DEVELOPMENT OF POLYMERIC ROD AS AN IMPLANTABLE DRUG DELIVERY SYSTEM FOR LIVER CANCER THERAPY

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ABSTRACT

Poly(ε-caprolactone)-random-poly(D,L-lactide)-block-poly(ethylene glycol)-block-poly (ε-caprolactone)-random-poly(D,L-lactide) or PLEC, a biodegradable and biocompatible polymer, was used as a material to develop an implantable drug delivery system for liver cancer treatment. The aims were to optimize delivery of an anticancer drug to target tissues in a proper manner, and to reduce the elimination of the drug as well. PLECs were synthesized in 6 different forms, varied by its molecular weight, 20 and 50 kDa and the proportion of D,L-Lactide (LA) to ε-caprolactone (CL) which are 0, 10 and 20% LA by mole. A monolithic system of polymeric rods was developed by fabricating PLECs into a cylindrical shape containing 30% trypan blue, which was used as a model drug. The releasing study showed that increasing of LA contents contributes to a higher trypan blue release rate. On the other hand, the trypan blue release rate was found to drop off as the molecular weight of polymer increased. A reservoir system of drug delivery was then further developed by coating a monolithic system with a thin polymer film 100 and 200 μm in thickness, resulting in a more constant release rate, as well as extending the duration of releasing. Hence, this research is applicable to the modelling of liver cancer drug delivery system.

KEY WORDS: DRUG DELIVERY SYSTEM/ BIODEGRADABLE POLYMER/
LIVER CANCER/ RESERVIOR SYSTEM/ POLYMERIC ROD

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การพัฒนาแท่งพอลิเมอร์ สำหรับส่งยารักษามะเร็ง ที่สามารถถูกฝังลงในก้อนมะเร็งตับโดยตรง
DEVELOPMENT OF POLYMERIC ROD AS AN IMPLANTABLE DRUG DELIVERY SYSTEM FOR LIVER CANCER THERAPY

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บทคัดย่อ

พอลิเมอร์ poly(ε-caprolactone)-random-poly(D,L-lactide)-block-poly(ethylene glycol)-block-poly(ε-caprolactone)-random-poly(D,L-lactide) หรือ PLEC ซึ่งสามารถย่อยสลายได้เองตามธรรมชาติได้ถูกนำมาใช้เป็นวัตถุดิบ สำหรับการพัฒนาระบบส่งยาเพื่อรักษามะเร็งในตับ ระบบส่งยานี้สามารถถูกฝังโดยตรงเข้าไปภายในตับ เพื่อลดความถี่ในการรับยาและลดอัตราการสูญเสียของยา PLEC ถูกสังเคราะห์ขึ้น 6 ชนิด มีความแตกต่างกันของมวลโมเลกุล (20 และ 50 kDa) และอัตราส่วนของ D,L-Lactide (LA) ต่อ ε-Caprolactone (CL) ในอัตราส่วน LA 0, 10 and 20 % ของโมลรวม PLEC จะถูกเตรียมเป็นรูปทรงกระบอกขนาดเล็ก ที่มีสี Trypan blue อยู่ร้อยละ 30 โดยน้ำหนัก สำหรับใช้เป็นตัวแทนของยา จากการศึกษาการปลดปล่อยของสีออกจากแท่งพอลิเมอร์ พบว่าเมื่อเพิ่มอัตราส่วนของ LA อัตราการปลดปล่อยสีเพิ่มขึ้น แต่เมื่อเพิ่มมวลโมเลกุลของพอลิเมอร์ ทำให้การปลดปล่อยของสีช้าลง นอกจากนี้ยังได้พัฒนาแท่งพอลิเมอร์แบบระบบกักเก็บ (Reservoir system) โดยการเคลือบแท่งพอลิเมอร์ในแบบแรกด้วยแผ่นฟิล์มพอลิเมอร์ ที่มีความหนา 100 และ 200 ไมโครเมตร พบว่าสามารถควบคุมอัตราการปลดปล่อยของสีให้มีอัตราแบบคงที่ ในระยะเวลาที่กำหนด ดังนั้นงานวิจัยนี้จึงสามารถนำมาใช้ประโยชน์สำหรับเป็นต้นแบบในระบบส่งยาเพื่อรักษามะเร็งตับ

80 หน้า

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CHAPTER I
INTRODUCTION

1.1 Background

Cancer is the main cause of death worldwide and 7.4 million people died because of cancer (13% of all deaths worldwide) in 2007 [1]. The death worldwide from cancer is rising continuously and the estimation of the death will be 12 million in 2030. In 2006 and 66,000 people were killed by cancer in Thailand [2]. In medical term, cancer is called malignant neoplasm which means the group of cells that has three malignant properties. The first property is that cancerous cells have uncontrollable growth because cells can avoid apoptosis and multiply unlimitedly. Second, they are called invasion cells because the cells press and destruct an adjacent tissues and the last property is the cells metastasize or spread to other places of body *via* lymph or blood vessels. The main cause of cancer mortality is liver cancer which causes 598,000 deaths per year worldwide [3]. Hepatocellular carcinoma (HCC or Hepatoma) is one type of liver cancer. The important risk factors of HCC are Hepatitis virus infection (HBV and HCV), cirrhosis and alfatoxin B1 [3].In Thailand, liver cancer is first main cause of death in male and the third in female [4]. Nowadays, Polymer technology has been used in many fields including medical fields. Polymer is wildly used in medical application because of its properties such as thermoplastic property, thermal property, hydrophilicity, biocompatibility and biodegradability. Polymeric drug delivery is one of the polymer applications in medical which is a new trend of drug technology [5, 6].

1.2 Problem Statement

The conventional route of liver cancer chemotherapy is the intravenous injection. This route needs to access several systems until the drug reach the target. The drug route starts at blood circulation and transports to the liver. Then it diffuses

through the solid tumor. Diffusion through the solid tumor is very slow because of interstitial hypertension [7-11], so the convection of drugs into the solid tumor decreased. According to this problem, most of anticancer drugs cannot fully affect to the targeted cancer cells because of drug loss during transportation process and the obstruction by physical barriers. Moreover, untargeted drugs produce side effects to adjacent tissues and organs. Furthermore, the unpredictable amount of drugs on the targeted organs leads to the ineffective dose or overdose to the patients

CHAPTER II
OBJECTIVE

2.1 Objective

The problem of conventional chemotherapy is low efficiency, fast clearance and high side-effect of anticancer drug, so the drugs delivery system is necessary and needed to be developed. The objective of this study is to fabricate the drug delivery system that can transports proper amount of anticancer drug to the tumor by directly intratumoral implantation (Figure 2.1).

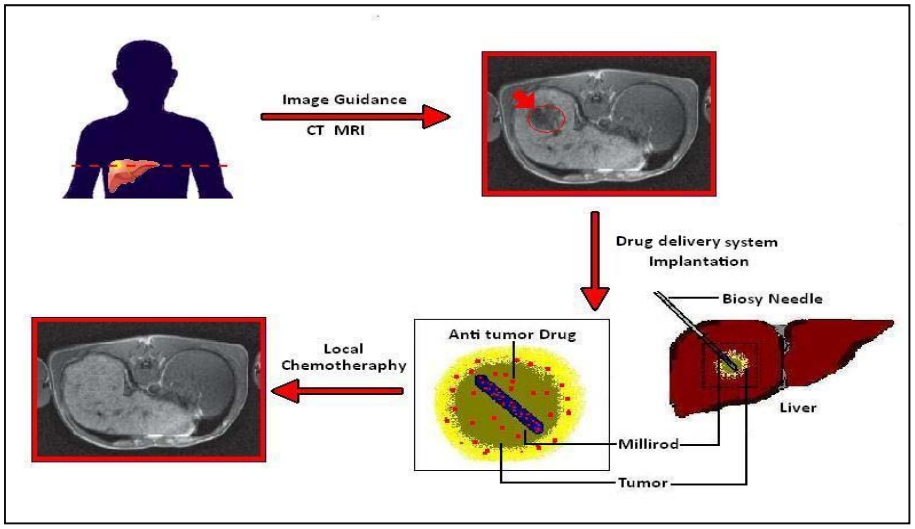


Figure 2.1 Schematic diagram of the utilization of polymeric rods for liver cancer therapy.

The advantages of this approach are as shown below.

1) This method is minimal invasive procedure which leaves a small wound after therapy and does not need to be removed because of the degradable property of polymer.

2) The optimum release rate dose and time.

3) This method can reduce the systemic drug concentration and decrease side effect of drug comparing to the conventional method.

In this study, the drug delivery system was developed using PLEC copolymer as a material and trypan blue dye as a representative of anticancer drug. Doxorubicin anticancer drug will be used in the next project as a result of the satisfied result of this study.

2.2 Scope of this study

PLEC copolymer is selected base on each component advantage. Poly(ε-caprolactone) (PCL), poly(D,L-lactide) (PLA) and poly(ethylene glycol) (PEG) have excellent abilities, including hydrophilicity, biocompatibility, low toxicity, absence of antigenicity and immunogenicity [12, 13]. PLEC copolymer is synthesized by ring opening polymerization. The ratio of CL:LA is varied to adjust the polymer properties. Next, PLEC is prepared as microspheres by the single emulsion procedure. Microparticles was mixed with trypan blue, molded into rod shape and coated with polymeric films. Then expected the trypan blue release from polymeric rods was performed in PBS buffer which the zero-order release.

2.3 Expected Outcome

The concentration of trypan blue release in PBS buffer was determined by UV-Vis spectroscopy at 586 nm which is the maximum absorption wavelength. The trypan blue would release at constant rate (zero-order release) over 2 weeks.

CHAPTER III
LITERATURE REVIEW

3.1 Polymer

Plastic, rubber, protein and nylon are the example of polymers which are common in daily. Polymer is from a Greek word “Poly” and “meros” which means “many part”, so the word polymer is defined as large structural molecules containing repeating units of monomer and the linkage between each monomer can be forming a long chain polymer. Accordingly, the polymer properties are controlled by ratio of monomer component. Cotton, wool, starch and protein are natural polymer products. The polymer products which are synthesized by human are nylon, plastic bags etc.

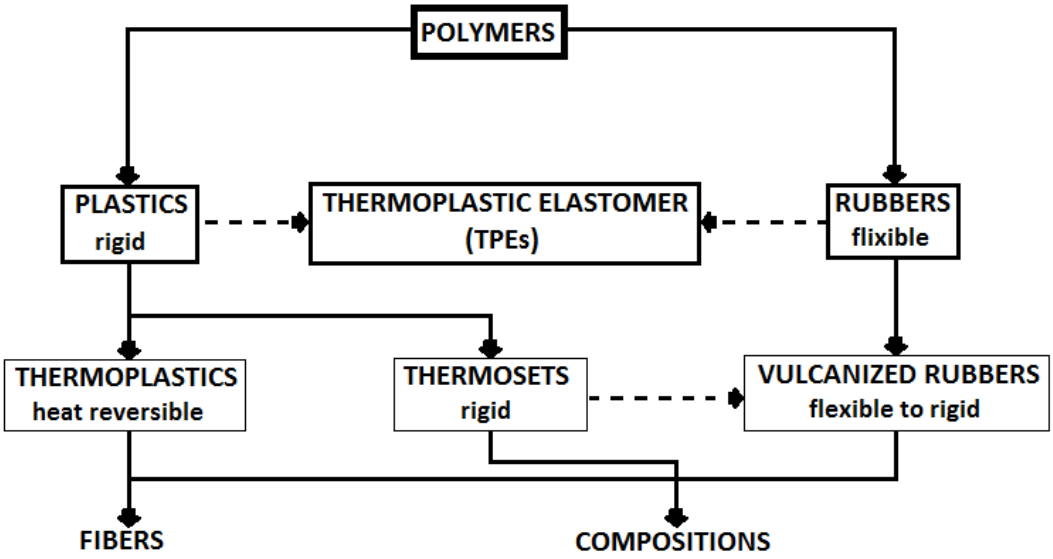


Figure 3.1 Classification of polymer by their properties.

3.1.1 Polymeric properties for drug delivery system

Nowadays, polymers can be applied for several research fields for example cosmetic and pharmaceutical research. The new technology of pharmaceutical uses polymer as a carrier or a transporter of drugs. The polymeric drug delivery system in pharmaceutical can control the dose of drug releasing and automatically seek the target cells. Polymeric properties can be modified to the drug delivery development as shown below.

3.1.1.1 Thermoplastic property

Polymer is melted by heat, so it can be formed in different shapes. The physical outlook of drug delivery system depends on route of administration for instance the nano-scale drug delivery is used for intravenous injection route that travels *via* vessel and gets to target organs. Another example is the solid tumor which evades in organ with high inner pressure, so constantly solid shape is proper.

3.1.1.2 Thermal property

Glass transition temperature (T_g) indicates the temperature which can break amorphous structure of polymer. The polymer will be melted over the melting temperature (T_m). The thermal property can be changed by various copolymer ratio.

3.1.1.3 Hydrophilicity

The hydrophilicity is defined as “having a strong affinity for water and tending to dissolve in water”. Polymeric solubility in water affects the degree of water absorption and releasing rate. The polymeric hydrophilicity can be controlled by ratio of monomer component.

3.1.1.4 Viscoelastic property

The polymer chain can move, so the drug molecules diffuse easily through polymeric matrix. And the same manner, the drug diffusion rate can be managed by ratio adjustment of monomer component which affects the movement of polymer chain.

3.1.1.5 Biocompatibility and Biodegradability

Non-toxic property is an important property of polymers. Moreover, the polymer is a self-degradable material. The post operation removing is not required.

3.1.2 Polymer in drug delivery system

Polyester has been extensively using in drug delivery applications. The polymer is synthesized by ring-opening polymerization which gives narrow distribution of molecular mass. The "Autocatalytic property" is designed in this polymer which is the bulk eroding degradation generates acid inside the polymer which leads to the inside out degradation.

3.1.2.1 Poly (ε-caprolactone) (PCL)

PCL is semicrystalline polymer with five methylene groups (-CH2) in its chain resulting in the high flexible property of PCL, drug molecules can easily diffuse through PCL polymeric matrix [14]. When PCL is used as copolymer, its hydrophobic property can prolong degradation time [15]. PCL has been using in many applications of drug delivery, Nasongkla, N., et al. formed PCL micelles and modified surface with $\alpha_v\beta_3$ ligand (cRGDfK) that delivered the Doxorubicin (anticancer drug) to targeted cancer cells [16]. Additionally, Gong, C. Y. et al. synthesized so-gel of PCL-PEG copolymer with temperature sensitivity [17].

3.1.2.2 Poly (lactide-co-glycolic acid) (PLGA**)**

The PLGA is mostly used in the drug delivery system application for cancer therapy [18-20]. PLGA properties can be controlled by copolymer adjustment such as increases ratio of glycolide which results in raising hydrophilicity and degradable rate. For instance, the commercial drug delivery product "Liporn Deport®" delivers Leuprolide hormone for prostate gland cancer and endometriosis, and it is verified by FDA. Qian, F., et al. fabricated PLGA millirod which contained doxorubicin and was implanted into rats liver [21].

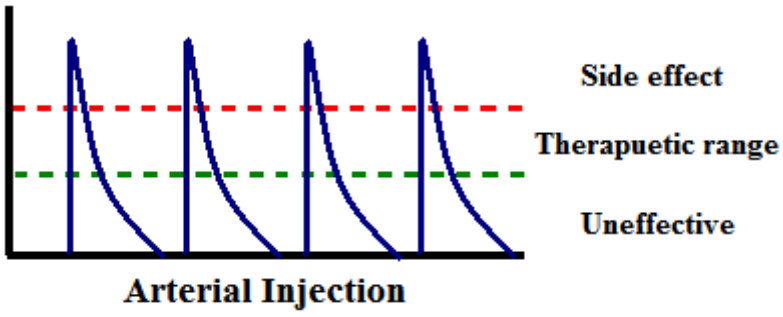
3.2 Drug delivery

Drug delivery is the administration of pharmaceutical compounds to reach a therapeutic effect in humans or animals [7]. The common route of administration are non-invasive peroral (through the mouth), topical (skin), transmucosal (nasal, buccal/sublingual, vaginal, ocular and rectal) and inhalation. Some of medications such as peptide and protein, antibody, vaccine, gene based and harmful drugs are limited for common routes. Consequently, the drug delivery system is developed to contain and transport drug to target organs for example the hormone delivery system was used for avoiding enzymatic degradation of peptide and the anticancer drug delivery system can reach therapeutic effect with less side effect [22, 23]. Hence, the therapeutic effect is achieved by pharmacokinetic modification which can control the drug release within therapeutic range as long as required. Moreover, the alternative ways of drug delivery increases drug's efficiency, safety and patient convenience.

3.2.1 Controllable dose

The drug delivery system that has high efficiency and safety should be drug with controllable dose in therapeutic range for an appropriate period of time. The comparison of 3 different routes is shown in Figure 3.2. The first figure shows the intravenous injection immediately arises and reduces of blood's drug concentration in short time (Figure 3.2a), the release profile is sustained decreasing in oral route of drug administration (Figure 3.2b) and the controllable drug delivery provide stable and predictable dose of drug in therapeutic range (Figure 3.2c).

(a)



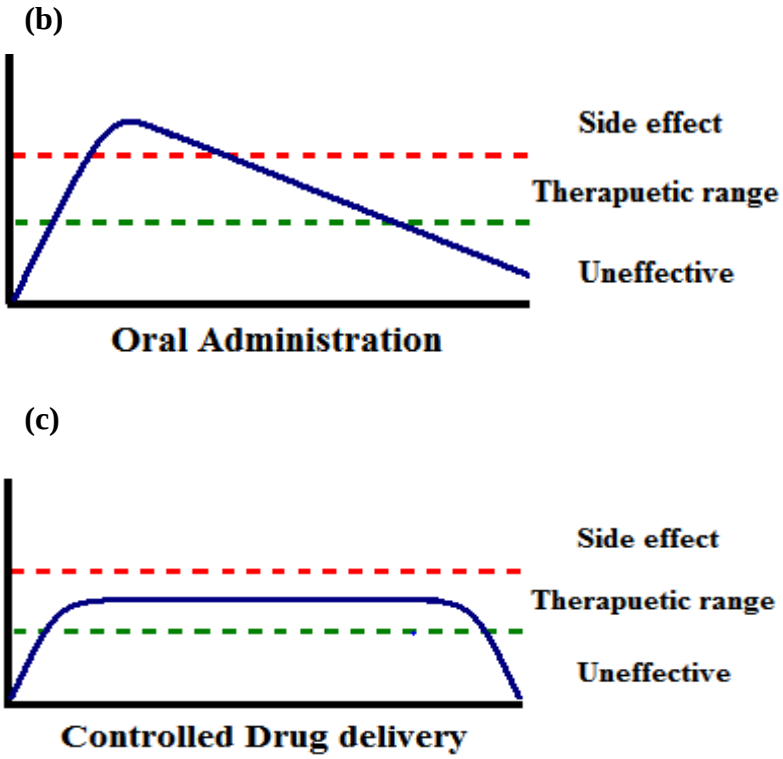


Figure 3.2 Dosing of different routes drug administration.

The pharmacokinetic is considered for drug delivery system development that includes drug absorption-distribution, elimination and side effect as shown below.

3.2.1.1 Drug absorption-distribution

The drug movement starts at the administered site, passes through several physiological systems to blood circulation and diffuses into extracircular spaces. The massive particles size and hydrophilic drug is the obstacle in drug movement and causes inefficiency therapy. The drug delivery system can avoid movement problems by contain drug and directly administer to target organ. The "Gliadel wafer" is used in BCNU delivery for brain tumor therapy by directly implants for conquers the blood brain barrier blocking [6, 24-26].

3.2.1.2 Drug elimination

Liver and renal are drug eliminator organs that use metabolism and excretion, respectively. Most of drugs have small molecules, so they are easily filtrates through glomerular filtration resulting in shorter drug half-life. The nano-particle drug delivery system is made of polymers or biological compounds such as

liposome or cellulose which have been developed in many aspects such as keeping drug inside, surrounded by targeting substance for auto-targeting, admonition *via* IV route, the protection from immune system and increase half-life of drug [27, 28].

3.2.1.3 Side effect

The anticancer drug is common used in chemo-therapy for cancerous cells elimination but it also kills normal adjacent cells. The conventional chemo-therapy via IV injection route is difficult to control dose in narrow therapeutic range and has a risk to reach overdoses which is harmful to patients. The controllable dose and intratumoral drug delivery systems developed in this study [29].

3.2.2 Zero-order release

The controllable and predictable drug delivery system should have steady drug release profile or zero order release which are defined as a drug release at constant rate and maintains the drug concentration with a desirable pharmaceutical dosage. Moreover, it minimizes the side effect but prolongs therapeutic concentration time which reduces uptake orders. It is explained by following equation.

$$\frac{dM(t)}{dt} = K_0 \qquad (1)$$

Where M(t) is the released drug at certain time
dMt/dt is the drug release rate

Other releasing orders are generated unsteady release rate. The first order produces high release rate on early period and fast decays until it stops or the rate of drug release depends on the gradient concentration which is described by following equation.

$$\frac{dM(t)}{dt} = K_1 (M_0 - M(t)) \qquad (2)$$

Where M_0 is the amount of drug input

The square root of time release shows the releasing rate that inversely proportion to the square root of time, so the drug releasing rate decreased by the time which is shown by following equation.

$$\frac{dM(t)}{dt} = \frac{K_{1/2}}{t^{-1/2}} \quad (3)$$

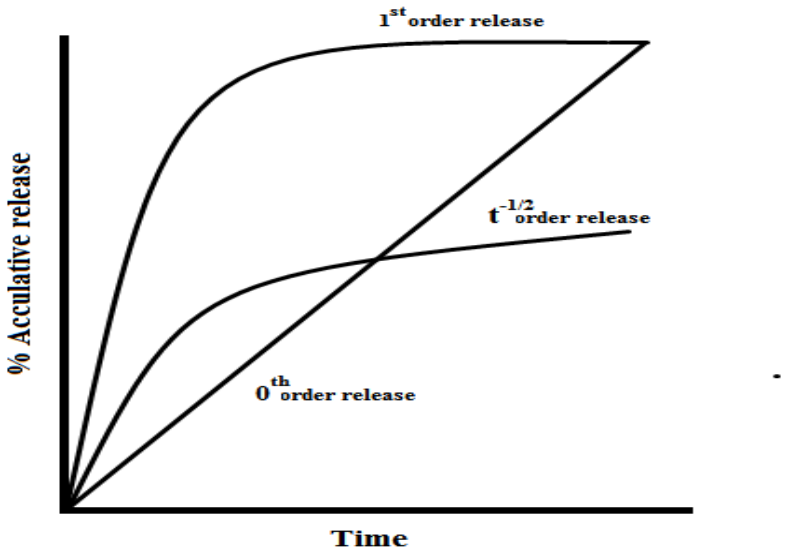


Figure 3.3 Curves of three types of drug release.

3.2.3 Reservoir drug delivery system

In this study, the polymeric cylindrical shape drug delivery system was developed. The drug release in drug delivery system depends on three mechanisms. First mechanism is diffusion mechanism that the drug inside the molded polymer diffuses out via polymeric matrices. The second mechanism is the osmosis or swelling mechanism causes solvent spread into the molded polymer and the last mechanism is polymeric degradation which changes physical and chemical structure of polymer and leads to drug release. Nevertheless, the first mechanism is the only mechanism that can control drug release by the design of the polymer matrix that allows the desirable

dose of drug passing through at constant rate. The degradable drug delivery system cannot control drug release so the osmosis and degradation mechanism are ignored.

In theoretical, the polymer matrix can be considered as a membrane that separates between the inner layer (drug and polymer matrix) and the outer layer (aqueous environment). The drug passes through polymeric matrices spaces in random manner. Therefore, the gradient concentration of membrane can be described by Fick's law as following.

$$J = - \frac{DdC_m}{Dx} \quad (4)$$

Where J is the diffusion flux which shows the continuous transportation of molecules passing the intermediate plain in equilibrium. ($g/cm^2.sec$)

D is the diffusion coefficient which depends on molecular size and mechanical properties. (cm^2/sec)

dC_m/dx is the change in concentration inside a membrane.

The concentration changing dC_m/d_x can rewrite in $K(C_{in}-C_{ex})/L$.

Therefore,

$$J = \frac{D\,K(C_{in}-C_{ex})}{L} \quad (5)$$

$$J = \frac{D\,K\Delta C}{L} \quad (6)$$

And,

$$J = P\,\frac{K\Delta C}{L} \quad (7)$$

Where K is the distribution coefficient

L is the membrane thickness

ΔC is the concentration gradient between inside and outside

P is the the permeability. (cm^2/sec)

Therefore,

$$\frac{dM_t}{dt} = \frac{AD\,K\Delta C}{L} \quad (8)$$

Where A is the surface area of drug delivery system

The assumption, external drug will be clearance by body so $C_{ex} = 0$ therefore $\Delta C = C_{in}$.

$$\frac{dM_t}{dt} = \frac{AD\,KC_{in}}{L} \quad (9)$$

From equation (6) and (9), the drug release rate is depending on following factor.

1. Diffusion coefficient (D)
   - Molecular size
   - Chemical property of drug and polymer
   - Interaction between drug and polymer
2. Drug concentration inside delivery system (C_{in})
3. Membrane thickness (L)
4. Permeability (P)
5. surface area (A)

In this case, the cylindrical shape of the polymeric drug delivery system is used as the main model. The traditional type of drug delivery system is “Monolithic system” where drugs are homogenously distributed all over a device. Therefore, diffusion coefficient (D) and drug concentration (C_{in}) are regulating factor of drug releasing rate. Chemical properties of the polymer will change regarding to the time. Drug concentration will decrease until drugs inside system reaches the depletion. The effect of D and C_{in} provide the first-order release which is a high release rate at early period and are reduced by gradient concentration. Unfortunately, the burst release always occurs at the beginning because of the presence of drug on the surface of the device [13, 30].

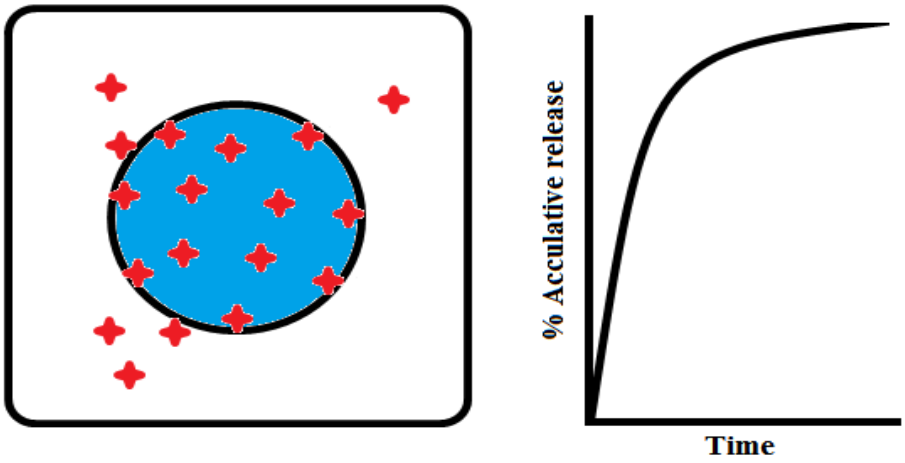


Figure 3.4 Monolithic cylindrical drug delivery system.

On the other hand, "Reservoir system" is fabricated by coating the monolithic system with polymeric film. A film acts as a drug store and allows drug to pass through at constant rate. The burst release is neglected and zero-order release will occur [31]. The adjustment of polymeric film properties such as hydrophilicity, glass transition temperature and membrane thickness can control drug releasing rate [30, 32, 33]. Additionally, the spin coating is designed for the reservoir system fabrication in this study.

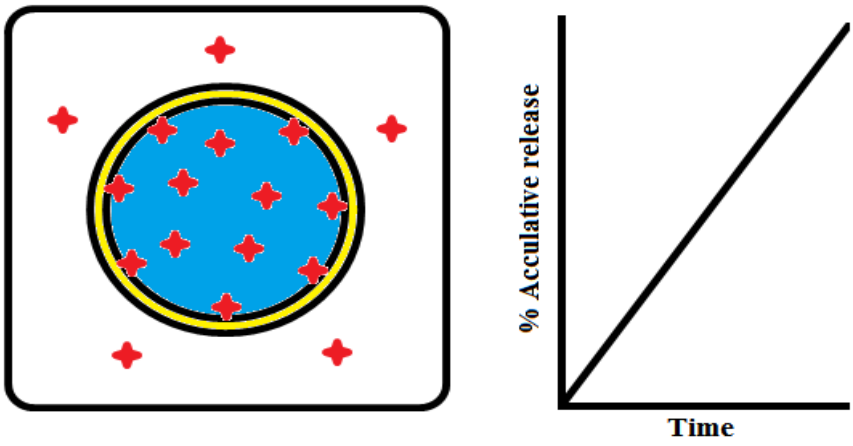


Figure 3.5 Reservoir cylindrical drug delivery system.

3.3 Previous works and study design

Research in an alternative drug delivery technology originated around 1980. In 1976 Langer and Folkman [34] reported that the ethylene vinyl acetate (EVAc) which is non-degradable copolymer for protein delivery resulted in a decrease of the release rate followed by first-ordered release kinetics. The polymer is usually used for drug delivery system in cancer therapy because of its suitable properties such as the viscoelastic property, so it can be formed into several shapes. Moreover, polymer can be digested by itself or biodegradation.

Langer and Brem [35, 36] developed polymer wafers which contained BCNU (carmustine), implanted in brain cavity (after tumor surjection) and released anticancer drug (BCNU) within 2-3 weeks. *In vivo*, rats with glioblastoma multiforme were treated by BCNU wafers could be prolonged the survival time to 62.3 days but rats which were treated by IP route had 27.3 days of survival time. In human trials, 19% and 6% of the patients in placebo group survived in 1 and 2 years respectively. But the groups that were treated by polymer wafers could survive 63% and 31% in 1 and 2 years respectively. In 1996, the BCUN polymer wafer was allowed by FDA to use as alternative treatment of brain cancer.

Dhanaraju, M. D., et al. [37], used poly (ε-caprolactone) (PCL) as intramuscular injectable drug delivery system. PCL microspheres were made by w / o /w double emulsion procedure which encapsulated the contraceptive steroids levonorgestrel (LNG) and ethinyl estradiol (EE). According to the demonstration, burst release was found on both in vitro and in vivo and complete degradation needed more than 20 weeks.

Poly (lactide-co-glycolic acid) or PLGA is common used in drug delivery applications because PLGA is biodegradable and non-toxic for living organism including human cells which called biocompatible. Qian, F., et al [38] fabricated polymeric millirod (10 mm length, 1.6 mm in diameter), contained trypan blue and shaped by heat-compression at 4.6 MPa about 2 hours which reproducible temperature is 90 ºC. Although, the burst release always occurs.

Another PLGA application was developed by Weinberg, B. D., et al. [5], millirods were made from PLGA and doxorubicin which were implanted into rabbit liver tumor. The results demonstrated that the treated tumors were smaller than non-

treated on both day 4 (0.17 ± 0.06 vs. 0.31 ± 0.08 cm^2, p = 0.048) and day 8 (0.14 ± 0.04 vs. 1.8 ± 0.8 cm^2, p = 0.025). In detail, DOX release half-time is 4 hr and drug releasing 71.3 ± 1.7% over 8 days period but 1.89 ± 0.03 released in 24 hr. Furthermore, over 1,000 ug/ml Doxorubicin is detained at core of tumor and it penetrated along 2.8 and 1.3 mm on day 4 and 8, respectively.

Liporn Deport® is commercial name of drug delivery system which is used to transport Leuprolide for prostate gland cancer and endometriosis. It is recognized by FDA. Products of polymer drug delivery have value about billion dollars US per year in USA.

PLGA has low Hydrophilicity. To manage the release rate, the excipient molecule such as NaCl crystal or sugar which is the compound with high hydrophilicity [31, 39, 40] is necessary. These excipient molecules have poor distribution in the mold which can cause inconstant release or disappointed release rate. Another problem of the drug delivery device is incomplete release of drug in device. This is due to the amount of drug is below the percolation threshold. The percolation threshold is defined as the minimum requirement of drug to generate the drug continuous phase inside the polymeric device. Above the percolation threshold, the drug can form interconnecting channel and generates continuous phase which leads to the complete release. In this study, PEG which is the component of block copolymer PLEC is designed to act like excipient molecules and distributes throughout the polymeric device.

The polymer in this study is ([poly (caprolactone)-random-poly (lactide)]-block-poly (ethylene glycol)-block-[poly (caprolactone)-random-poly (lactide)]) or PLEC. PLEC copolymer is synthesized by selecting the advantage of both PCL and PLA. PEG is selected as an initiator because of outstanding properties of PEG such as non-toxicity, hydrophilicity, biocompatible and low antigenicity [41, 42].

1) Epsilon-Caprolactone (PCL) is cyclic ester monomer with ring size of seven. It is a clear colorless liquid that is miscible with most organic solvents except aliphatic hydrocarbons. The ε-caprolactone has melting point and boiling point at -1 ºC and 253 ºC, respectively. The density is 1.030 g/L and molar mass is 114.14 g/mol [43]. PCL has been explored to use as drug delivery system. PCL is biocompatible for human tissues and can be metabolized by the body [44].

PCL has good permeability for drug diffusion because of its small molecules and high flexibility in polymer chain [12].

2) Poly-(D,L-lactide) (PLA) is the product that results from polymerization of D,L-lactide which is amorphous polymer. PLA has a glass transition temperature between 50-80 °C [45]. PLA segment raises biodegradable rate of PLEC polymer because it has moderate hydrophobicity that compensates high hydrophobic property of CL. Therefore, adjusting the ratio of CL/LA can control permeability and biodegradability [46-48].

3) Poly (ethylene glycol) (PEG) also known as poly (ethylene oxide) (PEO) or polyoxyethylene (POE). PEG is polymerized from ethylene oxide. PEG is insoluble in diethyl ether and hexane but soluble in methanol, benzene, dichloromethane and especially water, PEG dissolves very fast in water because of very high hydrophilicity [49]. PEG has been wildly used in cosmetic application because it has biocompatibility and absences of anti-genicity and immunogenicity [13, 41, 42].

Previous studies prepared PLEC in nano-scale polymeric drug delivery system. Furthermore, the zero-order release kinetics of PLEC release profile can be achieved by adjusting the ratio of hydrophilic/hydrophobic monomers (Lactide/Caprolactone) [13, 50]. Caprolactone has long chain of methylene group which provides good permeability. Lactide increases biodegradable rate. The cylindrical polymeric drug delivery system was chosen in this study, Because the cylindrical shape (10 mm length and 1.6 mm in diameter) fits with modified 14-gauge biopsy needle which is used as a drug implantation device in liver tumor [5]. Millirod is fabricated by heat-compression procedure at 4.6 MPa about 2 hours and 90 ºC [13, 50]. Moreover, a reservoir system is another approach to provide zero-order release. Qian, F., et al. [31] fabricated PLGA polymeric millirod which contained 5-fluorouracil and encased by polymeric membrane. The study found out that sustained release of 5-fluorouracil [rates between 0.1 and 0.4 mg/(day. cm of millirod)] in 2 or 5 weeks can achieve by polymeric membrane encased millirods [31].

In this study, PLEC block copolymer was synthesized by ring-opening polymerization in different ratio of Lactide/Caprolactone [13, 50-52] and fabricate in cylindrical shape that contains 30 % w/w trypan blue. Moreover, polymeric rods are

coated with PCL polymeric film by spin coating method to provide the reservoir system. The best ratio which provides zero-order release profile will be chosen as drug delivery system model to deliver doxorubicin for liver cancer therapy via implantable drug delivery system.

CHAPTER IV
MATERIALS AND METHODS

4.1 Materials

4.1.1 Chemical reagents

Poly (ethylene glycol) with a molecular weight 1000 Da was purchased from Aldrich. Caprolactone was dried with calcium hydride (CaH_2) and then distilled at reduced pressure. D,L-lactide (Aldrich) was re-crystallized from ethyl acetate and vacuum dried at room temperature. Toluene (Aldrich) was dried by refluxing over sodium and distilled under dry argon.

All other chemicals were reagent grade and purified by distillation which supplied by BioNEDD Lab, Biomedical engineering department, Faculty of engineer of Mahidol University.

4.1.2 Instruments

Freeze-drying machine (EYELA, Model:FDV-1200) and microscope (OLYPUS- CKX41) were from department of biomedical engineering, Faculty of engineer, Mahidol University.

NMR spectroscopy and Gel permeation chromatography (GPC) were supported by department of chemical, Faculty of science, Mahidol University.

UV-visible spectroscopy (Spectrumlab, Model: 752S) was from department of biomedical engineering, Faculty of engineer, Mahidol University.

Scanning electron microscope (HITASHI Model: S-2500) was from Center of nanoimaging (CNI), Faculty of science, Mahidol University.

Incubator-orbital shaker (Biosan ES-20/60), Centrifuge (Biosan LMC-3000) and sonicator (Sonic-VibraCell™, Model CV.18) and other instruments were supported by BioNEDD Lab, Biomedical engineering department, Faculty of engineer of Mahidol University

4.2 Methods

4.2.1 Synthesis of block copolymer of PEG and CL/LA

Block copolymers of PLEC ([Poly (caprolactone)-random-Poly (lactide)]-block-poly (ethylene glycol)-block-[Poly (caprolactone)-random-Poly (lactide)]) with different ratio of CL and LA are synthezied by “ring opening bulk polymerization”. PLEC polymerization procedure has been reported by Hu, Y., et. al. [13]. PEG is used as a macro-initiator and (Tin)octoate is used as a catalyst. PEG, CL and LA are weighed in two-neck round bottom flask under argon atmosphere. Then the air is vacated about 6 hours and substituted by Argon. The reaction flask is immerged in oil bath and heated at 140 C°. The reaction starts when 0.1 % (from the total weight of monomers) of the catalyst (stannous octoate) is added. The reaction is allowed to carry out for 48 hours. Solid PLEC is dissolved in acetone and precipitates by 1/10 cold methanol. The sediment of PLEC is separated and lyophilized by freeze-drying machine.

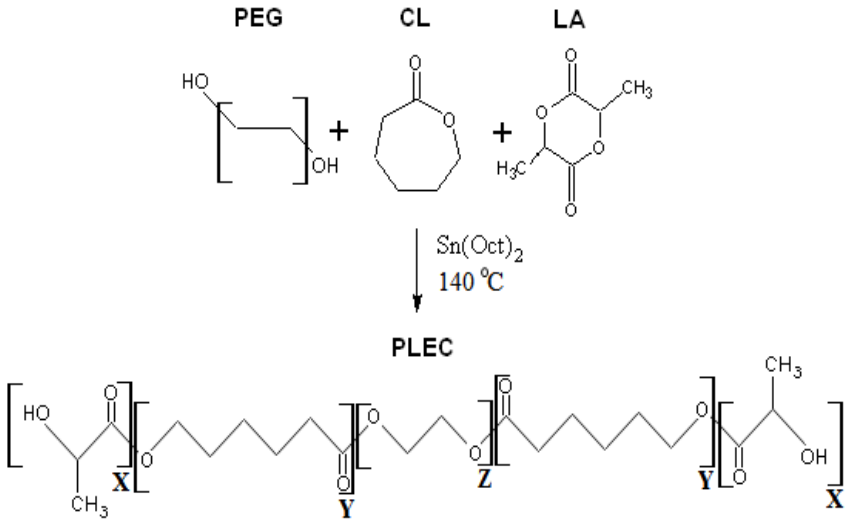


Figure 4.1 Scheme of PLEC polymer synthesis.

The PLEC block copolymers are synthesized in 6 different ratios. The molecular weights are 21 kDa and 51 kDa. Moreover, percentages of lactide are also varied at 0, 10 and 20 % mole for each molecular weight.

Table 4.1 Expected result of PLEC polymer synthesis.

PLEC	PEG (kDa)	CL		LA		MW of PLEC (kDa)
		(kDa)	% mol	(kDa)	% mol	
1	1	0	100	0	0	21
2	1	18	90	2	10	21
3	1	16	80	4	20	21
4	1	0	100	0	0	51
5	1	45	90	5	10	51
6	1	40	80	10	20	51

4.2.2 Chemical component characterization of PLEC

NMR spectroscopy is one of the principal techniques used to obtain physical, chemical, electronic and structural information about molecules due to the chemical shift and Zeeman Effect on the resonant frequencies of the nuclei. It is a powerful technique that can provide detailed information on the topology, dynamics and three-dimensional structure of molecules in solution and the solid state [53] .The MNR spectrum provides information on the number and type of chemical entities in a molecule. Therefore, the integral area under peak can calculate to chemical component of each monomer [54], peak “a” at 5.1 ppm (-CH), peak “b” at 4.0 ppm (-CH2) and peak “f” at 3.6 ppm (-CH2) are assigned to segment of LA, CL and PEG, respectively.

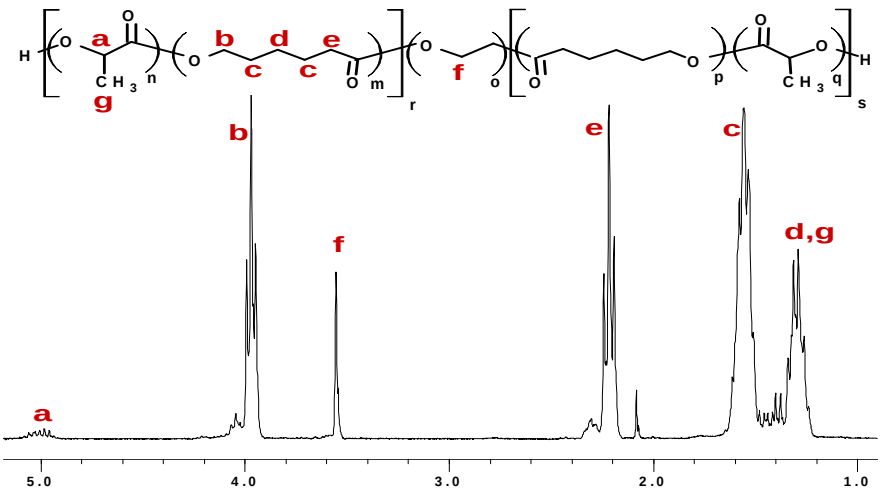


Figure 4.2 ^{1}H NMR spectrum of PLEC in CDCl3.

4.2.3 Molecular weight determination of PLEC

Molecular weight which is a fixed parameter for this study affects the degradable rate of millirod. Gel Permeation Chromatography (GPC) is a separation technique based on hydrodynamic volume (size in solution). Molecules are separated from one another based on difference of molecular size. This technique is widely used to determine molecular weight of PLEC. THF is used as a mobile phase.

4.2.4 PLEC microsphere preparation

PLEC is prepared into microsphere by single emulsion procedure which has been described by Qian, F., et al. [38]. The “Oil in water” is principle for this method. PLEC in methylene chloride acts like oil which isolates from water and forms droplet. PLEC 200 mg was dissolve in 2 ml methylene chloride. Then, slowly drop PLEC solution in 100 ml of 1% w/v poly (vinyl alcohol) while mixed by sonicator at 60 % amplitude (130 Watt 20 kHz) for 5 minutes. Next, the mixed solution is added in 300 ml of 1% w/v poly (vinyl alcohol) and stirred at 300 rpm. Methylene chloride allows to evaporate for 4 hours where PLEC droplets are formed into micro-particle. PLEC microspheres are collected by centrifugation, 3 times washed by water and lyophilized.

4.2.5 Size measurement of PLEC microsphere

PLEC microspheres are spread on the slide and taken picture through microscope at 40X magnification. Fifty particles are measured by using images processing software “Image J” and calculate the average size of particles**.**

4.2.6 PLEC Millirod molding

PLEC microspheres in 6 different ratios are prepared into polymeric rod by compression-heat molding. The preparation starts when mix PLEC microspheres are mixed with 30% w/w of trypan blue homogenously by vertex mixer. Then the mixture is filled the in mold which has cylindrical holes with 1.6 mm in diameter and 10 mm length. Anneal PLEC at 90 °C in 4.6 x 10^6 Pa pressure for 2 hours, cool down in room temperature before taking the mixture out off the mold.

4.2.7 Morphological observation of PLEC millirod

The observation of morphology and porous matrices of polymeric rod carried out by the Scanning Electron Microscope (SEM) which is a type of electron microscope that images the sample surface by scanning with a high-energy beam of electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition and other properties such as electrical conductivity.

4.2.8 PLEC millirod Spin coating

Reservoir system of PLEC polymeric rod is fabricated by a spin coating device. The poly (ε-caprolactone) (PCL) in acetone at 40 mg/ml is sprayed on the PLEC polymeric rod which is rotated at 100 rmp. Both end of spin-coated millirod is dipped in 400 mg/ml of PLEC solution [31].

4.2.9 Release study of PLEC millirod (*in vitro*)

Phosphate buffer saline (PBS) pH 7.4 at 37 °C is used as a buffer to study the release rate of trypan blue. A coated polymeric rod is divided into 3 pieces and weighted. Then put each millirod into the vial filled with 15 ml PBS. Vials are shaken by orbital shaker (Biosan ES-20/60) which is controlled at 120 rpm and at 37 °C of environment. At the sampling time, PBS in vials are removed out and refreshed with new PBS. The PBS samples are measured the concentration of trypan blue by UVWINLAB UV-Visible spectroscopy at 586 nm. Use the concentration of trypan blue in each time to provide the accumulative release percentage.

4.2.10 Water absorption study of PLECs polymeric rods

The water absorption is the main mechanism for drug release of PLEC, so the water absorption character may be lead to understanding and predicting of drug release. The PLEC rods are incubated in PBS and it is recorded the changing weight at the sampling time.

4.2.11 Degradation study of PLECs polymeric rods

The five month degradation study and it is investigated the physical and chemical result by SEM and GPC, respectively. The interesting results are porosity and morphology for SEM. While, GPC results are focus on molecular weight and the PDI changing.

CHAPTER V
RESULTS AND DISCUSSION

5.1 The synthesis of block copolymer of PEG and CL/LA

The PLEC block copolymers were synthesized in 6 different types which were varied both total molecular weight and copolymer ratio. The D,L-lactide (LA) monomer was fed at 0, 10 and 20 percentage by mole while the molecular weights were defined as 20 kDa (PLEC 1-3) and 50 kDa (PLEC 4-6). Then, nuclear magnetic resonance spectroscopy (NMR) and gel permeation chromatography (GPC) were used to characterize PLEC polymers. The results are shown in Table 5.1.

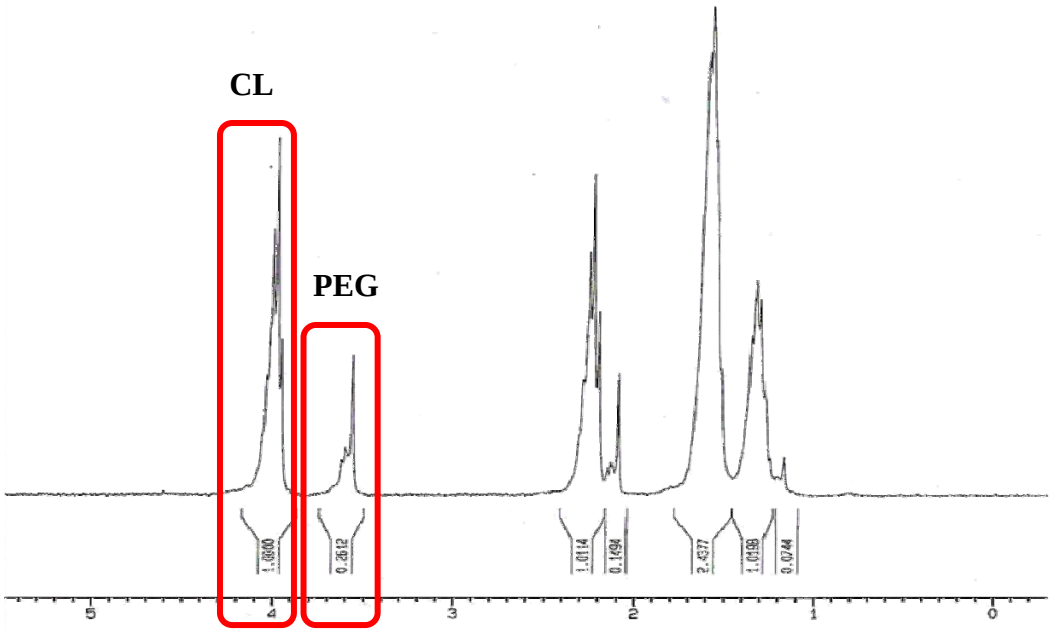
Table 5.1 Chemical composition of PLEC as determined by ^{1}H NMR and GPC

	LA		CL		MW of PLEC
PLEC	MW (kDa)	mol %	MW (kDa)	mol %	(kDa)
1	0.0	0.0	19.83	100.0	20.83
2	2.09	13.43	21.33	86.57	24.42
3	2.99	17.89	21.77	82.11	25.76
4	0.0	0.0	52.98	100.0	53.98
5	6.42	15.87	42.37	84.13	49.79
6	4.87	19.34	40.93	80.66	46.80

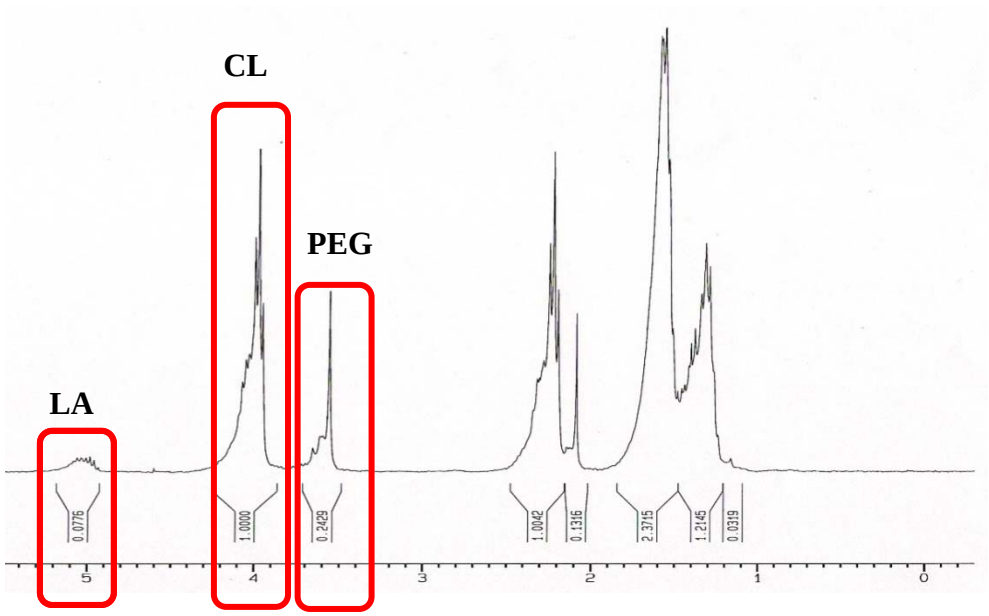
The ε-caprolactone (CL) is a semi-crystalline polymer. It forms crystalline regions or perfect packing structure. The D,L-lactide (LA) was polymerized into PLECs not only increases the hydrophilicity of polymer but also decreases crystalline structure of PLECs.PLEC 1 and 4 were without D,L-lactide where PLEC 2 and PLEC 3 contained 13.43 and 17.89 % D,L-lactide by moles, respectively. The molecular weights were at 20.83 kDa, 24.42 kDa and 25.76 kDa for PLEC 1, PLEC 2 and PLEC3, respectively.

The ^{1}H NMR spectrum represented the component of PLECs. The peak of CL ($-CH_2-$), PEG ($-CH_2-$) and LA (-CH-) were represented at 4.0, 3.6 and 5.1 ppm, respectively. The LA peaks at 5.1 ppm were found in both PLEC 2 (Figure 5.1b) and PLEC 3 (Figure 5.1c). Therefore, the LA's peak can be indicated the amount of LA from its integral peak area.

(a)



(b)



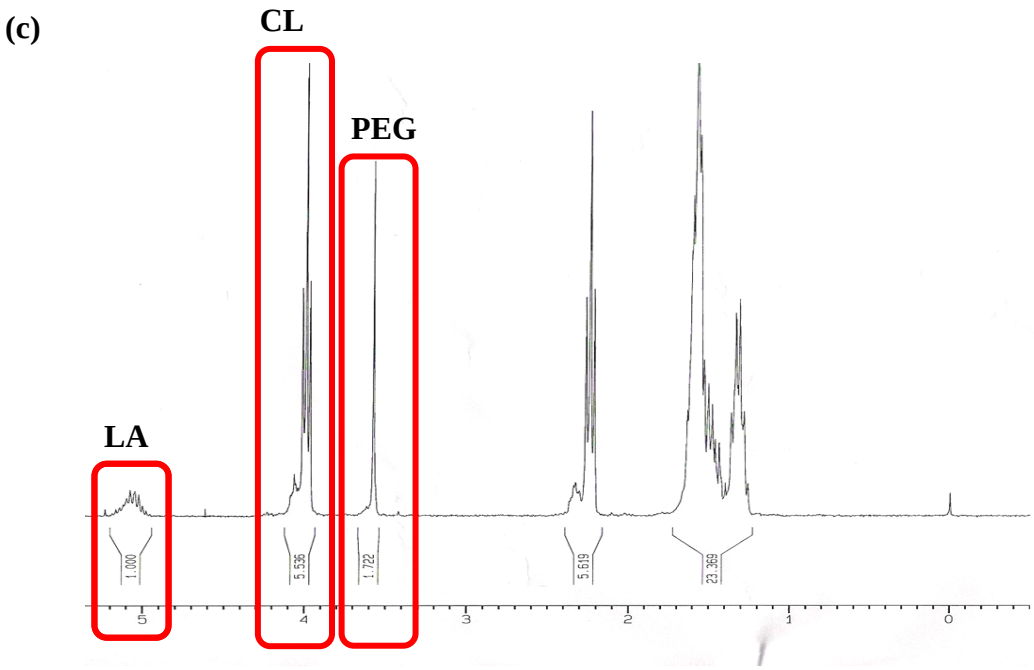
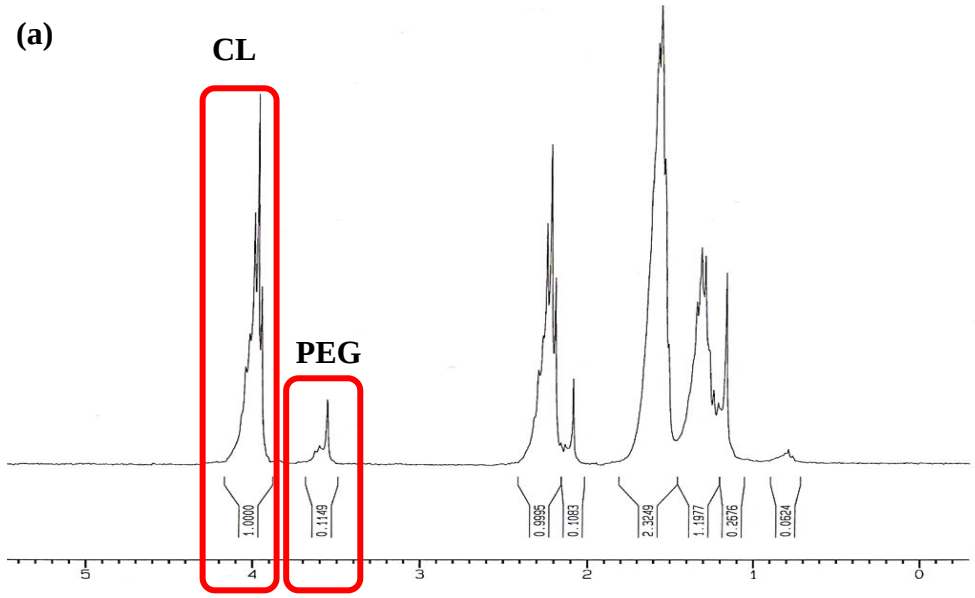
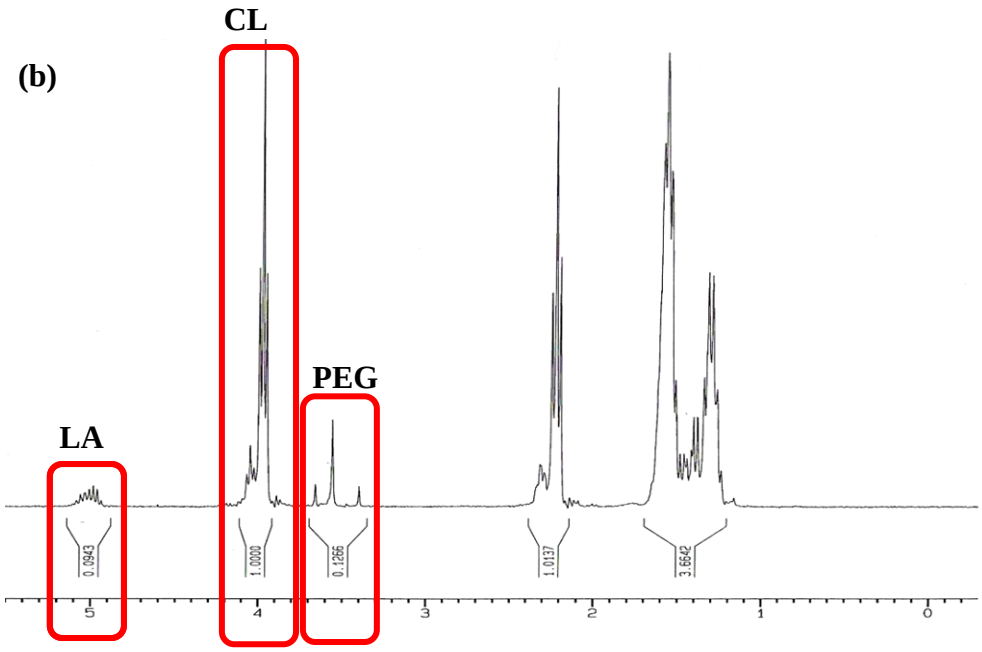


Figure 5.1 ^{1}H NMR spectrum of (a) PLEC 1, (b) PLEC 2 and (c) PLEC.

The molecular weight of another set of polymer increased from 20 kDa to approximately 50 kDa as showed in the last three rows of Table 2 (PLEC 4, PLEC 5 and PLEC 6). The percentage of LA and the molecular weight were identified by NMR and GPC technique in the same manner as first set. The PLEC 4, PLEC 5 and PLEC 6 were the percentages by mole of D,L-lactide at 0, 15.87% and 19.34%, respectively. The molecular weights were at 53.98, 49.79 and 46.80 kDa, respectively. In additional, the NMR spectrums were showed the component of PLEC and the LA's peak disappeared on spectrum of PLEC 4(Figure 5.2a).


(a)
CL
PEG
1.0000
0.1149
0.9995
0.1083
2.3249
1.1977
0.2676
0.0624
5
4
3
2
1
0


(b)
CL
PEG
LA
0.0943
1.0000
0.1265
1.0137
3.6642
5
4
3
2
1
0

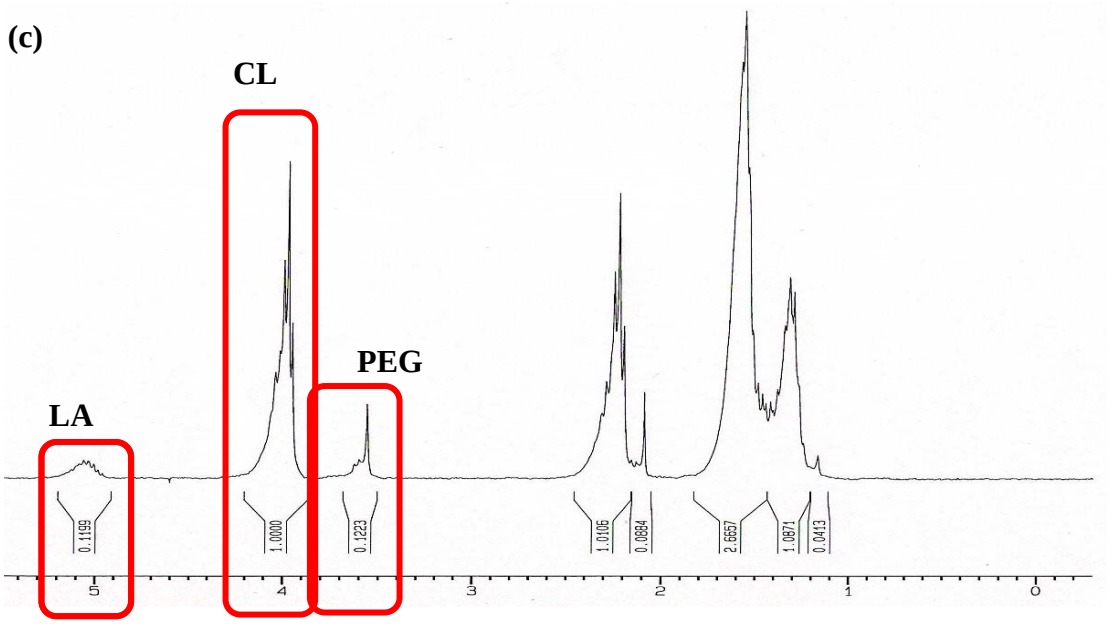


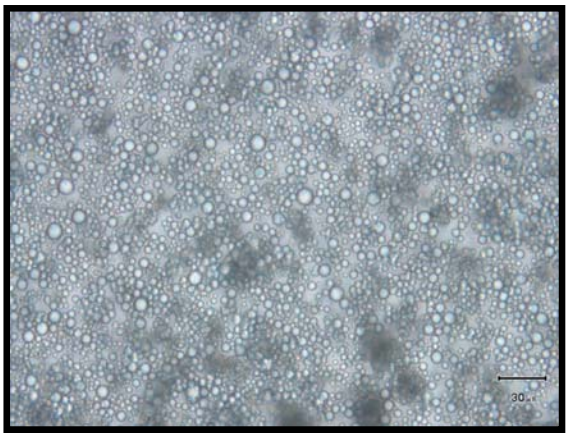
Figure 5.2 ^{1}H NMR spectrum of (a) PLEC 4, (b) PLEC 5 and (c) PLEC 6.

5.2 PLEC microsphere preparation

The micro-scale particles of PLECs were fabricated by the single emulsion procedure (oil in water method). The fine powder of PLEC (Figure 5.3a) was collected by centrifugation then dried with the lyophilization. The light microscope observation found in spherical shape without the micro-bubble inside particle (Figure 5.3b). The average size was controlled between 3-5 μm. The highly details of micro-particle with the scanning electron microscope (SEM) shown the micro-beat in different sizes (Figure 5.3c). It should be noted that the sample preparation process of SEM was leaded to the fusion particles.

(a)

(b)



(c)

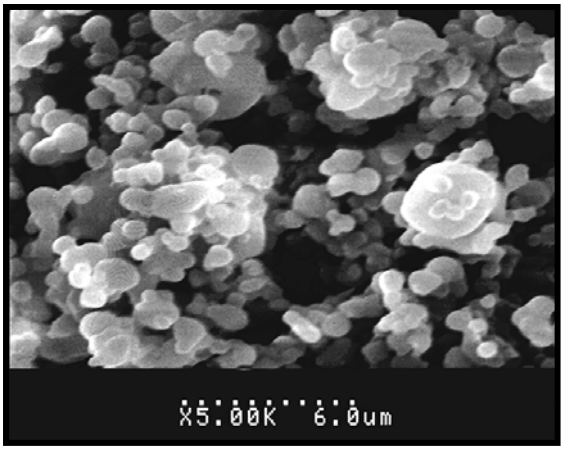


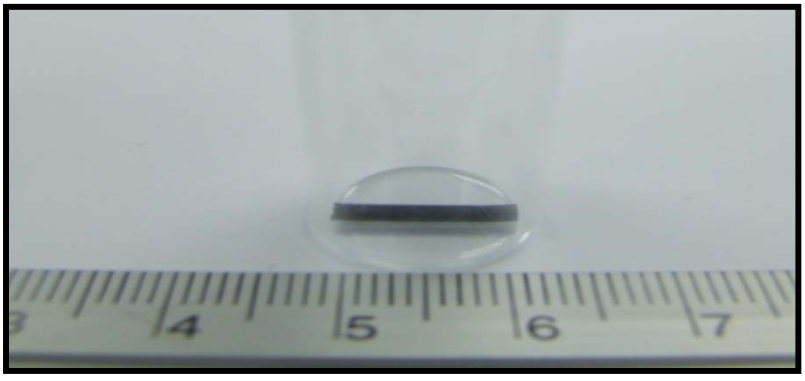
Figure 5.3 PLEC microparticles (a) Powder of PLEC. PLEC microparticles were analyzed by (b) Light microscope and (c) Scanning electron microspore (SEM).

5.3 PLECs Millirod molding

The PLEC millirods with trypan blue loaded (Monolithic system) were successfully fabricated. The PLEC microspheres and the trypan blue dye were homogeneously mixed. The compression-heat molding method was used to produce millirods from the mixing compound, resulting in solid-cylindrical shape of PLEC loaded with trypan blue (Figure 5.4b). Moreover, PLECs entire rods were made for water absorption study. The pure of PLEC microparticle were used and molded by the compression-heat molding method. The PLECs entire rods were colorless and solid (Figure 5.4c).The SEM investigation, the surface of PLEC loaded trypan blue rods were flatted from trypan blue (Figure 5.4d) while the PLEC entire rods were clearly surface (Figure 5.4e).

(a)

(b)



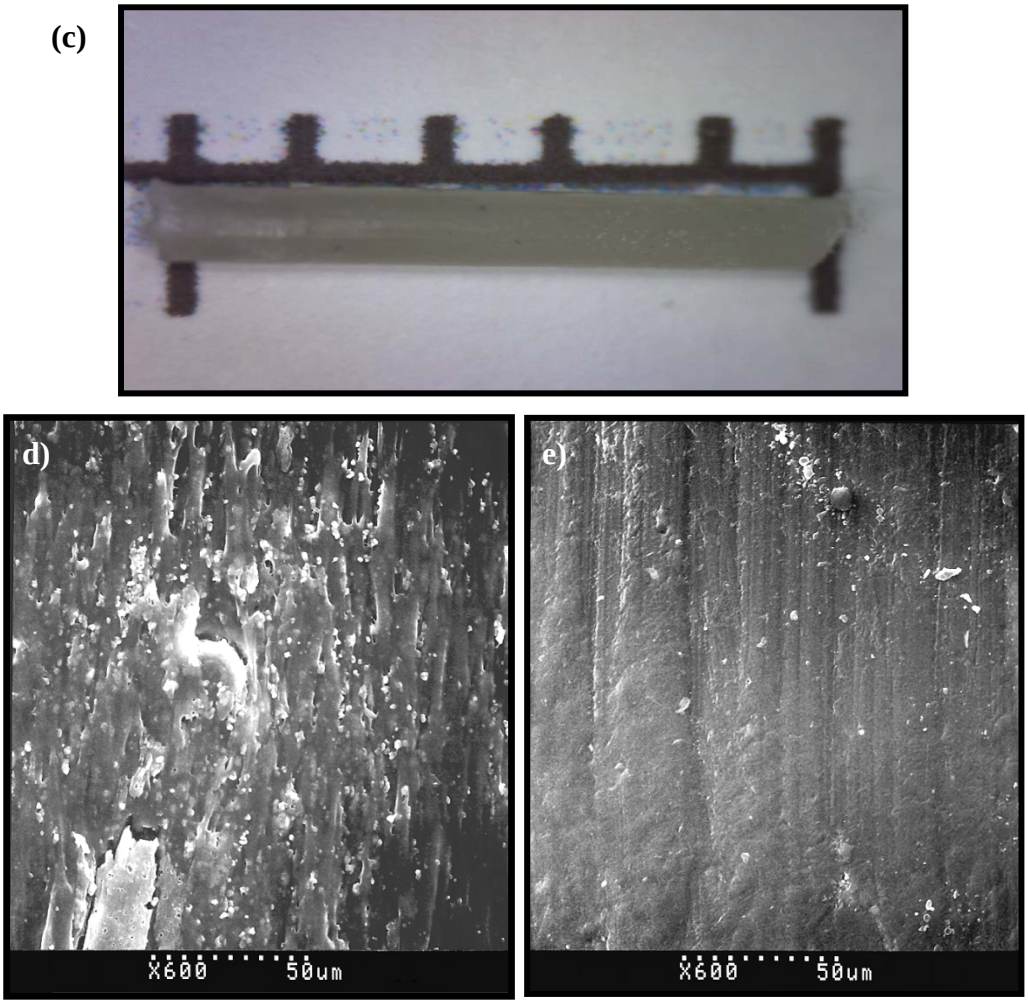


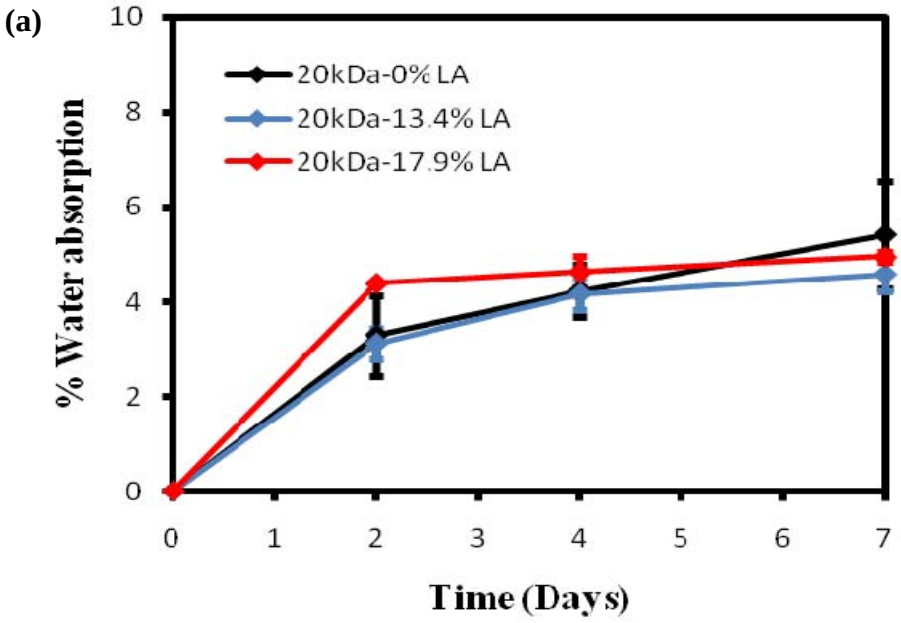
Figure 5.4 (a) Molder, (b) PLEC load trypan blue rod (Monolithic system) and (c) PLEC polymeric rod. Scanning electron microscope analyzed (d) the surface of PLEC loaded with trypan blue rods that presented trypan blue and (e) the surface of PLEC rod without trypan blue.

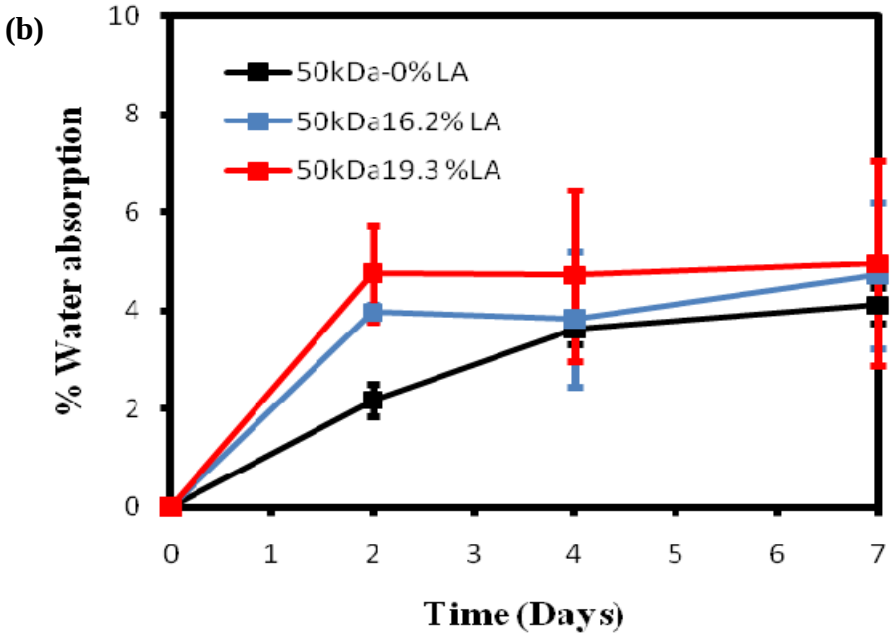
5.4 Water absorption study of PLECs polymeric rods

The water absorption is one of the mechanisms for drug release. In details, the water diffused into the polymeric matrix, so polymer is swelled through which allow drugs to pass. Moreover, the water absorption also affects the degradation rate of polymers. Water absorption of PLEC rods was studied for 7 days. In this experiment, polymeric rods were weighed (W_o) and immersed in PBS buffer pH 7.4 then incubated at 37 °C. At the sampling time, 3 pieces of the PLECs rod were withdrawn and wiped

to get rid of the water on the surface. Then, the clean samples were weighed (W_s) where the weight change represented the amount of the water in the rod [55]. The percentage of water absorption at each time was calculated by the equation 10. The water absorption result was performed by weight increasing (Ws-Wo) that represented the water in the rod.

$$\% \text{ water absorption} = (\underline{W_s - W_o})/ W_o \times 100 \quad (10)$$





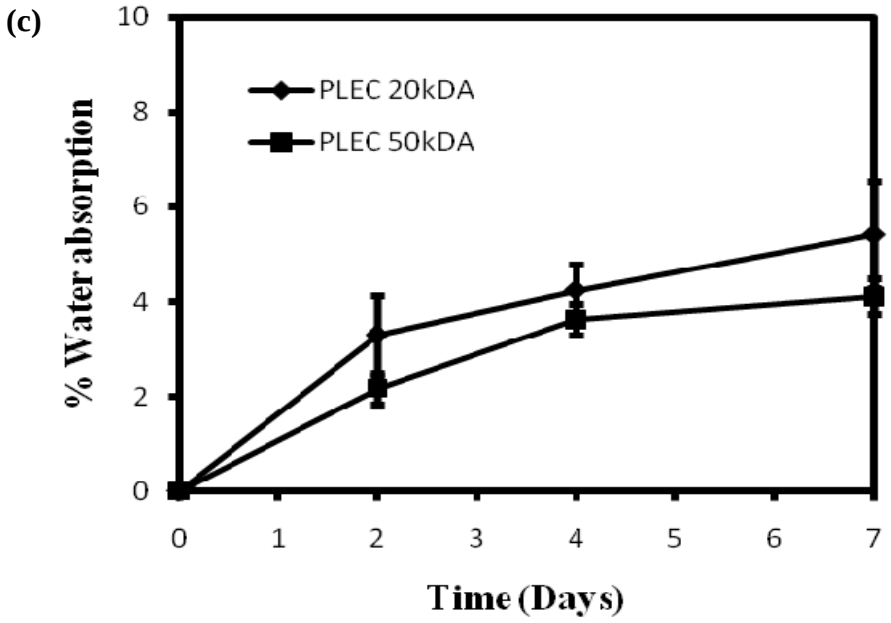


Figure 5.5 The percentage of water absorption of polymeric rods with different amount of D,L-lactide, and the PLEC molecular weight, (a) 20 kDa and (b) 50 kDa and (c) The effect of molecular weight on the percentage of water absorption from polymeric rods without D,L-lactide. It should be noted that water absorption was carried out only 7 days in order to avoid the interference from the rod degradation.

For PLECs 20 kDa (Figure 5.5a), PLEC 3 (17.9% LA) showed the highest water absorption rate which absorbed the water 4.95% from their original weight at day 7. PLEC 1 (0% LA) started to have higher water absorption than those of PLEC 2 and 3 after day 5 due to the onset of degradation [56]. This led to approximately the same water absorption after 5 days. For PLEC rods with 50 kDa (Figure 5.5b), PLEC 4 rods clearly demonstrated the lower absorption than those of PLEC 5 and 6 rods. This proved the effect of the high hydrophobicity of □-caprolactone which could slow down the drug release rate. Another factor affecting the water absorption is the crystallinity. It was reported that PLECs are the semicrystalline polymer that contain crystalline and non-crystalline region or so-called “amorphous”. Therefore, the water was easily absorbed into the amorphous region, so the water absorption rate increases as a function of percentage of D,L-lactide increases [57]. Moreover, D,L-lactide also affected to the PLEC degradation that accelerated the degradation process [58].

The ability of water absorption of PLEC 20 kDa (0% LA) was better than PLEC 50 kDa (0% LA) as a result of chain entanglement of polymers (Figure 5.5c). The highly chain entanglement hindered the water diffusion and subsequently delayed the swelling process. Because the entangle points act like a physical crosslinking, inhibiting the interaction between polymer chains and solvent preventing the polymer swelling [59].

5.5 Release study of the monolithic system of PLECs loaded trypan blue rods *(in vitro)*

The trypan blue release profiles of polymeric rods were studied. The PLEC rods in PBS buffer were periodically refreshed then the removed buffer was measured. After that, the accumulative concentration was calculated and plotted against the times.

5.5.1 The effect of D,L-lactide content on trypan blue release

The first-order release rate was produced by the monolithic system where the burst release was immediately occurred from the dye presented on the surface. Then, the water was uptaken into the rod and the dye was dissolved and diffused out, so the sustain release was observed afterwards. Finally, the trypan blue release rate decreased and approved zero before 2 weeks.

PLECs 20 kDa, PLEC 1, 2 and 3 rods produced short time zero-ordered release of 0.6 ,1 and 0.5 day, respectively. They had different release rate which depended on its D,L-lactide content (Figure 5.6a). PLEC 3 with 17.9% LA has the highest release rate but the PLEC 1 without LA produced the lowest release rate. The 50% release of PLEC rods with 0% (PLEC 1), 13.4% (PLEC 2) and 17.9% of LA (PLEC 3) were at 2.1, 1.2 and 0.4 days, respectively.

The release profiles of PLECs 50 kDa (PLEC 4, 5 and 6) rods were shown 3.7, 2.0 and 1.5 day of zero-ordered releases, respectively. The highest D,L-lactide content rods, i.e. PLEC 6 (19.3% LA) had the highest release rate. In contrast, the lowest release rate was found on PLEC without LA (PLEC 4). Moreover, the50% release of PLEC 4 (0% LA), PLEC 5 (13.4%LA) and PLEC 6 (17.9% LA) was 5.8, 2.0 and 1.6 days, respectively. The results of PLECs 50 kDa were confirmed the affect

of D,L-lactide content to the trypan blue release rate. The percentage of D,L-lactide is an important factor of the trypan blue release rate because it affects water absorption rate of polymer as discussed in section 4.

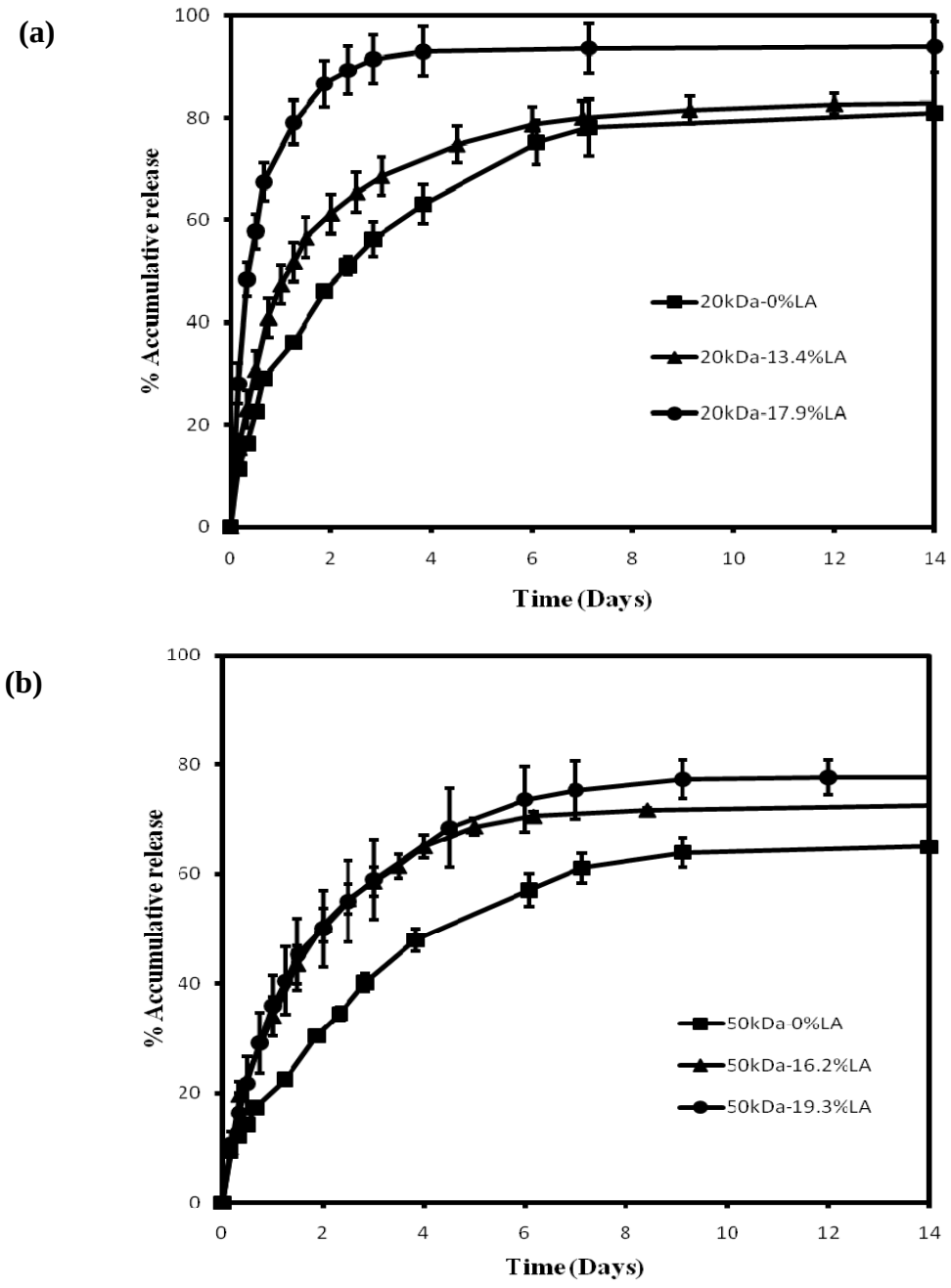


Figure 5.6 Trypan blue release profiles from polymeric rods containing different amount of D,L-lactide. The PLEC molecular weight is (a) 20 kDa and (b) 50 kDa. Trypan blue loading in all polymeric rods is 30 % w/w.

5.5.2 The effect of molecular weight on trypan blue release

Molecular weight also affects the trypan blue release rate as a results of chain entanglement that slows the water uptake and obstructs the dye diffusion, so the PLEC 1 (20kDa) has higher release rate than PLEC 4 (50 kDa) (Figure 5.7).

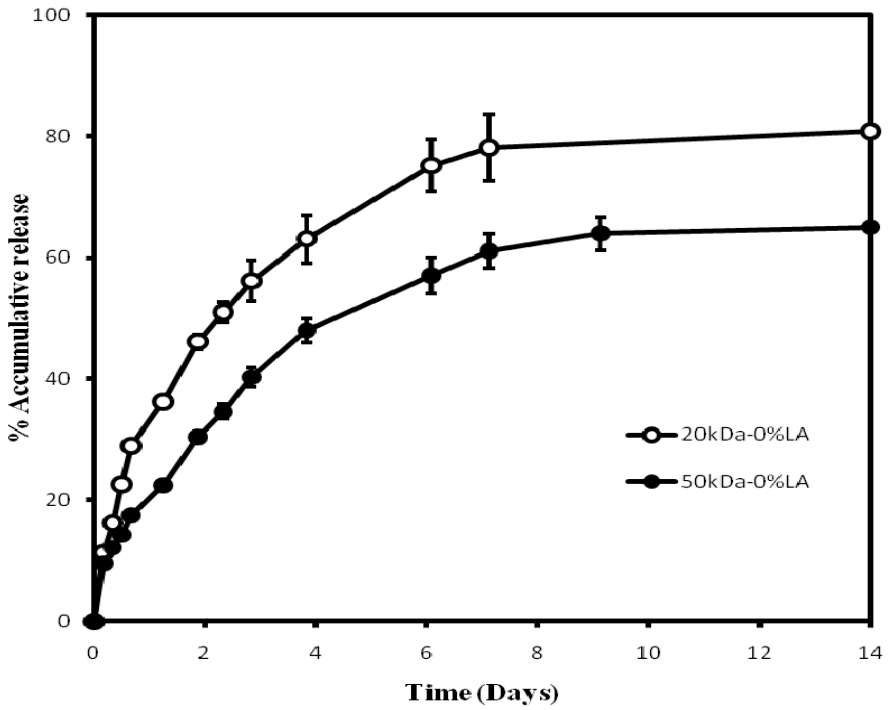


Figure 5.7 The effect of molecular weight on trypan blue release.

However, the monolithic system cannot produce the constant release (zero-ordered release) mainly due to the dye presented on the surface. This can be resolved by covering the surface in order to store the monolithic system inside. This system is so-called “the reservoir system”.

5.6 The preparation of the reservoir systems with spin coating method

The monolithic system cannot produce the zero-ordered release because of the dye on the surface that leads to the burst release. In this study, the reservoir system was used to solve this problem. The monolithic system was covered by a film which generated the dye storage system. Therefore, the dye can pass through the barrier film in constant rate and produces, the zero-ordered release.

The reservoir system was generated by spin coating method. The monolithic system of PLEC loaded trypan blue rods were sprayed by polymeric solution, while it was rotated at 100 rpm. The thickness of barrier film can be controlled by spinning time. The thickness was controlled approximately 100 μm and 200 μm which could be obtained by adjusting the spinning time at 20-25 second and 40-50 second, respectively.

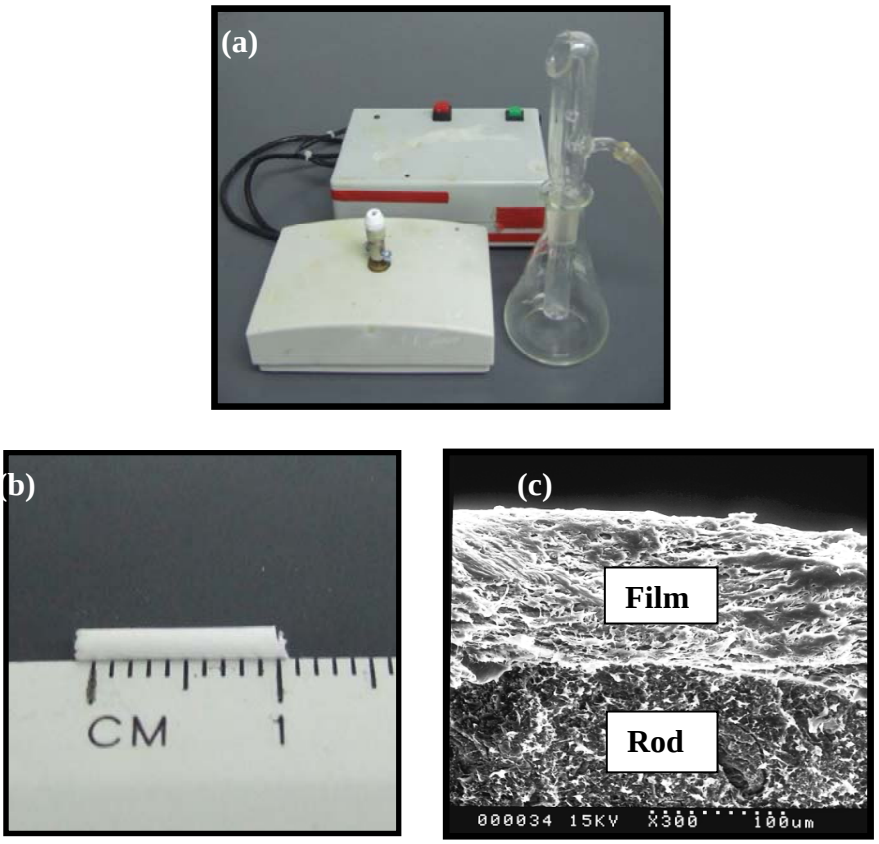


Figure 5.8 The picture of (a) coating device, (b) PLEC coating rod (reservoir system) and (c) SEM investigate the cross-section of PLEC coating rod

5.7 Release study of the reservoir system of PLECs loaded trypan blue rods *(in vitro)*

The release study of reservoir system with different thickness was showed in Figure 5.9 - 5.14. The thickness of barrier (polymeric film) was varied in order to obtain the suitable thickness that provided the zero-ordered release without the lag time. The trypan blue release profile was fitted with zero-ordered equation (eq. 1). The SigmaPlot® software was used for curve fitting and the coefficient of determination was over 95 % (r-square > 0.95). Moreover, the rate coefficient (K) indicated the release rate. The day of constant release and the release rate were summarized in Table 5.2.

5.7.1 PLEC 20kDa

The reservoir system of PLEC 1 (0% LA) showed the delayed release of trypan blue at the beginning time (Lag time). Beyond this time, the rod coated with 100 μm film thickness provided the zero-ordered release approximately seven days with the release rate of 8.5% per day. The thicker film (200 μm) could prolong the zero-ordered release approximately fourteen days with the release rate of 4.0% per day (Figure 5.9b). The 50% release was observed at 5.2 and 13.2 day for 100 and 200 μm film thicknesses, respectively (Figure 5.9a).

Eventhough, the 100 μm thickness reservoir system of PLEC 2 (13.4% LA) produced two days zero-ordered release with release rate of 25.8% per day and the three days of zero-ordered release with release rate of 13.6% per day was found in the 200 μm of thickness (Figure 5.10b). The 50% releases were found at 2.3 and 5.3 day on the thickness 100 and 200 μm, respectively (Figure 5.10a).

The 100 μm thickness reservoir system of PLEC 3 shown the shortest zero-ordered release (0.6 day) with the fastest of release rate (73.0% per day) and the 50% release at 0.9 day (Figure 5.11a). The 200 μm thickness produced the 3.8 days of zero-ordered release with 15.1% per day of release rate (Figure 5.11b) and the 50% release was 1.6 day. The thicker barrier could provide the longer zero-ordered release and decreased the release rate. Moreover, the release also increased as the LA content increased.

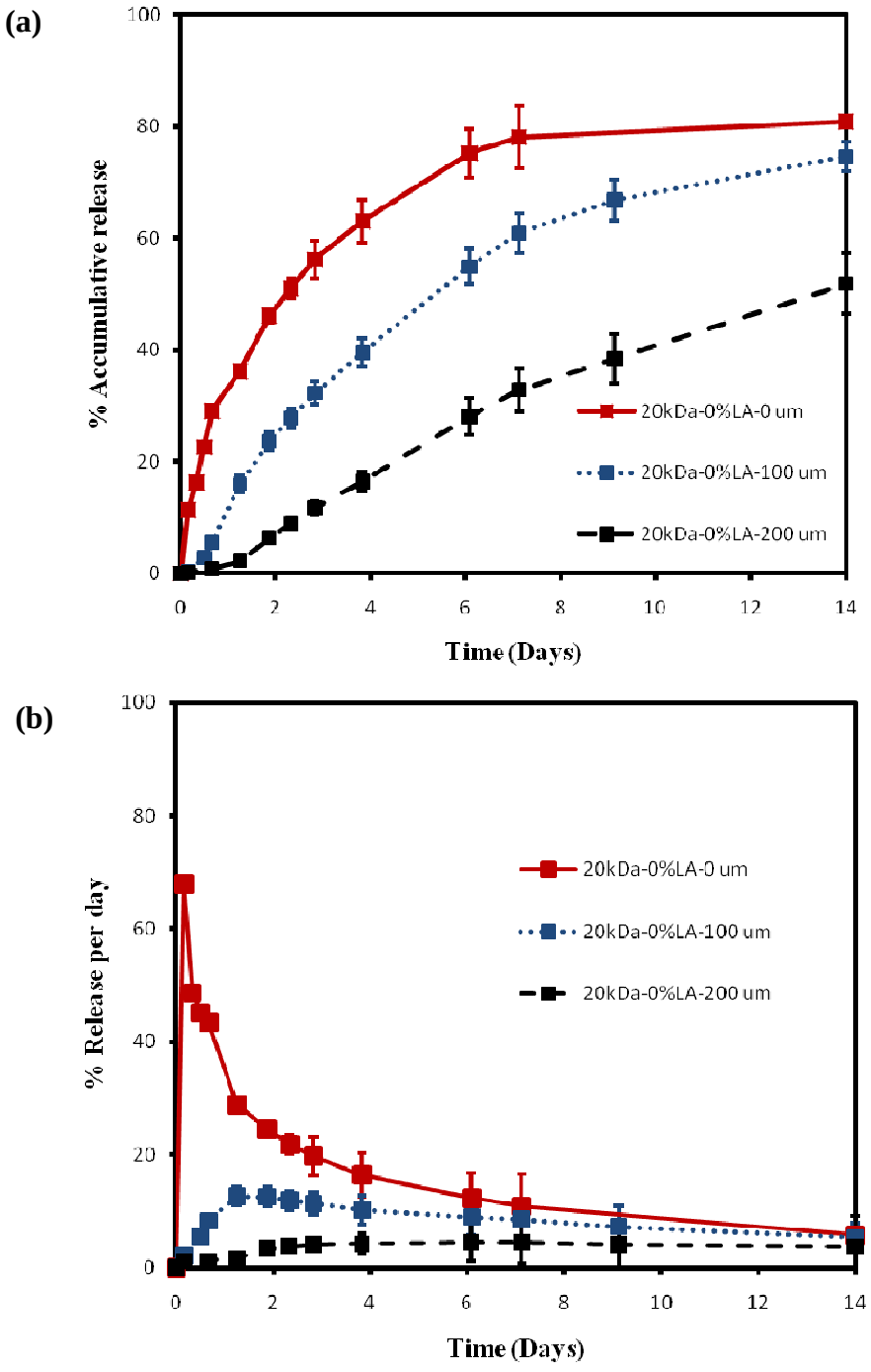


Figure 5.9 The PLEC1 (20 kDa, 0%LA) in reservoir system with different thickness coating (a) Trypan blue release profiles and (b) Percentage of trypan blue release per day.

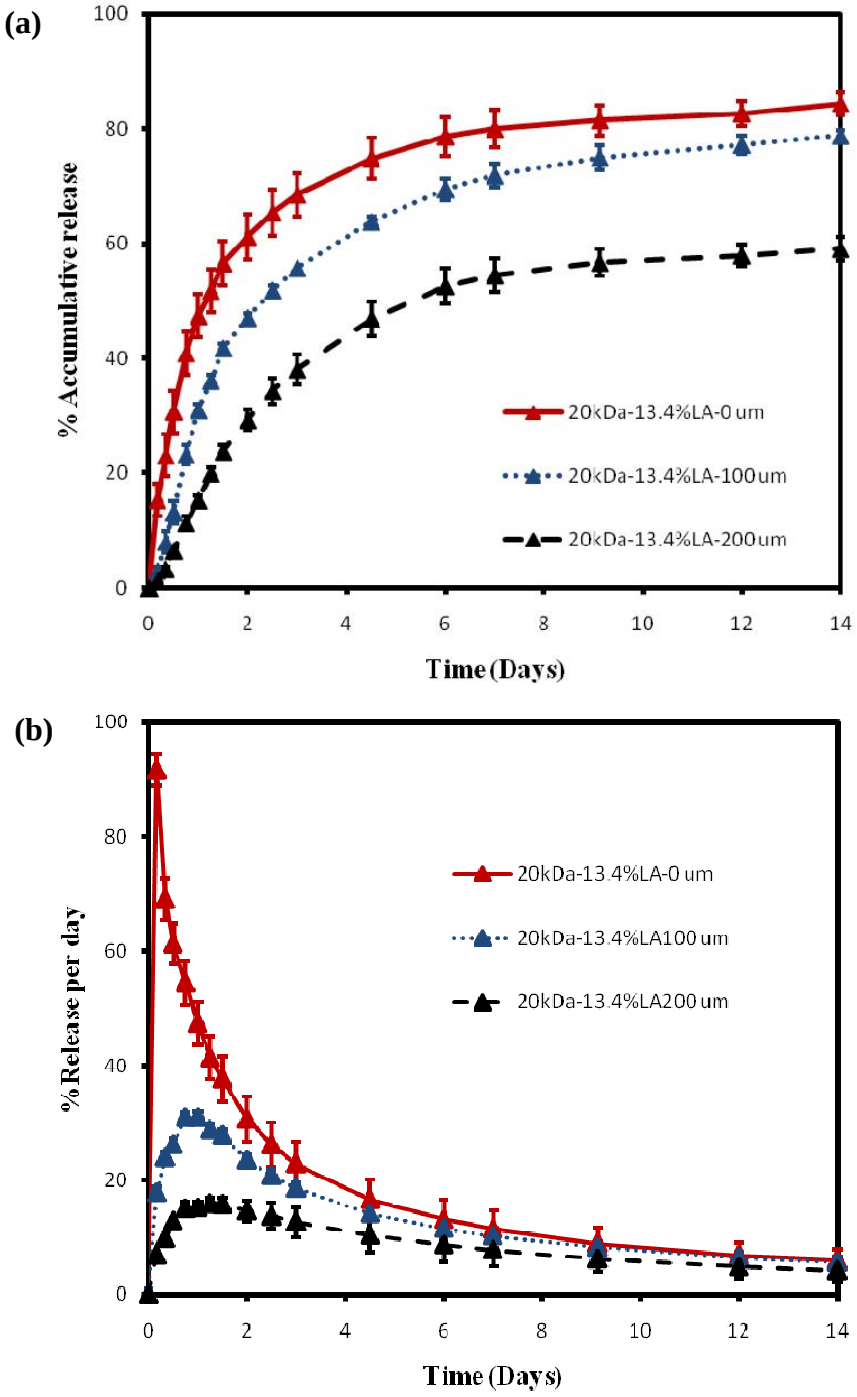


Figure 5.10 The PLEC1 (20 kDa, 13.4% LA) in reservoir system with different thickness coating (a) Trypan blue release profiles and (b) Percentage of trypan blue release per day.

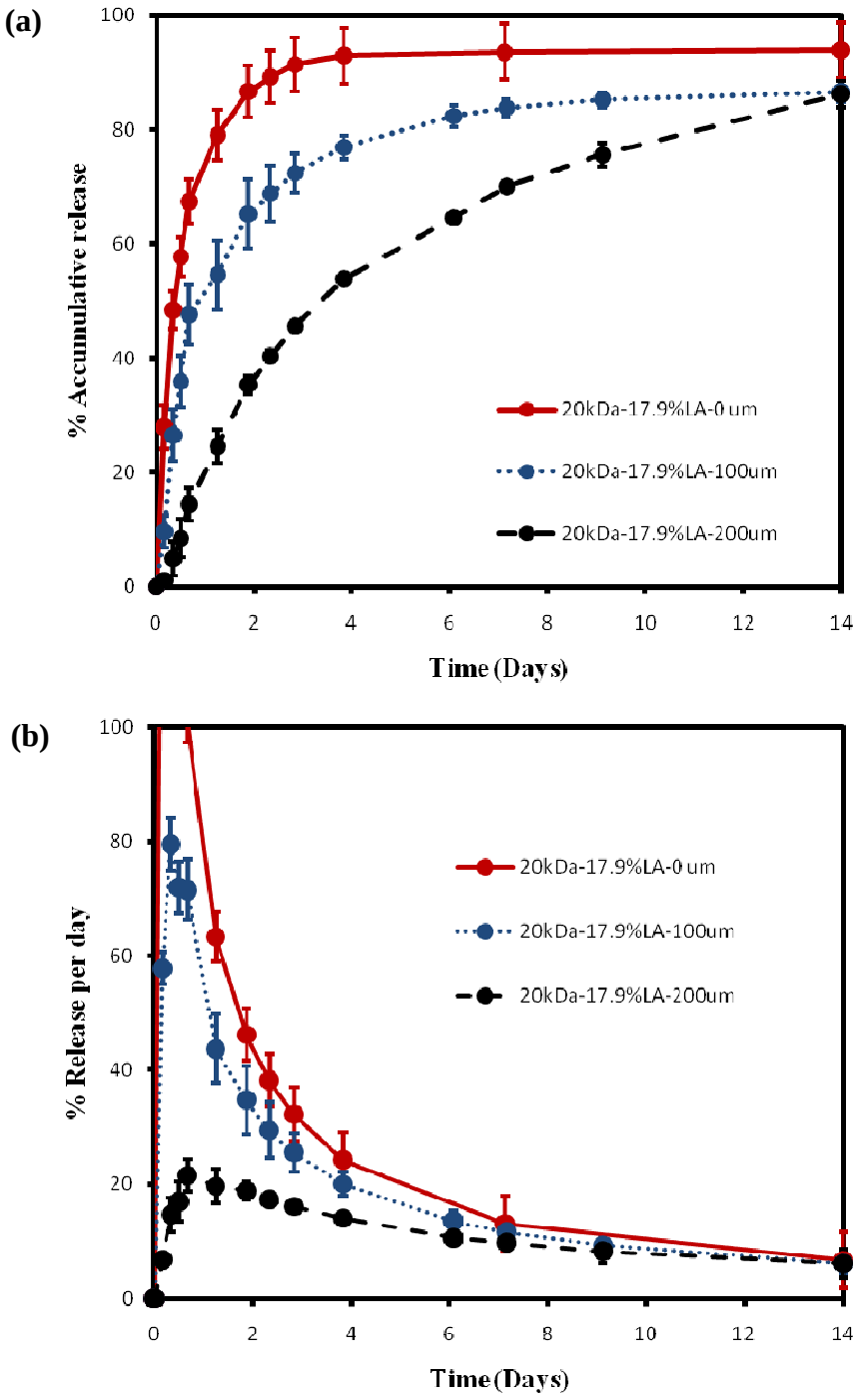


Figure 5.11 The PLEC1 (20kDa, 17.9% LA) in reservoir system with different thickness coating (a) Trypan blue release profiles and (b) Percentage of trypan blue release per day.

5.7.2 PLEC 50 kDa

PLECs 50 kDa were also improved the release performance by reservoir system. PLEC 4 (0% LA), the lag phase was occurred then followed by nine and fourteen days of zero-ordered release which released at 7.5 and 3.0% per days of 100 and 200 μm film thickness, respectively (Figure 5.12b). The 50% releases were found at 11.5 and over 14.0 day on the thickness 100 and 200 μm, respectively (Figure 5.12a).

The reservoir system of PLEC 5 (16.2% LA) was extended the 50% release to 6.6 day and more than 14.0 day for the thickness of 100 and 200 μm, respectively (Figure 5.13a). approximately six days of zero-ordered release with 8.8 and 5.8% per day of release rate were produced by 100 μm and 200 μm, respectively (Figure 5.13b).

The 100 μm thickness reservoir system of PLEC 6 (19.3% LA) could produce approximately three days zero-ordered release with the release rate at 12.1% per day and the nine days of zero-ordered release with 4.9% per day was found in the 200 (Figure 5.14b). The 50% releases were found at 7.2 day and over two weeks at the thickness 100 and 200 μm, respectively (Figure 5.14a). The zero-ordered release could be controled by the thickness of barrier and the LA content adjusting.

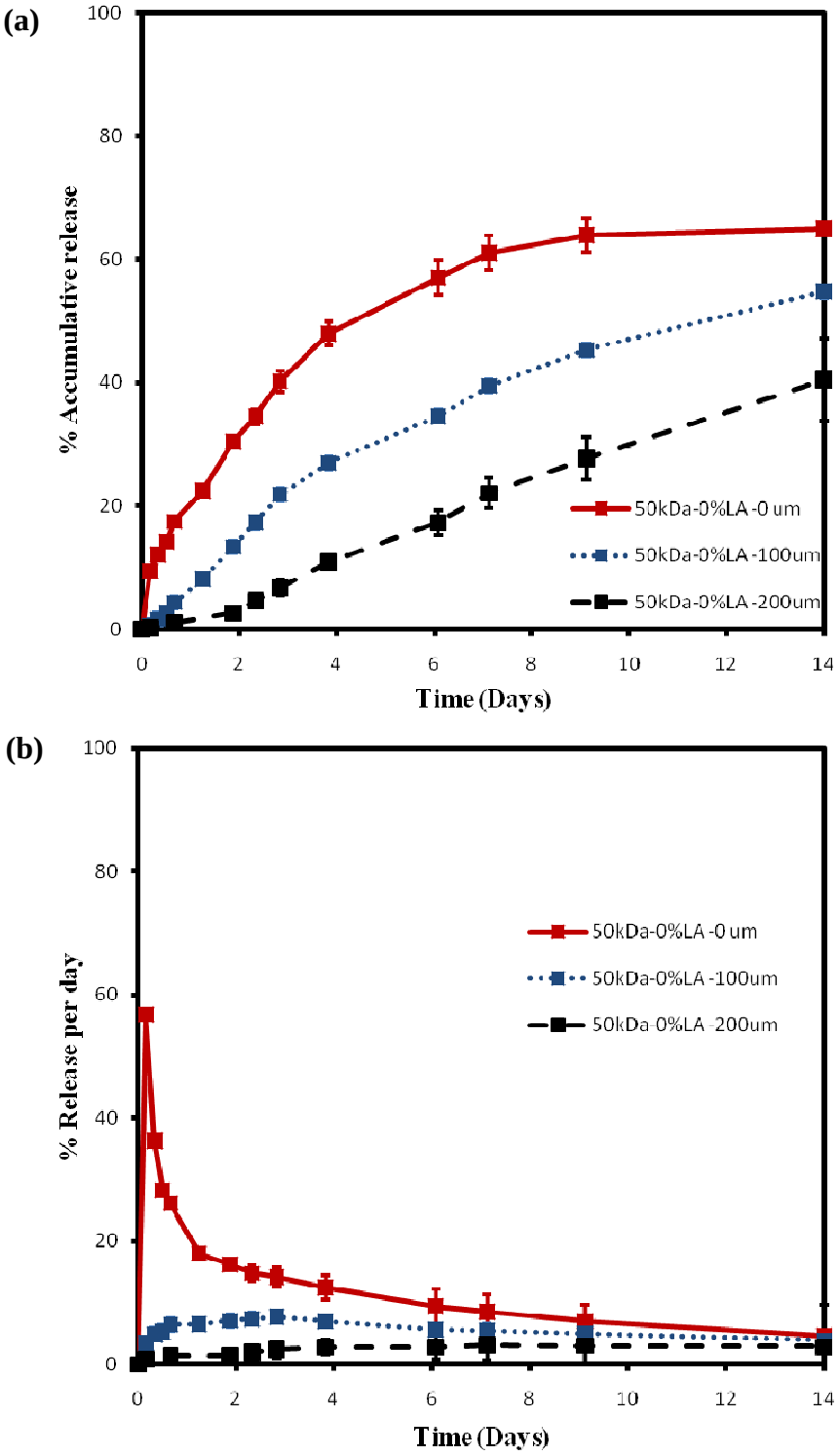


Figure 5.12 The PLEC4 (50 kDa, 0% LA) in reservoir system with different thickness coating (a) Trypan blue release profiles and (b) Percentage of trypan blue release per day.

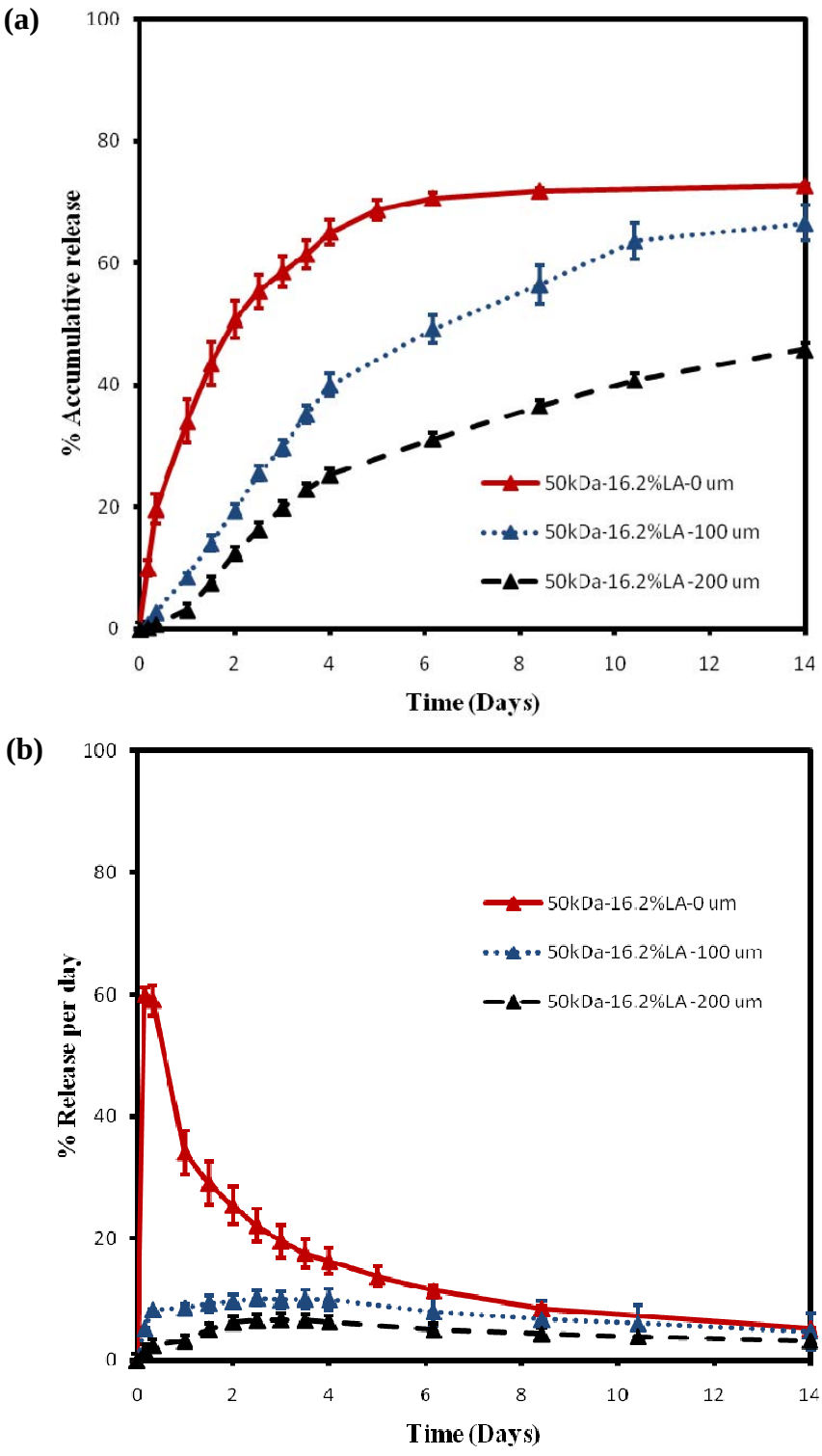


Figure 5.13 The PLEC5 (50 kDa, 16.2% LA) in reservoir system with different thickness coating (a) Trypan blue release profiles and (b) Percentage of trypan blue release per day.

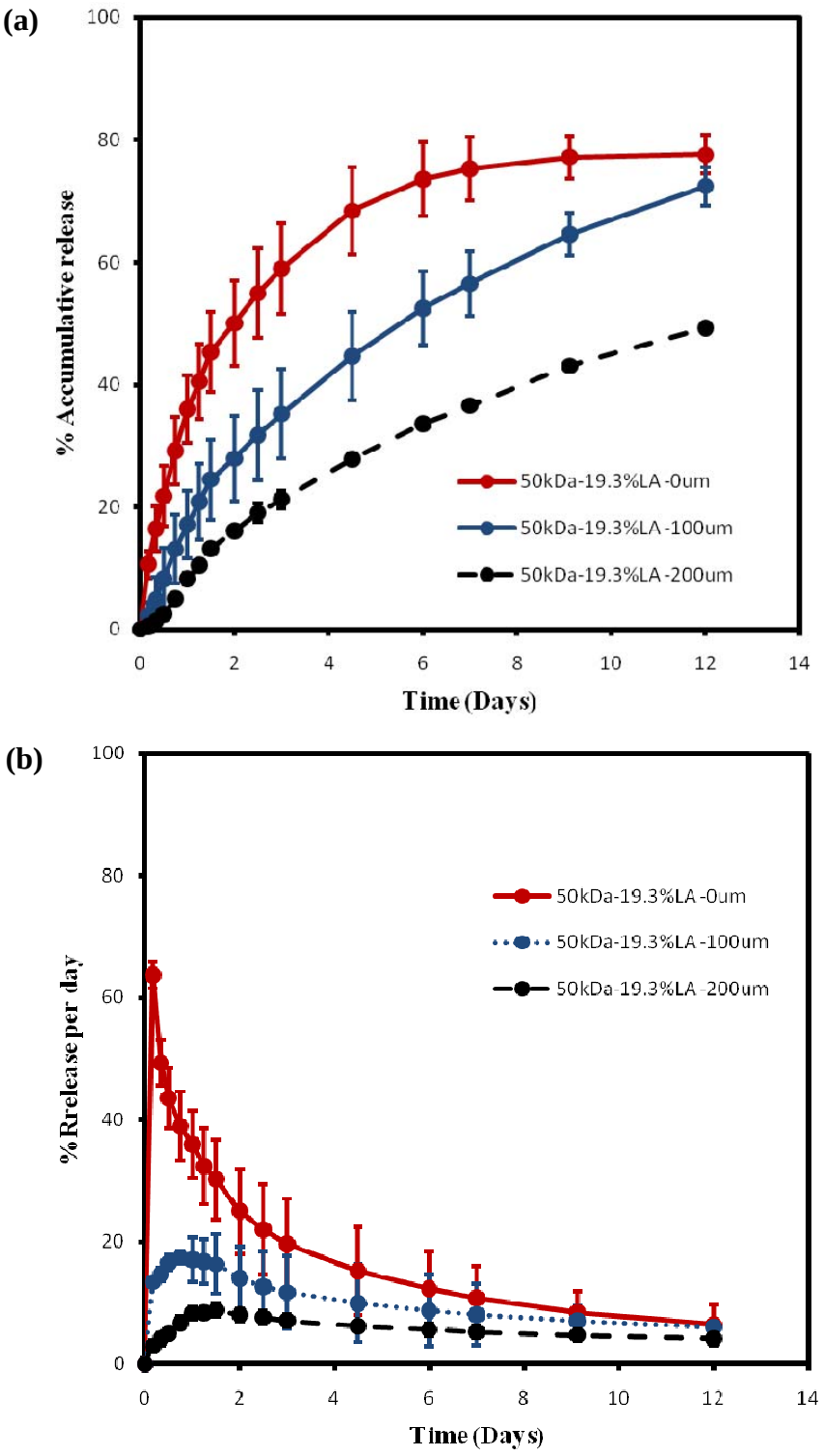


Figure 5.14 The PLEC6 (50 kDa, 19.3% LA) in reservoir system with different thickness coating (a) Trypan blue release profiles and (b) Percentage of trypan blue release per day.

The reservoir system successfully removed the burst release as a result of dye on surface. In this system, the polymeric film enveloped a rod and allowed the dye to pass with constant rate. It should be noted that, the diffusion rate depended on concentration gradient within the film, so the film properties played a key role in the release profile of trypan blue. The film thickness was one of the factors. The thicker barrier leaded to higher resistibility and subsequently lowered the release rate. The suitable thickness will lead to the longer constant release without the lag phase. From the result, it could be concluded that the thickness of 200 μm provided the lag phase for all PLECs. The thickness of 100 μm was the optimum coating which provided the constant release without the lag phase.

Table 5.2 The sumarization of release profiles

PLEC	LA content (% mol)		Total MW (kDa)	Thickness (um)	Half release (Days)	Zero-ordered release	
						Time (Days)	K (%/day)
1	0.0	0.0	20.83	0	2.1	0.6	41.5
				100	5.2	6.6	8.5
				200	13.2	14.0	4.0
2	2.09	13.43	24.42	0	1.2	1.0	45.6
				100	2.3	2.0	25.8
				200	5.3	3.0	13.6
3	2.99	17.89	25.76	0	0.4	0.5	116.2
				100	0.9	0.7	73.0
				200	1.6	3.8	15.1
4	0.0	0.0	53.98	0	5.8	3.8	15.1
				100	11.5	8.8	5.1
				200	>14	14.0	3.0
5	6.42	15.87	49.79	0	2.0	2.0	24.0
				100	6.6	6.2	8.8
				200	>14	6.2	5.8
6	4.87	19.34	46.80	0	1.6	1.5	28.9
				100	5.5	3.0	12.1
				200	>14	9.1	4.9

5.8 PLEC rods degradation study

The PLEC copolymers were developed for direct liver cancer therapy which implants into human body. These materials are considered as biodegradable materials so the time and characteristics of degradation are necessary to study. Moreover, the polymeric degradation may eventually affect the release profile of drugs. In this study, PLEC polymeric rods were incubated in 37°C PBS and were collected at 3 and 5 months. The morphology of rods and the molecular weight of polymer chain were observed by scanning electron microscope (SEM) and Gel permeable chromatography (GPC), respectively.

5.8.1 The morphological study

The morphological degradation study was focused on rod's surface and cross-section. Before incubated, the PLEC 1 and PLEC 4 rod (without LA content) showed the smooth surface (Figure 5.15 a1 and 5.17 a1). After 3 months, the PLEC 1 and 4 rods were found to have approximately the same dimension as that of day 0. Their surfaces clearly demonstrated the fractures throughout the samples. Interestingly, increasing the molecular weight to 53.9 kDa (PLEC 4 rods) showed much less fracture (Figure 5.15 a2 and 5.17 a2). However, the massive fractures were found in both PLEC 1 and PLEC 4 rods after 5 months (Figure 5.15 a3 and 5.17 a3).

The wrinkled surface was observed on PLEC 3 and PLEC 6 rod at starting (Figure 5.16a1 and 5.18a1). After 3 months, the surface of these rods shown the pit-like degradation pattern caused by the loss of amorphous domain (Figure 5.18 a2). [15] Subsequently, the appearance of porous was accelerated the water uptake, so the core of rods was extensive eroded as shown in Figure 5.19 b3 and d3 (SEM of cross-section). The higher magnification demonstrated the fine porous structure throughout the surface (Figure 5.18 b2). PLEC 3 rods presented the flake-debris pattern (Figure 5.16b1). In another hand, PLEC 1 and 4 (without LA content) showed no pit-like degradation pattern but the extensive fracture throughout the surface. These rods also showed the lower extend of degradation due to lower water absorption rate. It should be noted that both of PLEC 3 and PLEC 6 rods drastically lost their original dimension while PLEC 1 and 4 rods were still nearly intact.

PLEC 1 (0% LA - 20.83 kDa)

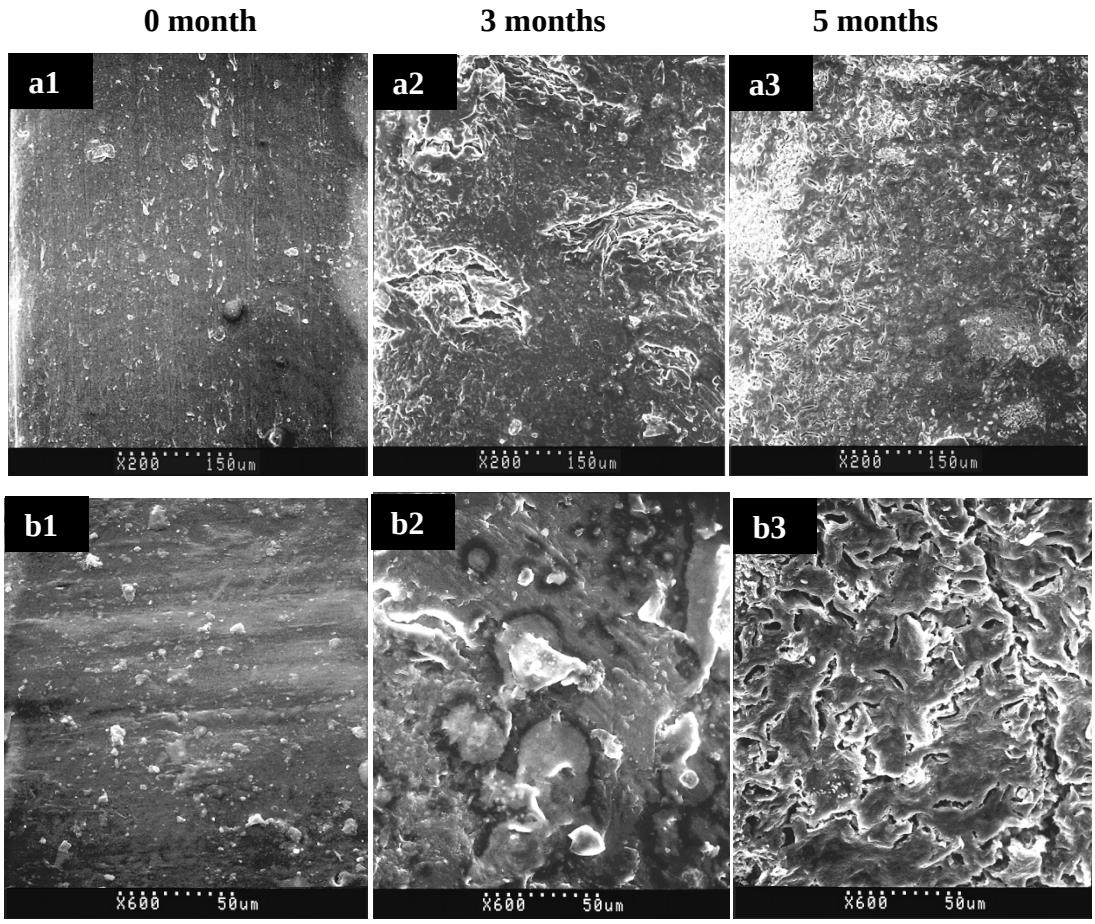


Figure 5.15 The surface degradation of PLEC 1 (0, 3 and 5 month) investigated by SEM (a1-a3) magnification x200 and (b1-b3) magnification x600.

PLEC 3 (17.9% LA - 25.8 kDa)

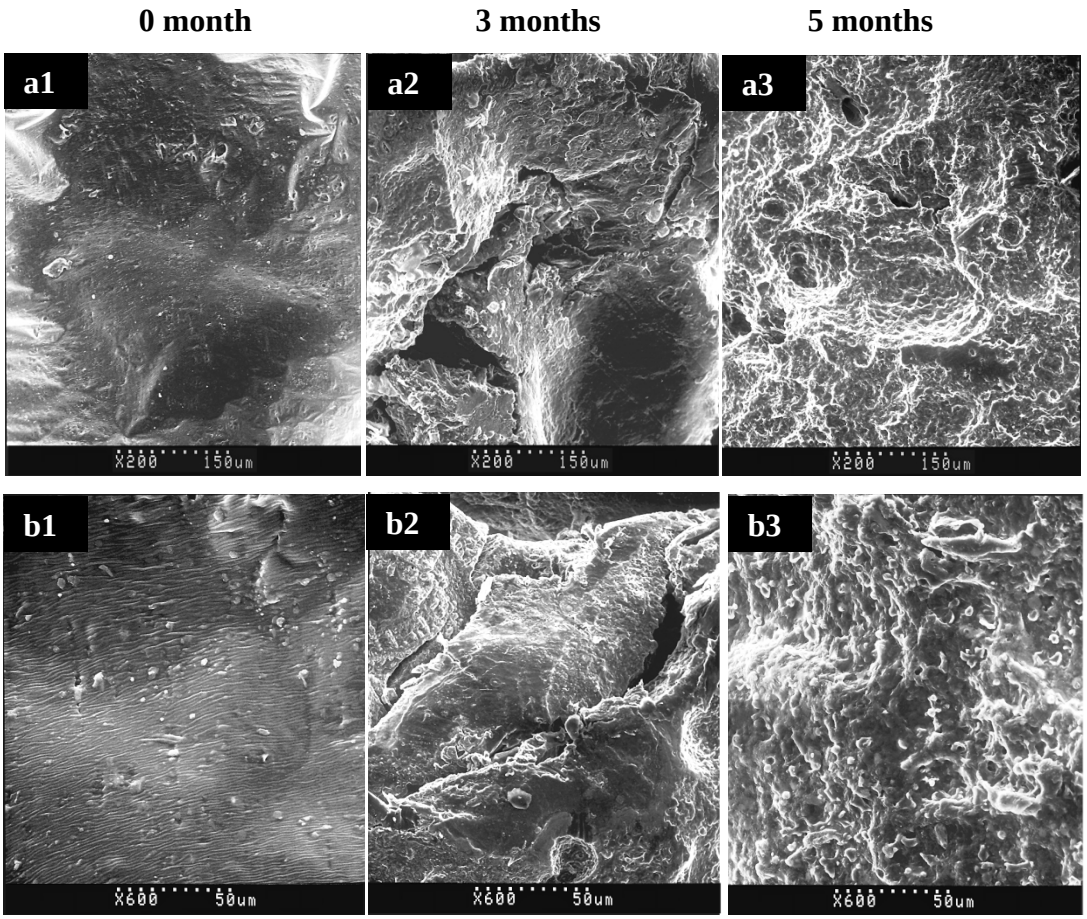


Figure 5.16 The surface degradation of PLEC 3 (0, 3 and 5 month) investigated by SEM (a1-a3) magnification x200 and (b1-b3) magnification x600.

PLEC 4 (0% LA – 53.9 kDa)

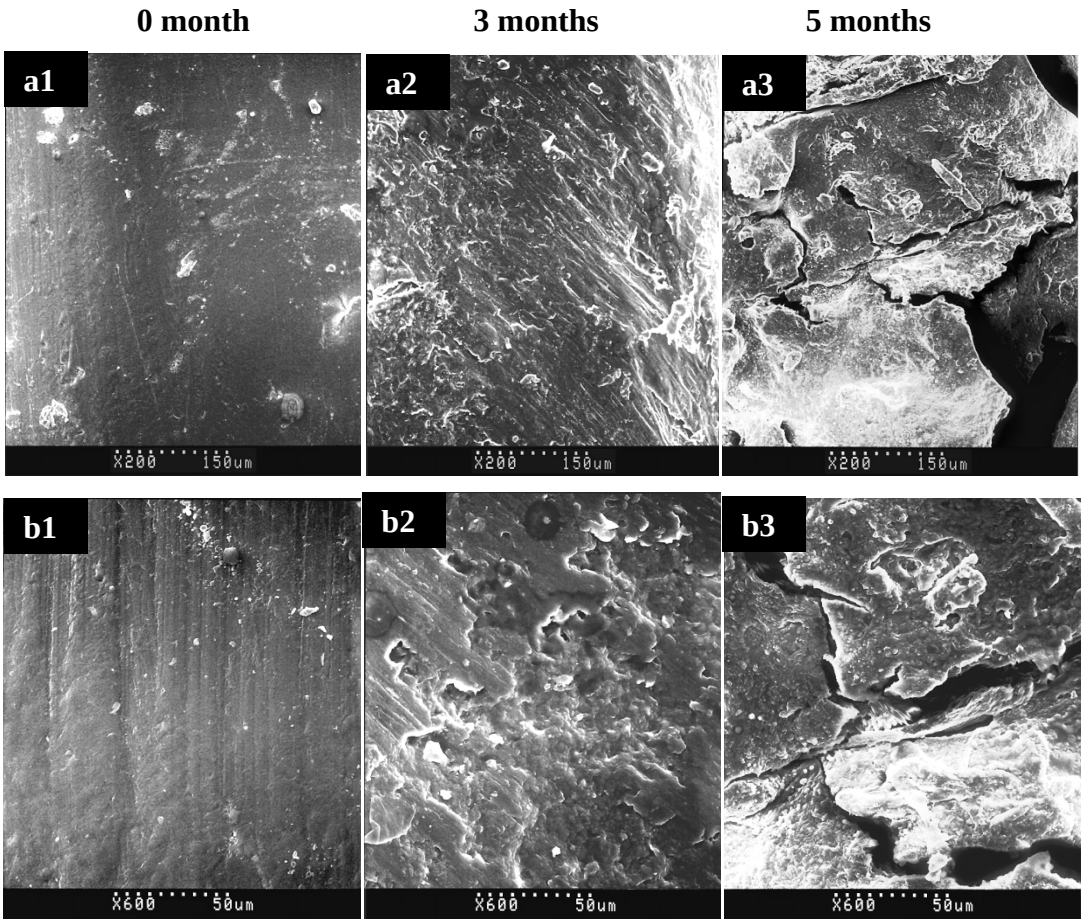


Figure 5.17 The surface degradation of PLEC 4 (0, 3 and 5 month) investigated by SEM (a1-a3) magnification x200 and (b1-b3) magnification x600.

PLEC 6 (19.4% LA - 46.80 kDa)

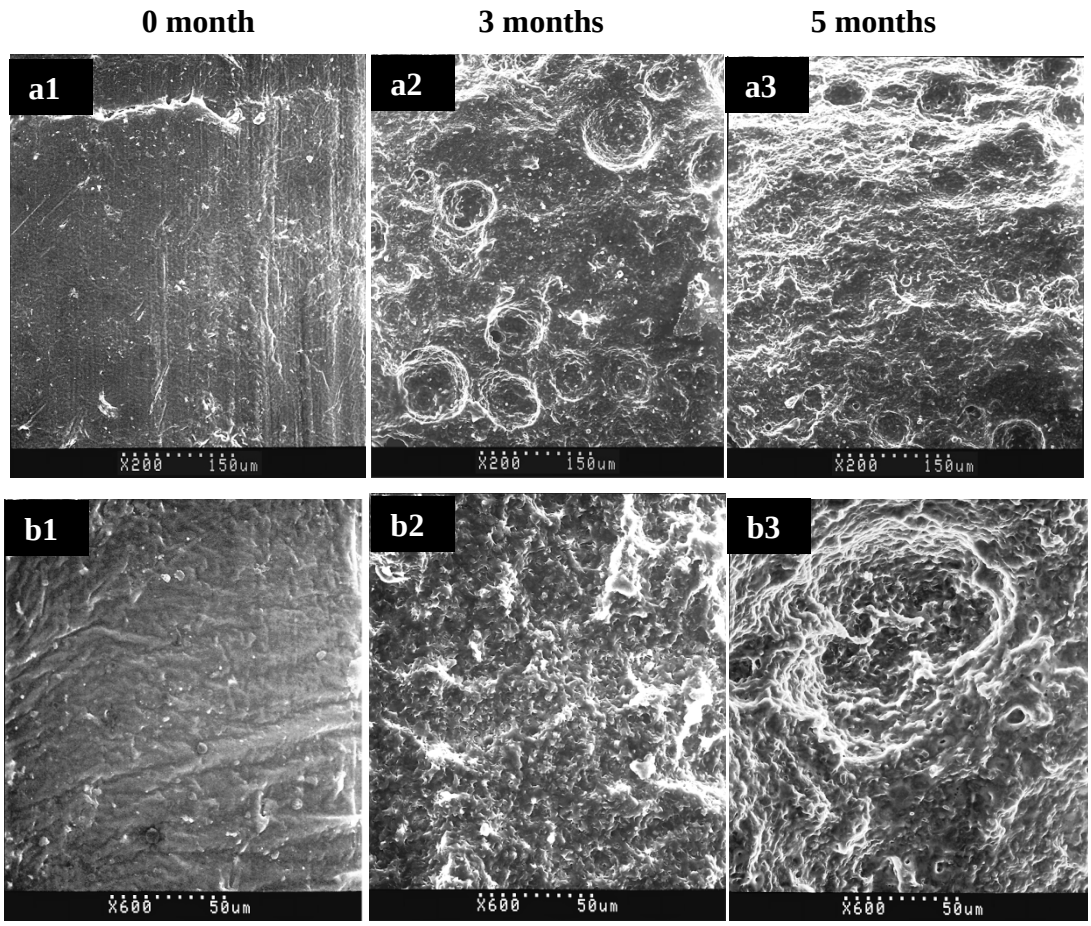
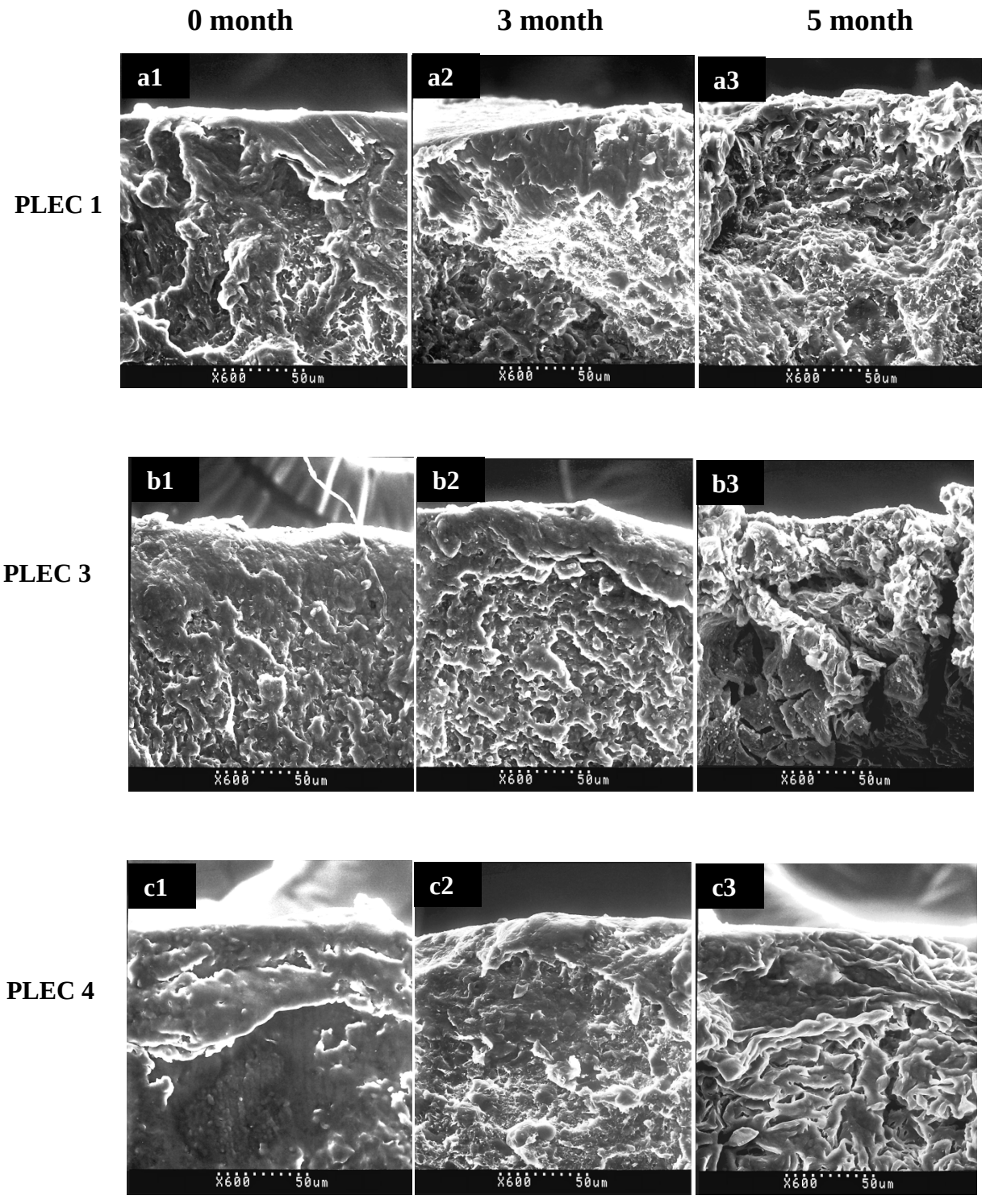


Figure 5.18 The surface degradation of PLEC 6 (0, 3 and 5 month) investigated by SEM (a1-a3) magnification x200 and (b1-b3) magnification x600.

Cross-section study was analyzed by SEM. At the time zero, the intact rods were observed from PLEC 1 and PLEC 4 (Figure 5.19 a1 and c1). PLEC 3 and PLEC 6 showed the porous core with intact edge (Figure 5.19 b1 and d1) demonstrating the autocatalytic effect in which an acid, by product, can accumulate inside the sample and causes the inside out degradation. Introduction of D,L-lactide increased the overall hydrophilicity of PLEC rod and subsequently raised the water absorption. After 5 month, the high extend of fracture and porous without intact edges was foundin rods from PLEC 1, PLEC 3 and PLEC 6 (Figure 5.19 a3, b3, and d3).


0 month
3 month
5 month
PLEC 1
PLEC 3
PLEC 4
a1
a2
a3
b1
b2
b3
c1
c2
c3
X600 50um

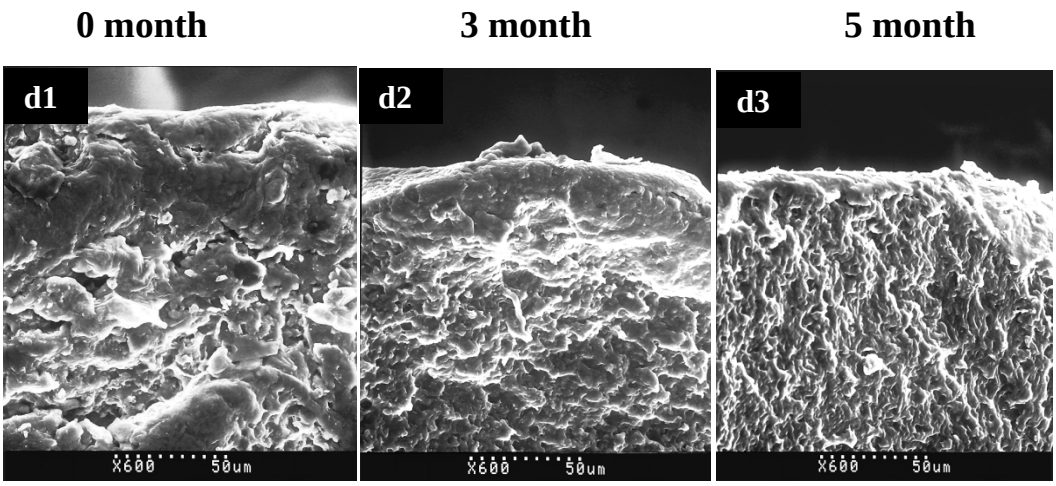


Figure 5.19 SEM of the cross-section of (a1-a3) PLEC 1 (28.4 kDa-0% LA), (b1-b3) PLEC 3 (25.8 kDa-17.9% LA), (c1-c3) PLEC 4 (53.9 kDa-0% LA) and (d1-d3) PLEC 6 (46.8 kDa-19.3% LA).

5.8.2 Polymer degradation

PLECs are classified as polyester therefore a chain was degraded by the cleavage of ester bond in CL and LA. [60] GPC was used to carry out the investigation of PLEC degradation. It was found that the average molecular weight decreased and the polydispersity (PDI values) increased by the time. The initiation average molecular weight (Mn) was 19,799 Da and decreased to 13,863 and 9,493 Da at month 3 and 5 (Figure 5.20b), respectively. The PDI original value was 1.56 then it spread to 1.63 and 1.68 at month 3 and 5 (Figure 5.20a), respectively. This demonstrated the degradation processing where polymeric chains of PLEC were separated into shorter pieces.

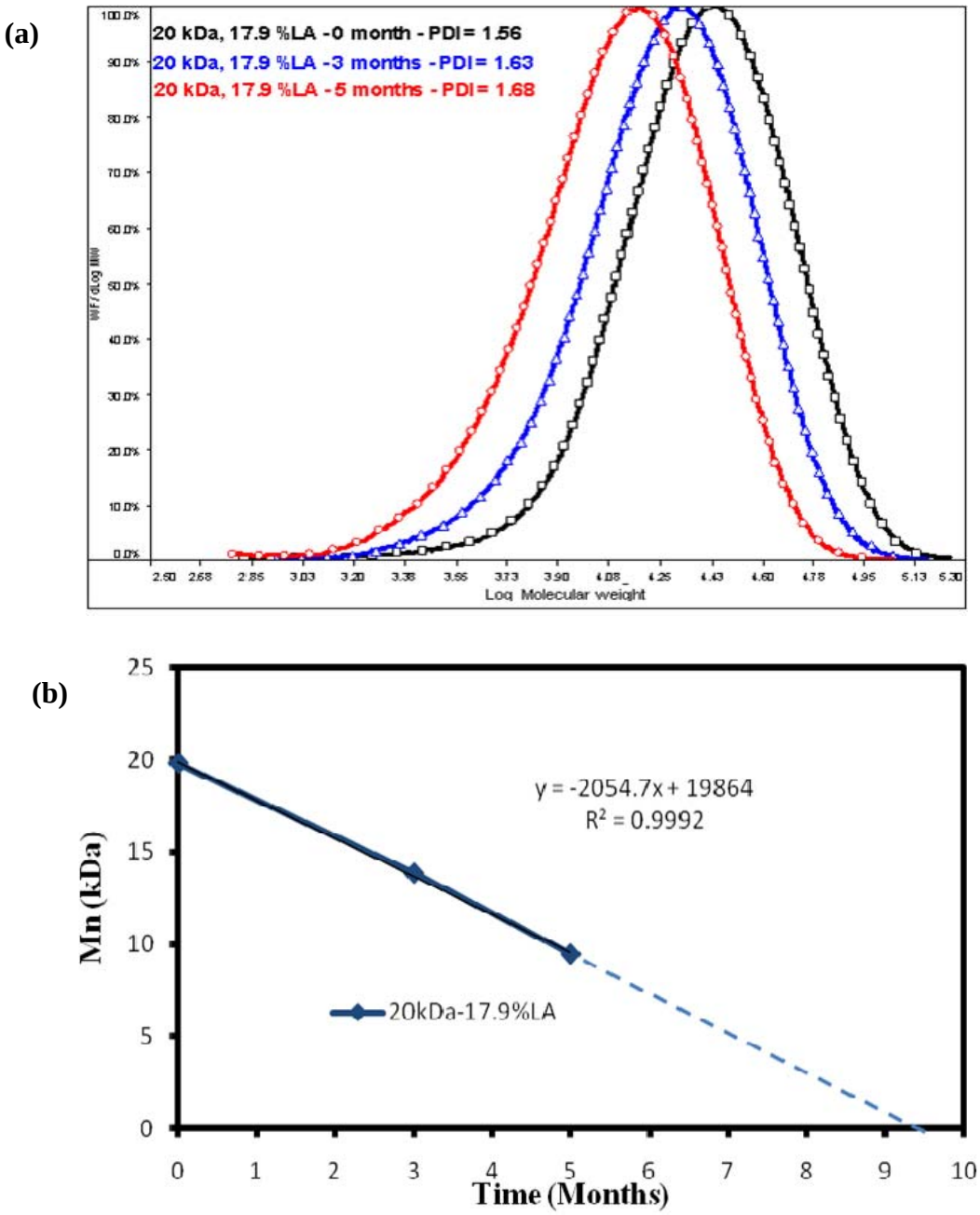


Figure 5.20 Degradation study (a) GPC (b) the plot of the average molecular weight (Mn) over time

CHAPTER VI
CONCLUSION

The six types of PLEC copolymers were synthesized with twoset of molecular weight and varied the LA content. PLECs were synthezied by the ring opening polymerization with the (Tin)octoate as a catalyst. PLEC microparticles were prepared by single emulsion method which provided the average size of 3-4 μm. the compression heat molding was used to fabricate 2 types of PLEC polymeric rods; 1) PLEC loaded trypan blue rods and 2) PLEC rods without trypan blue (the water absorption study).

The amorphous region (LA) and entanglement of polymer chain (MW) play in important rule of the water absorption rate, the water absorption rate increase as function of percentage of LA increase and molecular weight decrease. The effect of LA content and molecular weight were confirmed by the trypan blue release profile, the higher LA content and lower molecular weight were followed by higher trypan blue release rate (Sector 5). The conclusion, the water absorption is the main mechanism of trypan blue releasing form PLEC polymer which depended on the percentage of D,L-latide content and molecular weight.

The release profiles of the monolithic rods showed the first-ordered pattern, i.e., the burst release at the beginning followed by slow release. This problem can be resolved by covering the entire rods to prevent the burst release and controlled the drug release rate. The coating was carried out by spin-coating to control the thickness at 100 and 200 μm. Therefore, the dye was stored in the reservoir system and the release rate was controlled by film thickness. The thicker film decreased the release rate and caused the delay release (lag time). The burst release can be prevented and the zero-ordered release could be obtained.

SEM was carried out to study PLEC rods morphology change as a result of polymer degradation. The surface observation of PLEC 3 and 6 (with LA) shown the pit-like degradation pattern caused by the loss of amorphous domain. Subsequently,

the appearance of porous was accelerated the water uptake, so the core of rods was extensive eroded as shown Figure 5.19 b3 and d3 (SEM of cross-section). In another hand, PLEC 1 and 3 (without LA content) showed no pit-like degradation pattern but the extensive fracture through out the surface. These rods also showed the lower extend of degradation due to lower water absorption rate.

GPC indicated that the average molecular weight decreased as a function of time.PDIs (polydispersity) became progressively broader over time. In the degradation process, the PLEC polymeric chain was cut at ester bone between CL of LA linkages.

In the future, the anticancer drug (Doxorubicin) will be incorporated and move forward to *in vivo* test.

REFERENCES

1. WHO. *Cancer*. 2008 [cited; Available from: http://www.who.int/mediacentre/factsheets/fs297/en.

2.Chulabhorn_Cancer_Center. *Thailand cancer* 2008 [cited; Available from: http://www.cccthai.org/New-Toppic02/5-02-2551_Cancer_World/cancer-world-day2551.php.

3.But, D.Y., C.L. Lai, and M.F. Yuen, *Natural history of hepatitis-related hepatocellular carcinoma.* World J Gastroenterol, 2008. **14**(11): p. 1652-6.

4.Vatanasapt, V., S. Sriamporn, and P. Vatanasapt, *Cancer control in Thailand.* Jpn J Clin Oncol, 2002. **32 Suppl**: p. S82-91.

5.Weinberg, B.D., et al., *Antitumor efficacy and local distribution of doxorubicin via intratumoral delivery from polymer millirods.* J Biomed Mater Res A, 2007. **81**(1): p. 161-70.

6.*Gliadel wafers for treatment of brain tumors.* Med Lett Drugs Ther, 1998. **40**(1035): p. 92.

7.Au, J.L., et al., *Determinants of drug delivery and transport to solid tumors.* J Control Release, 2001. **74**(1-3): p. 31-46.

8.Gutmann, R., et al., *Interstitial hypertension in head and neck tumors in patients: correlation with tumor size.* Cancer Res, 1992. **52**(7): p. 1993-5.

9.Jain, R.K., *The next frontier of molecular medicine: delivery of therapeutics.* Nat Med, 1998. **4**(6): p. 655-7.

10.Leunig, M., et al., *Interstitial fluid pressure in solid tumors following hyperthermia: possible correlation with therapeutic response.* Cancer Res, 1992. **52**(2): p. 487-90.

11.Leunig, M., et al., *Angiogenesis, microvascular architecture, microhemodynamics, and interstitial fluid pressure during early growth of human adenocarcinoma LS174T in SCID mice.* Cancer Res, 1992. **52**(23): p. 6553-60.

12.Chengyun Ning, e.a., *In vitro mineralization of surface-modified porous polycaprolactone scaffolds in simulated body fluid.* Applied surface science, , , . **Volume 255, Issue 2**(15 Novenber 2008): p. pp. 429-431.

13.Hu, Y., et al., *Preparation and drug release behaviors of nimodipine-loaded poly(caprolactone)-poly(ethylene oxide)-polylactide amphiphilic copolymer nanoparticles.* Biomaterials, 2003. **24**(13): p. 2395-404.

14.Shogren, R., *A water vapor permeability of biodegradable polymers.* J Environ Polym Degrad, 1997. **5**(5).

15.Wang, Z., et al., *In vitro homogeneous and heterogeneous degradation of poly(epsilon-caprolactone/polyethylene glycol/L-lactide): The absence of autocatalysis and the role of enzymes.* J Biomed Mater Res A, 2006. **79**(1): p. 6-15.

16.Nasongkla, N., et al., *cRGD-functionalized polymer micelles for targeted doxorubicin delivery.* Angew Chem Int Ed Engl, 2004. **43**(46): p. 6323-7.

17.Gong, C.Y., et al., *Thermosensitive PEG-PCL-PEG hydrogel controlled drug delivery system: Sol-gel-sol transition and in vitro drug release study.* J Pharm Sci, 2009.

18.Zou, W., et al., *Studies on bioadhesive PLGA nanoparticles: A promising gene delivery system for efficient gene therapy to lung cancer.* Int J Pharm, 2009. **370**(1-2): p. 187-95.

19.Moffatt, S. and R.J. Cristiano, *PEGylated J591 mAb loaded in PLGA-PEG-PLGA tri-block copolymer for targeted delivery: in vitro evaluation in human prostate cancer cells.* Int J Pharm, 2006. **317**(1): p. 10-3.

20.Luo, G., et al., *RNA interference of MBD1 in BxPC-3 human pancreatic cancer cells delivered by PLGA-poloxamer nanoparticles.* Cancer Biol Ther, 2009. **8**(7): p. 594-8.

21.Qian, F., et al., *Quantification of in vivo doxorubicin transport from PLGA millirods in thermoablated rat livers.* J Control Release, 2003. **91**(1-2): p. 157-66.

22.Liu, X., et al., *Pulsatile release of parathyroid hormone from an implantable delivery system.* Biomaterials, 2007. **28**(28): p. 4124-31.

23.Hariharan, S., et al., *Design of estradiol loaded PLGA nanoparticulate formulations: a potential oral delivery system for hormone therapy.* Pharm Res, 2006. **23**(1): p. 184-95.

24.Bota, D.A., et al., *Interstitial chemotherapy with biodegradable BCNU (Gliadel) wafers in the treatment of malignant gliomas.* Ther Clin Risk Manag, 2007. **3**(5): p. 707-15.

25.McGirt, M.J., et al., *Gliadel (BCNU) wafer plus concomitant temozolomide therapy after primary resection of glioblastoma multiforme.* J Neurosurg, 2009. **110**(3): p. 583-8.

26.Quinn, J.A., et al., *Phase II trial of Gliadel plus O6-benzylguanine in adults with recurrent glioblastoma multiforme.* Clin Cancer Res, 2009. **15**(3): p. 1064-8.

27.Mahmud, A., X.B. Xiong, and A. Lavasanifar, *Development of novel polymeric micellar drug conjugates and nano-containers with hydrolyzable core structure for doxorubicin delivery.* Eur J Pharm Biopharm, 2008. **69**(3): p. 923-34.

28.Nasongkla, N., et al., *Multifunctional polymeric micelles as cancer-targeted, MRI-ultrasensitive drug delivery systems.* Nano Lett, 2006. **6**(11): p. 2427-30.

29.Kirsh, R., P.J. Bugelski, and G. Poste, *Drug delivery to macrophages for the therapy of cancer and infectious diseases.* Ann N Y Acad Sci, 1987. **507**: p. 141-54.

30.Tunon, A., et al., *Drug release from reservoir pellets compacted with some excipients of different physical properties.* Eur J Pharm Sci, 2003. **20**(4-5): p. 469-79.

31.Qian, F., N. Nasongkla, and J. Gao, *Membrane-encased polymer millirods for sustained release of 5-fluorouracil.* J Biomed Mater Res, 2002. **61**(2): p. 203-11.

32.Jonnalagadda, S. and D.H. Robinson, *A bioresorbable, polylactide reservoir for diffusional and osmotically controlled drug delivery.* AAPS PharmSciTech, 2000. **1**(4): p. E29.

33.Peyman, G.A., et al., *In vitro evaluation of polymeric matrix and porous biodegradable reservoir devices for slow-release drug delivery.* Ophthalmic Surg Lasers, 1996. **27**(5): p. 384-91.

34.Langer, R. and J. Folkman, *Polymers for the sustained release of proteins and other macromolecules.* Nature, 1976. **263**(5580): p. 797-800.

35.Wu, M.P., et al., *In vivo versus in vitro degradation of controlled release polymers for intracranial surgical therapy.* J Biomed Mater Res, 1994. **28**(3): p. 387-95.

36.Brem, H., et al., *Interstitial chemotherapy with drug polymer implants for the treatment of recurrent gliomas.* J Neurosurg, 1991. **74**(3): p. 441-6.

37.Dhanaraju, M.D., et al., *Characterization of polymeric poly(epsilon-caprolactone) injectable implant delivery system for the controlled delivery of contraceptive steroids.* J Biomed Mater Res A, 2006. **76**(1): p. 63-72.

38.Qian, F., A. Szymanski, and J. Gao, *Fabrication and characterization of controlled release poly(D,L-lactide-co-glycolide) millirods.* J Biomed Mater Res, 2001. **55**(4): p. 512-22.

39.Wang, F., G.M. Saidel, and J. Gao, *A mechanistic model of controlled drug release from polymer millirods: effects of excipients and complex binding.* J Control Release, 2007. **119**(1): p. 111-20.

40.Habib, Y.S., Augsburger, L.L., Shangraw, R.F., *Production of inert cushioning beads: effect of excipients on the physicomechanical properties of freeze-dried beads containing microcrystalline cellulose produced by extrusion-spheronization.* Int. J. Pharm, 2002. **233**: p. 67–83.

41.Richter, A.W. and E. Akerblom, *Antibodies against polyethylene glycol produced in animals by immunization with monomethoxy polyethylene glycol modified proteins.* Int Arch Allergy Appl Immunol, 1983. **70**(2): p. 124-31.

42.Herold, D.A., K. Keil, and D.E. Bruns, *Oxidation of polyethylene glycols by alcohol dehydrogenase.* Biochem Pharmacol, 1989. **38**(1): p. 73-6.

43.PCL, *Caprolactone.*

44.Athanasiou, K.A., G.G. Niederauer, and C.M. Agrawal, *Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers.* Biomaterials, 1996. **17**(2): p. 93-102.

45.PLC, *L-Lactide.*

46.Fu, Y.J., et al., *Characteristic and controlled release of anticancer drug loaded poly (D,L-lactide) microparticles prepared by spray drying technique.* J Microencapsul, 2001. **18**(6): p. 733-47.

47.Sternberg, K., et al., *In vitro study of drug-eluting stent coatings based on poly(L-lactide) incorporating cyclosporine A - drug release, polymer degradation and mechanical integrity.* J Mater Sci Mater Med, 2007. **18**(7): p. 1423-32.

48.Cho, H., D. Chung, and A. Jeongho, *Poly(D,L-lactide-ran-epsilon-caprolactone)-poly(ethylene glycol)-poly(D,L-lactide-ran-epsilon-caprolactone) as parenteral drug-delivery systems.* Biomaterials, 2004. **25**(17): p. 3733-42.

49.PEG, *PEG.*

50.Ge, H., et al., *Preparation, characterization, and drug release behaviors of drug nimodipine-loaded poly(epsilon-caprolactone)-poly(ethylene oxide)-poly(epsilon-caprolactone) amphiphilic triblock copolymer micelles.* J Pharm Sci, 2002. **91**(6): p. 1463-73.

51.Dechy-Cabaret, O., B. Martin-Vaca, and D. Bourissou, *Controlled ring-opening polymerization of lactide and glycolide.* Chem Rev, 2004. **104**(12): p. 6147-76.

52.Kaihara, S., et al., *Synthesis of poly(L-lactide) and polyglycolide by ring-opening polymerization.* Nat Protoc, 2007. **2**(11): p. 2767-71.

53.wikipedia, *NMR.*

54.YAN ZHANG , C.W., WULI YANG , BIN SHI , SHOUKUAN FU ,, *Tri-component diblock copolymers of poly(ethylene glycol)-poly(ε-caprolactone-co-lactide) : synthesis, characterization and loading camptothecin.* Colloid and polymer science ISSN 0303-402X CODEN CPMSB6 2005. **vol. 283**(no11): p. 1246-1252

55.Cho, H. and J. An, *The effect of epsilon-caproyl/D,L-lactyl unit composition on the hydrolytic degradation of poly(D,L-lactide-ran-epsilon-caprolactone)-poly(ethylene glycol)-poly(D,L-lactide-ran-epsilon-caprolactone).* Biomaterials, 2006. **27**(4): p. 544-52.

56.Takanari Muroya, *Degradation of cross-linked aliphatic polyester composed of poly(3-caprolactone-co-D,L-lactide) depending on the thermal properties.* Polymer Degradation and Stability, 2008. **94**: p. 285–290.

57.Huang MH, S.L., Coudane J, Vert M., *Synthesis and characterization of block copolymers of e-caprolactone and DL-lactide initiated by ethylene glycol or poly(ethylene glycol).* Macromol Chem Phys, 2003. **204.**: p. 1994-2001.

58.Bramfeldt, H., P. Sarazin, and P. Vermette, *Characterization, degradation, and mechanical strength of poly(D,L-lactide-co-epsilon-caprolactone)-poly(ethylene glycol)-poly(D,L-lactide-co-epsilon-caprolactone).* J Biomed Mater Res A, 2007. **83**(2): p. 503-11.

59.Stevents, M.P., ed. *Polymer chemistry and introduction,*. 1990, Oxford University Press: New York.

60.Li, S., *Hydrolytic degradation characteristics of aliphatic polyesters derived from lactic and glycolic acids.* J Biomed Mater Res, 1999. **48**(3): p. 342-53.

APPENDICES

APPENDIX A

Biodegradable polymeric implants as drug delivery systems for liver cancer therapy

Pat Akarajirathun and Norased Nasongkla*

***Abstract*—The narrow therapeutic window and adverse effect of anticancer drugs are the limitation for liver cancer therapy. Consequently, there are the demand for the drug delivery systems that can transport drugs directly into the tumor, reduce the systemic drug concentration and subsequently decrease the side effect. The biocompatible and biodegradable polymers are commonly used for the drug delivery applications. Therefore, the focus of this review is to provide the utilization of polymers to develop drug delivery systems for liver cancer therapy. A variety of drug delivery systems such as nanoparticles, microspheres, gel and rod are included in this review.**

I. Introduction

CANCER is the leading cause of death worldwide which account for 7.9 million death (13% of all deaths worldwide) in 2007 [1]. Among many kinds of cancers, liver cancer is the main cause of cancer mortality with 598,000 deaths per year worldwide [3]. This cancer is estimated to cause approximately 18,160 deaths in US from the expected 22,620 new cases in the year 2009.[2] Many countries such as Southeast Asia and Africa have 10–fold more cases than the developed countries. Common liver cancers are as follows: 1) hepatocellular carcinoma (hepatoma or HCC) is the primary and common malignancy among the liver cancer caused by the hepatitis B or C; 2) cholangiocarcinoma is caused from the chronic infection of a parasite (*Clonorchis sinensis*) which causes a major healthcare problem in Southeast Asia.

This review aims to provide the update and application of polymeric drug delivery systems that can introduce drug locally into liver tumors. Difficulty in conventional drug administration for liver cancer will be reviewed first, followed by the in-depth summary on the utilization of polymers as drug delivery systems. Current treatment for liver cancer includes surgical resection, systemic or regional chemotherapy, arterial embolization, cryotherapy and radiation therapy. For patients who are surgical candidates, resection of hepatic tumors can increase survival from a median of 6 months to 29-30 months, with up to a 40% five year survival. Even though surgical resection demonstrates that the local therapy can improve the outcome for a majority of patients, the resection may not be possible due to factors such as age, poor general health, multiple tumor sites and advanced cirrhosis. The high number of unresectable tumor cases demonstrates the necessity to develop a minimally invasive technique for the local tumor therapy.

The conventional route for liver cancer chemotherapy is the intravenous injection. For this route, drugs must access several systems until they reach the target cells. The passage starts at blood circulation and then drugs are transported to heart and subsequently high blood perfusion organs such as liver and lung. Then drugs must diffuse through the solid tumor where the diffusion is very limited because of interstitial hypertension [3-7]. According to these problems, most of anticancer drugs can not proficiently reach the targeted cancer cells and lose during transportation process. These drugs can produce

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side effects to adjacent tissues and organs. Furthermore, the unpredictable amount of drugs on targeted organs leads to the ineffective dose or overdose to patients.

II. Polymers in Drug Delivery Systems

Currently, polymers have been extensively used in many fields especially in medicine to solve problems mentioned above. Polymers are widely used in medical application because of its unique and outstanding properties such as thermoplastic property, thermal property, hydrophillicity, biocompatibility and biodegradability. The polymeric drug delivery is one of the polymer applications in medicine [8, 9].

Polyester has been used in drug delivery applications. This kind of polymers is synthesized by ring-opening polymerization which provides the narrow distribution of molecular weight. Polyester has a unique degradation characteristic so-called "autocatalytic effect" in which the bulk erosion generates acid inside the polymer leading to the inside out degradation.

Poly(ε-caprolactone) (PCL) is a semicrystalline polyester with five methylene groups ($-CH_2$) in its chain resulting in the high flexible property in the amorphous region. Consequently, drug molecules can diffuse through the amorphous region of PCL [10]. When PCL is used as copolymer, its hydrophobic property can prolong degradation time [11]. PCL has been used in many applications in drug delivery, Nasongkla, N. et al. formed PCL micelles and modified surface with $\alpha_v\beta_3$ ligand (cRGDfK) that delivered the Doxorubicin (an anticancer drug) to targeted cancer cells [12]. Additionally, Gong, C. Y. et al. synthesized sol-gel of PCL-PEG copolymer with temperature sensitivity [13].

Poly(lactide-co-glycolic acid) (PLGA) is mostly used in the drug delivery system application for cancer therapy [14-16]. PLGA properties can be controlled by adjusting the copolymer composition such as increasing glycolide content (raising hydrophillicity and degradation rate). For instance, the commercial drug delivery product "Luporn Deport®" delivers Leuprolide hormone for prostate gland cancer and endometriosis, and it is approved by FDA. Qian, F. et al. fabricated PLGA millirods which contained doxorubicin and was implanted into rat and rabbit liver tumors [17].

Drug delivery is the administration of pharmaceutical compounds to reach the therapeutic effect in humans or animals [3]. The common routes of drug administration are parental (through the mouth), topical (skin), transmucosal (nasal, buccal/sublingual, vaginal, ocular and rectal) and inhalation. Some of medications such as peptide/protein, antibody, vaccine, gene based and harmful drugs are limited for these common routes. Consequently, the drug delivery system is developed to contain and transport drugs to target organs, for example, the hormone delivery system was used to avoid the enzymatic degradation of peptides and anticancer drugs [18, 19]. Moreover, the therapeutic effect can be achieved by intratumoral administration which can directly introduce drugs to the cancer cells.

Drug delivery for liver cancer therapy can be developed in different approach as shown in the sequence from small to large: nanoparticles, microspheres, polymeric gel and polymeric rods. These drug delivery systems were designed to be able to deliver drug locally or even intratumorally. The advantages of these approaches are as shown below.

1) This method is a minimal invasive procedure which leaves only a small wound after therapy and does not need to be removed because of the biodegradable property of polymers.

2) The optimum release rate, dose and time.

3) This method can reduce the systematic drug concentration and decrease side effect2 of drug comparing to the conventional chemotherapy.

Nanoparticles

The nanotechnology has drawn the researcher attention in the recent decade and has been applied in many fields including drug delivery systems. The polymeric nanoparticles were developed for liver cancer treatment [20-23]. The anticancer drug was trapped in polymeric nanospheres which are made up of hydrophobic polymers. The cis-Dichlorodiamminoplatinum (II) (cisplatin) was widely used in clinical treatment for cancer chemotherapy but its high toxicity to neurons; gastric system and kidney hinders its application.

Li, X. et al.[24] reported the efficiency of cisplatin-loaded nanoparticles *via* intratumoral injection. The spherical core-shell structure of mPEG-block-PCL copolymers was used as carrier and incorporated with cisplatin. The mPEG4k-PCL20k contained 5.66% w/w cisplatin had 71.3 ± 0.4 nm in diameter and 5.28 ± 0.73%, 88.3 ± 9.3% and 93 ± 2.7% of drug loading content, encapsulation efficiency and nanoparticle yield,

respectively. The cisplatin loaded nanoparticles prolonged cisplatin release rate compared to the free cisplatin, and reduced the growth of hepatoma cell line up to 80%. ICR mice with murine Hepatoma cell line (H_{22}) were injected with saline, pure polymer, free cisplatin at 5, 10 and 20 mg/kg, respectively and also with the cisplatin loaded nanoparticles at 5, 10 and 20 mg/kg. It was found that the tumor volume of cisplatin group was smaller than that of non-cisplatin group (saline and pure polymer). The controlled release of cisplatin from the nanoparticles led to the smaller tumor volume of mice injected with cisplatin loaded nanoparticles (800 mm^3) compared to the free cisplatin group (1,200 mm^3).

MICROSPHERE

The initial burst, the fast drug release rate at the early time period due to the drug presentation on particle surface following by the slow release rate, is the inherited problem for the nanoparticle drug delivery system due to its high surface area and remains as the challenge for researchers. Microspheres with the same total weight theoretically have lower surface area compared to the nanoparticles are therefore selected as the alternative route to deliver drugs [25, 26]. Moreover, the drug encapsulation efficiency of Microspheres is higher [27]. Unfortunately, their microscale limits the transport through the blood vessel or intravenous injection.

Verrijk, R. et at.[28] demonstrated the utilization of PLGA microspheres loaded with cisplatin (cDDP). The male Wag/Rij rats were seeded with the cancer cell line CC531. Then, rats were treated with microspheric cDDP and free cDDP *via* mecenteric vain (venoportal). Not only the antitumor efficacy study was carried out but the kidney functional indicator (plasma urine and creatinine) and histology of kidney and liver were also considered to study the toxicity of cDDP. It was found that the smaller size of tumors was observed in the PLGA-cDDP group. The free cDDP group showed the higher damage of hepatocytes and glomerular and extensive increase of plasma urine and creatinine.

Another application of PLGA is the encapsulation of norcantharidin for chemoembolization[29]. PLGA is one type of polyester that has slow hydrolysis rate which subsequently leads to the slow release and takes a long time to degrade. Therefore, the alginate was combined with PLGA to increase the release rate. The higher alginate concentration could raise the norcantharidin loading content. For the *in vivo* test, male Sprague-Dawley rats which contained the hepatoma cell line (Walker 256) were treated by norcantharidin (10mg/kg) loaded PLGA with 3% w/w alginate, free norcantharidin (1.5mg/kg) and normal saline (1.5 ml/kg), respectively. The result showed that the norcantharidin loaded PLGA group had the highest survival rate at 126.8% for 31 ± 3.9 days.

The microsphere drug delivery was also developed for the intratumoral injection. Hanes, J. et at.[30] developed an injectable polymeric system containing interlukin-2 (IL-2). The complex coacervation of gelatin and chondroitin sulfate was chosen instead of PLGA to avoid the metabolic product of PLGA, *i.e.* lactic acid that can denature the proteins. Each BALB/C mouse was infused with 5 x 10^4 CT26 carcinoma cells. The IL-2 microsphere could inhibit the tumor growth which had tumor volumes of 14 ± 3 mm^3. Moreover, the healthy liver was showed upon autopsy analysis. On the other hand, empty microspheres had large tumor volume of 7,025 ± 2,354 mm^3.

The clinical trial of microspheres has been reported [31-33]. Kettenbach J. et al. showed the 15 patients from Hong Kong whose had 93.3% survival rate after treated with 75 mg/m^2 of PVA-DOX chemoembolization[34].

ROD

Tumor recurrence is a common problem in cancer therapy because of the residue cancer cells on boundary of tumor after surgery or ablation. Therefore, the ablation technique combination to the polymeric rods implantation has been demonstrated[35] to get rid of these cancer cells. Even though, the tumor ablation can increase the penetration of drugs, multiple polymeric rod implantations were reported for massive tumors to facilitate the drug distribution through out tumors[36]. Weinberg et at. reported the implantation of 4 doxorubicin-loaded PLGA millirods while the 3-D mathematical model stimulation was used to predict the results. In the 8^{th} day, the multiple implantations could maintain the DOX concentration in the tumor at 290 μg/g which was 3-fold higher than the single implantation. Moreover, the DOX concentration at the boundary of tumors which have a high risk of cancer recurrence was maintained at 99.3 μg/g that was 6-folds higher than the single implantation.

TABLE I
A VARIETY OF DRUG DELIVERY SYSTEMS FOR LIVER CANCER THERAPY

Formulations	Anticancer drug	Polymer	*In vitro* study	*In vivo* study	Re
Nanoparticles	Cisplatin	mPEG/ PCL	- Gastric (BGC-823) - Hepatoma (H22)	- ICR mices - Hepatoma (H22)	[2
Microspheres	Cisplatin	PLGA	-	- Wag/Rij rats - Carcinoma (CC531)	[2
Microspheres/ Chemo-embolization	Norcan tharidin	PLGA	- Cancer (SM-7721)	- Sprague / Dawley - Hepatoma (Walker 256)	[2
Microspheres/ Chemo-embolization	DOX	PVA	-	- Human HCC patiens	[3
Microspheres/ Injectable	IL-2	Gelatin/ Chon droitin	-	- BALB/C Mices - Carcinoma (CT26)	[3
Millirod	DOX	PLGA	-	- Rabbit - Carcinoma (VX2)	[3

III. CONCLUSION

A variety of drug delivery systems can be developed to locally deliver drugs to liver tumors. These systems can maintain the drug concentration within the therapeutic range, reduce the systemic drug concentration and avoid the side effects. The promising *in vivo* results have shed the light on the alternative procedure for liver cancer therapy.

REFERENCES

[1] WHO, "CANCER," 2008.

[2] A. JEMAL, R. SIEGEL, E. WARD, Y. HAO, J. XU, AND M. J. THUN, "CANCER STATISTICS, 2009," *CA CANCER J CLIN,* VOL. 59, P. 225, 2009.

[3] J. L. AU, S. H. JANG, J. ZHENG, C. T. CHEN, S. SONG, L. HU, AND M. G. WIENTJES, "DETERMINANTS OF DRUG DELIVERY AND TRANSPORT TO SOLID TUMORS," *J CONTROL RELEASE,* VOL. 74, PP. 31-46, JUL 6 2001.

[4] R. GUTMANN, M. LEUNIG, J. FEYH, A. E. GOETZ, K. MESSMER, E. KASTENBAUER, AND R. K. JAIN, "INTERSTITIAL HYPERTENSION IN HEAD AND NECK TUMORS IN PATIENTS: CORRELATION WITH TUMOR SIZE," *CANCER RES,* VOL. 52, PP. 1993-5, APR 1 1992.

[5] R. K. JAIN, "THE NEXT FRONTIER OF MOLECULAR MEDICINE: DELIVERY OF THERAPEUTICS," *NAT MED,* VOL. 4, PP. 655-7, JUN 1998.

[6] M. LEUNIG, A. E. GOETZ, M. DELLIAN, G. ZETTERER, F. GAMARRA, R. K. JAIN, AND K. MESSMER, "INTERSTITIAL FLUID PRESSURE IN SOLID TUMORS FOLLOWING HYPERTHERMIA: POSSIBLE CORRELATION WITH THERAPEUTIC RESPONSE," *CANCER RES,* VOL. 52, PP. 487-90, JAN 15 1992.

[7] M. LEUNIG, F. YUAN, M. D. MENGER, Y. BOUCHER, A. E. GOETZ, K. MESSMER, AND R. K. JAIN, "ANGIOGENESIS, MICROVASCULAR ARCHITECTURE, MICROHEMODYNAMICS, AND INTERSTITIAL FLUID PRESSURE DURING EARLY GROWTH OF HUMAN ADENOCARCINOMA LS174T IN SCID MICE," *CANCER RES,* VOL. 52, PP. 6553-60, DEC 1 1992.

[8] B. D. WEINBERG, H. AI, E. BLANCO, J. M. ANDERSON, AND J. GAO, "ANTITUMOR EFFICACY AND LOCAL DISTRIBUTION OF DOXORUBICIN VIA INTRATUMORAL DELIVERY FROM POLYMER MILLIRODS," *J BIOMED MATER RES A,* VOL. 81, PP. 161-70, APR 2007.

[9] "GLIADEL WAFERS FOR TREATMENT OF BRAIN TUMORS," *MED LETT DRUGS THER,* VOL. 40, P. 92, SEP 11 1998.

[10] R. SHOGREN, "A WATER VAPOR PERMEABILITY OF BIODEGRADABLE POLYMERS," *J ENVIRON POLYM DEGRAD,* VOL. 5, 1997.

[11] Z. WANG, S. WANG, R. GUIDOIN, Y. MAROIS, AND Z. ZHANG, "IN VITRO HOMOGENEOUS AND HETEROGENEOUS DEGRADATION OF POLY(EPSILON-CAPROLACTONE/POLYETHYLENE GLYCOL/L-LACTIDE): THE ABSENCE OF AUTOCATALYSIS AND THE ROLE OF ENZYMES," *J BIOMED MATER RES A,* VOL. 79, PP. 6-15, OCT 2006.

[12] N. NASONGKLA, X. SHUAI, H. AI, B. D. WEINBERG, J. PINK, D. A. BOOTHMAN, AND J. GAO, "CRGD-FUNCTIONALIZED POLYMER MICELLES FOR TARGETED DOXORUBICIN DELIVERY," *ANGEW CHEM INT ED ENGL,* VOL. 43, PP. 6323-7, NOV 26 2004.

[13] C. Y. GONG, P. W. DONG, S. SHI, S. Z. FU, J. L. YANG, G. GUO, X. ZHAO, Y. Q. WEI, AND Z. Y. QIAN, "THERMOSENSITIVE PEG-PCL-PEG HYDROGEL CONTROLLED DRUG DELIVERY SYSTEM: SOL-GEL-SOL TRANSITION AND IN VITRO DRUG RELEASE STUDY," *J PHARM SCI,* FEB 2 2009.

[14] W. ZOU, C. LIU, Z. CHEN, AND N. ZHANG, "STUDIES ON BIOADHESIVE PLGA NANOPARTICLES: A PROMISING GENE DELIVERY SYSTEM FOR EFFICIENT GENE THERAPY TO LUNG CANCER," *INT J PHARM,* VOL. 370, PP. 187-95, MAR 31 2009.

[15] S. MOFFATT AND R. J. CRISTIANO, "PEGYLATED J591 MAB LOADED IN PLGA-PEG-PLGA TRI-BLOCK COPOLYMER FOR TARGETED DELIVERY: IN VITRO EVALUATION IN HUMAN PROSTATE CANCER CELLS," *INT J PHARM,* VOL. 317, PP. 10-3, JUL 6 2006.

[16] G. LUO, C. JIN, J. LONG, D. FU, F. YANG, J. XU, X. YU, W. CHEN, AND Q. NI, "RNA INTERFERENCE OF MBD1 IN BXPC-3 HUMAN PANCREATIC CANCER CELLS DELIVERED BY PLGA-POLOXAMER NANOPARTICLES," *CANCER BIOL THER,* VOL. 8, PP. 594-8, APR 2009.

[17] F. QIAN, N. STOWE, E. H. LIU, G. M. SAIDEL, AND J. GAO, "QUANTIFICATION OF IN VIVO DOXORUBICIN TRANSPORT FROM PLGA MILLIRODS IN THERMOABLATED RAT LIVERS," *J CONTROL RELEASE,* VOL. 91, PP. 157-66, AUG 28 2003.

[18] X. LIU, G. J. PETTWAY, L. K. MCCAULEY, AND P. X. MA, "PULSATILE RELEASE OF PARATHYROID HORMONE FROM AN IMPLANTABLE DELIVERY SYSTEM," *BIOMATERIALS,* VOL. 28, PP. 4124-31, OCT 2007.

[19] S. HARIHARAN, V. BHARDWAJ, I. BALA, J. SITTERBERG, U. BAKOWSKY, AND M. N. RAVI KUMAR, "DESIGN OF ESTRADIOL LOADED PLGA NANOPARTICULATE FORMULATIONS: A POTENTIAL ORAL DELIVERY SYSTEM FOR HORMONE THERAPY," *PHARM RES,* VOL. 23, PP. 184-95, JAN 2006.

[20] P. XU, E. A. VAN KIRK, W. J. MURDOCH, Y. ZHAN, D. D. ISAAK, M. RADOSZ, AND Y. SHEN, "ANTICANCER EFFICACIES OF CISPLATIN-RELEASING PH-RESPONSIVE NANOPARTICLES," *BIOMACROMOLECULES,* VOL. 7, PP. 829-35, MAR 2006.

[21] H. ARAKI, T. TANI, AND M. KODAMA, "ANTITUMOR EFFECT OF CISPLATIN INCORPORATED INTO POLYLACTIC ACID MICROCAPSULES," *ARTIF ORGANS,* VOL. 23, PP. 161-8, FEB 1999.

[22] E. C. Gryparis, M. Hatziapostolou, E. Papadimitriou, and K. Avgoustakis, "Anticancer activity of cisplatin-loaded PLGA-mPEG nanoparticles on LNCaP prostate cancer cells," *Eur J Pharm Biopharm,* vol. 67, pp. 1-8, Aug 2007.

[23] K. Avgoustakis, A. Beletsi, Z. Panagi, P. Klepetsanis, A. G. Karydas, and D. S. Ithakissios, "PLGA-mPEG nanoparticles of cisplatin: in vitro nanoparticle degradation, in vitro drug release and in vivo drug residence in blood properties," *J Control Release,* vol. 79, pp. 123-35, Feb 19 2002.

[24] X. Li, R. Li, X. Qian, Y. Ding, Y. Tu, R. Guo, Y. Hu, X. Jiang, W. Guo, and B. Liu, "Superior antitumor efficiency of cisplatin-loaded nanoparticles by intratumoral delivery with decreased tumor metabolism rate," *Eur J Pharm Biopharm,* vol. 70, pp. 726-34, Nov 2008.

[25] A. L. Lewis, M. V. Gonzalez, A. W. Lloyd, B. Hall, Y. Tang, S. L. Willis, S. W. Leppard, L. C. Wolfenden, R. R. Palmer, and P. W. Stratford, "DC bead: in vitro characterization of a drug-delivery device for transarterial chemoembolization," *J Vasc Interv Radiol,* vol. 17, pp. 335-42, Feb 2006.

[26] A. L. Lewis, R. R. Taylor, B. Hall, M. V. Gonzalez, S. L. Willis, and P. W. Stratford, "Pharmacokinetic and safety study of doxorubicin-eluting beads in a porcine model of hepatic arterial embolization," *J Vasc Interv Radiol,* vol. 17, pp. 1335-43, Aug 2006.

[27] R. M. Ribeiro-Costa, A. J. Alves, N. P. Santos, S. C. Nascimento, E. C. Goncalves, N. H. Silva, N. K. Honda, and N. S. Santos-Magalhaes, "In vitro and in vivo properties of usnic acid encapsulated into PLGA-microspheres," *J Microencapsul,* vol. 21, pp. 371-84, Jun 2004.

[28] R. Verrijk, I. J. Smolders, N. Bosnie, and A. C. Begg, "Reduction of systemic exposure and toxicity of cisplatin by encapsulation in poly-lactide-co-glycolide," *Cancer Res,* vol. 52, pp. 6653-6, Dec 1 1992.

[29] X. Liu, W. S. Heng, Paul, Q. Li, and L. W. Chan, "Novel polymeric microspheres containing norcantharidin for chemoembolization," *J Control Release,* vol. 116, pp. 35-41, Nov 2006.

[30] J. Hanes, A. Sills, Z. Zhao, K. W. Suh, B. Tyler, F. DiMeco, D. J. Brat, M. A. Choti, K. W. Leong, D. M. Pardoll, and H. Brem, "Controlled local delivery of interleukin-2 by biodegradable polymers protects animals from experimental brain tumors and liver tumors," *Pharm Res,* vol. 18, pp. 899-906, Jul 2001.

[31] J. Bruix, M. Sherman, J. M. Llovet, M. Beaugrand, R. Lencioni, A. K. Burroughs, E. Christensen, L. Pagliaro, M. Colombo, and J. Rodes, "Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver," *J Hepatol,* vol. 35, pp. 421-30, Sep 2001.

[32] J. M. Llovet, M. I. Real, X. Montana, R. Planas, S. Coll, J. Aponte, C. Ayuso, M. Sala, J. Muchart, R. Sola, J. Rodes, and J. Bruix, "Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial," *Lancet,* vol. 359, pp. 1734-9, May 18 2002.

[33] K. Malagari, K. Chatzimichael, E. Alexopoulou, A. Kelekis, B. Hall, S. Dourakis, S. Delis, A. Gouliamos, and D. Kelekis, "Transarterial chemoembolization of unresectable hepatocellular carcinoma with drug eluting beads: results of an open-label study of 62 patients," *Cardiovasc Intervent Radiol,* vol. 31, pp. 269-80, Mar-Apr 2008.

[34] J. Kettenbach, A. Stadler, I. V. Katzler, R. Schernthaner, M. Blum, J. Lammer, and T. Rand, "Drug-loaded microspheres for the treatment of liver cancer: review of current results," *Cardiovasc Intervent Radiol,* vol. 31, pp. 468-76, May-Jun 2008.

[35] F. Qian, A. Szymanski, and J. Gao, "Fabrication and characterization of controlled release poly(D,L-lactide-co-glycolide) millirods," *J Biomed Mater Res,* vol. 55, pp. 512-22, Jun 15 2001.

[36] B. D. Weinberg, R. B. Patel, H. Wu, E. Blanco, C. C. Barnett, A. A. Exner, G. M. Saidel, and J. Gao, "Model simulation and experimental validation of intratumoral chemotherapy using multiple polymer implants," *Med Biol Eng Comput,* vol. 46, pp. 1039-49, Oct 2008.

APPENDIX B

Effect of Molecular Weight and Copolymer Composition on the Drug Release from Polymeric Rods

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***Abstract*—The copolymers of poly(ε-caprolactone)-random-poly(D,L-lactide)-block-poly(ethylene glycol)-block-poly(ε-caprolactone)-random-poly(D,L-lactide) (PLEC) were used as materials to develop implantable polymeric rods for liver cancer chemotherapy. PLECs were synthesized and varied the molecular weight and the copolymer ratio between ε-caprolactone and D,L-lactide (CL:LA). PLECs were fabricated in a cylindrical shape and contained a trypan blue, a hydrophilic dye molecule. PLECs with three different LA:CL ratio (LA 0, 15 and 26.5 % mole) and two molecular weight (21 and 50 kD) were synthesized. It was found that increasing amount of LA:CL ratio led to a higher trypan blue release rate. In contrast, drug release rate was lower when the molecular weight of PLECs increased from 21 kDa to 50 kDa.**

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IV. Introduction

Cancer is the main cause of death worldwide and 7.4 million people died because of cancer (13% of all deaths worldwide) in 2007[1]. The death worldwide from cancer is rising continuously and the estimation of the death will be at 12 million in 2030. Among various kinds of cancers, liver cancer is one of the deadliest form and accounts for 598,000 deaths per year worldwide [3]. Surgical resection, systemic or regional chemotherapy, arterial embolization, cryotherapy and radiation therapy are the current treatment for liver cancer. Resection of hepatic tumors can increase five year survival up to 40% from the population and a median of survival time increases from 6 months to 29-30 months. Unfortunately, resection may not be possible for a majority of patients due to factors such as poor general health, age, multiple tumor sites and advanced cirrhosis. Moreover, most of anticancer drugs for chemotherapy can not capably reach the targeted cancer cells and lose during transportation process. Subsequently, adjacent tissues and organs will have side effects from these drugs. In addition, the ineffective dose or overdose to patients can be caused by an unpredictable amount of drugs on targeted organs. Tumor recurrence is common problem in cancer therapy because the residue cancer cells are on boundary of tumor after surgery or ablation[2].

The problems mentioned above leads to the need for the minimally invasive technique for the local tumor therapy. Currently, polymers have been extensively used in a broad spectrum of applications including in medicine. Polymers are wildly used in medical applications not only because of their unique properties such as

thermoplastic property, biocompatibility, and thermal property but also their tunable hydrophilicity and biodegradability. Polymeric drug delivery systems are one of the polymer applications in medicine which is a new trend in pharmaceutical technology [3, 4]. The conventional route of liver cancer chemotherapy is the intravenous injection. This route needs to access several systems until the drug reach the target cells or organs. Initially, drugs are administered to the blood circulation and transported to the liver. Then, they must diffuse through the solid tumor which is very slow because of the interstitial hypertension [5-9]. According to this problem, most of anticancer drugs cannot fully affect the targeted cancer cells because of drug loss during the transportation process and the obstruction by physical barriers.

To solve this problem, drug delivery systems are necessary and needed to be developed. The objective of this study is to fabricate polymeric rods that can transports proper amount of anticancer drugs to liver tumors by directly intratumoral implantation. PLEC copolymer is selected base on each component advantage. Poly(ε-caprolactone) (PCL), poly(D,L-Lactide) (PLA) and poly(ethylene glycol) (PEG) have excellent properties including , biocompatibility, low toxicity, absence of antigenicity, immunogenicity [10, 11], especially an adjustable hydrophilicity and degradation time leading to a controllable drug release rate. PLEC copolymers are synthesized by ring opening polymerization. The ratio of CL:LA was varied to adjust the polymer properties. PLECs were prepared as microspheres by the single emulsion procedure. Then, the fine powder of PLEC microparticles were mixed with trypan blue, a hydrophilic dye, molded into a rod shape. Finally, the trypan blue release from polymeric rods was carried out in phosphate buffered saline (PBS) which mimics the anticancer drugs release *in vivo* [2].

V. MATERIALS AND METHODS

Materials

Poly(ethylene glycol) (PEG, MW = 1000 Da) was purchased from Sigma (USA). ε-Caprolactone monomer (CL, from Aldrich) was dried with calcium hydride (CaH_2) and then distilled at reduced pressure. D,L-Lactide (LA, from Aldrich) was recrystallized from ethyl acetate. Stannous (II) octoate ($Sn(Oct)_2$, from Aldrich). Toluene was dried by refluxing with sodium and distilled under dry argon. Other chemicals reagents were reagent grade and purified by distillation.

Synthesis of block copolymer of PEG and CL/LA (PLEC)

[Poly(ε-caprolactone)-random-Poly(D,L-lactide)]-block-poly(ethylene glycol)-block-[Poly(ε-caprolactone)-random-Poly (D,L-lactide)] with different ratio of CL and LA were synthesized by "ring opening bulk polymerization"[11]. PEG was used as a macro-initiator and (Tin)octoate was used as a catalyst. PEG, CL and LA were weighed in two-neck round bottom flask under argon atmosphere. Then the air was vacated about 6 hours and substituted by argon. The reaction flask was immerged in oil bath and heated at 140 C°. The reaction started when 0.1 % (from the total weight of monomers) of the catalyst (stannous octoate) was added. The reaction was allowed to carry out for 48 hours. Solid PLECs was dissolved in acetone and precipitates by cold methanol. The sediment of PLECs was separated and lyophilized. The nuclear magnetic resonance spectroscopy (NMR) technique was used to measure the molecular weight and copolymer composition of polymers.

Preparation of PLEC microsphere

PLECs were prepared as microspheres by single emulsion procedure using "Oil in Water (o/w) procedure [2]. PLECs in methylene chloride act like an oil phase which is isolated from aqueous phase and forms microscale droplets. PLEC 200 mg was dissolved in 2 ml of methylene chloride. Then, PLEC solution was slowly added drop wise in 100 ml of 1% w/v poly(vinyl alcohol) while mixed by sonicator at 60 % amplitude (130 Watt 20 kHz) for 5 minutes. Next, the mixed solution is added in 300 ml of 1% w/v poly (vinyl alcohol) and stirred at 300 rpm. Methylene chloride was allowed to evaporate for 4 hours while PLEC droplets solidified to form microparticles. PLEC microspheres were collected by centrifugation, 3 times washed by water and lyophilized and obtained as fine powders.

Fabrication of PLEC polymeric rod

PLEC microspheres were prepared as polymeric rods by compression heat molding [2]. PLEC microspheres were mixed with 30% w/w of trypan blue and homogenously mixed by vertex mixer. Then the mixture was filled in the mold which has cylindrical holes with 1.6 mm in diameter and 10 mm in length. PLECs were allowed to anneal at 90 °C with the pressure of 4.6×10^6 Pa for 2 hours. The mold was cooled down to room temperature and polymeric rods were then taken out off the mold.

In vitro release study of trypan blue

Phosphate buffer saline (PBS) pH 7.4 at 37 °C was used as a buffer to study the release of trypan blue. Polymeric rods were weighted and immerged into the vial filled with 15 ml PBS. Vials were shaken by orbital shaker which is controlled at 90 rpm and 37 °C. At a certain time point, PBS in vials were removed out and refreshed with new PBS. The solution was analyzed for the concentration of trypan blue by UV-Visible spectroscopy at the wavelength of 586 nm. The concentration of trypan blue at each time point was carried out in triplicate and was used to calculate the percent accumulative release of trypan blue.

VI. Results and Discussion

The PLEC copolymers were synthesized in 6 different CL:LA ratios. The molecular weights was controlled approximately 21 kDa (PLEC 1, 2 and 3) where the percentages of D,L-lactide were varied at 0, 13.4 and 26.5 % mole, respectively. Moreover, PLECs with the molecular weight of 50 kDa were synthesized and the percentages of D,L-lactide were varied at 0, 15.9 and 19.3% by mole (PLEC 4 to 6).

TABLE 1
CHEMICAL COMPOSITION OF PLEC AS DETERMINED BY ^{1}H NMR.

PLEC	LA		CL	
	MW (kDa)	mol %	MW (kDa)	mol %
1	0	0	19.8	100
2	2.1	13.4	21.3	86.6
3	3.8	26.5	16.7	73.5
4	0	0	45.1	100
5	3.4	15.9	42.4	84.1
6	6.4	19.3	42.4	80.7

The ^{1}H NMR spectrum provides the information on the number and type of chemical composition in molecules. Therefore, the integral area under peaks can be used to calculate the chemical components of each component [12]. The chemical shift at 4.0 and 5.1 ppm are the methine proton of D,L-lactide (-C**H**) and methylene proton (-C**H$_2$**) of ε-caprolactone, respectively. These two peaks were used to calculate the proton ratio by referring to four methylene protons (-C**H$_2$**C**H$_2$**O-) of poly(ethylene glycol) at 3.6 ppm. PLECs were used as raw materials to fabricate polymeric rods. PLECs microspheres are fabricated by the single emulsion procedure. The average size is controlled between 3 – 5 μm. The microscopic size of microparticles leads to the homogeneous distribution of trypan blue throughout PLEC polymeric rods.

PLEC polymeric rods were fabricated by homogeneous mixing of microspheres and the fine powder of trypan blue. The mixture was then molded into cylindrical shape by the compression heat molding. PLEC was allowed to anneal at 90 ^{o}C and 4.6 MPa compressive pressures for 2 hours. The size of PLEC polymeric rods was 10 mm in length and 1.6 mm in diameter. Since, the LA segment raised the hydrophilicity of PLECs which increased the water absorption rate, so the higher LA content provided a faster release. It was found that PLEC 3 (26.5 % LA) was the fastest release and PLEC 1 (0 % LA) was the slowest release for PLECs with 21 kDa (Figure 1). Furthermore, PLEC 1, 2 and 3 provided the zero-order release over one day (Figure 1 inset) where the time for the 50 percents trypan blue release of PLEC 1, 2 and 3 were 58, 43 and 36 hours, respectively. However, rising amount of LA content in PLEC copolymers will not necessary increase the release rate by increasing the hydrophilicity of copolymers but in contrast it leads to the decrease in the polymer chain flexibility which eventually hinders the diffusion of trypan blue through the polymer matrix [13]. This situation can be confirmed by the increase in the glass transition temperature (T_g) of the copolymer compared to homopolymer of poly(ε-caprolactone) [14].

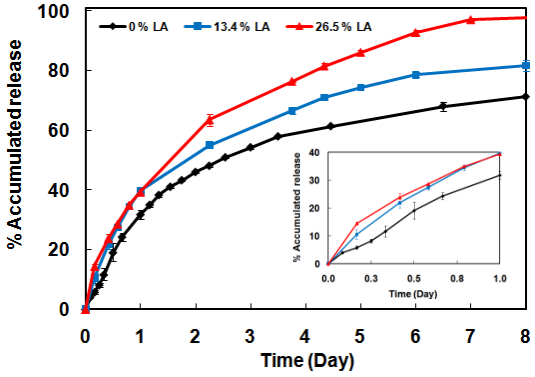

Fig. 1. The trypan release profile from PLECs 21 kDa with 30% trypan blue loading and different LA:CL mole ratio. (The inset showed the first day release.)

For PLECs with MW of 50 kDa (Figure 2), PLEC 6 (19.3% LA) had the fastest trypan blue release as a result of the highest LA content. The zero-order release within 5 days was found only in PLEC 4 while the burst release was observed in PLEC 5 and 6. The time for 50 percent release of PLEC 4, 5 and 6 are 246, 36 and 9 hours, respectively.

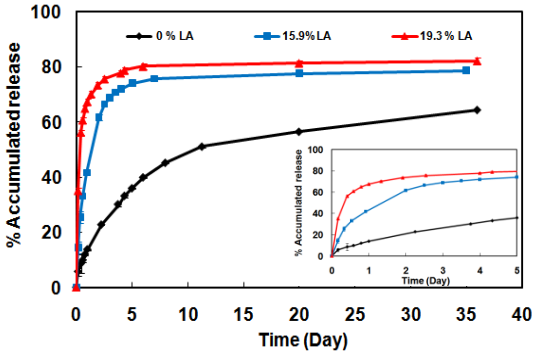

FIG. 2. THE TRYPAN RELEASE PROFILE FROM PLECS 50 KDA WITH 30% TRYPAN BLUE LOADING AND DIFFERENT LA:CL MOLE RATIO. (THE INSET SHOWED THE FIVE DAY RELEASE.)

Effect of molecular weight on the release profiles was demonstrated in Figure 3. Trypan blue release from the higher molecular weight (PLEC 4, 46.1 kDa) was slower than that of lower molecular weight (PLEC 1, 20.8 kDa) which corresponded to more limited diffusion of trypan blue molecule through the polymer matrix and the more chain entanglement of a higher molecular weight polymer. Both of copolymers showed the incomplete release which was occurred by crystallinity entrapment [15]. This phenomenon was not observed in PLEC 3 which had a higher content of LA and lower molecular weight. Usually, it can be solved by addition of an excipient molecule which creates the interconnecting channels through out the polymer matrix and provides the complete release [16].

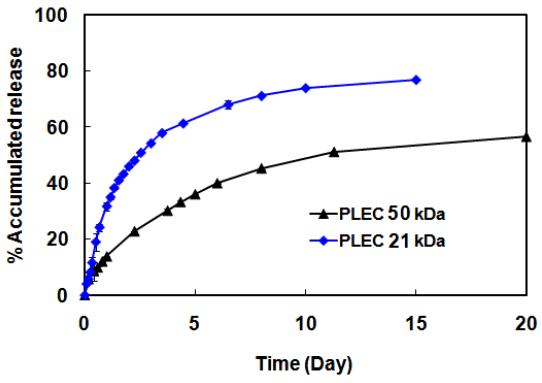

Fig. 3. The trypan release profile of PCL with 30% trypan blue loading and different molecular weight (21 and 50 kDa).

VII. CONCLUSION

By adjusting the ratio of poly (D,L lactide) and poly(ε-caprolactone), the drug release rate can be controlled. The higher LA:CL ratio results in the more hydrophilic property, increases the water absorption rate and decreases the crystallinity of PLECs. These mentioned properties facilitate the drug and subsequently causes a faster release rate. Furthermore, the chain entanglement which also effects on the diffusion directly depends on the molecular weight of polymer, so the higher molecular weight leads to the release rate reduction.

To have the excellent release profile, the drug release should be a zero ordered release where the release rate is constant over time. This can be obtained by the reservoir system which is the future work of this research.

VIII. REFERENCES

[1] WHO, "Cancer," 2008.

[2] F. Qian, A. Szymanski, and J. Gao, "Fabrication and characterization of controlled release poly(D,L-lactide-co-glycolide) millirods," *J Biomed Mater Res,* vol. 55, pp. 512-22, Jun 15 2001.

[3] B. D. Weinberg, H. Ai, E. Blanco, J. M. Anderson, and J. Gao, "Antitumor efficacy and local distribution of doxorubicin via intratumoral delivery from polymer millirods," *J Biomed Mater Res A,* vol. 81, pp. 161-70, Apr 2007.

[4] "Gliadel wafers for treatment of brain tumors," *Med Lett Drugs Ther,* vol. 40, p. 92, Sep 11 1998.

[5] J. L. Au, S. H. Jang, J. Zheng, C. T. Chen, S. Song, L. Hu, and M. G. Wientjes, "Determinants of drug delivery and transport to solid tumors," *J Control Release,* vol. 74, pp. 31-46, Jul 6 2001.

[6] R. Gutmann, M. Leunig, J. Feyh, A. E. Goetz, K. Messmer, E. Kastenbauer, and R. K. Jain, "Interstitial hypertension in head and neck tumors in patients: correlation with tumor size," *Cancer Res,* vol. 52, pp. 1993-5, Apr 1 1992.

[7] R. K. Jain, "The next frontier of molecular medicine: delivery of therapeutics," *Nat Med,* vol. 4, pp. 655-7, Jun 1998.

[8] M. Leunig, A. E. Goetz, M. Dellian, G. Zetterer, F. Gamarra, R. K. Jain, and K. Messmer, "Interstitial fluid pressure in solid tumors following hyperthermia: possible correlation with therapeutic

response," *Cancer Res,* vol. 52, pp. 487-90, Jan 15 1992.

[9] M. Leunig, F. Yuan, M. D. Menger, Y. Boucher, A. E. Goetz, K. Messmer, and R. K. Jain, "Angiogenesis, microvascular architecture, microhemodynamics, and interstitial fluid pressure during early growth of human adenocarcinoma LS174T in SCID mice," *Cancer Res,* vol. 52, pp. 6553-60, Dec 1 1992.

[10] e. a. Chengyun Ning, "In vitro mineralization of surface-modified porous polycaprolactone scaffolds in simulated body fluid.," *Applied surface science, , , ,* vol. Volume 255, Issue 2, pp. pp. 429-431.

[11] Y. Hu, X. Jiang, Y. Ding, L. Zhang, C. Yang, J. Zhang, J. Chen, and Y. Yang, "Preparation and drug release behaviors of nimodipine-loaded poly(caprolactone)-poly(ethylene oxide)-polylactide amphiphilic copolymer nanoparticles," *Biomaterials,* vol. 24, pp. 2395-404, Jun 2003.

[12] C. W. YAN ZHANG , WULI YANG , BIN SHI , SHOUKUAN FU ,, "Tri-component diblock copolymers of poly(ethylene glycol)-poly(ε-caprolactone-co-lactide) : synthesis, characterization and loading camptothecin," *Colloid and polymer science ISSN 0303-402X CODEN CPMSB6* vol. vol. 283, pp. 1246-1252 2005.

[13] H. Ge, Y. Hu, X. Jiang, D. Cheng, Y. Yuan, H. Bi, and C. Yang, "Preparation, characterization, and drug release behaviors of drug nimodipine-loaded poly(epsilon-caprolactone)-poly(ethylene oxide)-poly(epsilon-caprolactone) amphiphilic triblock copolymer micelles," *J Pharm Sci,* vol. 91, pp. 1463-73, Jun 2002.

[14] J. Rich, T. Jaakkola, T. Tirri, T. Narhi, A. Yli-Urpo, and J. Seppala, "In vitro evaluation of poly(epsilon-caprolactone-co-DL-lactide)/ bioactive glass composites," *Biomaterials,* vol. 23, pp. 2143-50, May 2002.

[15] M. D. Dhanaraju, D. Gopinath, M. R. Ahmed, R. Jayakumar, and C. Vamsadhara, "Characterization of polymeric poly(epsilon-caprolactone) injectable implant delivery system for the controlled delivery of contraceptive steroids," *J Biomed Mater Res A,* vol. 76, pp. 63-72, Jan 2006.

[16] F. Qian, N. Nasongkla, and J. Gao, "Membrane-encased polymer millirods for sustained release of 5-fluorouracil," *J Biomed Mater Res,* vol. 61, pp. 203-11, Aug 2002.

APPENDIX C

Development of Tri-component Copolymer Rods as Implantable Drug Delivery System for Liver Cancer

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Abstract— Tri-component copolymer of poly(ε-caprolactone)-random-poly(D,L-lactide)-block-poly(ethylene glycol)-block-poly(ε-caprolactone)-random-poly(D,L-lactide) (PLEC) prepared in a cylindrical rod shape was developed as the implantable drug delivery system. PLEC was successfully synthesized and the copolymer ratio was controllable. Trypan blue was selected as a model drug and the release profiles of trypan blue were carried out at 20 and 30 % trypan blue loading. The release rate of trypan blue was found to directly depend on the amount of lactide in PLEC and the amount of trypan blue.

Keywords— Drug delivery system, cancer, block copolymer, polymeric rod, implantation

I. Introduction

Cancer is the second highest cause of death in the US today, and more than half a million people die from this disease each year.1 Liver cancer is one of the most deadly forms of cancer. Current treatment for liver cancer includes surgical resection, systemic or regional chemotherapy, arterial embolization, cryotherapy and radiation therapy. Even though surgical resection demonstrates the improvement in the therapeutic outcome, for the majority of patients, resection may not be possible due to factors such as age, poor general health, multiple tumor sites and advanced cirrhosis. A small portion of cancer cells can survive a standard treatment, especially at locations close to blood vessels. Subsequently, the remaining cancer cells cause the tumor recurrence that requires further treatment. Therefore, it is necessary to develop intratumoral drug delivery as a local drug therapy for the treatment of liver cancers. This controlled release drug delivery system is required to deliver drugs at a predetermined rate for a prolonged period of time.[1,2] A successful drug delivery device for local chemotherapy has to be able to precisely control the concentration of anticancer drugs within their narrow therapeutic windows. Polymeric rods will be fabricated in the shape of a cylindrical rod (1.6 mm in diameter), which will be implanted directly into the tumor tissue. This therapy has potential advantage in that the local delivery can reduce drug dosage, toxicity and side effects usually associated with systemic chemotherapy.

Polymers have been used as materials for a variety of drug delivery systems, for example, polymer implant [3], and nanoparticles [1,4-8]. In this study, polyester was selected as a material for this drug delivery due to its well-known biocompatibility and biodegradation.[9,10] Poly(ε-caprolactone) (PCL) is the excellent biodegradable polymer with good biocompatibility and non-toxicity.[5] However, the degradation rate of homo-PCL is very slow, and its biodegradation half time is longer than one year owing to its strong crystallinity.[11,12] It was reported that poly(D,L-lactide) (PLA) is more susceptible to hydrolysis than PCL. Thus the degradation half time of PCL can be reduced. Poly(ethylene glycol) (PEG) can be introduced to adjust the hydrophilicity of the polymer matrix. To combine these properties, copolymerization of these polymers is commonly regarded as a convenient approach to achieve the desired properties of the polymer. The tri-component copolymer of poly(ε-caprolactone)-random-poly(D,L-lactide)-block-poly(ethylene glycol)-block-poly(ε-caprolactone)-random-poly(D,L-

lactide) (PLEC) was used to entrap drugs in a cylindrical polymeric rods. The rate of drug release will be controlled by varying the overall drug loading content and copolymer composition.

II. Materials and Methods

Materials

Stannous (II) octoate ($Sn(Oct)_2$, from Aldrich) was used as received. Poly(ethylene glycol) (PEG, MW = 1000 Da) was purchased from Sigma (USA). ε-Caprolactone monomer (CL, from Aldrich) was dried in calcium hydride and distilled under vacuum. D,L-Lactide (LA, from Aldrich) was recrystallized from dry toluene. Other reagents were used as received.

Polymerization of poly(ε-caprolactone)-random- poly(D,L-latide)-block-poly(ethylene oxide)- block-poly(ε-caprolactone)-random-poly(D,L- latide) (PLEC)

PLEC with different compositions was synthesized by ring-opening polymerization of LA and CL in the presence of PEG (Mw = 1000) as macro-initiator and stannous octoate as catalyst.[13],[6] LA and PEG were weighted in the dry two-necked round bottomed flask and the mixture was dried under reduced pressure for 1 h. Then dehumidified argon was purged through the system. Dry CL and toluene (used as a solvent) were introduced into the flask through the dry glass syringe. The mixture was dried by azeotropic distillation under reduced pressure to remove the trace of water. The flask was immersed in an oil bath and maintained at 140 °C. Stannous octoate was introduced into a polymerization flask and the reaction was carried out for 48 h. The product was obtained by precipitating the toluene solution of the raw product by cold methanol. This synthesis can control the copolymer composition by monomer feeding ratio. Thus, the desired hydrophilicity, degradation rate and drug release rate can be obtained.

Preparation of PLEC microspheres

PLEC microspheres were prepared by a solvent evaporation method. PLEC was dissolved in methylene chloride and added into 1% poly(vinylalcohol) aqueous solution drop by drop while homogenized at 8000 rpm for 4 min to form an oil/water emulsion. The emulsion system was stirred over night to remove the methylene chloride. Finally, microspheres were corrected by centrifugation, washed with distilled water, and freeze-dried to give a powder like sample.

Fabrication of polymeric rods

Polymeric rods were prepared using a compression-heat-molding procedure that has been developed recently.21 Briefly, lyophilized trypan blue particles were mixed with PLEC microspheres then the well-mixed particles were placed in a mold and fabricated at a compression pressure of 4.6 x 10^6 Pa and temperature of 90 °C for 2 hours.

Trypan blue release study

Polymeric rods are weighed prior to the drug release. The rods are placed in 20 mL glass vials and submerged in 15 mL phosphate-buffered saline (PBS) pH 7.4. The vials are placed in an orbital shaker at 37 °C, and a buffer solution was removed periodically for UV measurement at its maximum absorption wavelength (λ_{max} = 586 nm). The cumulative mass of the released agent is calculated by summing the individual sample mass after each removal. The release profile is obtained by plotting the amount of released agent as a function of time.

III. Results and Discussion

PLEC was synthesized by ring opening polymerization of D,L-lactide and ε-caprolactone at 140 °C. Poly(ethylene glycol) (HO-PEG-OH, Mn = 1,000 Da) was used as a macro-initiator. The degree of polymerization of CL and LA in PLEC was calculated by comparing integral intensity of characteristic resonance of the CL proton at 3.9 ppm (-C(=O)-$(CH_2)_4$C**H**$_2$-O-), LA proton at 5.1 ppm (-C(=O)-C**H**(-CH_3-)) and PEG resonance at 3.55 ppm (-OC**H**$_2$C**H**$_2$-) in the ^{1}H NMR spectrum (Figure 1).

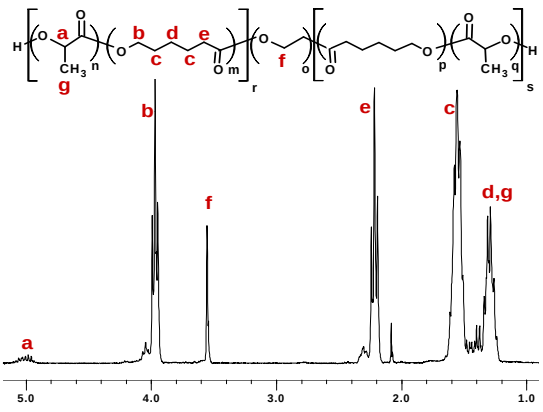


Fig. 1 ^{1}H NMR spectrum of PLEC in $CDCl_3$.

PLECs with different chemical composition were prepared by varying the molar ratio of LA/CL monomer as shown in Table 1. Therefore, PLEC with different properties such as drug release rate, hydrophilicity and degradation rate can be produced. The LA fraction is gradually increased from 0 (PLEC 1) upto approximately 26.5 % (PLEC 3) with similar molecular weight. The molecular weight of PLEC was controlled approximately 20 kD. For all PLEC, a unimodal distribution was observed in the size exclusion chromatograms (data not shown).

Table 1 Chemical composition of PLEC as determined by ^{1}H NMR

PLEC	PEG	LA		CL		MW of PLEC
	kDa	MW (kDa)	mol %	MW (kDa)	mol %	(kDa)
1	1000	0	0	22.47	100	23.47
2	1000	1.86	10.92	24.02	89.08	26.87
3	1000	3.8	26.54	16.66	73.46	21.46

PLEC was successfully fabricated into microparticles with the single emulsion procedure. An analysis by light microscope showed that these particles have the average diameter at 3.77 ± 2.02 μm based on the image analysis of 50 particles. The micro-scale size allows a homogenous dispersion with lyophilized trypan blue particles which consequently leads to a homogenous distribution of trypan blue throughout polymeric rods.

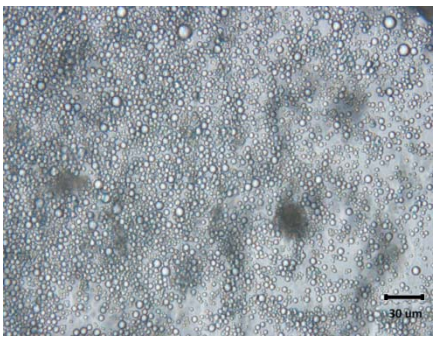


Fig. 2 Light microscope analysis of PLEC microparticles.

The PLEC microparticles and trypan blue were vigorously mixed by vortex mixing. The mixture were then molded into a rod shape at 90 °C and 4.6 MPa. After 2 h, the mold was allowed to cool down and the polymeric rods were obtained. PLEC polymeric rods were varies percent of thypan blue in 20% and 30 % total weight.

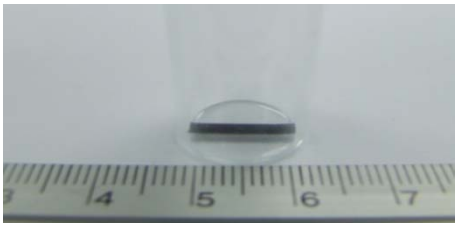
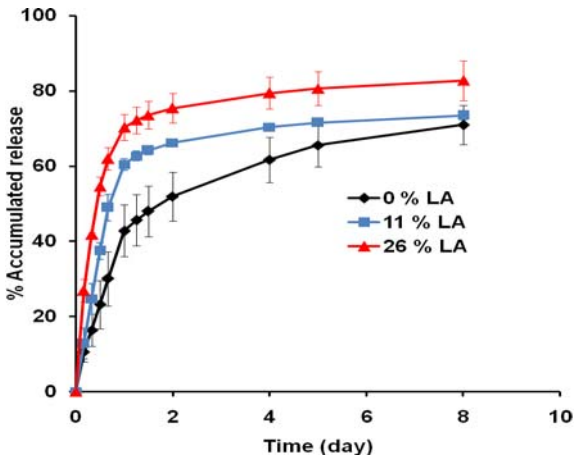
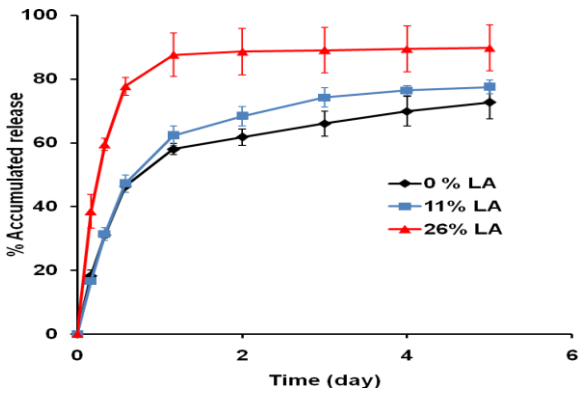


Fig. 3 PLEC polymeric rod contains thypan blue.

The release studies of thypan blue, each rod separated into 3 pieces and submerged in 15 mL PBS buffer which are placed in an orbital shaker at 37 °C. Buffer solution was periodically refreshed, removed buffer solution was UV measurement at wavelength 586 nm. Thypan blue concentration in each period was calculated and plot graph of accumulative concentration with times.

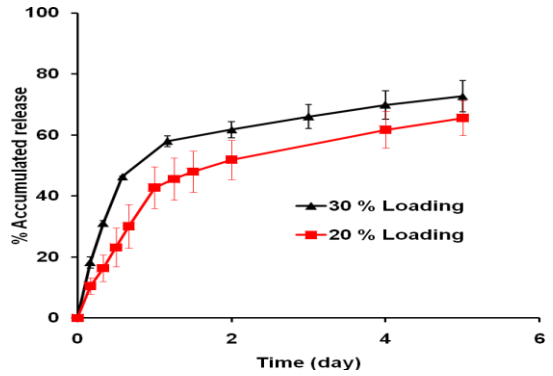


(A)

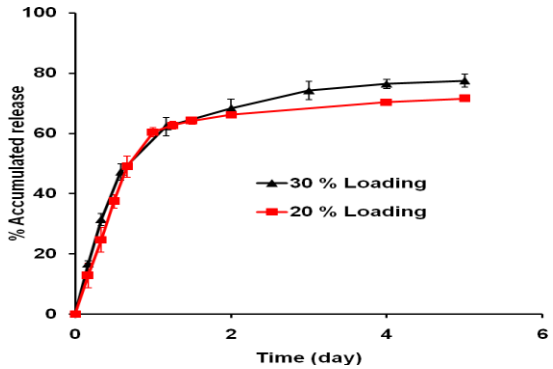


(B)

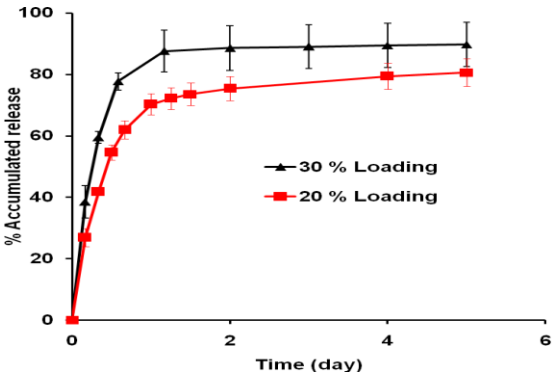
Fig. 4 Trypan blue release profiles (A) polymeric rods with MW 20 kDa and 20% trypan blue loading, (B) polymeric rods with MW 20 kDa and 30% trypan blue loading.



(A)



(B)



(C)

Fig. 5 Effect of trypan blue loading on release profiles of polymeric rods with A) LA 0%, B) LA 11% and C) LA 26%.

IV. Conclusion

The thypan blue release faster as ratio of poly(D,L-lactide) was increased. The PEG component is leading to completely release without another excipient. The molecular weight of PLEC cans affecting to release rate of thypan blue as trend to prolong the release rate and permanent entrapped was occurred that cause of incompletely release.

References

1 Nasongkla N, Bey E, Ren J, Ai H, Khemtong C, Guthi JS, Chin SF, Sherry AD, Boothman DA, Gao J: Multifunctional polymeric micelles as cancer-targeted, mri-ultrasensitive drug delivery systems. Nano Lett 2006;6:2427-2430.

2 Baker R: Controlled release of biologically active agents. New York, Wiley, 1987.

3 Qian F, Nasongkla N, Gao J: Membrane-encased polymer millirods for sustained release of 5-fluorouracil. J Biomed Mater Res 2002;61:203-211.

4 Gao J, Nasongkla N, C K: Crgd-encoded, mri-visible polymeric micelles for tumor-targeted drug delivery; in Amiji M (ed Nanotechnology for cancer therapy. Boca Raton, CRC Press, 2007.

5 Nasongkla N, Shuai X, Ai H, Weinberg BD, Pink J, Boothman DA, Gao J: Crgd-functionalized polymer micelles for targeted doxorubicin delivery. Angew Chem Int Ed Engl 2004;43:6323-6327.

6 Shuai X, Ai H, Nasongkla N, Kim S, Gao J: Micellar carriers based on block copolymers of poly(epsilon-caprolactone) and poly(ethylene glycol) for doxorubicin delivery. J Control Release 2004;98:415-426.

7 Sutton D, Nasongkla N, Blanco E, Gao J: Functionalized micellar systems for cancer targeted drug delivery. Pharm Res 2007;24:1029-1046.

8 Sutton D, Wang S, Nasongkla N, Gao J, Dormidontova EE: Doxorubicin and beta-lapachone release and interaction with micellar core materials: Experiment and modeling. Exp Biol Med (Maywood) 2007;232:1090-1099.

9 Okada H: One- and three-month release injectable microspheres of the lh-rh superagonist leuprorelin acetate. Adv Drug Deliv Rev 1997;28:43-70.

10 Shameem M, Lee H, DeLuca PP: A short term (accelerated release) approach to evaluate peptide release from plga depot-formulations. AAPS PharmSci 1999;1:E7.

11 Schindler A, Jeffoat R, Kimmel GL: Contemporary topics in polymer science. New York Plenum Press, 1977.

12 Karjalaimen T: Mechanical properties of e-caprolactone and lactide copolymers after hydrolysis in vitro. J Appl Polym Sci 1996;59:5.

13 Lang MD, Wang SG: Synthesis and identification of pcl/peo/pla tri-components copolymer. . J Biomater Sci,Polym Ed 1999;4:12.

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