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Glycerol-modified Poly-ε-caprolactone Nanoparticles for Drug Delivery Application

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The most common methods of administering drugs are oral and intravenous, which have major limitations such as low specificity, toxicity, and high degradability. While studies on drug-carrier nanoparticles of poly-ε-caprolactone (PCL) were conducted, this study sought to modify the poly-ε-caprolactone polymer using glycerol as drug-carrier that can encapsulate and release less hydrophobic drugs. In this study, poly-ε-caprolactone was synthesized as well as crosslinked with glycerol simultaneously using Novozym 435 as the biocatalyst. This glycerol modified poly-ε-caprolactone was further converted into nanoparticles of size 100-400 nm by nanoprecipitation technique. The potential of glycerol modified poly-ε-caprolactone (GMPCL) to encapsulate and release propafenone was studied and the results were compared with that obtained from poly-ε-caprolactone nanoparticles. Findings reveal that these nanoparticles are most stable in pH 7 buffer as compared to pH 4 and pH 10 buffers. 10% (v/v) glycerol modified PCL could encapsulate 62 μg of drug in 30 minutes, whereas PCL without glycerol encapsulated 24 μg of drug in same time interval. Propafenone got released at a controlled rate at pH 7.4 and 37.4 °C from all the formulations. Maximum release was observed in the case of 10% (v/v) glycerol modified nanoparticles where 31% of encapsulated drug got released in 30 minutes whereas 29 % got released from PCL counterpart in the same time interval.

Keywords: *modified poly-ε-caprolactone, propafenone, nanoparticles, glycerol, drug delivery*

INTRODUCTION

Oral and intravenous (IV) routes are the common methods of administering drugs inside the body due to convenience and higher patient compliance (Kayser, et al., 2005). However, both of these methods have major limitations. The therapeutic efficacy of drugs delivered orally is limited due to the exposure of drugs to stringent physical conditions and metabolic processes inside our body. On the other hand, the IV method suffers low specificity for injectable drugs. Only a small amount of drug reaches the target tissue, necessitating a large amount of drug to be injected to a patient to obtain the desired therapeutic efficacy, which has side effects. Recently, it has been found that drug delivery

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using nanoparticles, utilizing degradable and absorbable polymers, provides a more efficient, less risky solution to many drug delivery challenges (Francis, et al., 2004). Nanoparticles are vehicles that carry and deliver drugs to target sites in the body. They can be administered via oral, intravenous and pulmonary routes or formulated in ointments and ocular products (Li and Wood, 1986). In nanoparticle formulations, the drug can either be integrated in the polymer matrix or attached to the particle surface. Many studies have discussed the advantages of using polymeric nanoparticles for drug delivery application over other drug formulations such as solvent-based, liposomal, and micro-emulsions (Leroux et al., 1996; Soppimath et al., 2001). In general, these polymeric nanoparticles increase drug solubility and bioavailability, can be tissue-specific, and improve the drug's therapeutic efficacy with decreased toxic effects. Due to the stability of drug-loaded polymeric nanoparticles, there is controlled and sustained release of drugs for a required duration and maintenance of bioactivity before the drug reaches the target (Gref et al., 2000). Nowadays, the technology has become a fascinating field ensuing to the development of novel polymeric drug delivery systems.

Distinctively, polymers have gained wide attention as materials for nanoparticle preparation, primarily because of their high biodegradability, biocompatibility, diversity, and multiplicity of functional groups. Aliphatic polyesters such as polylactide, polyglycolide and poly-ε-caprolactone and their copolymers are some of the most widely studied biodegradable polymers for controlled drug delivery (Anderson and Shive, 1997; Soppimath et al., 2001; Kissel et al., 2002). However, due to their hydrophobic nature, they have a tendency to become thermodynamically unstable and aggregate when they are reduced to the nanometer range for drug delivery application (Peppas, 1995; Kataoka et al., 2001). To address this challenge, nanoparticles formed by self-assembling of amphiphilic polymers are increasingly studied as drug carriers (Sÿachl et al., 2007). PCL is extensively studied as a biodegradable polymer for biomedical applications due to its biocompatibility and high permeability to low molecular weight substances at body temperature (Sinha et al., 2004). However, the biomedical applications of PCL are limited by its less hydrophilic nature. Incorporation of a hydrophilic moiety such as glycerol to PCL makes the system amphiphilic and conducive for the delivery of hydrophilic as well as hydrophobic drugs. Glycerol is an abundant and low-cost substance that has been utilized in the biomedical field due to its low toxicity (Wolinsky et al., 2007). Pluronic F68, a difunctional block copolymer terminating in primary hydroxyl groups is the surfactant used in the synthesis of nanoparticles. This surfactant's nonionic and non-toxic characteristics make it suitable as a stabilizer in the fabrication of polymeric nanoparticles for biomedical applications. Novozym 435 is a biocatalyst immobilized on a macroporous crosslinked resin of poly(methyl methacrylate). It is used as a highly enantioselective catalyst in the synthesis of optically active alcohols, amines and carboxylic acids. Over the last ten years, most groups have used it for electrophilic ring opening polymerization (Kobayashi et al., 2001).

This study aims at synthesizing nanoparticulate glycerol modified PCL and to evaluate its potential towards encapsulation and release of drug propafenone. Glycerol modified PCL will be synthesized using biocatalyst Novozym 435.

EXPERIMENTAL

Chemicals. The chemicals used in this experiment were as follows: ε-caprolactone monomer (≥99%) from Fluka, toluene and glycerol of reagent-grade from Merck and used without further purification, methanol of reagent-grade supplied by Lab-Scan; HPLC-grade chloroform from J.T. Baker and used in GPC analysis, novozym-435 purchased from Novozymes and dried under vacuum with P_2O_5 for 2 days prior every use and stored in vials and refrigerated when not in used, molecular sieves washed with technical-grade

acetone and oven dried prior to use, propafenone hydrochloride (≥99%) from Sigma Aldrich and used as received, and deionized water for all aqueous solutions.

Instruments. The major instruments used were gel permeation chromatograph (Perkin Elmer GPC equipped with PE Series 200 LC Pump, PE NELSON 900 Series Interface, Applied Biosystems 785A Programmable Absorbance Detector) to determine the molecular weight of the polymer, scanning electron microscope (JEOL JSM 5310) to image the size of the nanoparticles, and UV-Vis Spectrophotometer (Shimadzu 2401 PC UV Spectrophotometer) to quantify the amount of drug loaded and released.

Gel Permeation Chromatograph. Average molecular weights of PCL and GMPCL polymers were determined using Perkin Elmer Gel Permeation Chromatograph, and PLgel 5μ Mixed-C column. HPLC-grade chloroform was used as eluent, and the UV detector was set at 250 nm. Flow rate was 0.75 mL/min for the first calibration and 0.50 mL/min for the second calibration. Minimum pressure of the column was set at 0 psi, while maximum pressure was set at 1750 psi for the first calibration and 1900 psi for the second calibration. Elution time was set at 45.0 mins.

Scanning Electron Microscope. The polymeric nanoparticles prepared were first coated with gold and then imaged using Scanning Electron Microscope. All SEM images were captured at 15,000 and 20,000x magnification.

UV-Vis Spectrophotometer. Propafenone in sample solutions was quantified using UV-Vis analysis. Two calibration plots were constructed. A stock solution of 1000 ppm propafenone was prepared by dissolving 0.1g of propafenone hydrochloride with deionized water in 100 mL volumetric flask. Appropriate volumes of stock solutions were placed in 25-mL volumetric flasks and diluted with deionized water to prepare standard solutions with concentrations of 5, 30, 50, 70, and 100 ppm. The 5ppm solution was run first in the UV-Vis spectrophotometer to obtain the absorption spectrum of propafenone. From the absorption spectrum, the lambda max was obtained at 250 nm. This wavelength was used in getting the absorbance as a function of concentration in succeeding UV-Vis experiments. Fresh samples of standard and sample solutions were used in the analysis and quartz cells served as the sample containers.

Methodology. *Synthesis of Poly (ε-caprolactone) (PCL) and Glycerol-Modified Poly (ε-caprolactone) (GMPCL) Polymer*. The procedure for the synthesis of PCL and GMPCL polymer was adopted from the previous work of Melgar and Chakraborty (Melgar and Chakraborty, 2009). In a one-pot synthesis, PCL was polymerized by adding the monomer ε-caprolactone (CL) (54 mmol) into large test tubes together with 0.60 g of dried Novozym 435 (10:1 v/w monomer:catalyst), 6mL of the solvent toluene (1:1 v/v monomer:solvent), 5-6 beads of molecular sieves and a small magnetic stirrer. The test tube was covered with a septum cap and purged with N_2 gas for 30 seconds. The mixture was polymerized for 12 hours with constant stirring at 70 °C using a temperature-controlled water bath. Similar procedure was followed for the synthesis of GMPCL polymer at different cross linker density. To prepare 5%, 10% and 20% GMPCL, 0.38g glycerol (5%v/v glycerol:monomer), 0.76g glycerol (10%v/v glycerol:monomer), and 1.5g glycerol (20%v/v glycerol:monomer), respectively, were combined and the reactions were performed as above. After polymerization, the molecular sieves and enzyme were removed by vacuum filtration. The remaining filtrate was precipitated in large amount of methanol and white solid products were subsequently air-dried.

Synthesis of Polymeric Nanoparticles by Nanoprecipitation. PCL and GMPCL nanoparticles were synthesized by nanoprecipitation (Al Khouri, et al., 1986). The polymer was first dissolved in acetone with a ratio of 0.5% w/v polymer:acetone solution. The organic solution (75 mL) was poured in a controlled manner into an aqueous solution of Pluronic F68 surfactant (150 mL) with constant stirring. The acetone

was removed from the mixture under reduced pressure using vacuum for 30 minutes. Afterwards, nanoparticles solutions were transferred to centrifuge tubes and centrifuged at 4600 rpm for 30-45 minutes until the nanoparticles settled at the bottom of the tubes. The supernatant was decanted and white, solid nanoparticles were collected and freeze dried. Samples were freeze-dried at 475-550 × 10^{-3} mbar at -48 to -50 °C for 10-16 hours until nanoparticles were dry. White, powderish and completely dried surfactant-coated nanoparticles were obtained and then stored in vials at room temperature.

Determination of the Stability of PCL and GMPCL Nanoparticles. This experiment was performed in pH 4, pH 10 and in deionized water. Acetate buffer was used for pH 4 and borate buffer for pH 10. A 0.005 g of nanoparticles was weighed in small vials, and into it was poured 1 mL of the buffer solutions. At room temperature, these dispersions were stirred for different time intervals (0.5, 1, 2, 4, 6, 12, 24 and 48 hours). The dispersions were then freeze-dried, removing the aqueous buffer. Solid residues obtained were dissolved in chloroform and filtered using 0.45 μm PTFE syringe membrane filter. A 50 μL of the filtrate was used in GPC analysis to determine the average molecular weights of polymer.

Loading of Drug. A 2.65 × 10^{-4} mmol of propafenone was dissolved in 0.5 mL deionized water. This solution was added to 0.5 mL dispersion of nanoparticles (1 mg/mL) in deionized water previously stirred for two hours. The systems were placed in small scintillating vials and were allowed to equilibrate at different times (0.17, 0.33, 0.5, 1, 2, 4, 6, and 7 hours). The samples were then transferred to ultrafiltration devices and centrifuged at 10,409 rpm for 30 min using a refrigerated centrifuge. Thereafter, the supernatant around 1 mL was withdrawn from the containers using a micropipette and transferred to another vial. The supernatant were diluted with 3 mL deionized water and analyzed for drug-content using UV-Vis Spectrophotometer at 250 nm. % Drug Loading was computed according to the formula:

$$\% \text{ Drug Loading} = \frac{\text{drug loaded in nanoparticle}}{\text{initial amount of drug}} \times 100$$

where drug loaded in nanoparticle = initial amt. of drug loaded – amt. of drug in supernatant

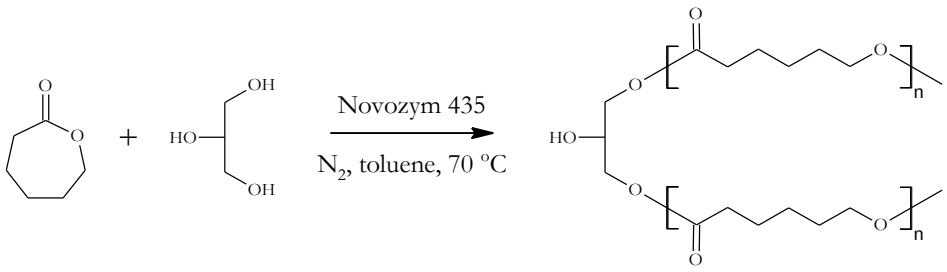
Releasing of Drug. Drug release of propafenone-loaded PCL, 5% GMPCL and 10% GMPCL nanoparticles were investigated in pH 7.4 buffer solution at 37 °C at different equilibration time (0.5, 1, 2, 4 and 6 hours). Drug-loaded nanoparticles of 6.2, 5.6 and 2.4 (w/w)% for 10%(v/v) GMPCL nanoparticles, 5%(v/v) GMPCL nanoparticles and PCL nanoparticles, respectively, were dispersed in 1 mL of pH 7.4 phosphate buffer solution. The systems were stirred using a magnetic stirrer in a temperature-controlled water bath maintained at 37 °C to simulate physiological condition. After a certain time interval, samples were transferred to ultrafiltration devices and centrifuged at 10,409 rpm for 30 minutes at room temperature using a refrigerated centrifuge. Thereafter, the supernatant around 1 mL was withdrawn from the containers using a micropipette and transferred to a vial. They were diluted with 3 mL deionized water and analyzed for drug-content using UV-Vis Spectrophotometer. The amount of drug released was monitored as a function of stirring time. % Drug Release was computed according to the formula:

$$\% \text{ Drug Release} = \frac{\text{drug in supernatant}}{\text{drug in the nanoparticle}} \times 100$$

RESULTS AND DISCUSSION

Synthesis of Polymeric Nanoparticles by Nanoprecipitation. The synthesis of PCL and GMPCL yielded glycerol-modified poly (ε-caprolactone) branched polymer, one of the proposed products is shown in Scheme 1.

As shown in Figure 1, 10% (v/v) GMPCL polymer is composed of five different peaks that correspond to high molecular weight polymer chains from 8.20 × 10^5 g/mol to around 200 g/mol. Using different cross linker densities, two GMPCL polymers of high MW were produced.



Scheme 1. *Biocatalytic Synthesis of 10% (v/v) Glycerol-modified Poly (ε-caprolactone). Above represents one of the proposed products.*

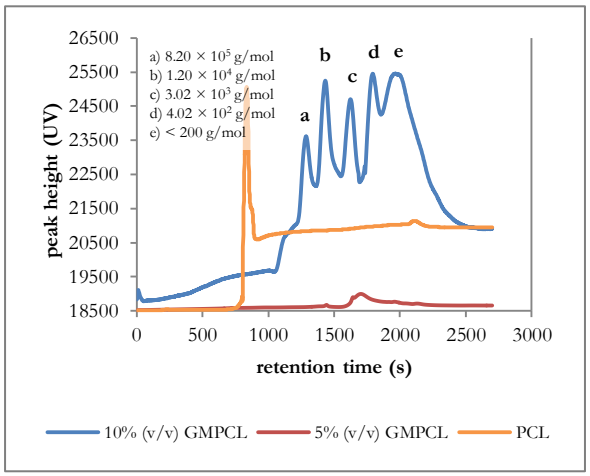


Figure 1. *Gel Permeation Chromatography Profile of PCL and GMPCL Polymer.*

When PCL and modified GMPCL polymers were synthesized and formed into nanoparticles, the size was determined using SEM. As shown in Figure 2, smooth and spherical nanoparticles with a size range of 100 to 400 nm were formed.

Determination of the Stability of PCL and GMPCL Nanoparticles. The molecular weight of the polymeric nanoparticles under different pH and degradation time was determined by GPC analysis. This investigation was performed in order to evaluate the stability of the branched structure at different pH and to get an insight of the release mechanism of the drug from GMPCL nanoparticles. Figure 3 shows the GPC profile of PCL, 5% (v/v) GMPCL and 10% (v/v) GMPCL nanoparticles in pH 4 and 10. As revealed, at 30 minutes of degradation, the molecular weights of 10% (v/v) GMPCL polymer were reduced to less than 200 g/mol, in pH 4 buffer. Similar loss in molecular weight was also observed in pH 10 buffer also, indicating polymer degradation in pH 4 and pH 10. Loss of the characteristic branched structure further supported degradation. Low molecular weight fragments were similarly produced in both PCL and 5% (v/v) GMPCL polymers after 30 minutes. Polymer degradation occurred with time in acidic and basic media, which can be attributed to acid- and base-catalyzed hydrolysis of the PCL-PCL ester linkage. The nanoparticles were not stable at pH 4 and 10 and might not be efficient as controlled release vesicle at these pHs. However, when deionized water (pH~6) was used as dispersion medium, 10% (v/v) GMPCL nanoparticles has shown stability until 12 hours degradation time. As shown in Figure 4, the characteristic branched structure of the polymer disappeared only after 12 hours, implying delayed onset of degradation.

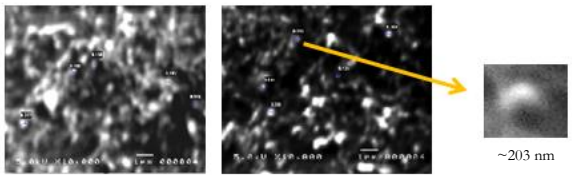


Figure 2. *Scanning Electron Microscope Image of Nanoparticles (A) PCL and (B) 10% (v/v) GMPCL.*

Loading of Drug. Propafenone, an anti-arrhythmic drug, was loaded into the nanoparticles using deionized water in varying drug-polymer equilibration times and initial amounts of drug used in the encapsulation process. As shown in Figure 5, the maximum drug loading obtained were 62%, 56% and 36% for 10% (v/v) GMPCL, 5% (v/v) GMPCL and PCL nanoparticles, respectively.

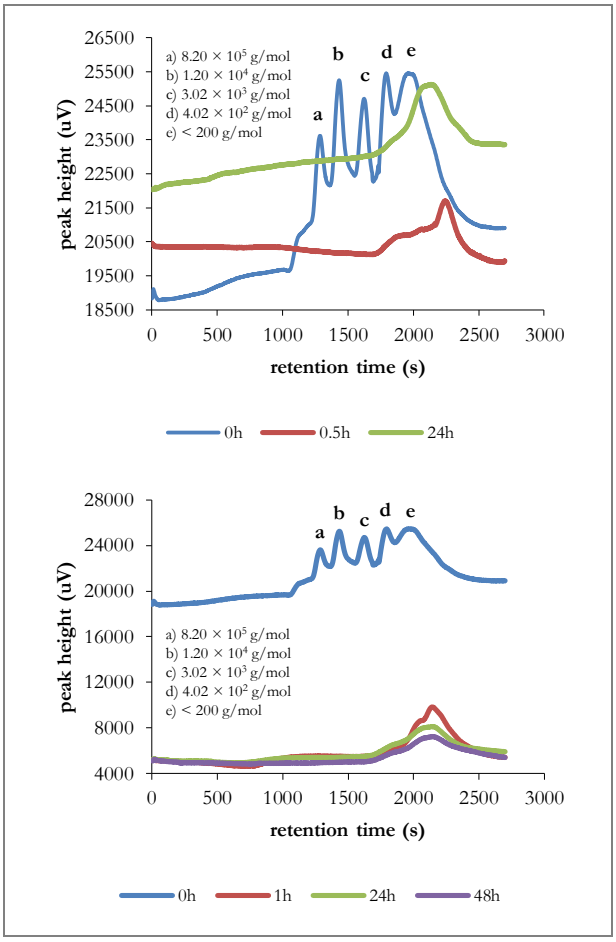


Figure 3. *Gel Permeation Chromatographic Profiles of 10% (v/v) GMPCL Nanoparticles in pH 4 (top) and pH 10 (bottom).*

The highest drug loading was obtained at 1 hour equilibration for PCL nanoparticles and 30 minutes for both 5% (v/v) GMPCL and 10% (v/v) GMPCL nanoparticles. Hence, GMPCL nanoparticles are more efficient in encapsulating propafenone as compared to PCL nanoparticles, which can be attributed to the increased affinity of GMPCL towards the drug.

As illustrated in Figure 6, a combination of hydrophobic interactions (between the non-polar aromatic groups of propafenone and hydrophobic PCL chains in GMPCL) and increased hydrogen bonding attractions (between the hydroxyl and carbonylic portions of GMPCL and -OH, -NHR, -CO groups in propafenone) might have allowed more drug molecules to be encapsulated in GMPCL nanoparticles than in PCL nanoparticles. Furthermore, the amount of drug loaded in the nanoparticles decreased in succeeding

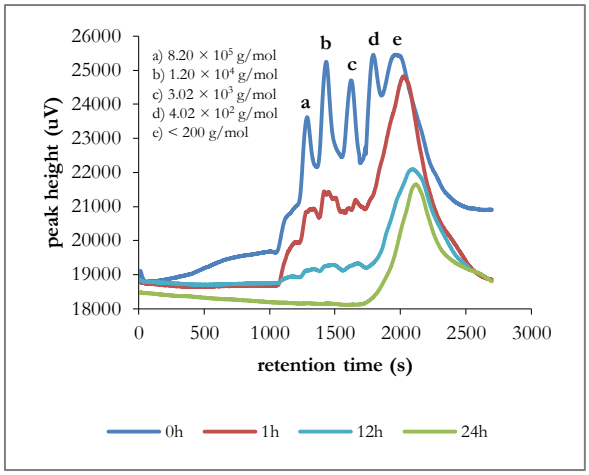


Figure 4. *Gel Permeation Chromatographic Profiles of 10% (v/v) GMPCLNanoparticles in deionized water.*

equilibration times after the nanoparticles obtained their maximum drug loading. This finding can be attributed to two occurring processes, namely supersaturation and attainment of equilibrium. Initially, when the drug was combined with the nanoparticles, the drug molecules got entrapped and adsorbed at the surface of the nanoparticles. With time supersaturation occurred, hence the observed maximum drug loading. However, after some time, drug molecules diffused to the outer solution to establish equilibrium of the systems.

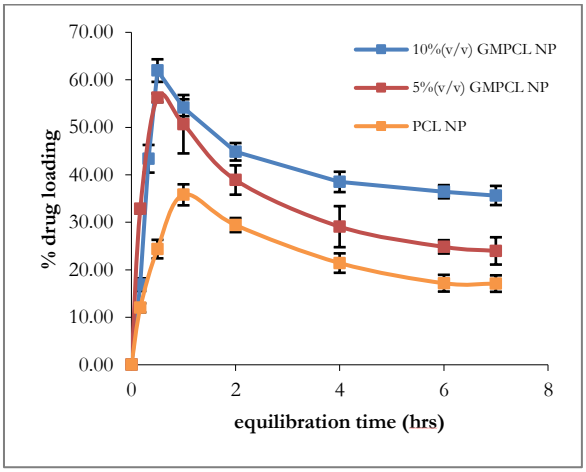


Figure 5. *Drug Loading Profile of Glycerol-modified Poly-ε-caprolactone Nanoparticles in Deionized Water with 0.1 mg initial amount of propafenone in 1 mL nanoparticle dispersion. Error bars indicate standard deviation calculated from three trials.*

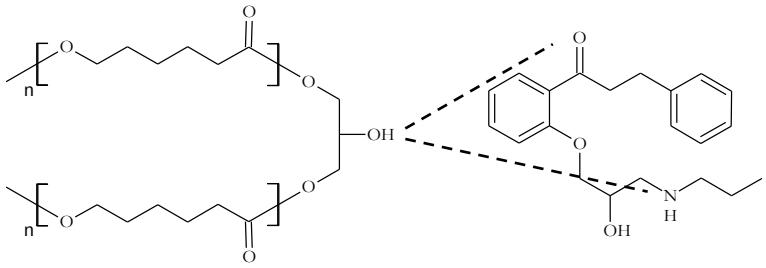


Figure 6. *Proposed Interaction Between Glycerol-modified Poly-ε-caprolactone and Propafenone.*

The 10% (v/v) GMPCL nanoparticles were used in the investigation of drug loading as a function of initial propafenone concentration. Figure 7 shows a significant increase from 28.2% to 66.9% in drug loading when propafenone concentration was increased from 50 to 250 ppm. It is predicted that subsequent increase of the initial amount of drug will not further increase drug loading as the nanoparticles are already saturated with propafenone.

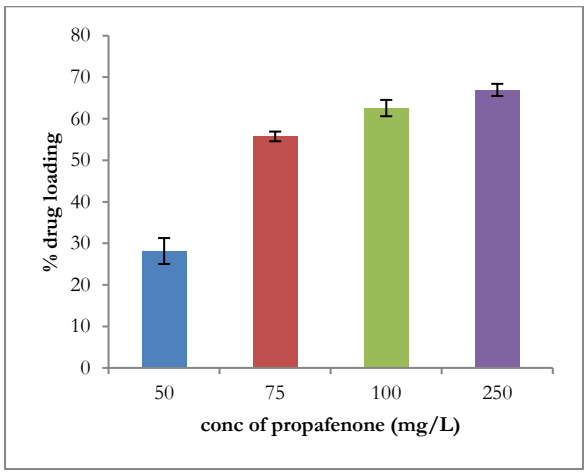


Figure 7. *Drug Loading of 10% GMPCL Nanoparticles at 1 mg/mL nanoparticle dispersion and equilibrated in 30 minutes. Error bars indicate standard deviation calculated from three trials.*

Releasing of Drug. The amounts of propafenone released to the solution from drug-loaded polymeric nanoparticles as a function of equilibration time were monitored *in vitro* (pH 7.4 PBS, 37°C). As shown in Figure 8, 10% (v/v) GMPCL, 5% (v/v) GMPCL, and PCL nanoparticles released 31%, 24% and 29%, drug respectively, in 30 minutes equilibration. Highest amount of propafenone released from 10% (v/v) GMPCL nanoparticles can be attributed to increased hydrophilic interactions of the polymer to the aqueous buffer, resulting in enhanced swelling. When the polymeric material swells, drug molecules diffuse out of the pores of the nanoparticles.

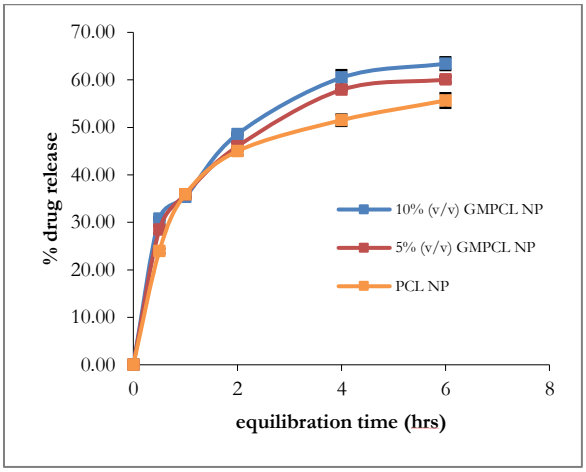


Figure 8. *Drug Release Profile of Polymeric Nanoparticles.*

CONCLUSION

Novel polymeric nanoparticles were prepared from poly-ε-caprolactone and glycerol-modified poly (ε-caprolactone) pre-formed polymers via a nanoprecipitation method. These nanoparticles were spherical in shape with size less than 400 nm. Gel permeation chromatograms indicate that the nanoparticles degraded rapidly in pH 4 and 10 as significant weight loss was observed within 30 minutes of immersion of the polymer in buffer solutions. The degradation is hastened in acidic and basic media. The drug-loading profile was evaluated and results show that the nanoparticles could encapsulate propafenone. (10) % (v/v) GMPCL nanoparticles encapsulated 62% of free drug in 30 minutes equilibration. Furthermore, the use of

10% (v/v) GMPCL nanoparticles resulted in a substantial increase in drug loading when the initial amount of drug was increased in the process. Drug-release simulating physiological conditions (pH 7.4 and 37 °C) showed that 10% (v/v) GMPCL nanoparticles had the highest amount of propafenone released.

Thus this study established that polymeric nanoparticles fabricated from benign components such as CL, glycerol, Novozym 435, and pluronic F68 are conducive for drug delivery application. They are suitable for drug delivery purposes at physiological conditions.

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REFERENCES

Al Khouri N, Roblot-Treupel L, Fessi H, Devissaguet JP, Puisieux F. Development of a new process for the manufacture of polyisobutylcyanoacrylate nanocapsules. Int J Pharm. 1986; 28:125-32.

Anderson JM, Shive MS. Biodegradation and biocompatibility of PLA and PLGA microspheres. Advanced Drug Deliver Rev. 1997; 28:5-24.

Francis M, Cristea M, Winnik FM. Polymeric micelles for oral drug delivery: Why and how. Pure Appl Chem. 2004; 76:1321-1335.

Gref R, Luck M, Quellec P, Marchand M, Dellacherie E. 'Stealth' corona-core nanoparticles surface modified by polyethylene glycol (PEG): influences of the corona (PEG chain length and surface density) and of the core composition on phagocytic uptake and plasma protein adsorption. Colloids Surf B. 2000; 18:301-313.

Kataoka K, Harada A, Nagasaki Y. Block copolymer micelles for drug delivery: design,characterization and biological significance. Adv Drug Deliver Rev 2001; 47:113-131.

Kayser O, Lemke A, Hernandez-Trejo N. The impact of nanobiotechnology on the development of new drug delivery systems. Curr Pharm Biotechnol. 2005; 6:3-5.

Kissel T, Li Y, Unge F. ABA-triblock copolymers from biodegradable polyester A-blocks and hydrophilic poly(ethylene oxide) B-blocks as a candidate for in situ forming hydrogel delivery systems for proteins. Adv Drug Deliver Rev. 2002; 54:99-124.

Kobayashi, S.; Uyama, H.; Kimura, S. Enzymatic Polymerization. Chem Rev. 2001, 101(12):3793-3818.

Leroux JC, Allemann E, Da Jaeghere F, Doelker E, Gurny R. Biodegradable nanoparticles - from sustained release formulations to improved site specific drug delivery. J Control Release. 1996; 39:339-350.

Li VHK, Wood RW. Ocular drug delivery of progesterone using nanoparticles. J Microencapsul. 1986; 3:213-218.

Melgar ZK, Chakraborty S. Biocatalytic synthesis and characterization of glycerol-modified branched poly-ε-caprolactone [thesis]. [Philippines]: Ateneo de Manila University; 2009. 35 p.

Peppas LB. Recent advances on the use of biodegradable microparticles and nanoparticles in the controlled drug delivery. Int J Pharm. 1995; 116:1-9.

Sinha VR, Bansal K, Kaushik R, Kumria R, Trehan A. Poly-caprolactone microspheres and nanospheres:an overview. Int J Pharm. 2004; 278:1-23.

Soppimath K, Aminabhavi T, Kulkami A, Rudzinski W. Biodegradable polymeric nanoparticles as drug delivery devices. J Control Release. 2001; 70:1-20.

Sÿachl R, Uchman M, Mateˇjı´cˇek P, Procha´zka K, Sÿteˇpa´nek M. Preparation and characterization of self-assembled nanoparticles formed by poly(ethylene oxide)-block-poly(epsilon-caprolactone) copolymers with long poly(epsilon-caprolactone) blocks in aqueous solutions. Langmuir. 2007; 23:3395-3400.

Wolinsky JB, William R, Colson YL, Grinstaff MW. Poly(carbonate ester)s based on units of 6-hydroxyhexanoic acid and glycerol. Macromolecules. 2007; 40:7065-7068.