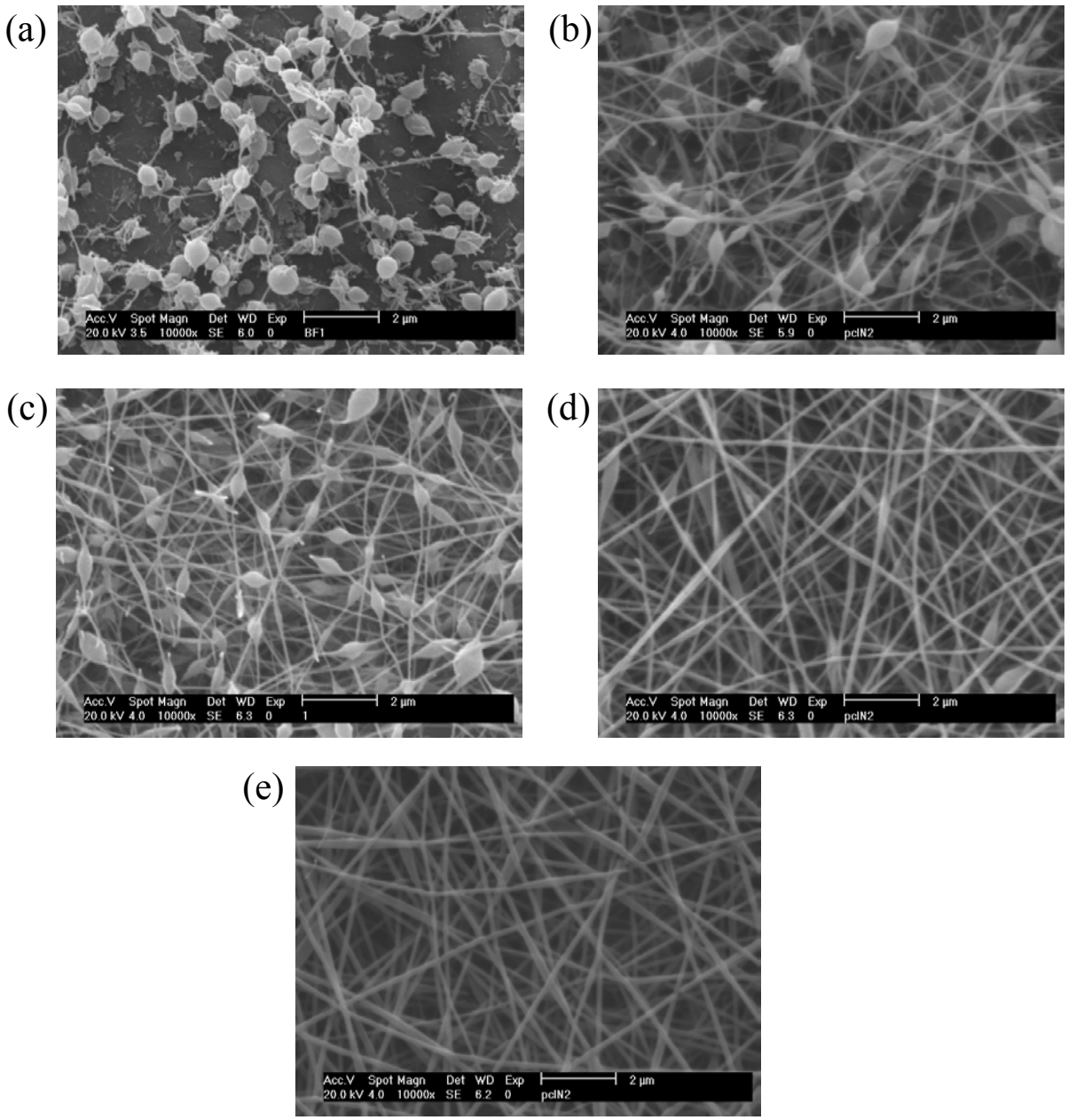


Figure 4.1 Effect of solution concentration on morphology by SEM (a) 2% (b) 3% (c) 4% (d) 4.5% and (e) 5% of the overall solution, based on the total weight of PEO/Alg/β-CD.

The results can be explained that the formation of nanofibers required an optimal solution concentration. The polymer solution must have a concentration high enough to cause polymer entanglements yet not so high that the viscosity prevents polymer motion induced by the electric field [19]. The solution concentration relates both viscosity and surface tension of the solution which matter in the nanofiber formation. At low concentration, the mixture of beads and fibers were observed as the influence of surface tension, the polymer jet split into droplet before reaching the collector. Whereas, increasing concentration affect the decreaced

surface tension which the sperical beads were changed to spindle-like and finally fibers. In addition, it has been found that the obtained nanofiber have been increased diameter when concentration was raised (Table 4.1). Due to at the high concentration has a high viscosity which exhibit longer stress relaxation time to prevent the fractured jets during electrospining [31].

Table 4.1 Effect of solution concentration on nanofibers size

Concentration (%)	Fiber morphology	Diameter size (nm)
2	Beads	-
3	Beaded nanofiber	62-105
4	Beaded nanofiber	98-120
4.5	Spindle beaded nanofiber	110-150
5	Nanofiber	120-190

4.1.2 Effect of mass ratio of PEO/alg/β-CD

To study the effect of PEO/alg/β-CD ratio on the nanofiber morphology, the other parameters i.e. applied voltage, flow rate, working distance, needle gauge and solution concentration were kept at 20 kV, 1.3 ml/hr, 18 cm, 26g and 5% concentration, respectively. The effect of PEO:alg:β-CD ratio on the morphology of nanofiber were evaluated at 1:1:3, 1:3:1, 3:1:1, 2:2:1, 1:2:2 and 2:1:2.

As shown in Fig 4.2 (a), the 1:1:3 of PEO/alg/β-CD ratio showd beads morphology. This results can be attributed to the viscosity of solution is too low to form the nanofiber due to the most amount in this component is β-cyclodextrin which is oligomer and low molecular weight. The beads-free nanofiber would be formed by using the molecular weight is not too low and too high because the low molecular weight led to form beads, while the high molecular weight probably gives fiber. Thus, the beads and droplet decreased when the solution has higher viscosity as higher molecular weight. When the high molecular weight segment, alginate, is increased in the soltuion instead β-cyclodextrin at 1:2:2 and 1:3:1 of PEO/alg/β-CD ratio and fixed the ratio of PEO at 1. It is found that the nanofibers have rarely been occurred due to the strong inter/intramolecular forces of alginate is a hindering the

electrospun formation (Fig 4.2(b) and (c)). Poly (ethylene oxide) would be increased to replace alginate in the solution because it decreased these inter/intramolecular forces which is an inhibitor formation of nanofiber through secondary bonding with alginate molecules. Therefore, the bead formation was completely eliminated and the bead-free sodium alginate/PEO/β-CD nanofibers were obtained at 3:1:1(Fig 4.2(d)) with the diameter size of 185-334 nm (Table 4.2).

To reduce the amount of poly (ethylene oxide), the others segment were added the suitable proportion to form nanofiber. The others of SEM image (Fig 4.2 (e) and (f)) demonstrated the various amount alginate and β-cyclodextrin on the nanofiber formation. When the PEO:alg:β-CD ratio was adjusted from 3:1:1 to 2:2:1, the spindle-like nanofibers were slightly observed due to the alginate segment raised replusive forces of the polymer solution which effect the polymer jet will break up into droplets (Fig 4.2 (e)) and affected on the decreasing diameter size of 105-143 nm. While the 1:2:2 ratios (Fig 4.2 (b)), the beads were almostly occurred since the co-spinning agent, poly (ethylene oxide), was too decrease and the alginate was too much, the electrospun cannot be produced. However, the PEO:Alg:β-CD ratio was 2:1:2, the fiber can be electrospun with the diameter size of 120-190 nm.

The results can be attributed to the 2:1:2 mass ratio of PEO/Alg/β-CD was suffciently the amount of poly (ethylene oxide) to reduce replusive forces of alginate and β-cyclodextrin also help decrease the number of beads. In addition, the PEO component has significantly influenced on viscosity of the solution which affected on nanofiber sizes. Increasing the amount of PEO showed the increasing viscosity. The high viscosity can swell which provided increased diameter. Therefore, the nanofiber sizes have been increased diameter when the amount of PEO was raised (Table 4.2).

The amount of β-cyclodextrin concerned with the entrapment of small molecule i.e. estradiol. The increased entrapment were obtained the increasing of β-cyclodextrin. Thus, the 2:1:2 of PEO:Alg:β-CD ratio was efficiently used to prepare nanofiber for drug delivery application.

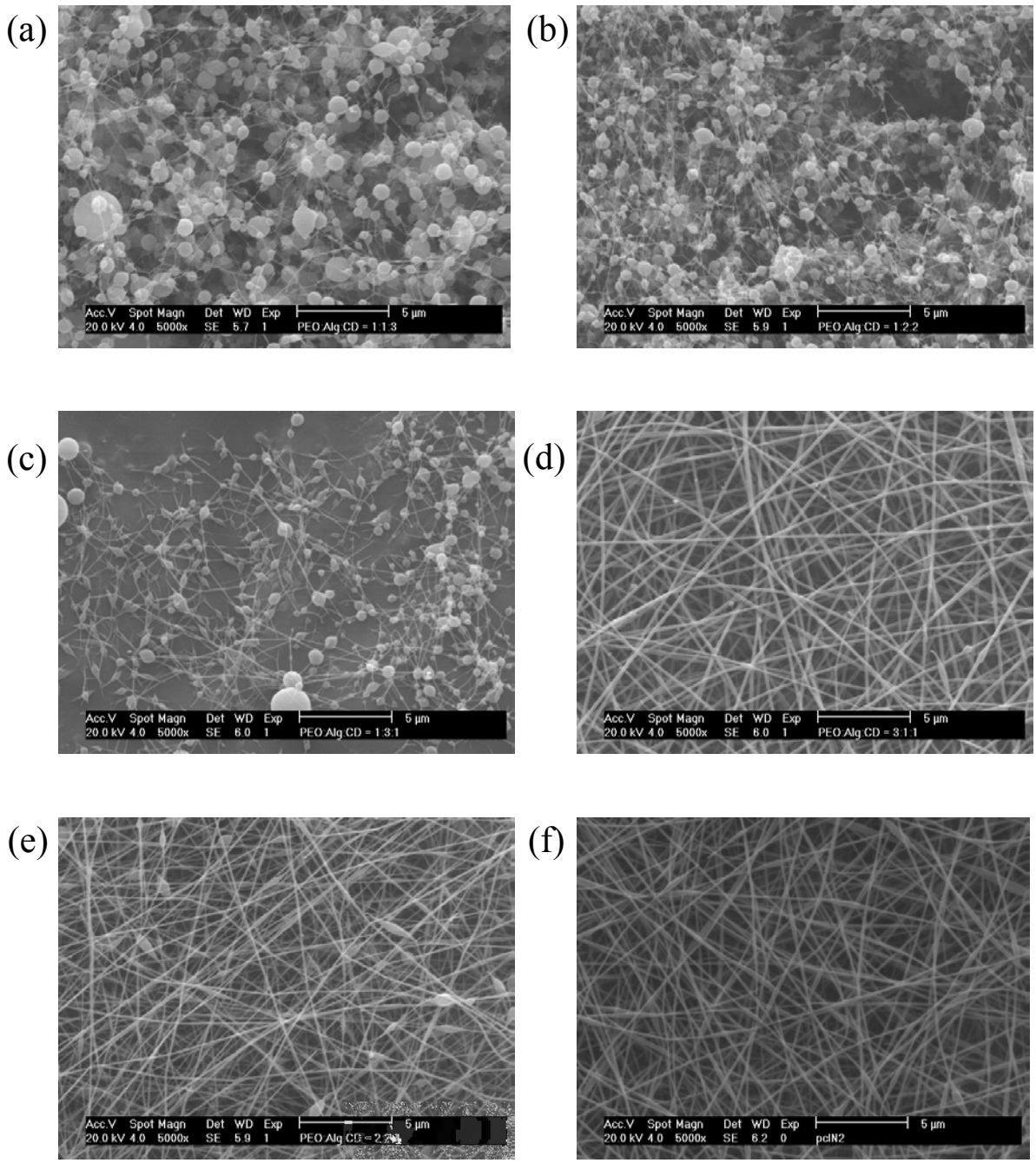


Figure 4.2 Effect of PEO/alg/β-CD ratio on morphology (a) 1:1:3 (b) 1:2:2 (c) 1:3:1 (d) 3:1:1 (e) 2:2:1 and (d) 2:1:2

Table 4.2 Effect of PEO/alg/β-CD on nanofibers size

PEO:alg:β-CD ratio	Fiber morphology	Diameter size (nm)
1:1:3	Beads	-
1:2:2	Beads	-
1:3:1	Beaded nanofiber	82-131
3:1:1	Nanofiber	185-334
2:2:1	Spindle beaded nanofiber	105-143
2:1:2	Nanofiber	120-190

4.1.3 Effect of applied voltage

The influence of applied voltge on morphology of the nanofibers was studied. The applied voltage was evaluated at 16 kV, 18 kV, 20 kV and 23 kV, while the flow rate, needle guage, working distance, solution concentration and PEO/alg/β-CD ratio were kept at 1.3 ml/hr, 26g, 18 cm, 5% concentration and 2:1:2 of the PEO/alg/β-CD ratio.

The effect of applied voltage is an important process parameter by the increasing applied voltage change the shape of the obtained Taylor cone and fiber jet. At lower applied voltage, the Taylor cone was formed at the tip of pendent drop and the volume of the pendent drop was reduced when applied voltage was increased until the tip of needle occur Taylor cone [36]. The voltage is sufficiently high for a stable jet will form near the tip of the Taylor cone, if the applied voltage is not high enough the jet will break up into droplets, a phenomenon called Rayleigh instability [40].

The applied voltage was increased from 16 to 20 kV, the diameter size of nanofiber decreased from 185-386 to 120-190 nm (Table 4.3). But the applied voltage higher than 20 kV, the high voltage affects to increase of charges on the surface of solution. The diameter sizes were increased to 185-234 nm.

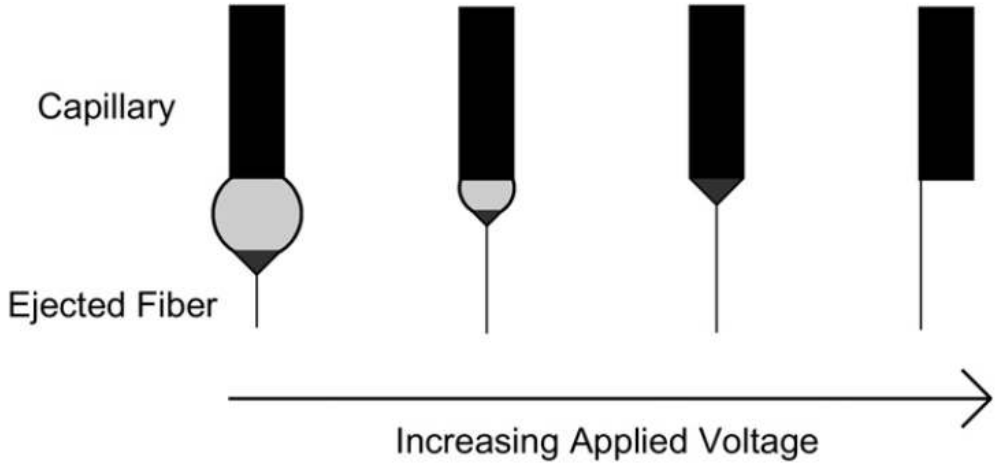
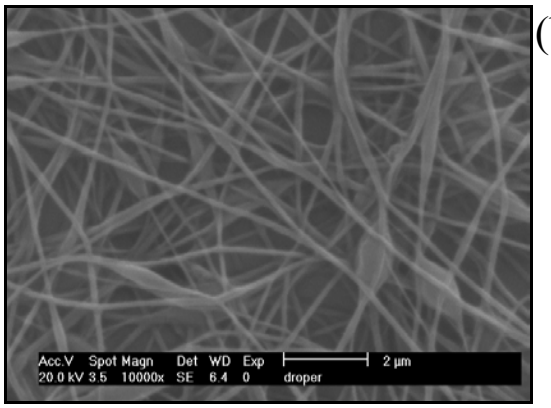
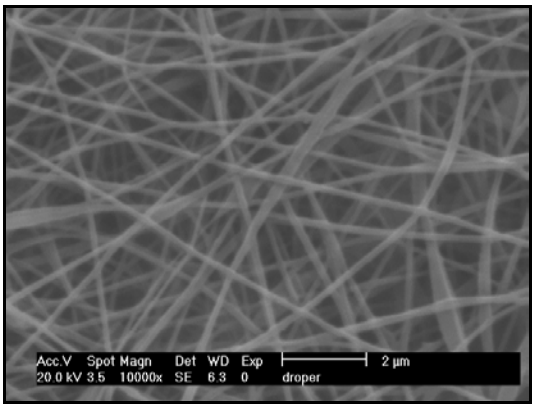
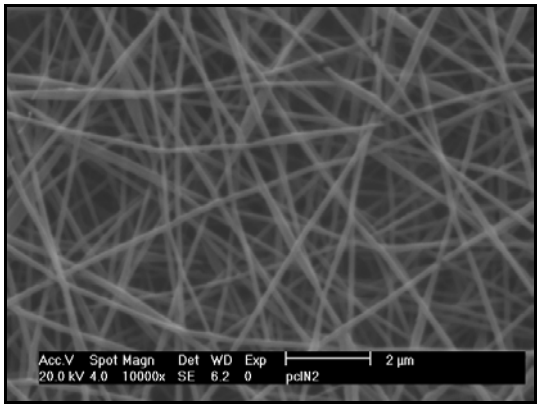


Figure 4.3 Effect of varying the applied voltage on the formation of the Taylor cone [36]

The spindle-like morphology was observed at low applied voltage i.e. 16, 18 and 23 kV and the bead-free nanofiber would be occurred when increasing the applied voltage at 20 kV (Figure 4.4). Whereas, the higher voltage may be produced the large diameter nanofibers and the beads formation. This behaviour can be explained that the high applied voltage affected greater streching of the jet because there is a lot of charge on the solution, so the electrostatic repulsive force on the jet increased (Fig 4.3), while In addition the higher applied voltage causes the evaporation of solvent from the nanofibers is rapid.

(a) 

(b) 

(c) 

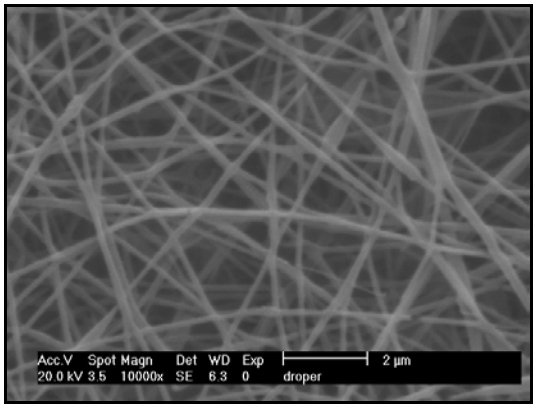
(d) 

Figure 4.4 Effect of applied voltage on morphology of nanofibers by SEM (a) 16 kV (b) 18 kV (c) 20 kV and (d) 23 kV

Table 4.3 Effect of applied voltage on nanofibers size

Applied voltage (kV)	Fiber morphology	Diameter size (nm)
16	Spindle beaded nanofiber	185-386
18	Spindle beaded nanofiber	131-282
20	nanofiber	120-190
23	Spindle beaded nanofiber	185-234

4.1.4 Effect of flow rate

The effect of the flow rate on nanofibers formation was studied by varying a flow rate of 1.3, 1.5 and 2 ml/hr and the others parameter were constant at applied voltage 20 kV, working distance 18 cm, needle gauge 26g, 5% solution concentration and 2:1:2 of PEO/alg/β-CD ratio.

At 1.3 ml/hr, the bead-free nanofibers were obtained in Fig 4.5 (b) and (c). Whereas, the flow rate of 2 ml/hr, the spindle beaded nanofiber were obtained (Figure 4.5 (a)). When the flow rate was raised from 1.3 to 1.5 ml/hr, the nanofiber diameter increased in size of 120-190 to 140-250 nm (Table 4.4) and when the flow rate was applied at 2 ml/hr, the diameter size was not significantly changed The incident can be illustrated that a lower flow rate, the solvent can evaporate enough time from the solution jet, the swelling of polymer solution is decreased which obtained small nanofiber diameter. Whereas, the higher flow rate

provided the large nanofiber diameter and resulted in beaded nanofiber due to the solvent did not get the drying time prior to reaching the collector.

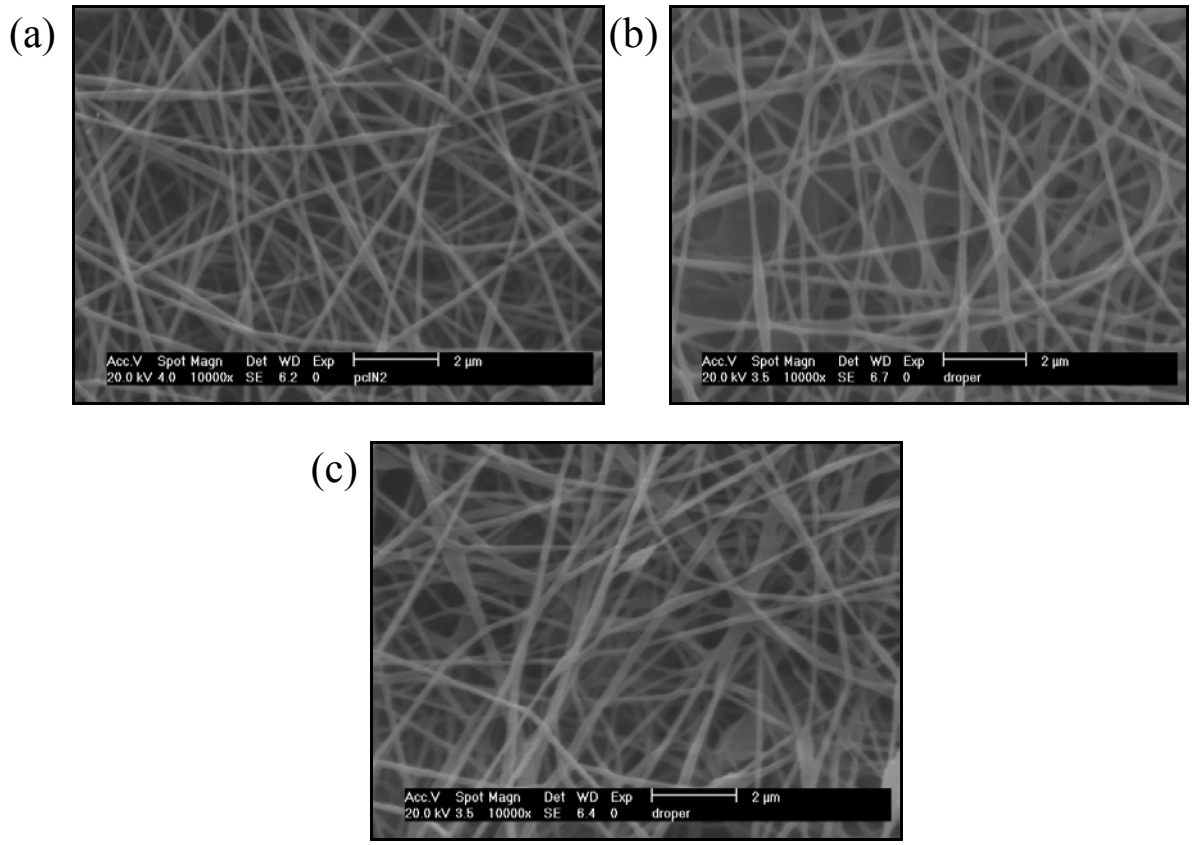


Figure 4.5 Effect of flow rate on morphology of nanofibers (a) 1.3 ml/hr (b) 1.5 ml/hr and (c) 2 ml/hr

Table 4.4 Effect of flow rate on nanofibers size

Flow rate (ml/hr)	Fiber morphology	Diameter size (nm)
1.3	Nanofiber	120-190
1.5	Nanofiber	140-250
2	Spindle beaded nanofiber	130-280

4.1.5 Effect of working distance

The effect of working distance on nanofiber formation was studied by using the difference of working distance values of 16, 18 and 20 cm, while the applied voltage, flow

rate, needle guage, solution concentration and PEO/alg/β-CD ratio were kept at 20 kV, 1.3 ml/hr, 26g, 5%concentration and 2:1:2 of the PEO/alg/β-CD ratio.

As the working distance 16 cm, the morphology of nanofibers was a spindle beaded nanofiber (Figure 4.6 (a)). While working distance 18 and 20 cm, the bead-free nanofibers were obtained (Figure 4.6 (b) and (c)). Moreover, the diameter of nanofibers were increased in size 80-130 to 150-220 nm when the working distance increased from 16 to 20 cm.

The results can be simply attributed to the formation of nanofiber is required an optimal working distance for the evaporate time of solvent from the nanofibers as well as effect of flow rate. The smaller and beaded nanofibers were observed when the working distance is too close. Whereas the larger diameter nanofibers were provided at the working distance is increased.

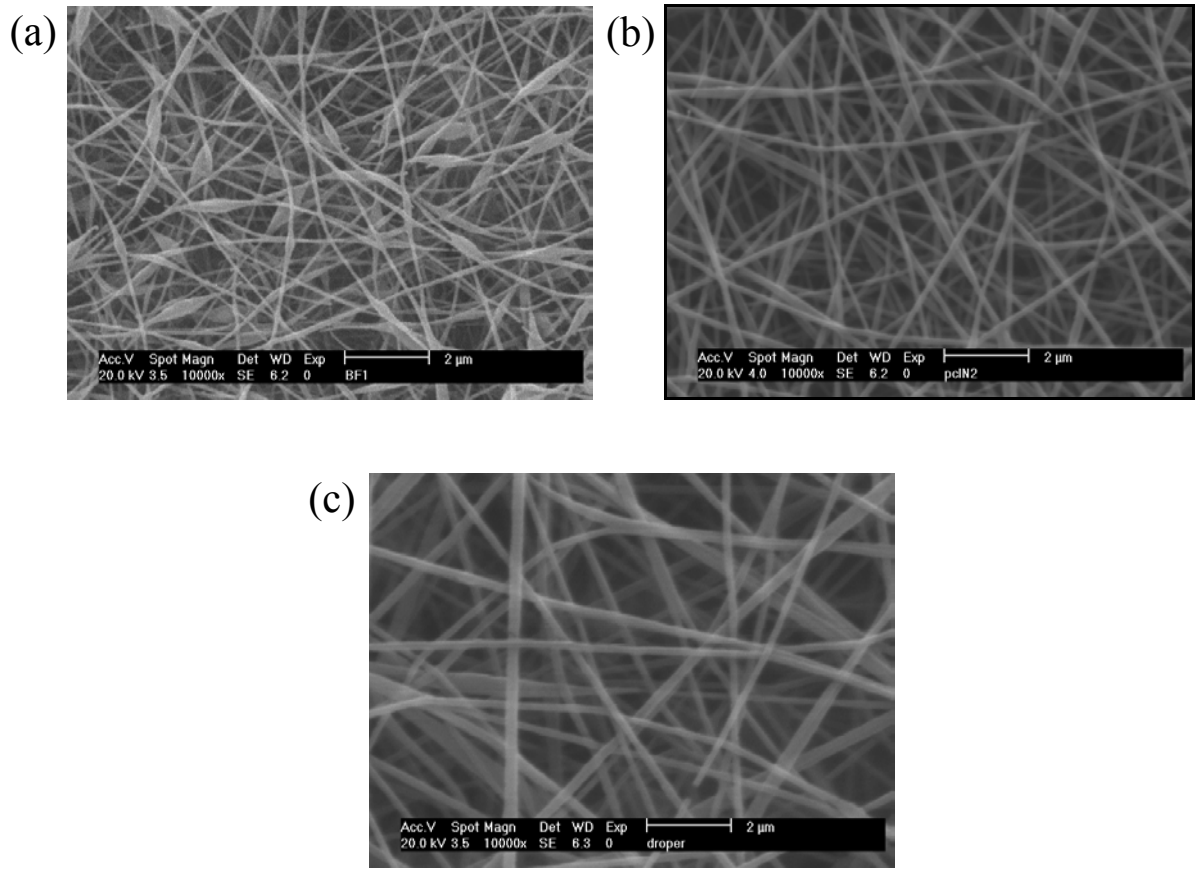


Figure 4.6 Effect of working distance on morphology of nanofibers (a) 16 cm (b) 18 cm and (c) 20 cm

Table 4.5 Effect of working distance on nanofibers size

Working distance (cm)	Fiber morphology	Diameter size (nm)
16	Spindle beaded nanofiber	80-130
18	Nanofiber	120-190
20	Nanofiber	150-220

4.1.6 Effect of percentage of estradiol/β-CD complex

Estradiol/β-cyclodextrin complex loaded nanofibers were prepared by the results obtained from the optimization studies. In order to study the effect of estradiol/β-cyclodextrin complex on the formation of nanofiber, the estradiol/β-cyclodextrin complexes were investigated at 0, 2, 4, 6, 8 and 10% complexes in the solution. All the other parameters were kept constant at 5% concentration, applied voltage 20 kV, flow rate 1.3 ml/hr, working distance 18 cm, needle gauge 26g and 2:1:2 of PEO:alg:β-CD ratio.

The varying of percentage of estradiol/β-CD complex on morphology and size of nanofiber were presented in Table 4.6. The nanofiber without estradiol (0% complex) exhibited the diameter size of 120-190 nm.At lower than 6% complex, the nanofiber cannot be formed to nanofiber. Whereas the complex was increased from 6% to 10% complex, the nanofiber diameter decreased in size 80-130 to 60-110 nm.

All condition, the beads and beaded nanofiber were observed when compared with a non-loaded drug nanofiber (Figure 4.7 (a)-(f)). Varying the drug content did not significantly influence to decreasing of number of beads. This can be explained that the electrospinning process needs homogeneous solution to form nanofiber while the addition of estradiol/β-cyclodextrin complexes into the solution, resulted in decreasing of the solubility of the solution. The obtained solution is a suspension solution which is not suitable to form nanofiber.

In case of the 6% complex added provided the spindle-like nanofiber which is the best morphology after added estradiol (Fig 4.7 (d)). Thus, this condition was selected for subsequence drug delivery experiments.

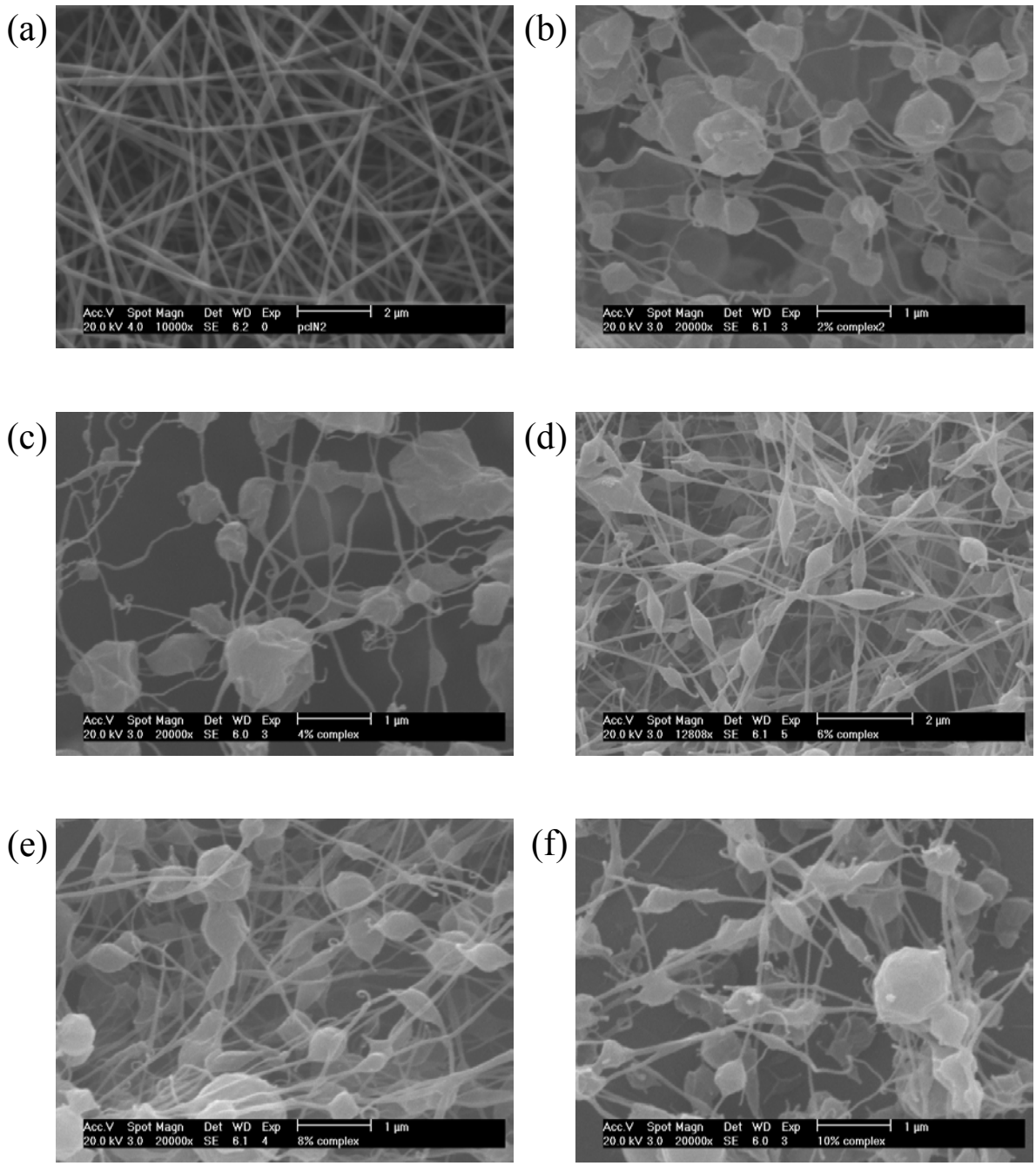


Figure 4.7 Effect of percentage of estradiol/β-CD complex in β-CD part (a) 0% (b) 2% (c) 4% (d) 6% (e) 8% and (f) 10% complex

Table 4.6 Effect of percentage of estradiol/β-CD complex in β-CD part

Est/β-CD Complex (%)	Fiber morphology	Diameter size (nm)
0	Nanofiber	120-190
2	Beads	-
4	Beads	-
6	Spindle beaded nanofiber	80-130
8	Beaded nanofiber	75-150
10	Beaded nanofiber	60-110

4.2 Characterization of complexes and obtained nanofibers with and without estradiol

4.2.1 Fourier Transform Infrared Spectroscopy (FT-IR)

4.2.1.1 Estradiol/β-CD complex

The FT-IR spectrum of estradiol showed the absorption peak of O-H stretching at 3447 $cm^{-1}$and the absorption O-H of the phenol molecules at 3236 cm^{-1}. The symmetric and asymmetric peaks of C-H stretching (alkane) were revealed at 2961, 2936 and 2862 cm^{-1}. The absorption peak of C-C multiple bond stretching (alkene) and C-C multiple bond stretching (aromatic) showed at 1680 and 1585 cm^{-1} [41]. In addition, the adsorption peak of cycloalkane was presented at 819 cm^{-1}.

The FT-IR spectrum of β-cyclodextrin demonstrated the absorption band at 3399 cm^{-1} was assigned to the O-H stretching vibration. The absorption peak of antisymmetric stretching vibration of the C-O-C glycosidic bridge of the β-CD at 1157 cm^{-1}. And the absortion peak of the couple C-C in β-cyclodextrin molucules at 1028 cm^{-1}.

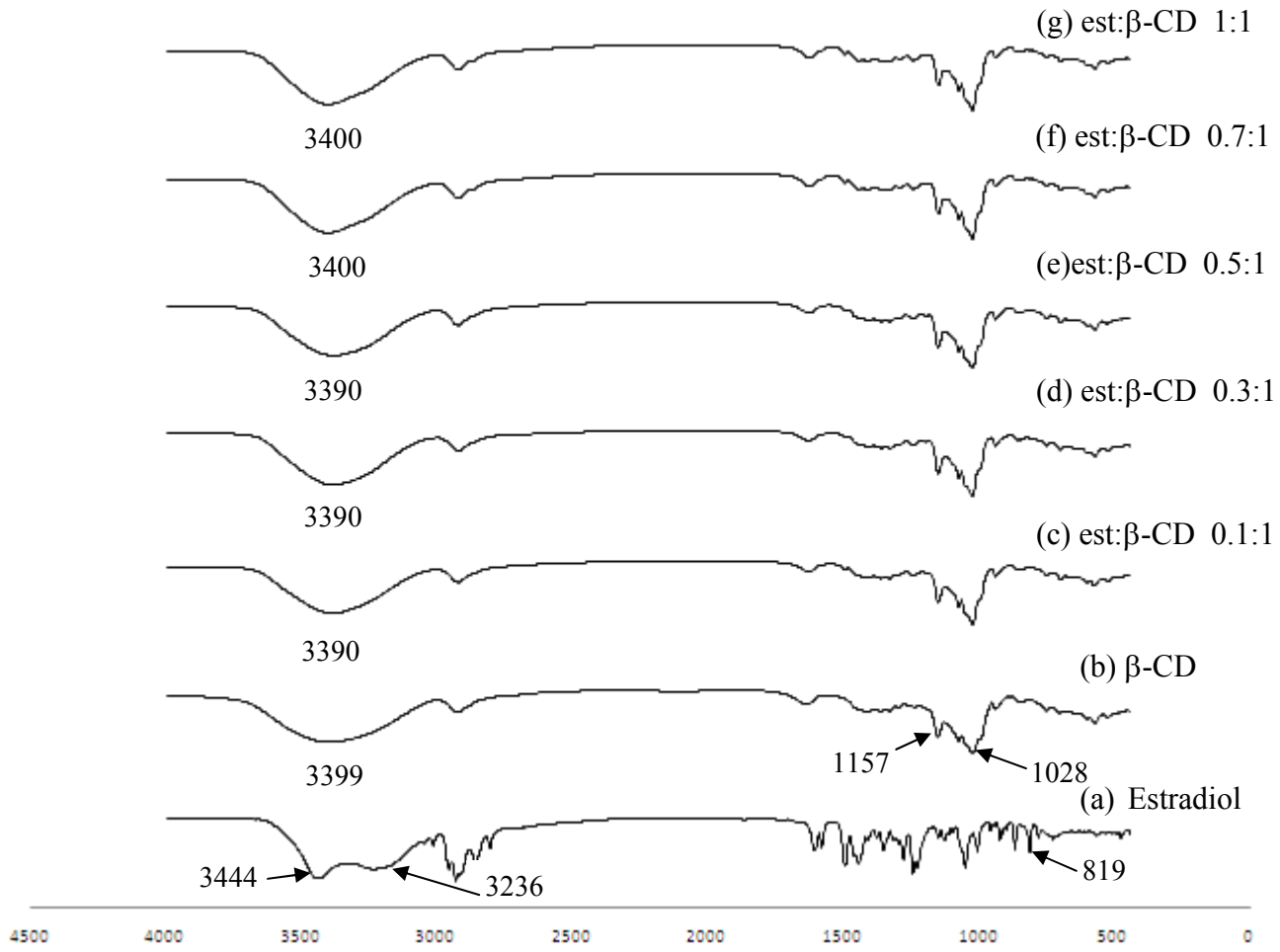


Figure 4.8 FT-IR spectra of (a) estradiol, (b) β-CD, varying estradiol/β-cyclodextrin complexes (c) 0.1:1, (d) 0.3:1, (e) 0.5:1, (f) 0.7:1 and (g) 1:1.

The FT-IR spectrum of estradiol/β-cyclodextrin complexes with different molar ratio of estradiol were showed in Figure 4.8 (c)-(g). The absorption peaks revealed at 3390 cm^{-1} corresponding to O-H stretching vibration of β-cyclodextrin. Some characteristic peaks of estradiol were found to be overlapping in the region as the β-cyclodextrin due to the both structure's compounds have not significantly different and amount of estradiol in complexes was less than β-cyclodextrin. The obtained FT-IR spectrum graphically showed the absorption peak of β-cyclodextrin and did not presented a new peak which indicated no chemical interaction in the formed complex.

4.2.1.2 PEO/Alg/β-CD nanofiber

The FT-IR spectrum of alginate (Figure 4.9 (a)) showed a distinct peak of –OH absorption band at 3433 cm^{-1}. The absorption band at 1610 and 1418 cm^{-1}were attributed to the symmetric and asymmetric peak of the carboxylate groups (COO^-) stretching vibration. The absorption band at 1031 cm^{-1} resulted the C-O-C stretching in the intra- and intermolecular between polymer's repeating units.

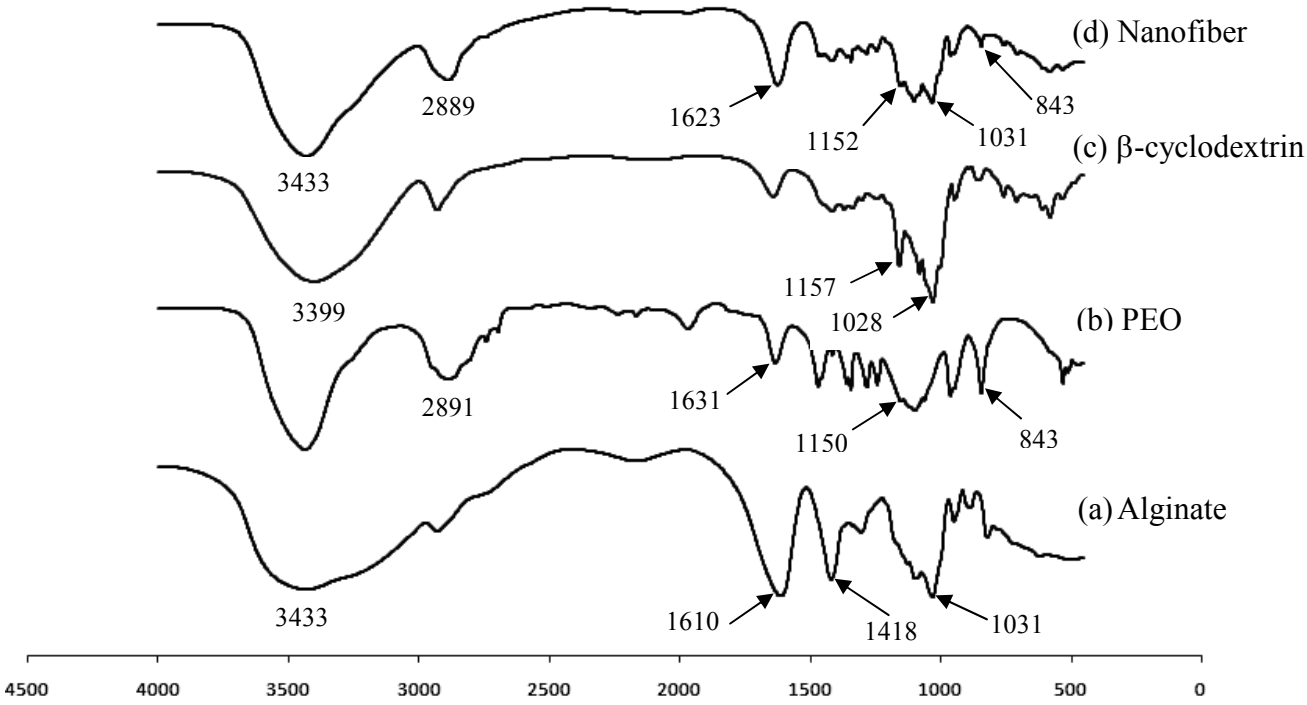


Figure 4.9 FT-IR spectra of (a) alginate, (b) PEO, (c) β-CD and (d) PEO/Alg/β-CD nanofibers.

The FTIR spectrum of poly(ethylene oxide) or PEO (Figure 4.9 (b)) revealed the absorption peak at 2891 and 2741 cm^{-1} corresponding to the –OH stretching of the PEO. The peaks showed at 1150 and 842 cm^{-1} were attributed to the C-O-C bending vibration of the PEO.

The FTIR spectrum of β-cyclodextrin or β-CD (Figure 4.9 (c)) showed the absorption peak at 1155 cm^{-1} was assigned to the antisymmetric stretching vibration of the C-O-C glycosidic bridge of the β-CD. The peak at 1030 cm^{-1} showed the couple C-C of the β-CD.

The characteristic absorption bands of nanofiber (Figure 4.9 (d)) showed at 2889 and 843 cm^{-1} corresponding to the OH stretching and C-O-C bending vibrations of the PEO. The peak at 1622 cm^{-1} showed the presence of the COO^- asymmetric stretching of alginate. The peaks presented at 1151 and 1031 cm^{-1} corresponding to the antisymmetric stretching vibration of the C-O-C glycosidic bridge and coupled C-C/C-O stretching vibrations of the β-CD. These results confirmed the successful fabrication of the PEO/Alg/β-CD nanofibers.

4.2.1.3 Nanofiber without and with estradiol/β-CD complex

The FT-IR spectrum of nanofiber with different amount of estradiol/β-CD were presented in Figure 4.10 (b)-(f). The 0.1:1 complex loaded nanofiber (Figure 4.10(b)) showed the absorption peak at 2886 and 842 cm^{-1} corresponding to the OH stretching and C-O-C bending vibration of PEO. The peak at 1606 cm^{-1} corresponded to the COO^- asymmetic stretching of alginate. The absorption peak at 1149 and 1025 cm^{-1} showed the antisymmetric stretching vibration of the C-O-C glycosidic bridge and coupled C-C/C-O stretching vibration of β-CD. Whereas the peak of estradiol were overlayed by the absorption band of PEO, β-CD and alginate due to the absorption band of hydroxy and methyl group of estradiol are similar with the functional group of the PEO, β-CD and alginate. For the other ratios (Figure 4.10 (c)-(f)) presented the similar absorption peak of the 0.1:1 complex loaded nanofiber.

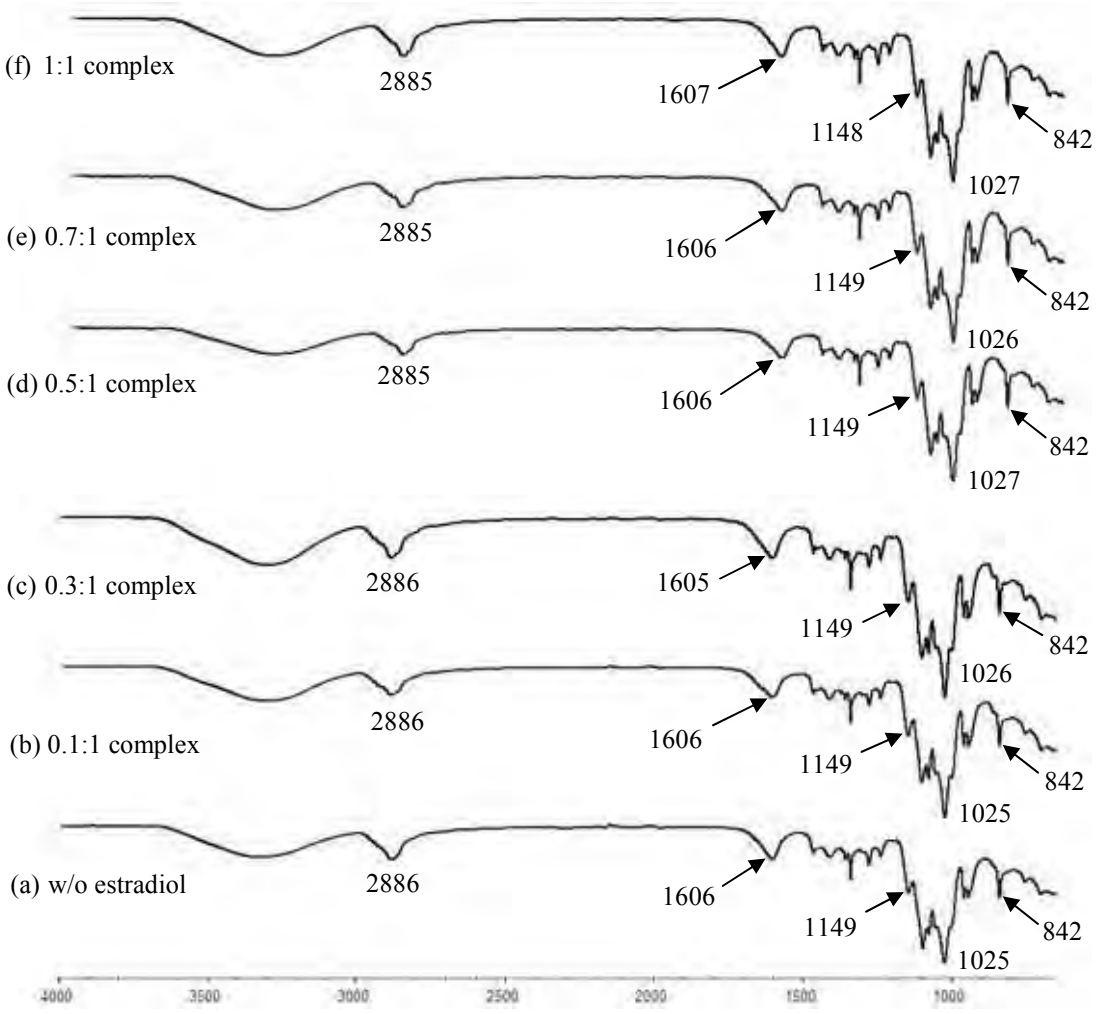


Figure 4.10 FT-IR spectra of (a) nanofiber without estradiol, nanofiber containing estradiol/β-cyclodextrin complexes (b) 0.1:1, (c) 0.3:1, (d) 0.5:1, (e) 0.7:1 and (f) 1:1

4.2.2 X-ray Diffraction

The crystalline state of polymer and drug was characterized by X-ray diffraction. The X-ray diffractogram of the estradiol/β-cyclodextrin complexes were shown in Figure 4.11. The XRD pattern of PEO/alg/β-cyclodextrin nanofiber with and without estradiol/β-cyclodextrin complexes were presented in Figures 4.12 and 4.13.

4.2.2.1 Estradiol/β-CD complex

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The diffractogram of β-cyclodextrin (Figure 4.11 (a)) revealed a crystalline diffraction peak at 2θ = 6.28°, 12.5° and 17.4 ° [42] and the estradiol (Figure 4.11 (b))

showed a crystalline state in XRD pattern at 2θ = 11.6°, 13.22°, 18.3°, 22.7° and 26.5°. The diffractogram of the various estradiol/β-cyclodextrin complex ratio (Figure 4.11 (c)-(g)) displayed the peak in position of 6.2° and 17.04° of β-cyclodextrin. The intense peak of estradiol in the position of 2θ = 11.6°, 13.22°, 18.3°, 22.7° and 26.5° were observed. In addtion, the new diffraction peak were found at 2θ = 5.84° which indicated the formation of inclusion complex between estradiol and β-cyclodextrin.

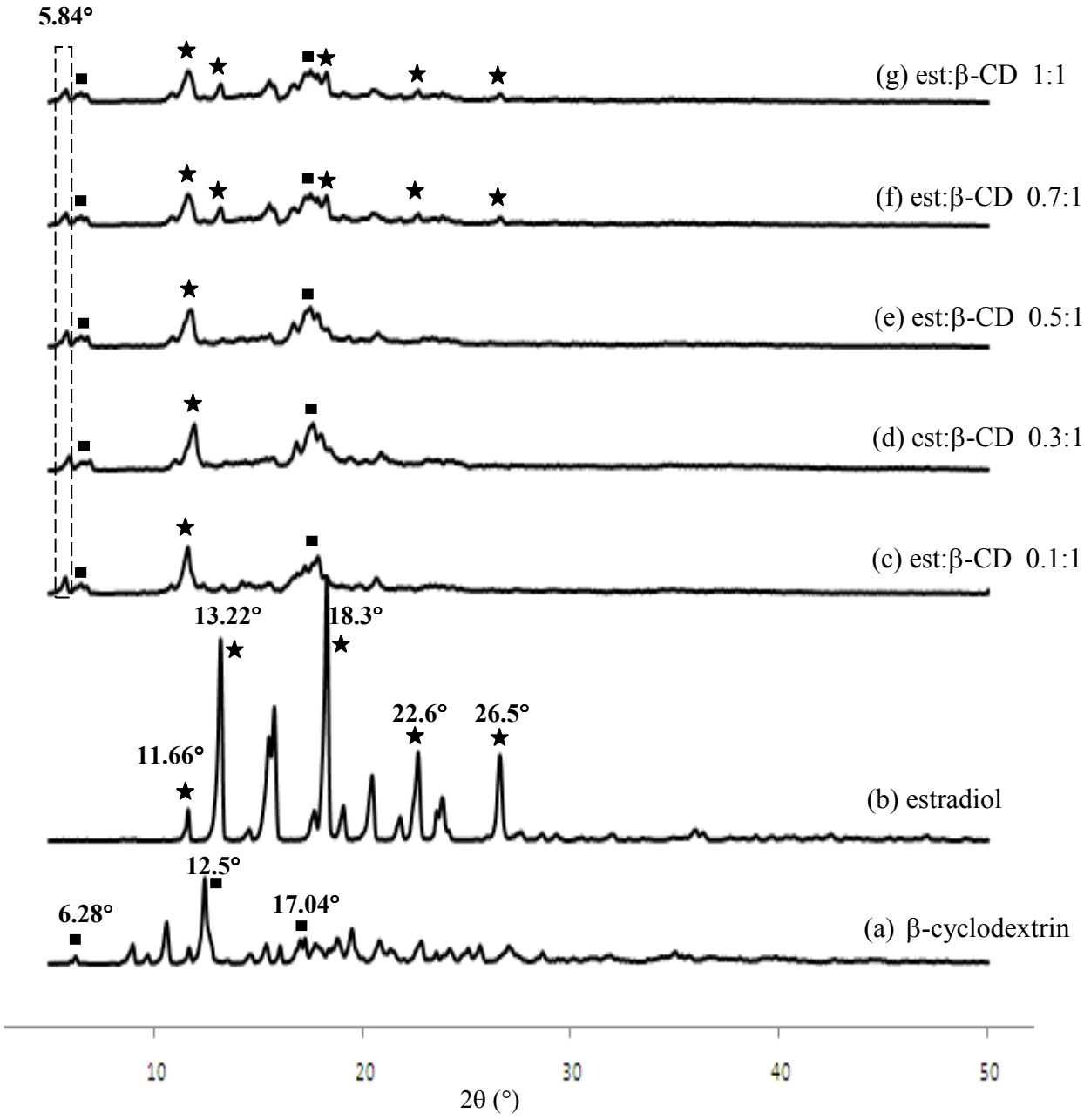


Figure 4.11 X-ray diffractograms of (a) β-CD, (b) estradiol, estradiol/β-cyclodextrin complexes (c) 0.1:1, (d) 0.3:1, (e) 0.5:1, (f) 0.7:1 and (g) 1:1.

4.2.1.2 PEO/Alg/β-CD nanofiber

The diffractogram of PEO (Figure 4.12 (a)) which is a semi-crystalline polymer exhibited the diffraction peaks at 2θ = 19º and 23º [8]. The diffractogram of β-CD showed a crystalline diffraction peak at 2θ = 12.5° (Figure 4.12 (b)) and the diffractogram of alginate (Figure 4.12 (c)) revealed a broad peak at 2θ = 13.9° [27]. The XRD patterns of the PEO/alginate/β-cyclodextrin nanofibers showed diffraction peaks at 2θ = 12.5°, 19° and 23° which indicated the presence of PEO and β-cyclodextrin and the crystallinity of PEO and β-CD were slightly disrupted with alginate. This result could be explained that the amount of PEO and β-CD which had higher than the alginate, exhibited the appearance of the diffraction peaks and dispersed into the mixtures.

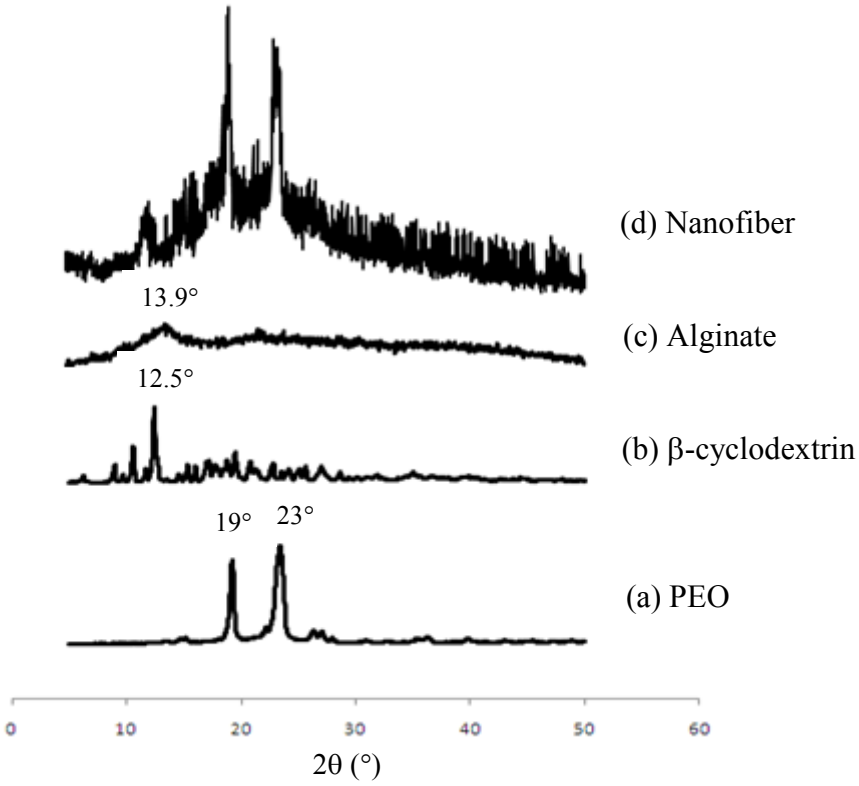


Figure 4.12 X-ray diffractograms of (a) PEO, (b) β-CD, (c) Alginate and (d) PEO/Alg/β-CD nanofibers.

4.2.1.3 Nanofiber without and with estradiol/β-CD complex

The XRD pattern of nanofiber without estradiol/β-CD complex (Fig 4.13 (a)) revealed a crystalline diffraction peak of PEO at 2θ = 19° and 23° and showed sligthly a crystalline diffraction peak of β-CD at 2θ = 6.28°. The XRD pattern of nanofiber with different ratio of estradiol/β-CD complex were presented in Figure 4.13 ((b)-(f)). The nanofiber with estradiol/β-CD showed the same diffraction peak of PEO and β-CD but the diffraction peak of estradiol (at 2θ = 22.7° and 26.5°) disappearred in the XRD patterns. The result can be explained that the estradiol within the nanofiber have a little amount which were overlayed by the broad peak of alginate.

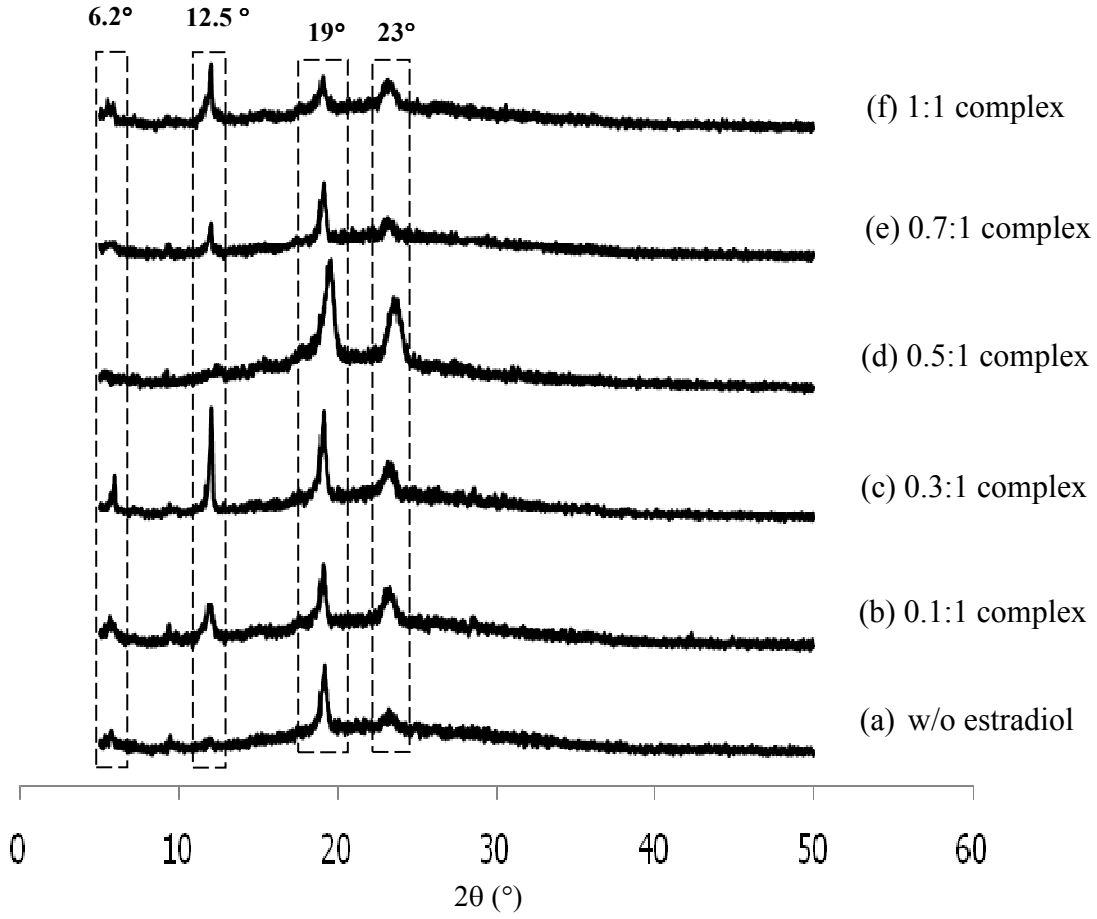
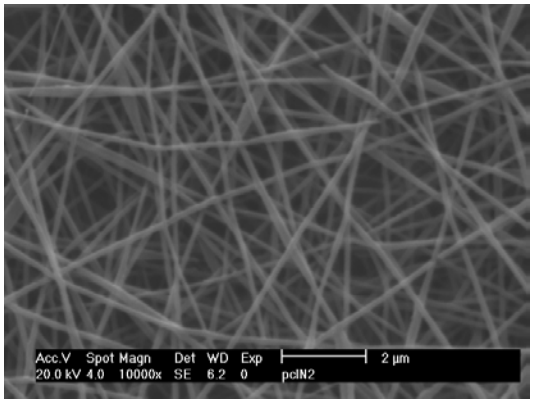


Figure 4.13 X-ray diffractograms of (a) nanofiber without estradiol/β-CD complex, estradiol/β-CD nanofiber (b) 0.1:1, (c) 0.3:1, (d) 0.5:1, (e) 0.7:1, and (f) 1:1.

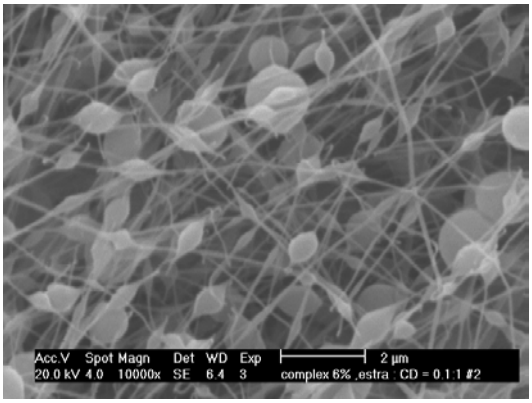
In addition, the nanofibers with various estradiol/β-CD complexes also were characterized by SEM. The morphology and size of nanofibers can be clearly explained the

results from the X-ray diffractograms. As the various estradiols into nanofiber, the morphology of the nanofiber with 0.1:1 estradiol/β-CD complexes were beaded nanofiber and the spindle beaded nanofiber were obtained at higher 0.1:1 complex (Figure 4.14 (b)-(f)). As shown in Table 4.7, the increasing of estradiol from 0.1 to 1 molar, the diameter size of nanofibers had not been significantly changed. Whereas, the increased estradiol, the beads size on nanofiber were decreased. The results can be attributed to the suspension of estradiol/β-CD complex provided to form beads on the nanofiber and the non-entraped estradiol affected the decreasing of the solubility of PEO/AlG/β-CD solution which the high beads were formed. In addition, SEM revealed the distribution of beads on the nanofiber were anisotopic, the resulting of X-ray diffractogram did not trend with the increasing of estradiol.

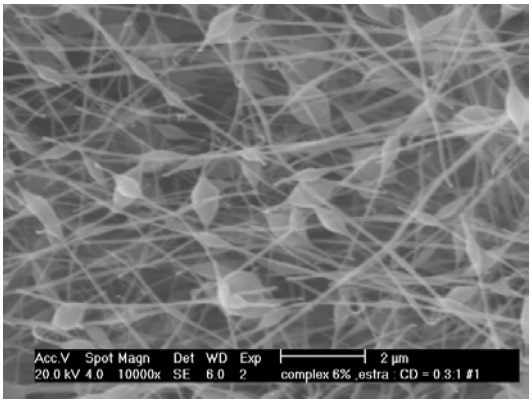
(a)



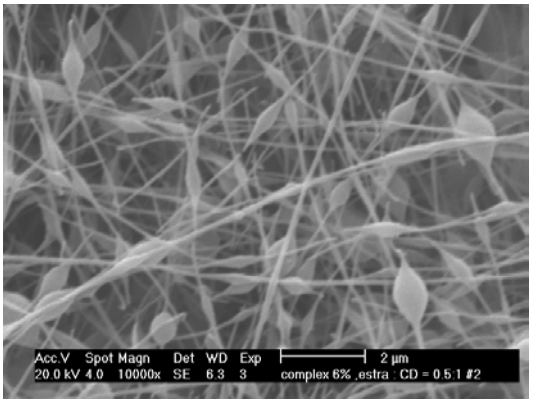
(b)

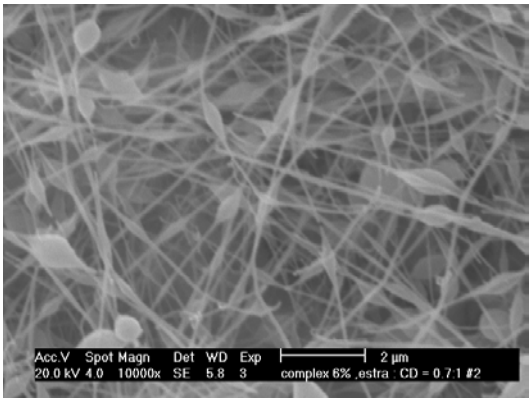


(c)



(d)



(e) 

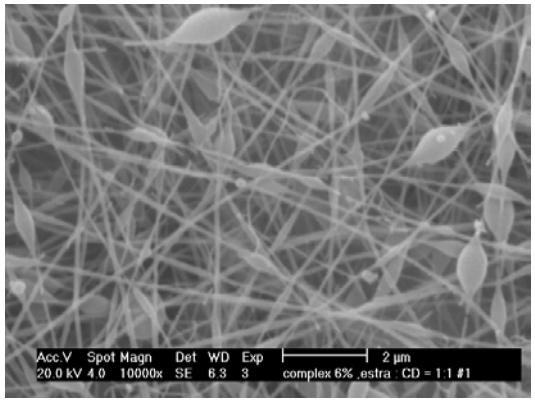
(f) 

Figure 4.14 SEM images of estradiol/β-CD complex ratios (a) 0:1, (b) 0.1:1, (c) 0.3:1, (d) 0.5:1, (e) 0.7:1, and (f) 1:1

Table 4.7 The effect of estradiol/β-CD complex ratios on nanofiber and beaded within nanofiber size

Ratio of estradiol/β-CD (molar/molar)	**Fiber morphology**	**Diameter of nanofiber (nm)**	**Diameter of Beads (nm)**
0:1 (w/o estradiol)	nanofiber	120-190	-
0.1:1	Beaded nanofiber	77-124	454-1480
0.3:1	Spindle beaded nanofiber	87-147	399-991
0.5:1	Spindle beaded nanofiber	97-162	380-980
0.7:1	Spindle beaded nanofiber	85-145	346-730
1:1	Spindle beaded nanofiber	98-197	263-724

4.3 Evaluation of drug encapsulation efficiency (%EE)

The encapsulation efficiency of estradiol /β-cyclodextrin complexes were analyzed using UV/Vis spectroscopy at λ_{max} = 203 nm. The percentage of encapsulation efficiency of the estradiol/β-cyclodextrin complex were shown in Table 4.7

The effect of estradiol amount on encapsulation efficiency was investigated by increasing estradiol to β-cyclodextrin molar ratio from 0.1:1 to 1:1. When the estradiol to β-cyclodextrin molar ratio increased, the encapsulation efficiency of estradiol/β-cyclodextrin complexes were increased from 35.71% to 93.82%. It may be explained that at the 0.1:1 of

estradiol/β-CD complex, the amount of estradiol to β-cyclodextrin was low which the β-cyclodextrin cavity can entrap the higher estradiol amount. The obtained encapsulation efficiency of this ratio is too low. Thus, the lower than 0.1:1 estradiol/β-CD ratio, they were selected for the further experiments

Table 4.8 Encapsulation efficiency of estradiol loaded β-cyclodextrin complexes

Ratio of estradiol:β-CD(mol/mol)	%EE
0.1:1	35.71±2.61
0.3:1	75.68±1.82
0.5:1	82.62±0.99
0.7:1	92.26±0.69
1:1	93.82±0.42

4.4 Drug release study of PEO/alg/β-CD nanofibers in phosphate buffer medium

The drug release from the PEO/alg/β-CD nanofibers was analyzed using UV-Vis spectroscopy and described as a graph of cumulative released of drug as a function of time. The release rates of the nanofibers having different estradiol content were shown in Figure 4.13 and Appendix B.

The *in vitro* release of PEO/alg/β-CD nanofibers having different estradiol amount was investigted in 25% (v/v) ethanol/phosphate buffered saline at 37±1 °C. The molar ratio of estradiol/β-cyclodextrin complexes at 0.3:1 (mol/mol) exhibited the 50% of estradiol released within 1 day which was suggested that estradiol release showed a rapidly release (initial burst). This result can be attributed to the drug in physically encapsulate into the core through non-inclusion interactions were quickly released from the nanofiber surface in initial incubation [43]. After the initial burst, the drug was associated with β-cyclodextrin by inclusion complex were sustained release for 6 days. The others molar ratio showed the release rate were slower. The molar ratio of estradiol/β-cyclodextrin complexes at 1:1 (mol/mol) is the lowest release rate. This stoichiometry mean one drug molecule forms a complex with one β-cyclodextrin molecule which would allow maximum contact between the hydrophobicportion of the organic substrate with the apolar cavity of β-cyclodextrin [44,

45]. It seems that this molar ratio were strongly encapped within nanofiber. Due to the nanofiber were improve insoluble properties using immerse in 3% $CaCl_3$ before releasing which estradiol might be incorporated to two layer.

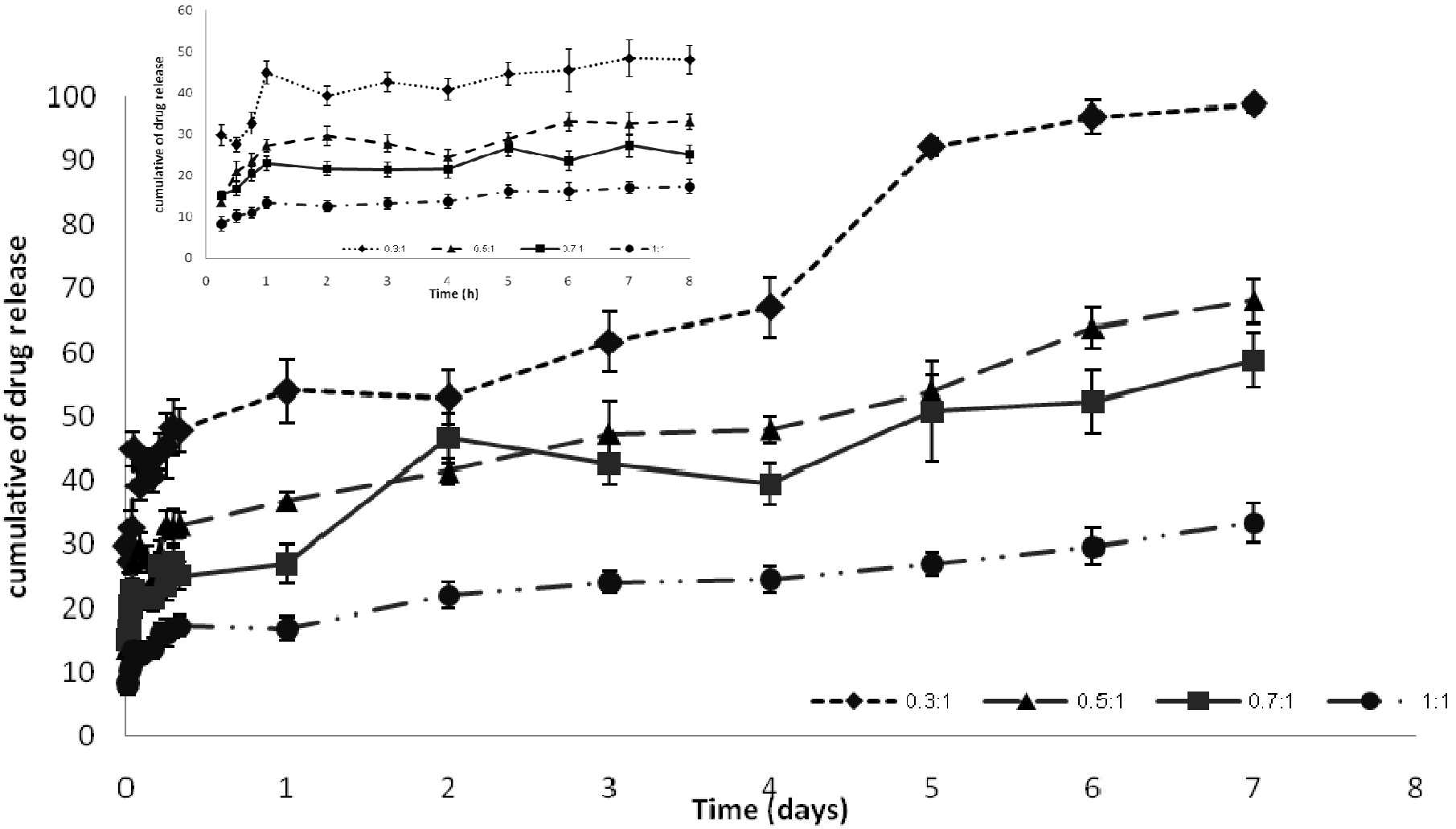


Figure 4.15 Release profiles of different estradiol/β-cyclodextrin complexes from PEO/alg/β-CD nanofibers at 25% EtOH/phosphate bufferd saline pH 7.4, 37°C (± 1SD, three repeat experiments)

4.5 *In vitro* study of estradiol permeation through cellulose acetate membrane

The *in vitro* cellulose acetate membrane permeation profiles of estradiol were presented at 25% EtOH/phosphate buffered saline pH 7.4, 37±1°C. The different molar ratio of estradiol/β-cyclodextrin complexes are studied as 0.3:1, 0.5:1, 0.7:1 and 1:1 (Fig 4.14).

The permeation of estradiol from PEO/Alg/(β-CD complex) nanofiber was calculated in term of mean cumulative amount of permeated estradiol across a unit area of membrane (Q_p) at each sampling time for 7 days. The cumulation in each sample increased with time, indicating that estradiol was continuously permeated from the through the cellulose acetate membrane to accumulate in the 25% EtOH/phosphate buffered saline. The linear relationship between amount of drug permeating through cellulose acetate membrane (Q_p) and time (day) as the drug flux.The flux of estradiol (μg/cm^2.day) at steady state are presented in Table 4.8. The permeation behaviors of each molar ratio of estradiol/β-cyclodextrin complexes were exhibited linear relationship with time (r^2 =0.9844, 0.9089, 0.9735 and 0.9729 for 0.3:1, 0.5:1, 0.7:1 and 1:1 respectively). The linear relationship suggested that the estradiol were continuously released from PEO/Alg/(β-CD complex) nanofibers and resulted in continuous drug permeation through celloluse acetate membrane in the 7 days period. Increasing amount of estradiol in complex led to increasing permeating flux. The permeating flux of 0.3:1 estradiol/β-cyclodextrin complex is 5.7321 μg/cm^2.day and 0.5:1 estradiol/β-CD molar ratio is 5.8582 μg/cm^2.day. The highest permeating flux is 10.431 μg/cm^2.day of 0.7:1 estradiol/β-CD molar ratio but not significant different with 1:1 estradiol/β-CD molar ratio.

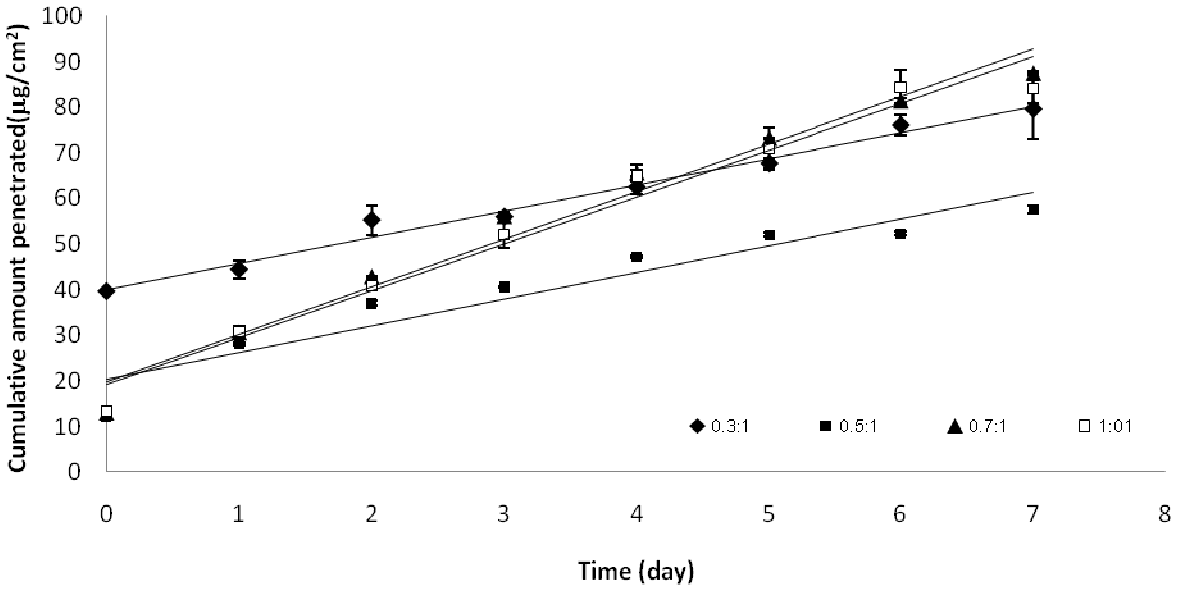


Figure 4.16 Permeation profiles of different estradiol/β-cyclodextrin complexes from PEO/alg/β-CD nanofibers through cellulose membrane at 25% EtOH/phosphate bufferd saline pH 7.4, 37°C (± 1SD, three repeat experiments)

Table 4.9 Linear relation between amounts of estradiol permeating through one area division of cellulose membrane (Q_p) and times

Ratio of estradiol/β-CD complex	Linear relationship	r^2	F (μg/cm^2.day)
0.3:1	Q_p = 5.7321t+40.044	0.9844	5.7321
0.5:1	Q_p = 5.8582t+20.382	0.9089	5.8582
0.7:1	Q_p = 10.431t+19.71	0.9735	10.431
1:1	Q_p = 10.305t+19.129	0.9729	10.305

CHAPTER V

CONCLUSION AND SUGGESTIONS

5.1 Conclusion

The results of this study demonstrated that the preparation of estradiol/β-cyclodextrin complex by co-precipitate method produce high entrapment drug efficiency over 75% and the electrospinning process is a simple technique for the production of poly (ethylene oxide)/alginate/β-cyclodextrin nanofibers. The morphology and size of these nanofibers can be manipulated by controlling the system parameters such as solution concentration, molar ratio of PEO/Alg/β-CD and the process parameter such as voltage, flow rate and working distance. The preliminary studies of the effects of both parameters on the morphology and size of nanofiber showed the PEO/Alg/β-CD nanofibers were successfully prepared using system and process parameters as follows: voltage of 20 kV, flow rate of 1.3 ml/h, needle gauge of 26 g and working distance of 18 cm. The PEO/Alg/β-CD nanofibers are almostly smooth surface with a diameter within 120-190 nm. While, the PEO/Alg/β-CD containing estradiol/β-CD complexes were prepared to beaded nanofiber or spindle nanofibers due to the insolubility of estradiol strongly affected on the solubility of mixed solution. However, the PEO/Alg/β-CD nanofibers containing estradiol/β-CD complexes provided the prolonged release over a period of 7 days. The permeating release of estradiol through cellulose acetate membrane also showed a sustained release for 7 days with flux of 10.431 $\mu g/cm^2$.day.

5.2 Suggestions

5.2.1 Nanofibers Improvement

From this study, the nanofibers with estradiol/β-CD complex presented the spindle-beaded nanofibers which were produced from the insoluble of complexes within polymer mixture solution. Due to the free-beaded nanofibers fabrication need homogeneous solution, the β-CD should be replaced with modified β-CD e.g. hydroxypropyl-β-cyclodextrin (HPβCD) and methyl-β-cyclodextrin (MβCD) as have been reported that the solubility of estradiol was increased when used HPβCD or MβCD as a host molecule.

5.2.2. Pharmaceutical application

5.2.2.1 As alginate has very good haemostatic and wound healing properties and biocompatibility, the synthetic of fabrication parameter should be especially studied using alginate as a main part for preparation of alginate nanofibers. The gelation of alginate can be formed using calcium salt.

5.2.2.2 The estradiol dosage of 25 μg/days is a minimum required dosage that gave high effective and minimal adverse effect. This can be performed by

1) Increasing the estradiol content in nanofiber.
2) Increasing solubility of complexes using HPβCD or MβCD, these compounds should be increased the higher estradiol content and released a longer operation time.
3) In addition, the increasing thickness of patch provide increase drug loading and improve the slower diffusion of estradiol which could be provided the prolonged release up to 30 days.

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APPENDICES

Appendix A

Calibration curve

The concentration versus absorbance data of estradiol in methanol at 280 nm and in 25% (v/v) ethanol/phosphate buffered saline pH 7.4 at 203 nm are presented in Table 1A and 2A. They show a linear relationship with the correlation coefficient = 0.9993 and 0.9852.

Table 1A Absorbance of estradiol in methanol determined at 203 nm.

Concentration of estradiol in methanol (μg/ml)	Absorbance
1	0.0622
5	0.1450
10	0.2269
20	0.3896
50	0.8132

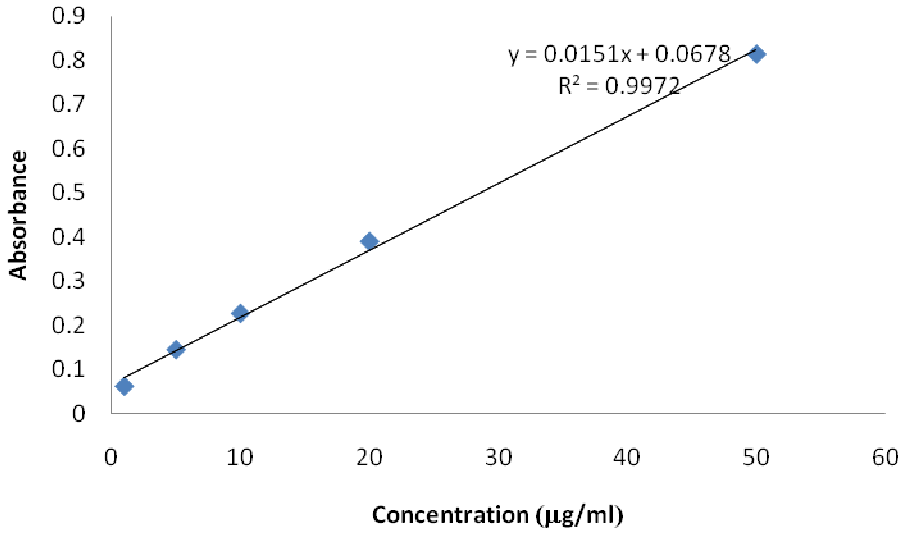

Figure 1A Standard calibration curve of estradiol in methanol

Table 2A Absorbance of estradiol in 25% (v/v) ethanol/ phosphate buffered saline determined at 203 nm.

Concentration of estradiol in methanol (μg/ml)	Absorbance
0	0.0013
2	0.2431
4	0.4676
6	0.7093
8	0.8963
10	1.1838

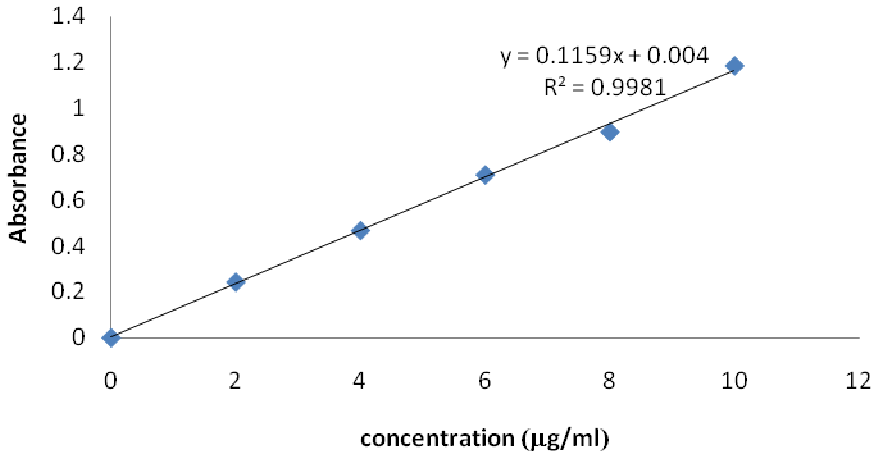

Figure 2A Standard calibration curve of estradiol in 25% (v/v) ethanol/phosphate buffered saline

Appendix B

Percentage of Cumulative Drug Release

Table 1B % Cumulative of estradiol release from PEO/Alg/(β-CD complex) containing 0.3:1 of estradiol/β-CD ratio

Time (day)	% Cumulative estradiol release				
	1	2	3	Mean	SD
0.010417	27.37	32.32	29.56	29.75	2.48
0.020833	26.01	29.31	27.02	27.44	1.69
0.03125	30.65	35.71	31.48	32.61	2.72
0.04167	42.23	47.70	44.65	44.86	2.74
0.08333	36.86	41.56	39.50	39.31	2.36
0.125	40.37	45.07	42.47	42.64	2.36
0.1667	37.88	42.98	41.31	40.73	2.60
0.20833	41.93	47.51	44.08	44.51	2.81
0.25	41.95	51.42	43.10	45.49	5.17
0.29167	44.00	52.90	48.01	48.30	4.46
0.3333	45.18	51.81	46.87	47.95	3.44
1	50.56	59.75	51.94	54.08	4.95
2	49.09	57.36	52.44	52.96	4.16
3	58.34	67.10	59.88	61.77	4.67
4	61.94	71.25	68.01	67.07	4.72
5	93.81	91.29	91.66	92.25	1.36
6	96.68	94.36	99.53	96.86	2.59
7	97.78	98.64	100.00	98.81	1.12

Table 2B % Cumulative of estradiol release from PEO/Alg/(β-CD complex) containing 0.5:1 of estradiol/β-CD ratio

Time (day)	% Cumulative estradiol release				
	1	2	3	Mean	SD
0.010417	13.66	14.47	12.68	13.60	0.90
0.020833	20.40	23.70	19.03	21.04	2.40
0.03125	23.64	25.34	21.44	23.47	1.95
0.04167	26.64	28.80	26.09	27.18	1.43
0.08333	28.88	32.14	27.69	29.57	2.31
0.125	26.82	30.05	26.35	27.74	2.02
0.1667	23.60	26.61	23.08	24.43	1.91
0.20833	27.98	30.72	27.99	28.90	1.58
0.25	31.28	35.63	32.35	33.08	2.27
0.29167	31.41	35.83	30.47	32.57	2.86
0.3333	32.43	35.19	31.51	33.04	1.92
1	36.03	38.41	36.14	36.86	1.34
2	40.78	43.53	40.00	41.44	1.85
3	52.96	45.99	42.88	47.28	5.16
4	47.07	50.34	46.52	47.97	2.06
5	53.19	56.86	51.66	53.90	2.67
6	63.42	67.36	61.08	63.95	3.17
7	67.79	71.63	64.92	68.11	3.37

Table 3B % Cumulative of estradiol release from PEO/Alg/(β-CD complex) containing 0.7:1 of estradiol/β-CD ratio

Time (day)	% Cumulative estradiol release				
	1	2	3	Mean	SD
0.010417	15.04	16.36	14.24	15.21	1.07
0.020833	15.95	18.75	15.63	16.78	1.72
0.03125	19.98	22.22	19.01	20.40	1.65
0.04167	22.17	24.79	21.90	22.95	1.60
0.08333	20.99	23.64	20.40	21.68	1.73
0.125	20.81	23.58	20.09	21.50	1.84
0.1667	21.14	24.01	19.62	21.59	2.23
0.20833	25.93	29.02	25.10	26.68	2.07
0.25	23.14	26.06	21.49	23.56	2.31
0.29167	26.06	30.55	25.33	27.31	2.83
0.3333	23.71	27.67	23.97	25.11	2.21
1	25.94	30.46	24.67	27.02	3.05
2	42.59	46.97	50.33	46.63	3.88
3	41.55	46.28	40.10	42.65	3.23
4	38.10	43.15	37.25	39.50	3.19
5	47.74	59.71	45.00	50.82	7.82
6	51.55	57.63	47.74	52.31	4.99
7	53.88	60.16	62.18	58.74	4.33

Table 4B % Cumulative of estradiol release from PEO/Alg/(β-CD complex) containing 1:1 of estradiol/β-CD ratio

Time (day)	% Cumulative estradiol release				
	1	2	3	Mean	SD
0.010417	6.45	9.73	8.84	8.34	1.69
0.020833	8.49	11.39	10.83	10.24	1.54
0.03125	9.80	12.36	10.90	11.02	1.29
0.04167	12.16	14.81	13.32	13.43	1.33
0.08333	11.22	13.87	12.83	12.64	1.34
0.125	12.03	14.68	13.32	13.34	1.32
0.1667	11.99	15.33	14.04	13.79	1.68
0.20833	14.61	17.61	16.26	16.16	1.50
0.25	14.01	18.46	16.10	16.19	2.22
0.29167	15.50	18.43	17.29	17.07	1.47
0.3333	15.72	19.08	17.21	17.34	1.68
1	15.00	18.58	17.08	16.89	1.80
2	19.96	24.21	21.77	21.98	2.13
3	22.26	25.83	24.26	24.12	1.79
4	22.36	26.60	24.84	24.60	2.13
5	25.28	29.06	26.75	27.03	1.91
6	26.74	32.31	29.97	29.67	2.80
7	30.16	36.34	33.52	33.34	3.09

Appendix C

In vitro estradiol permeation through membrane

Table 1C Cumulative amount of permeated estradiol through a unit area of cellulose acetate membrane (Q_p), μg/cm^2 of estradiol/β-CD ratio = 0.3:1

Time(day)	$Q_p(1)$	$Q_p(2)$	$Q_p(3)$	Mean	SD
0	39.7163	39.4744	37.8323	39.0077	1.0251
1	46.3447	44.4301	42.4577	44.4108	1.9436
2	58.6397	54.6605	52.3826	55.2276	3.1669
3	57.1148	55.6379	55.0581	55.9369	1.0605
4	63.8353	62.5063	60.9894	62.4437	1.4240
5	68.9510	67.4525	66.4936	67.6324	1.2385
6	78.5959	75.3489	74.1602	76.0350	2.2961
7	83.7977	72.1276	82.7556	79.5603	6.4580

Table 2C Cumulative amount of permeated estradiol through a unit area of cellulose acetate membrane (Q_p), μg/cm^2 of estradiol/β-CD ratio = 0.5:1

Time(day)	$Q_p(1)$	$Q_p(2)$	$Q_p(3)$	Mean	SD
0	13.1780	12.5003	12.4324	12.7036	0.4122
1	27.8885	28.2542	28.0116	28.0514	0.1861
2	36.4205	37.0014	37.4979	36.9733	0.5392
3	40.4640	40.7743	40.7141	40.6508	0.1645
4	46.9607	47.4131	47.4152	47.2630	0.2618
5	52.5188	52.0654	51.5667	52.0503	0.4762
6	52.9101	52.3547	51.8429	52.3692	0.5337
7	58.5148	57.2618	57.1004	57.6257	0.7743

Table 3C Cumulative amount of permeated estradiol through a unit area of cellulose acetate membrane (Q_p), μg/cm^2 of estradiol/β-CD ratio = 0.7:1

Time(day)	Q_p(1)	Q_p(2)	Q_p(3)	Mean	SD
0	13.1772	12.4689	13.0150	12.8870	0.3711
1	30.6088	30.4994	30.5792	30.5624	0.0566
2	42.7814	42.8256	42.8413	42.8161	0.0311
3	56.1476	55.8161	56.0830	56.0156	0.1757
4	66.1777	64.7142	65.7850	65.5590	0.7575
5	73.1926	73.2757	72.9309	73.1331	0.1799
6	81.8352	80.7959	81.4592	81.3635	0.5262
7	87.7984	86.9396	87.5238	87.4206	0.4386

Table 4C Cumulative amount of permeated estradiol through a unit area of cellulose acetate membrane (Q_p), μg/cm^2 of estradiol/β-CD ratio = 1:1

Time(day)	Q_p(1)	Q_p(2)	Q_p(3)	Mean	SD
0	14.2716	13.2433	12.7292	13.4147	0.7854
1	29.5612	30.2667	32.2997	30.7092	1.4219
2	41.2038	42.0370	39.7074	40.9827	1.1805
3	53.6018	54.1265	48.8383	52.1888	2.9135
4	67.2976	65.1566	62.1792	64.8778	2.5705
5	71.3101	75.2162	66.2709	70.9324	4.4846
6	84.2271	88.1948	80.7233	84.3817	3.7382
7	80.4355	86.5743	85.2583	84.0894	3.2320

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