# SIZE TUNABLE CORE-SHELL POLYMER NANOPARTICLES FOR CISPLATIN DELIVERY TO CANCER CELLS

Thesis Report submitted towards the partial fulfilment of

B.S. - M.S. dual degree program


By

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**31st March 2016**

## CERTIFICATE

This is to certify that this dissertation entitled "**Size tunable core-shell polymer nanoparticles for cisplatin delivery to cancer cells**" towards the partial fulfilment of the B.S.-M.S. dual degree programme at the Indian Institute of Science Education and Research, Pune represents original research carried out by "**Khushboo Singh** at IISER Pune" under the supervision of "Dr. M. Jayakannan, Associate Professor, Department Of Chemistry, IISER Pune" during the academic year 2015-2016.

Date: 31-March-2016

Place: Pune

Signature

## DECLARATION

I hereby declare that the matter embodied in the report entitled **"Size tunable core-shell polymer nanoparticles for cisplatin delivery to cancer cells"** are the results of the investigations carried out by me at the Department of Chemistry, Indian Institute of Science Education and Research Pune, under the supervision of **Dr. M. Jayakannan** and the same has not been submitted elsewhere for any other degree.

Date: 31/03/16

Place: Pune

Signature

# ACKNOWLEDGEMENTS

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-Khushboo

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## ABSTRACT

In this work, we synthesized biodegradable and biocompatible di-block copolymers based on γ-substituted caprolactone and polyethylene glycol. The caprolactone monomer was ring opened by an initiator, polyethylene glycol monomethyl ether ($M_w$ 2000 g/mol), in the presence of catalyst stannous(II) 2-ethylhexanoate ($Sn(oct)_2$) to yield the polymer $PEG_{2000}$-b-$CPCL_x$. Seven polymers were synthesized by varying the number of substituted caprolactone units (the drug core) and keeping the polyethylene glycol shell the same. The polymers were characterized by $^{1}$H-NMR, $^{13}$C-NMR and Gel Permeation Chromatography (GPC) techniques. Thermo gravimetric analysis (TGA) and Differential Scanning Calorimeter (DSC) was used to study the thermal properties of the polymer. Upon characterization of these polymers, the side chain carboxylates in the polymer were stitched with the anti-cancer drug, cisplatin. The cisplatin-polymer conjugates were subjected to IR analysis. Dynamic light scattering (DLS) and Field Emission Scanning Electron Microscopy (FESEM) were used to characterize the self-assembled core-shell nanoparticles. Drug loading content (DLC) and drug loading efficiency (DLE) was estimated by TGA and by absorption spectroscopy (OPD assay). Following this, the stability of these particles in water and PBS buffer was studied. The aim is to understand the differences in therapeutic efficiency and DLC of these core variable, core-shell nanoparticles.

# INTRODUCTION

## 1.1 Introduction

The drugs that are in use today are mostly small molecular weight compounds such as peptides, synthetic organic compounds and metal based drugs. Each drug is effective only in a certain therapeutic window, which below a certain threshold is inactive while it is toxic above another threshold[1] (Fig 1.1).Biological drug candidates when exposed to blood plasma undergo destabilization due to proteolytic enzymes in the blood, leading to the loss of therapeutic activity. Owing to the small molecular weight of the drugs, these molecules have fast metabolism rate, therefore short half-life, and faster clearance rate from the body, low therapeutic selectivity and adverse biodistribution. These small molecule drugs diffuse into the healthy cells throughout the body and so only a very small amount of the drug reaches the target site. Consequently, to enhance their activity, the drugs are administered at a frequency greater than needed. The disadvantages of the conventional methods of therapeutics include narrow therapeutic index, excess dosage leading to their accumulation in healthy tissues and unwanted side reactions such as nephrotoxicity, neurotoxicity, gastrointestinal toxicity and cardiotoxicity.

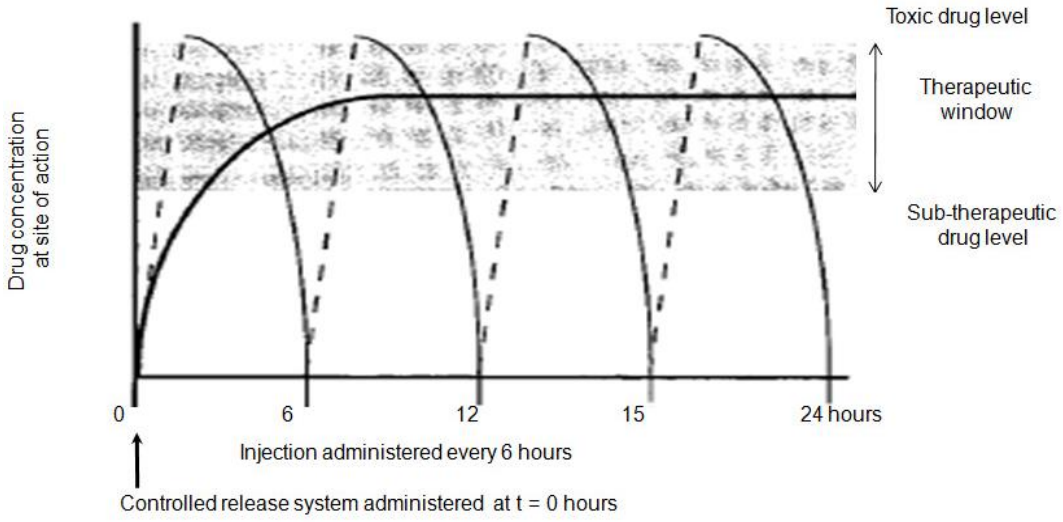



*Figure 1.1 Comparison of conventional therapeutics and drug delivery system (Chem. Rev. **1999**, 99, 3181-3198)*

## 1.2. Polymers as drug delivery systems

Significant amounts of effort in the past decade were aimed at overcoming the limitations of the conventional methods of therapeutics and improve upon the potential of existing drugs through the development of drug delivery vehicles. Polymer based drug delivery systems were developed for the efficient delivery of these drugs to tumor tissues, giving rise to a new field of research altogether that is now termed as “Polymer Therapeutics”. Polymer therapeutics is sub-divided into five major categories: polymeric drugs, polymer-drug conjugates, polymer–protein conjugates, polymeric micelles and polyplexes (complexes of polymers and poly (nucleic acids)). The polymeric nanoparticles can either be physically loaded with drug molecules or the drug can be chemically conjugated to the protein/ synthetic polymer/ polysaccharide.

Ringsdorf in 1975 proposed a model of drug delivery system using synthetic polymer chains as the backbone (see Fig. 1.2).[2] The polymer chain contains multiple chemical functional groups that allow attachment of drug via a cleavable linker that in response to stimuli can break to release the drug at the site of interest. The polymer chain can be chemically modified to contain a targeting group such as an antibody/ sugar moiety that are specific to receptors/ proteins of a particular disease and therefore imparts selectivity to the polymeric vehicle in addition to enhanced cellular uptake by the cells. The polymer can contain a solubilizing group that can increase the aqueous stability of low solubility therapeutic reagents or insoluble drugs whilst modifying the bioavailability of the polymeric vehicle. The polymer scaffold protects the drug from the harsh conditions of the body and increases their concentration at the target site, thereby, improving the therapeutic index of the drug. The polymeric system also protects the drug from enzyme degradation, phagocytosis and endocytosis. Polymers as drug carriers, thus, increase the pharmacokinetics of the drug; enhance the circulation time in the blood and the pharmacological efficacy.[3]

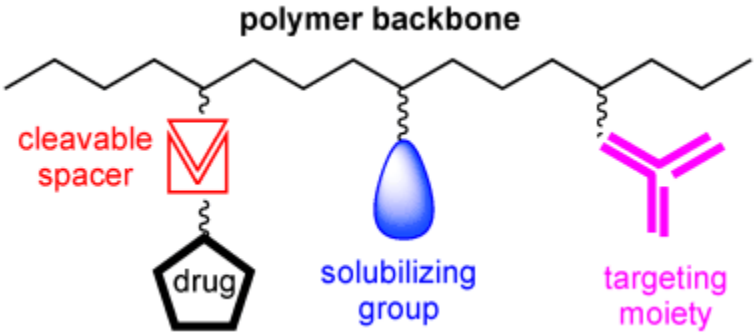



*Figure 1.2.Ringsdorf's model for polymer-drug conjugate for drug delivery*

An ideal polymeric scaffold must fulfill certain necessities before it can be used for drug delivery application.[4] They are the following:

- Polymers must be water soluble
- Polymers must be non-toxic and non-immunogenic
- Must be bio-degradable and easily excreted from the body
- Precise bio-distribution of drug must be known
- Must have predictable release profile for the drug
- Targeted delivery to diseased tissue

## 1.3. Enhanced Permeability and Retention (EPR) Effect[2]

The use of macromolecules such as polymers for the delivery of anti-cancer drugs whilst the polymer lacked a chemical entity specific to receptors on the tumor cells was first done by Maeda et al and Jain et al. Low molecular weight drugs diffuse equally through the endothelial cells of the normal tissue and the cancer tissues whereas polymeric vehicles are seen to selectively accumulate at the site of tumor as compared to healthy tissues. A detailed pathophysiological study of the tumor tissue revealed that the endothelial cells are not very closely packed, are poorly aligned, are irregular in shape and are leaky. As a result, when there is an exchange of nutrient from the blood capillaries to the tumor cells, the macromolecular micelle can pass through the gaps between the cells thereby reaching the site of tumor cells. Since the tumor tissues have a defective lymphatic drainage system, the clearance of these macromolecules does not occur, leading to an increased accumulation (10-50 times greater than in normal

tissues) of polymer-drug conjugates at the site of tumor. This process of passive targeting is called as Enhanced Permeability and Retention effect or EPR effect (see Fig. 1.3.). The uptake of polymeric delivery systems is closely linked with the size of the macromolecule. The renal threshold of particle molecular weight is about 30kDa. Therefore, polymers with molecular weight greater than renal threshold of 30kDa to 200kDa is readily taken up by tumor cells by EPR effect. These polymeric nanoparticles thus have enhanced circulation time in the body and greater exposure to cancer tissues. This forms the motivation for the use of polymers as drug delivery systems.

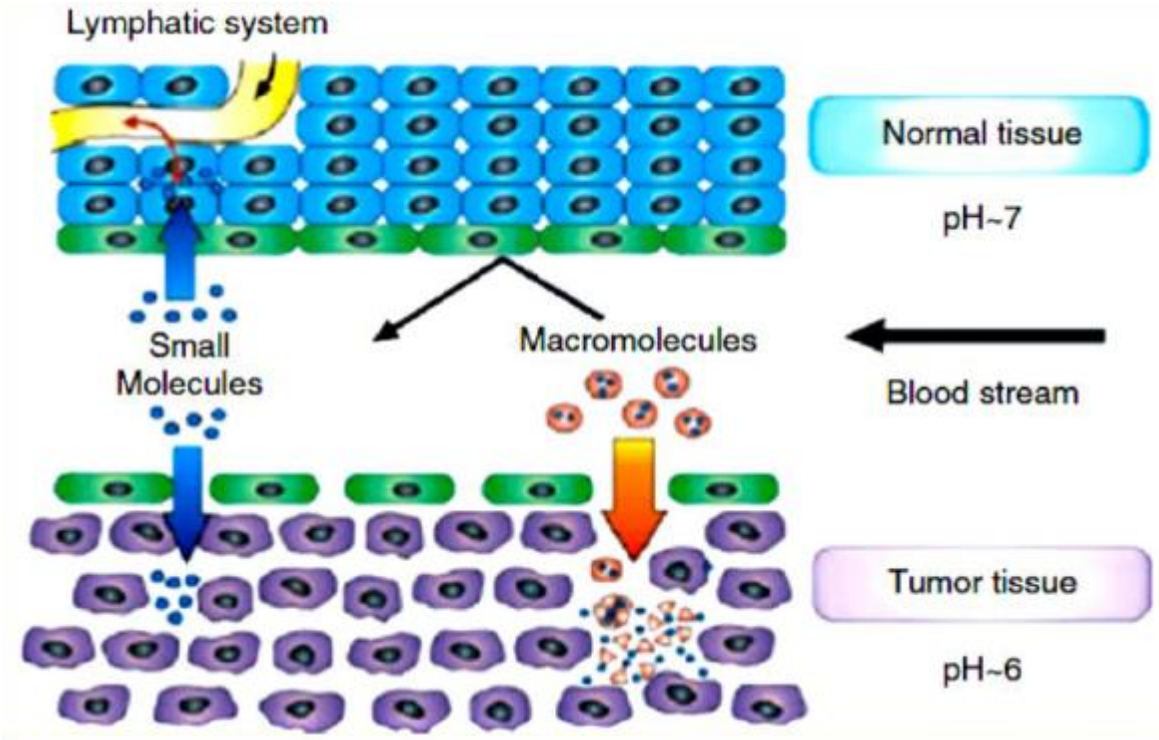



*Figure 1.3. Passive Targeting by EPR effect*

## 1.4. Drug delivery based on polycaprolactones

Pluronics or block copolymers of ethylene oxide and propylene oxide formed micellar structures in water and hence were used for drug delivery applications. The micelles formed from pluronics were capable of loading various anti-cancer drugs such as doxorubicin, amphotericin B, epirubicin and indomethacin. Also, these polymeric micelles proved to be less toxic than the low molecular weight surfactants. The hydrophobic pluronics also inhibit P-glycoproteins leading to tumor resistant cancer

cells. However, since these polymers were non-biodegradable, these particles resulted in long term toxicities in the cells. Polyesters, on the other hand, have been widely explored for their application in biomedical research. Polyesters also find applications in medicine as biodegradable sutures, resorbable prostheses, artificial skin and for drug delivery.[5] Polycaprolactone is aliphatic polyester that can be molded to have varying mechanical strength when blended with other monomer. Importantly, polycaprolactones due to its properties such as biodegradability, biocompatibility, miscibility with other polymers and synthesis of monomers from natural resources have been used for applications in drug delivery to cancer cells. Owing to the biodegradability of the polycaprolactones by esterases in the cells, caprolactones form an important area of study for their application in drug delivery.

There are two ways in which PCL is synthesized: [6]

**A.** By condensation of 6-hydroxycaproic acid and

**B.** By ring opening polymerization (ROP) of ε-Caprolactone.

However, ROP of cyclic lactones (Fig. 1.4), caprolactone, is a more preferred way of making PCL due to its controlled rate of polymerization, higher molecular weight build up and narrow polydispersity. The ring strain in the caprolactone monomer is the driving force for the ring opening polymerization of the lactones.


*Figure 1.4 General Scheme for Ring Opening Polymerization (ROP)*

Based on the catalyst involved in the process of polymerization, four mechanisms for ROP have been proposed. They are the following:

1. Cationic ROP
2. Anionic ROP
3. Monomer activated ROP
4. Co-ordination Insertion ROP

ROP by metal alkoxides and carboxylates such as Stannous-(II)-ethylhexanoate [$Sn(Oct)_2$] proceeds by co-ordination insertion mechanism[6]. (See Fig. 1.5) Catalysts such as $Sn(Oct)_2$ react with the alcohol initiator to generate tin alkoxide that causes the initiation of ring opening polymerization. The monomer first co-ordinates to the metal catalyst followed by the insertion of the monomer into the metal oxygen bond of the catalyst. This is the propagation step of the polymerization. Ring opening polymerization is better known as 'living polymerization' since the molecular weight of the polymer increases linearly with the increase in the degree of polymerization. The living nature of the polymerization produces high molecular weight polymers with narrow polydispersity.

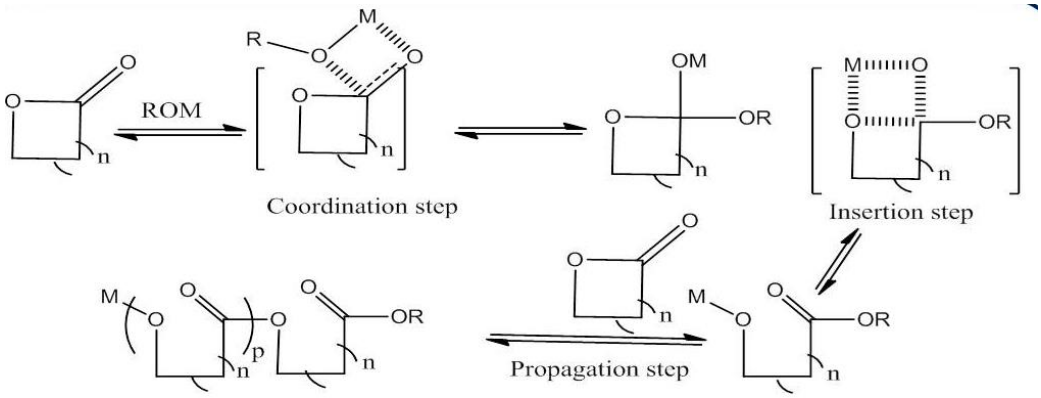



*Figure 1.5. Coordination Insertion Mechanism for ROP*

PCL due to its bio-compatibility has been exploited for the synthesis of polymers that can self-assemble in water to form either a micelle or a vesicle. PCL alone does not assemble in water owing to its enormous amount of hydrophobicity and therefore needs to be attached to hydrophilic groups such as polyethylene glycols (PEG) that impart amphiphilic character to the polymer. The PEG chains form a protective corona around the hydrophobic caprolactone core resulting in the formation of self-assemblies such as micelles and vesicles. These structures can then serve as carriers for drugs via physical encapsulation.

Since polycaprolactones lack a chemical site for attachment of drugs and targeting moieties, efforts in the past were directed on the development of substituted caprolactone (Fig. 1.6.). The figure below is a summary of a few substituted caprolactones that have been synthesized.[6] However, none of these monomers can be

employed for the appendage of a drug/ targeting molecule on the polymer. Bapu et al from our group developed a new γ-substituted caprolactone monomer that is, on one hand, stimuli responsive (pH) while on the other allows for chemical conjugation of cisplatin to the polymer.[7]

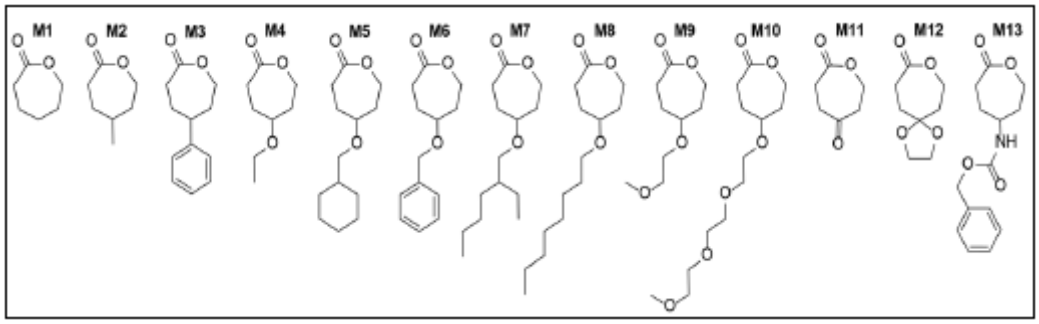



*Figure 1.6.Functionalized Caprolactone Monomers*

1.5. Block copolymers for cisplatin delivery

Block co-polymeric micelles for drug delivery were first used by Ringsdorf in 1975. The micelles formed by block co-polymers comprise of a hydrophobic core with a hydrophilic corona that forms a protective layer, separating the hydrophobic drug in the core compartment from the external medium. The driving force for the formation of micelle is the minimization in free energy due to coming together of the hydrophobic core against the aqueous medium forming the core and the interaction of the hydrophilic layer with water. This stability of the micelle can be measured in terms of critical micelle concentration (CMC). The lower the CMC of a polymer, better are its chances to serve as an efficient drug delivery system. Low CMC micelles ensure that it does not disrupt when it is diluted by large amount of blood in the body.

Block co-polymers have been chemically modified to yield tumor selective drug delivery systems. Additionally, they can be very easily modified to allow for efficient drug loading capabilities of the micelle via physical encapsulation or by chemical modification. Kataoka[8] and group (in 2005) reported a folic acid (FA) micelle, Folic acid-Polyethylene glycol-poly(aspartate hydrazone doxorubicin) [FA-PEG-PASP-DOX],to yield FA receptor targeted delivery of the micelle to the cancer cells. The hydrophilic component of the block co-polymeric micelle can be formed by polyethylene glycol (PEG). Polyethylene

glycols have been approved by FDA for drug delivery[8]. PEG is believed to reduce aggregation of the micelle, increasing the stability of the micelle and therefore enhances the blood circulation time of the drug present in the micelle. The hydrophobic component of the micelle is formed by polycaprolactone (PCL).

Platinum drugs are used for the treatment of many kinds of cancer.[9,10] The most routinely used platinum, anti-cancer drug is cisplatin.[11] Although there are other platinum drugs, only few have been approved for clinical trials, making cisplatin a very potent therapeutic agent. Cisplatin inhibits cell division by inducing apoptosis via activation of signaling transduction pathways.[12] (Fig. 1.7) However, patients receiving cisplatin suffer from plenty of side-effects such as nausea, vomiting, damage to kidney, damage to neuron and hearing disability to name a few. It is therefore important to look for means of selectively delivering cisplatin to tumor cells stressing the importance of drug delivery systems for cisplatin delivery.

**2.** Cellular interactions of CDDP: (**1**) reactive oxygen species; (**2**) DNA; (**3**) TNF; (**4**) mitochondria; (**5**) p53; (**6**) calcium signaling; (**7**) caspases; (**8**) multidrug resistant proteins.

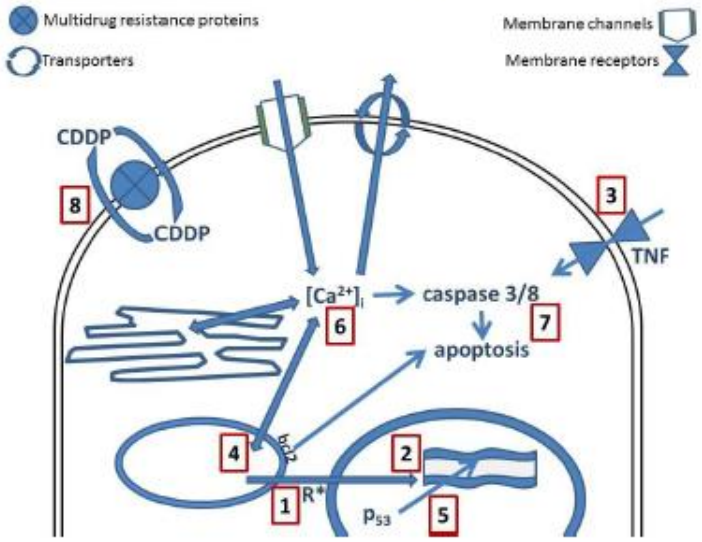



*Figure 1.7.Cellular Interaction of cisplatin*

N-(2-hydroxypropyl)methacrylamide [HPMA] –drug conjugates have been synthesized to conjugate anti-cancer drugs that combine the passive targeting of polymeric vehicles and are small enough to be eliminated by renal filtration.[13] For instance, HPMA-

cisplatin AP5280 is stitched with cisplatin at the malonate end (Fig 1.8). Another such polymer-drug conjugate is AP5346 (Prolindac) that is in the clinical stages for the treatment of cancer. This polymer contains DACHPt attached to the HPMA polymer via a pH sensitive amido malonate linkage. The polymer-drug conjugates AP 5280 and AP5346 are shown in Fig. 1.8 below.

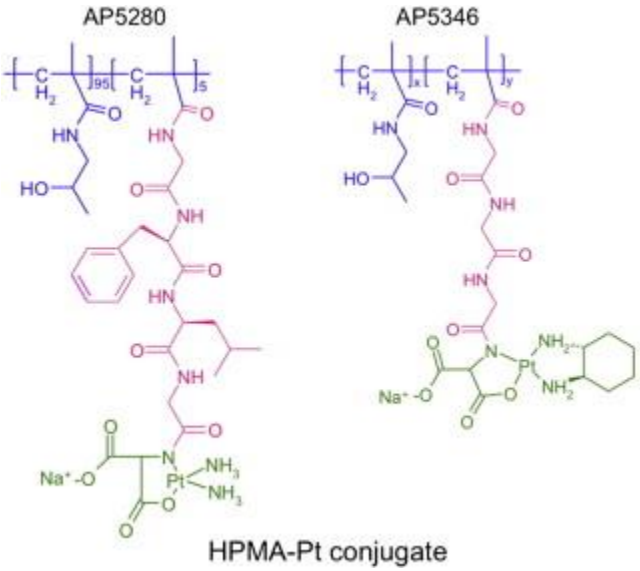



*Figure 1.8. Polymer-Platinum conjugates*

Bapu et al from our group developed a new polymer scaffold based on γ-carboxylic substituted caprolactone and hydrophilic polyethylene glycol [$PEG_x$-b-$CPCL_{100}$].[14] The carboxylic end was used to chemically conjugate cisplatin to the polymer chain. The drug loaded nanoparticle was then evaluated for its anti-cancer activity. In another paper by Bapu et al (Fig. 1.9), caprolactone based core-shell nanoparticles were synthesized by keeping the cisplatin core of the particle fixed while varying the PEG shell.[15] The role of polyethylene glycol shell in the stability of the nanoparticle was investigated. A controlled release of cisplatin from the nanoparticle core under esterase as an intracellular stimulus was observed. The release studies also showed that the PEG shell protects cisplatin against attack by cytosolic thiols such as glutathione and prevents GSH detoxification of the drug. In this manner, greater amount of platinum drug was available for tumor treatment which on the other hand is a daunting task to achieve when free cisplatin drug is injected thereby overcoming a major limitation in

platinum drug delivery. This thesis work is therefore based on this piece of work by Bapu et al.

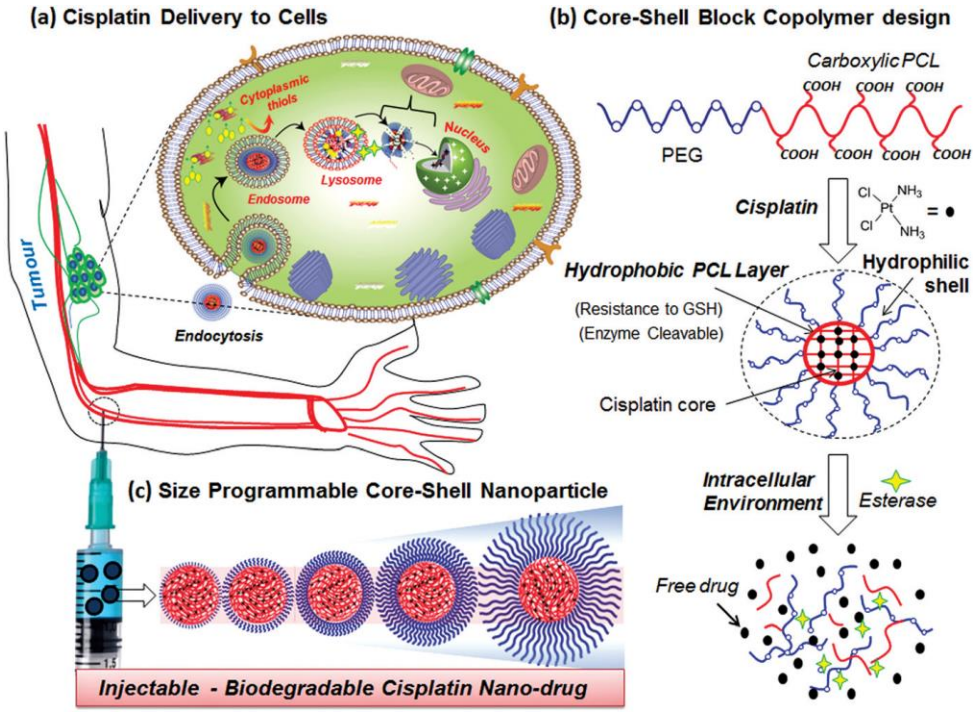



*Figure 1.9 Core-shell polymer-cisplatin nanoparticles for drug delivery to cancer cells.*

## 1.6 Aim of the Thesis

As discussed in the paper, PEG-2000 has the best release profile. This thesis work is aimed to develop new variable core and fixed shell cisplatin polymer conjugate for drug delivery (Fig. 1.10). We, therefore, employed polyethylene glycol monomethyl ether ($M_w$ 2000 g/mol) as an initiator and synthesized caprolactone based diblock copolymers by ring opening polymerization of γ-substituted caprolactone. The self assembly of these di-block copolymers resulted in micellar structures. We then varied the drug core by varying the CPCL units in the polymer while maintaining the size of the shell constant (using PEG 2000 as an initiator).

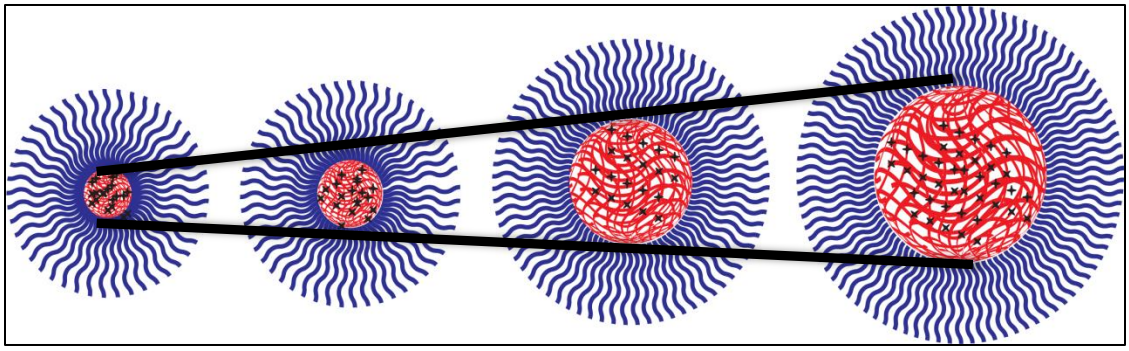


*Figure 1.10 Core-shell platinum nanoparticles with fixed shell and variable core.*

Changing the core of the particle is accompanied by changes in two important chemical entities.

1. The number of carboxylic units available for cisplatin stitching
2. The number of ester linkages in the backbone of the copolymer

Increasing the number of carboxylates in the system allows us to enhance the drug loading content of the polymer- drug conjugate. The problem that we attempt to address is: Does an increase in the number of carboxylic units strictly follow a linear relationship with drug loading capacity of the nanoparticles? The cytotoxicity studies for these nanoparticles in the normal and cancer cells will aid in answering yet another important question-what is the minimum amount of cisplatin drug needed to obtain 100% killing in tumor cells. The increase in the core length also implies an increase in the number of ester linkages, making it important to study the release profiles of these particles using esterase as stimuli. Release studies will also help us to study whether a particle undergoes burst release or controlled release, possibly influencing the death of the cells.

## 2. METHODOLOGY

2.1 Materials: 1, 4-Cyclohexanediol, tert-butyl acrylate, potassium tertiary butoxide, pyridinium chlorochromate (PCC), molecular sieves (4 Å), m-chloroperbenzoic acid (m-CPBA), polyethylene glycol mono-methyl ether with $M_w$=2000 g/mol, tin (II)-2-ethylhexanoate ($Sn(oct)_2$), trifluoroacetic acid (TFA), doxorubicin hydrochloride (DOX. HCl), triethylamine, cisplatin (CP) and silver nitrate were purchased from Sigma Aldrich chemicals. Anhydrous sodium sulphate, sodium bicarbonate, sodium thiosulphate pentahydrate and sodium hydroxide were locally purchased.

Solvents**:** tetrahydrofuran (THF), petroleum ether, dichloromethane (DCM), dimethylsulfoxide (DMSO) and dimethylformamide (DMF) were used.

2.2 Instrumentation: All the samples for $^{1}H$ and $^{13}C$-NMR were prepared in $CDCl_3$ solvent using trimethylsilane as the standard. Bruker 400MHz spectrophotometer was used for recording the NMR spectrum. Gel Permeation Chromatography (or Size exclusion chromatography) data was obtained by using a Viscotek VE 1122 pump, Viscotek VE 3580 RI, 3210 UV-Vis and Light scattering detectors. The polymers were dissolved in HPLC grade THF and the method file obtained after calibration using polystyrene standards was employed for the data acquisition. The thermal stability of the polymers were determined by using Perkin-Elmer thermal analyzer STA 6000 model, where the polymers were heated 10°C/min under nitrogen atmosphere. The polymers were then subjected to differential scanning calorimeter (DSC) using TA Q20 polymers were analyzed using a TA Q20 DSC, where they were first melted to remove pre-history. Following this, heating-cooling cycle takes place at 10°C /min under $N_2$ to generate DSC thermograms. The absorption studies and release assays were carried out by using Perkin-Elmer Lambda 45 UV-Visible Spectrophotometer. FT-IR study of the polymer and cisplatin stitched polymer was done by using Thermo Scientific Nicolet 6700 spectrometer. Dynamic Light Scattering (DLS) study of the self-assembled particles was done by using Malvern Instrument -Nano ZS- 90 setup using 633nm laser as the source with the detector collecting the scattered light at 90° angle. The DLS yields information on the correlation function that in turn provides the value of diffusion coefficient (D), the Stock Einstein equation is then used to calculate the diameter of the

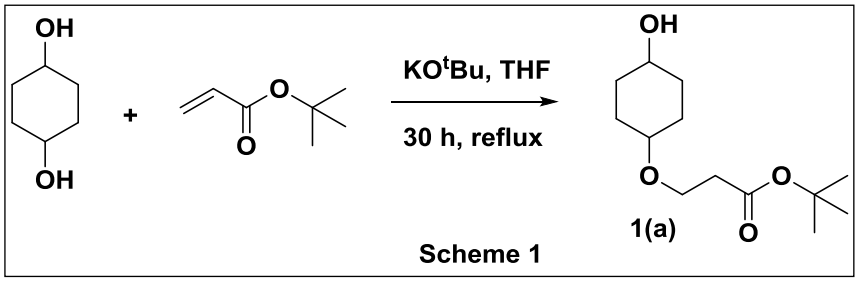
nanoparticles. The samples for FESEM- analysis were prepared at 0.05mg/ml concentration, filtered using 0.45 µm filter and were drop-casted on silicon wafers followed by air drying. The instrument used for recording FESEM images was Zeiss Ultra Plus scanning electron microscope.

## 2.3 GENERAL PROCEDURE

Multi-step synthesis of γ-substituted caprolactone

2.3.1 Synthesis of t-Butyl-3-((4-hydroxycyclohexyl)oxy)-propanoate (1a):

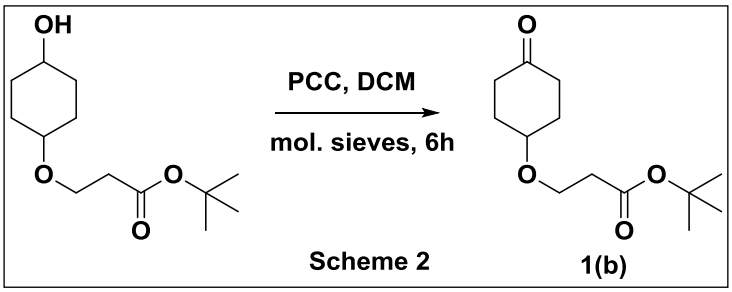
To a round bottom flask equipped with magnetic bar, 1,4-cyclohexane diol (30.00g, 258.6 mmol) was weighed and dissolved in distilled tetrahydrofuran (THF, 400 mL). To this solution, catalytic amount of potassium tert-butoxide (0.30 g, 2.67 mmol) was added under inert environment. A solution of tert-butyl acrylate (26.52g, 206.9mmol) in THF (50ml) was drop-wise added to the reaction mixture and the resulting solution was refluxed for 30 hours under nitrogen. Upon the completion of the reaction, THF from the reaction was evaporated in-vacuo and the reaction mixture was dissolved in dichloromethane (DCM). The reactant diol being insoluble in DCM is filtered off from the reaction using Buchner funnel. Pure product was obtained by carrying out column chromatography in ethyl acetate and petroleum ether (3:17 v/v). Yield=45% (22.64g).**$^{1}$H-NMR** (400 MHz, $CDCl_3$), δ ppm: 3.64 (m, 3H, O−$CH_2$− and O−CH), 3.29−3.39 (m, 1H, CH−OH), 2.4 (t, 2H, −$CH_2$CO−), 1.96−1.81 (m, 4H, OCH($CH_2$)$_2$), 1.64−1.32 (m, 4H, CO($CH_2$)$_2$), 1.45 (s, 9H, −C($CH_3$)$_3$).**$^{13}$C-NMR** (100 MHz, $CDCl_3$) δ ppm: 171.01, 80.42, 69.49, 63.84, 63.60, 32.54, 30.32, 29.20 and 27.48. **FT-IR** ($cm^{-1}$): 3422, 2979, 2937, 2863, 1731, 1462, 1393, 1366, 1215, 1158, 1116 and 1134.




Scheme 1

2.3.2 Synthesis of t-Butyl-3-((4-oxocyclohexyl)oxy)-propanoate (1b):

Compound 1a (22.64g, 92.8 mmol) was added to a flask and dissolved in dry dichloromethane (400mL) under inert conditions. This was followed by addition of pyridinium chlorochromate (PCC, 30.0g, 139.2 mmol) and molecular sieves (4Å). The reaction mixture was stirred at room temperature for 6 hours. Pure product (1b) was obtained by column chromatography, eluted in ethyl acetate and hexane (1:5 v/v). Yield=87 % (22.46 g).**$^{1}$H NMR**(400 MHz, $CDCl_3$) δ ppm: 3.56 (m, 3H, O−$CH_2$ and O−CH), 2.58 (t, 2H, −$CH_2$−CO), 2.64 (m, 2H, −(C=O)$CH_2$−), 2.26 (m, 2H, −(C=O)$CH_2$−), 2.09 (m, 2H, −(CO)$CH_2$−), 1.90 (m, 2H, −(CO)$CH_2$−), 1.45 (s, 9H, −$C(CH_3)_3$).**$^{13}$C-NMR** (100 MHz, $CDCl_3$) δ ppm: 211.40, 170.99, 80.56, 72.74, 64.02, 37.02, 36.56, 30.40 and 20.04. **FT-IR** ($cm^{-1}$): 2974, 2874, 2360, 1716, 1456, 1419, 1393, 1368, 1316, 1239, 1212 and 1109.

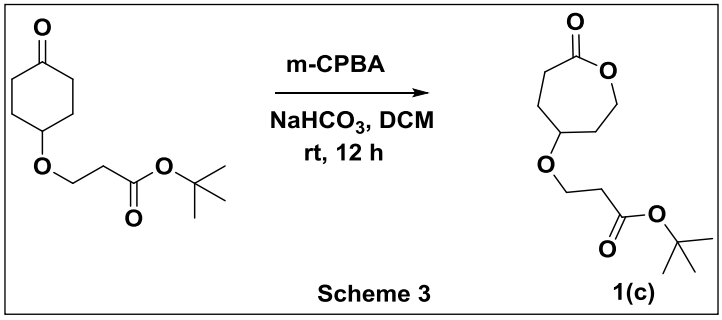



Scheme 2 1(b)

2.3.3 Synthesis of t-Butyl-3-((7-oxooxepan-4-yl)oxy)-propanoate 1(c):

Compound 1b (19.50 g, 80.5 mmol) was dissolved in 600 mL DCM followed by addition of $NaHCO_3$ (10.16g, 121 mmol). To this reaction mixture, m-CPBA (20.84g, 121 mmol) was added with constant stirring. The reaction was stirred at room temperature for 12 hours. Upon the completion of the reaction, DCM form the mixture was evaporated and the resultant was washed with saturated solution of sodium bicarbonate+ sodium thiosulphate to remove the excess peracid and m-chlorobenzoic acid formed. The organic layer was combined, dried over sodium sulphate and evaporated under vaccum. The crude product was purified by column chromatography eluted in ethyl acetate and petroleum ether (1:5 v/v) to yield a colorless liquid as pure product. Yield=80% (17.99 g).**$^{1}$H NMR** (400 MHz, $CDCl_3$) δ ppm: 4.40 (dd, 1H, COOCH), 4.06 (dd, 1H, COOCH), 3.60 (m, 4H, $OCH_2$, OCH, and COCH), 2.98 (dd, 1H, COCH), 2.48 (t,

2H, $COCH_2$), 2.42−1.81 (m, 4H, OCH−$(CH_2)_2$), 1.46 (s, 9H, $C(CH_3)_3$).**$^{13}$C-NMR** (100 MHz, $CDCl_3$) δ ppm: 176.35, 171.19, 81.01, 75.24, 64.24, 63.66, 36.80, 34.15, 28.40, 27.84 and 27.61. **FT-IR** ($cm^{-1}$): 2928, 1727, 1446, 1393, 1367, 1253, 1155, 1110 and 1058.



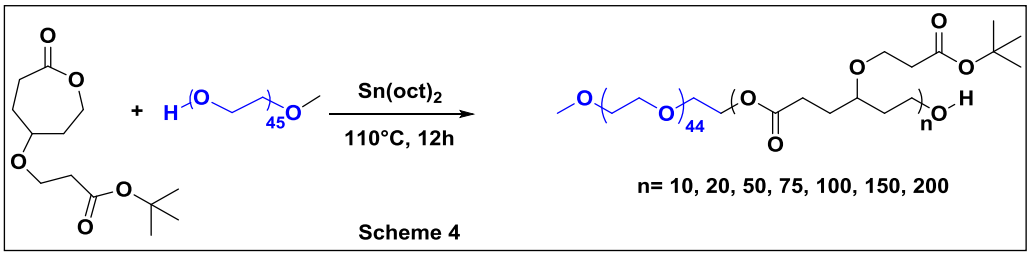

Scheme 3 1(c)

2.3.4 Ring Opening Polymerization (ROP) of Substituted Caprolactone (CL)

Synthesis of $PEG_{2000}$-b-$BPCL_x$ di-block copolymers: γ-Substituted Caprolactone was refluxed in toluene and dried prior to use for ROP. The initiator used for the polymerization is polyethylene glycol mono methyl ether (PEG 2000). The ratio of monomer to initiator ([M]/ [I]) was fixed to be 50. In a clean, flame dried schlenk tube, the initiator polyethylene glycol (0.075g, 0.0388mmol) was weighed and the monomer (0.5g, 1.94 mmol) was added. Following this, catalytic amount of $Sn(Oct)_2$ (7.8 mg, 0.0194 mmol) was added in inert conditions and the resulting mixture was subjected to high vacuum for about 35 minutes to remove traces of moisture in the mixture. The reaction mixture was then stirred at 110°C, whilst under vacuum, for 12 h. The mixture was then precipitated in petroleum ether. The polymer obtained was re-dissolved in minimum amount of THF and re-precipitated in THF to yield pure polymer. The polymer was then characterized by$^1$H-NMR. The peak at 3.63 ppm belonging to –OC**$H_2$**C**$H_2$**O- of the PEG was compared with the peak for methylene at 4.13 ppm (-OC**$H_2$**-) from the polyester backbone. The peak at 3.63 ppm merges with –OC**$H_2$**$CH_2$COO- (42 * 2H)of the substitution on the polymer with 180 protons of the polyethylene glycol. The difference in the peaks at 3.63 and 4.13 ppm equals the number of repeating units in the polymer. Yield= 80% (0.4g) .**$^1$H-NMR** (400MHz, $CDCl_3$) δ ppm: 4.13 (s, 2H), 3.63 (m, 5.6H), 3.44 (m, 1H), 3.37 (s, 0.03H), 2.45 (t, 2H), 2.37 (t, 2H), 1.75-1.85 (m, 4H),

1.44 (s, 9H).**$^{13}$C-NMR** (100MHz, $CDCl_3$) δ ppm:173.77, 170.91, 80.58, 75.60, 70.79, 64.94, 61.38, 36.62, 33.06, 29.84, 29.05 and 28.24. **FT-IR ($cm^{-1}$)**: 2972, 2930, 2876, 1727, 1457, 1363, 1251, 1156, 1099, 1063, 957, 898, 846, 759, 587 and 535.

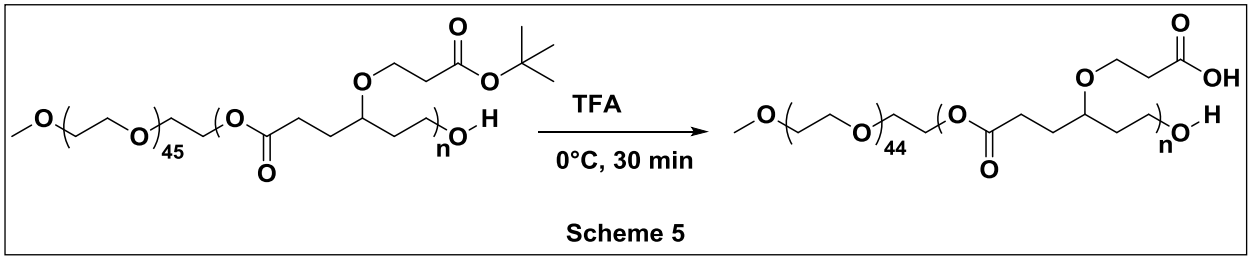
The advantage of ROP over other techniques of polymerization derives from the fact that the number of repeating units of caprolactone monomer in the resulting polymer can be varied by varying the ratio of the concentrations of the monomer to that of initiator ([M]/[I]). For instance, maintaining the ratio of [M]/[I] as 100 would yield a polymer with 100 repeating units of the caprolactone and one of PEG. The number of repeating units in the polymer was estimated using $^1$H-NMR. Seven such polymers were synthesized by varying the [M]/ [I] ratio from 10, 20, 50, 75, 100,150 and 200. These polymers were characterized by $^1$H-NMR and IR spectroscopy. An average yield of 80-85% was obtained.



Scheme 4

2.3.5 Deprotection of $PEG_{2000}$-b-$BPCL_x$ block co-polymer

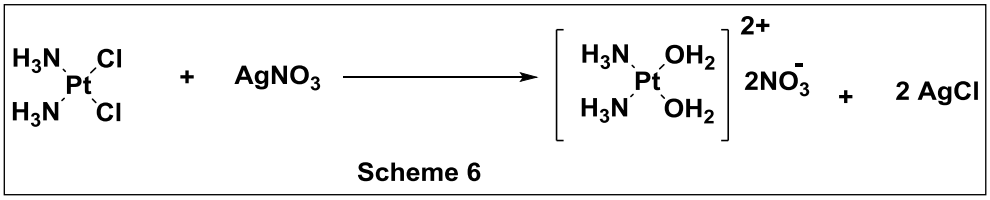
To a small round bottom flask, polymer ([M]/ [I]=50, 200 mg) was weighed and trifluoroacetic acid (TFA) was slowly added to the flask at 0°C. The polymer was dissolved in TFA by sonication. The reaction was stirred for 30 minutes at 0°C. The TFA from the reaction mixture was removed by repeated washing with toluene to yield carboxylic acid groups on the polymer backbone. The polymer was characterized by $^1$H and $^{13}$C- NMR. The polymers with 10, 20, 100 and 200 substituted caprolactone units were deprotected using the same procedure and characterized by $^1$H –NMR. **$^1$H-NMR** (400 MHz, $CDCl_3$) δ ppm: 4.14 (t, 2H), 3.70 (m, 2H), 3.64 (m, 3.6H), 3.48 (m, 1H), 2.57 (t, 2H), 2.38 (t, 2H) and 1.78 (m, 4H).**$^{13}$C-NMR** (100MHz, $CDCl_3$) δ ppm: 173.73, 170.91, 80.58, 75.60, 70.79, 64.94, 61.38, 36.62, 33.06, 29.84, 29.05 and 28.84. **FT-IR**($cm^{-1}$): 2926, 1723, 1352, 1249, 1171, 1095, 950, 839, 581, 546 and 519.




Scheme 5

2.3.6 Synthesis of Aquated cisplatin

Cisplatin (30mg, 0.0997 mmol) was dissolved in water (10 mL) at room temperature. To this solution, silver nitrate (33.86 mg, 0.199 mmol) was added and the reaction was stirred for 24 hours under dark conditions. It was observed that the solution turned turbid due to the formation of silver chloride salt. The salt was removed from the solution by centrifugation at 10,000 rpm for 30 minutes and the resulting aquated cisplatin was filtered through 0.45µmsyringe filter. The sample was lyophilized and used for stitching to carboxylic containing polymers.




Scheme 6

2.3.7 Stitching cisplatin to the polymer via chemical conjugation

$PEG_{2000}$-b-$CPCL_x$ polymer (10 mg) was dissolved in water and 1mg/ml solution of NaOH was drop-wise added to this solution until pH 7 was reached. The solution was stirred for 30 minutes at room temperature followed by the addition of cisplatin aqua complex. The solution was stirred at ambient temperature for 24 h under dark conditions. The solution was transferred into a semi-permeable membrane bag (MWCO= 3.5 kD) and dialyzed against large amount of water for 48 h. The water in the container was periodically replaced with fresh water to allow removal of un-encapsulated cisplatin. The solution was filtered using 0.45 µm filter and lyophilized.

2.3.8 Ortho-Phenylenediamine (OPD) Calorimetric Assay for Cisplatin Encapsulation

OPD Assay was employed to calculate the amount of cisplatin encapsulated into the polymer nanoparticles. 100 µL of cisplatin stitched polymer in water (1mg/mL) was taken in a vial and 3 mL of OPD solution in DMF (1.2 mg/mL) was added to the vial. The solution was heated at 100°C for 2 h. After 2 h, the solution was bought to room temperature and the absorbance of each of these samples was measured at 706 nm. The absorbance was then used to calculate the amount of drug encapsulated in the polymer. The following equations were then used to calculate the drug loading content (DLC) and drug loading efficiency (DLE).

**DLC (%) = (weight of the drug in the polymer/ weight of polymer)*100**

**DLE (%) = (weight of the drug in the polymer/ weight of drug in feed)*100**

2.3.9 In-vitro Drug Release Study for Cisplatin Stitched Polymers

The polymer micelle containing cisplatin (1mL) was taken in a dialysis bag and was dialyzed, against 10 mL PBS solution, with constant stirring at room temperature. At regular intervals of time, 100 µL of the dialysate was taken and replaced with an equal amount of fresh PBS. The aliquots were then treated with OPD solution to determine the amount of cisplatin released. The cumulative release is then calculated using the formula:

**Cumulative Release (%) = $C_n$ *$V_o$/ m *100** where,

$C_n$ is the amount of polymer in the dialysis bag

$V_0$ is the total volume of PBS in the beaker

M is the amount of drug in the polymeric micelle

2.3.10 Encapsulation of Doxorubicin in $PEG_{2000}$-b-$BPCL_x$

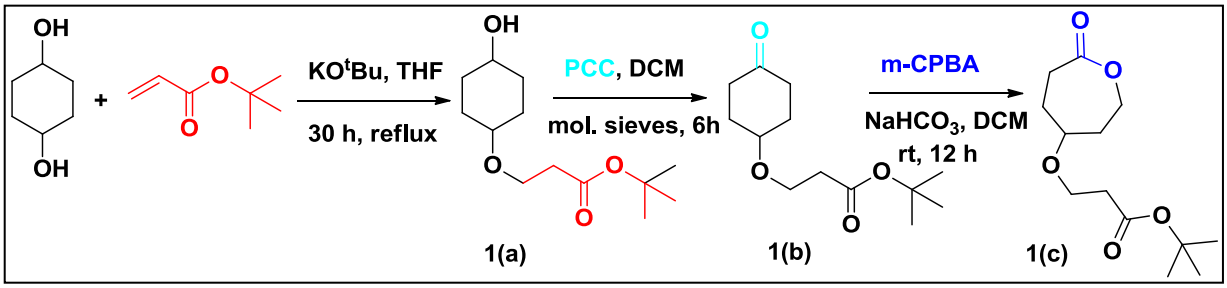
The anti-cancer drug doxorubicin was encapsulated into the polymeric micelle. Doxorubicin hydrochloride (2.5 mg) was dissolved in DMSO (2.5 mL) and triethylamine was added to neutralize the HCl. The solution was stirred for 30 minutes to yield a wine

red solution of doxorubicin in DMSO. 0.5 mL of pre-synthesized doxorubicin (1mg/mL) in DMSO was added to a solution of the di-block copolymers (10 mg) in DMSO (2mL).Self assembly of the polymers was done by slow drop-wise addition of water (3mL) into the above solution. The resulting solution was stirred at room temperature under dark conditions for 4 hours. To remove DMSO and un-encapsulated DOX, the solution was transferred into a semi-permeable membrane and dialyzed against large amount of fresh water for 48 hours. During this period, water was periodically changed after every hour for the first eight hours and for every two hours subsequently. The dialyzed solution was filtered through 0.45um syringe filter. The DOX content in the polymer was determined by using absorption spectroscopy. 2mg/ml concentration of the solution was used for absorption spectroscopy and the absorption was measured at 490nm. The molar extinction coefficient for DOX used is 11500. DLE and DLC for doxorubicin loaded micelles were calculated using the equations stated in section OPD Assay.

## RESULTS AND DISCUSSION

### 3.1 Synthesis and Characterization of monomer and their polymers

The monomer for the polymer was synthesized in multiple steps as stated in the experimental section. *Scheme 3.1*




*Scheme 3.1. Multi-step synthesis of γ-substituted caprolactone monomer*

In the first step 1, 4-Cyclohexane diol underwent Michael addition reaction with tert-butyl acrylate in presence of potassium tert-butoxide as the base to yield mono-coupled Michael addition product. The product was confirmed by NMR spectroscopy. (Fig. 3.1) The peak appearing at 1.41 ppm belongs to -COO**$(CH_3)_3$** and is in accordance with mono coupled product. Also the peak observed at 3.35 ppm, corresponding to proton attached to carbon adjacent to alcohol group, is split into two due to two chair conformations of the substituted cyclohexane ring. In the subsequent step the mono-coupled product, containing a secondary alcohol, was converted into a ketone employing PCC oxidation reaction. From $^1$H-NMR the peak that appeared at 3.35 ppm belonging to –**CH** proton adjacent to alcohol disappeared upon alcohol conversion to ketone. We infer from this that the ketone has been formed. In the last step, six member substituted ketone was subjected to Bayer-Villiger oxidation in presence of m-chloroperbenzoic acid (m-CPBA) that in turn produced γ-substituted caprolactone monomer. A new set of peaks appeared at 4.49 ppm and 4.06 ppm indicating the incorporation of the oxygen into the ring.

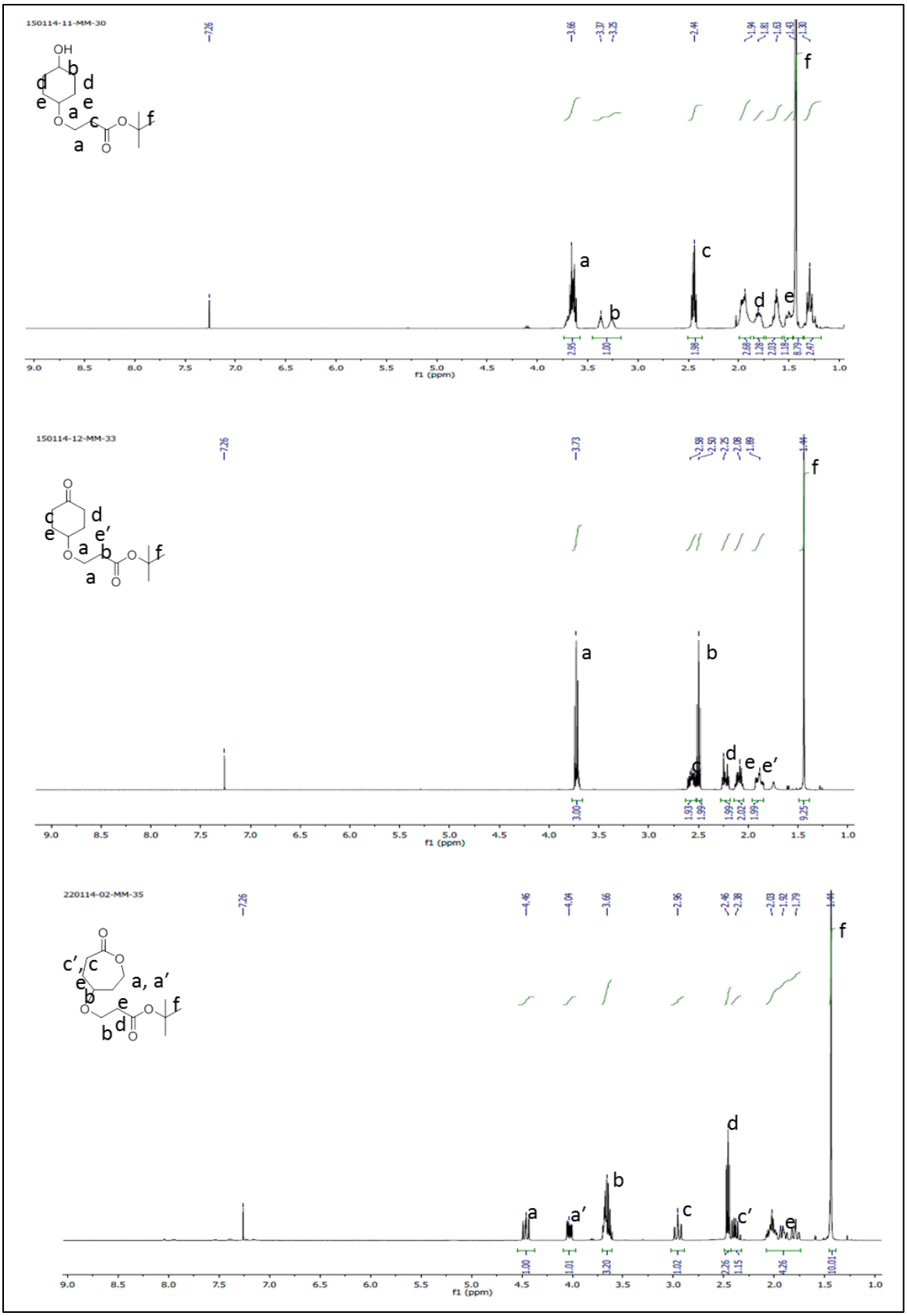



*Figure 3.1. Characterization of the multi-step monomer synthesis*

Ring Opening polymerization (ROP) is a preferred way of synthezing polymers owing to the robustness of the method and low polydispersity index with controlled molecular weight. A typical ROP procedure begins by adding the monomer, γ-substituted caprolactone [1(c)], into a clean, dry schlenk tube. The monomer was then mixed with initiator and catalytic amount of Stannous-(II)-ethylhexanoate followed by subjection to vacuum for an hour. The polymerization reaction was carried out at 110°C for 12 hours. Substituted caprolactone in itself is hydrophobic and hence hydrophilic polyethylene glycol monomethyl ether ($M_w$= 2000 g/mol) served to be the initiator in our reactions. This yielded polymers that are amphiphilic in nature and would ensure self assembly of polymers in water. The polymer once formed, was dissolved in minimum amount of THF and precipitated in hexane.

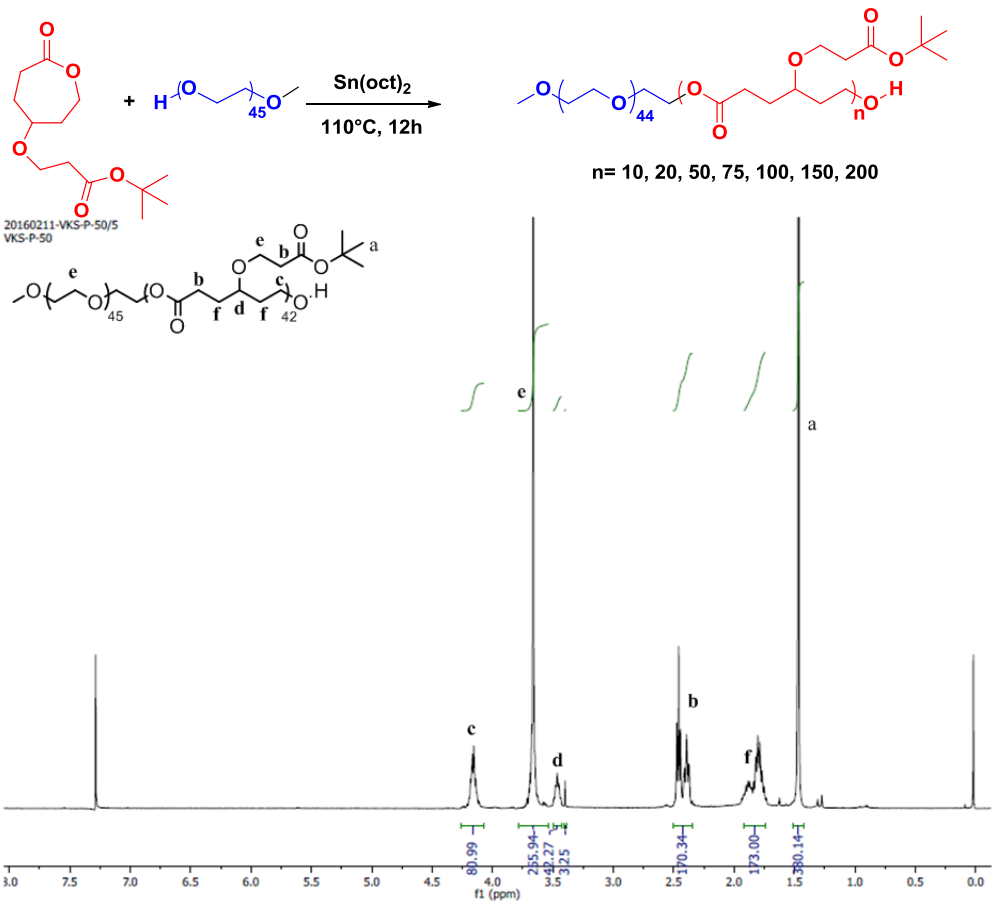



*Figure3.2. Characterization of polymer by $^1$H-NMR*

The characterization of the polymers was done by $^{1}$H-NMR. (Fig. 3.2) Varying the concentration of the initiator with respect to that of monomer could in principle lead to polymers containing a fixed number of caprolactone units in the backbone of the polymer. For instance, [M]/ [I] = 100 would yield a polymer that contains 100 caprolactone units and one unit of initiator in one polymer chain. Polymers with different number of caprolactone units [$PEG_{2000}$-b-$BPCL_x$; x=10, 20, 50, 75, 100, 150, 200] were synthesized and characterized by $^{1}$H-NMR (Fig.3.2). The graph for the caprolactone unit in feed vs. number of caprolactone units in the polymer is shown below (Fig. 3.3.) The stacked plot of NMR for the polymers with x= 10, 50, 75, 100 and 200 is shown in Fig. 3.4.

The polymers were then characterized by Gel Permeation Chromatography (GPC) in THF as the solvent and polystyrene as the standard. The GPC analyses showed mono-modal distribution of the polymer with narrow polydispersity and high molecular weights (Fig. 3.5). The chromatogram also revealed that the molecular weight of the polymer increases with increase in the number of caprolactone units in the polymer. However, the retention time is not very different for polymers with 100, 150 and 200 repeating units of caprolactone due to the inability of the polystyrene standards to distinguish between the hydrodynamic volumes of these polymers. GPC also underestimates the molecular weight of the polymers in comparison to that of $^{1}$H-NMR. The $M_w$ and $M_n$ for these polymers are tabulated in Table 3.1.

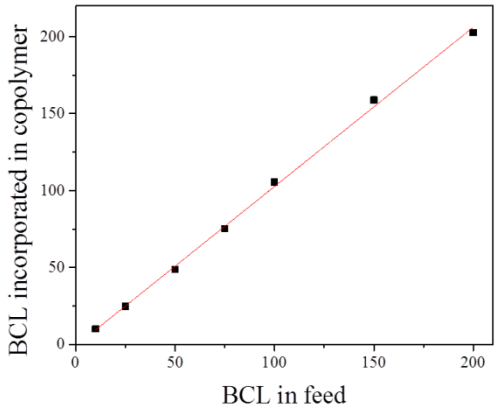



*Figure 3.3. Incorporation vs. Feed of monomer in the polymer*

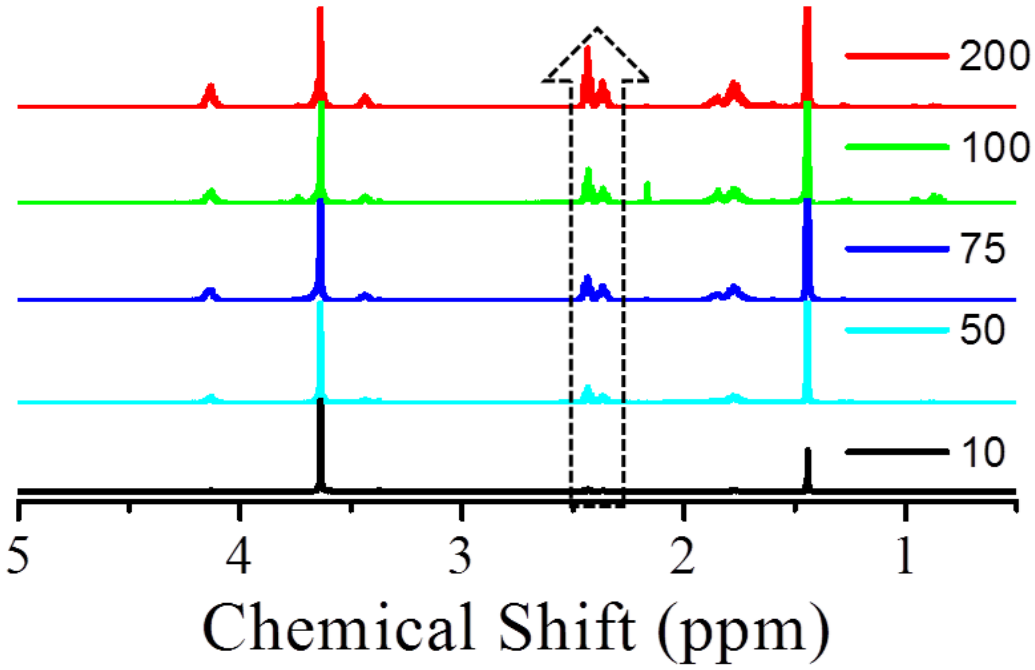



*Figure 3.4. Stacked NMR plot of protected polymers*

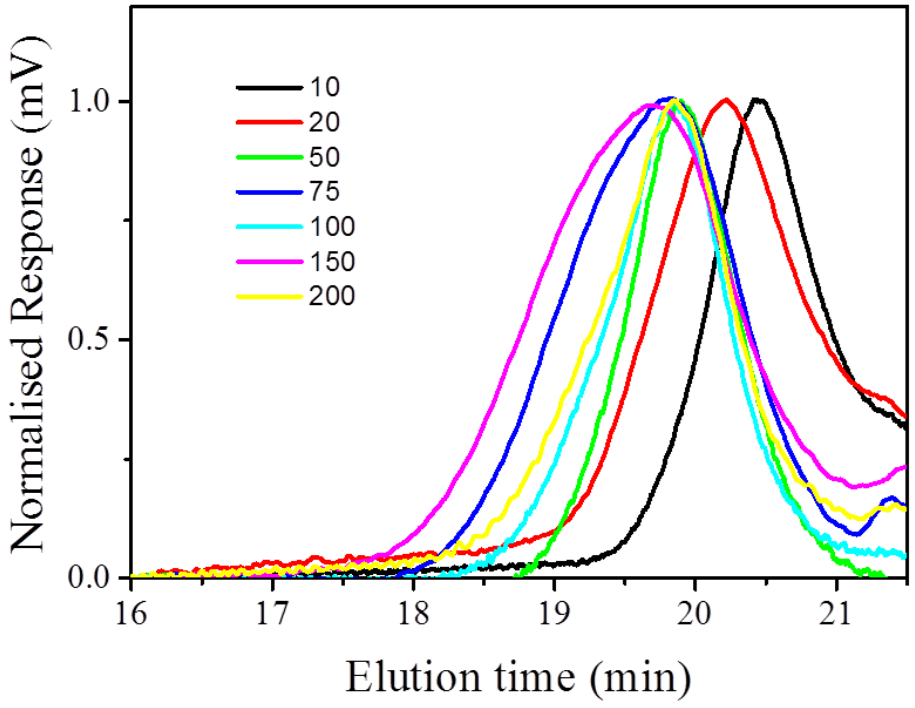



*Figure 3.5. Characterization of polymer by Gel Permeation Chromatography (GPC)*

*Table 3.1.Molecular weights from $^{1}$H-NMR and GPC*

| $PEG_{2000}$-b-$CPCL_x$ | | $^{1}$H-NMR | GPC | | |
|---|---|---|---|---|---|
| Feed | Incorporated | $M_n$ | $M_n$ | $M_w$ | PDI |
| 10 | 10 | 2600 | 4000 | 5100 | 1.2 |
| 25 | 25 | 6500 | 5800 | 7700 | 1.3 |
| 50 | 49 | 12600 | 7100 | 9000 | 1.2 |
| 75 | 75 | 19300 | 9100 | 16400 | 1.3 |
| 100 | 105 | 27100 | 8900 | 13400 | 1.2 |
| 150 | 159 | 41000 | 10100 | 15300 | 1.5 |
| 200 | 203 | 52400 | 9200 | 12300 | 1.3 |

The tertiary butyl group of these polymers was de-protected using trifluoroacetic acid (TFA) at 0°C. The polymer so obtained was characterized using $^{1}$H-NMR. Deprotection of these units was confirmed by the absence of singlet peak at 1.44 ppm corresponding to tertiary butyl group. The NMR of the de-protected polymer is compared to protected polymer in the Fig. 3.6. The stacked NMR for all the de-protected polymers is shown in Fig. 3.7.

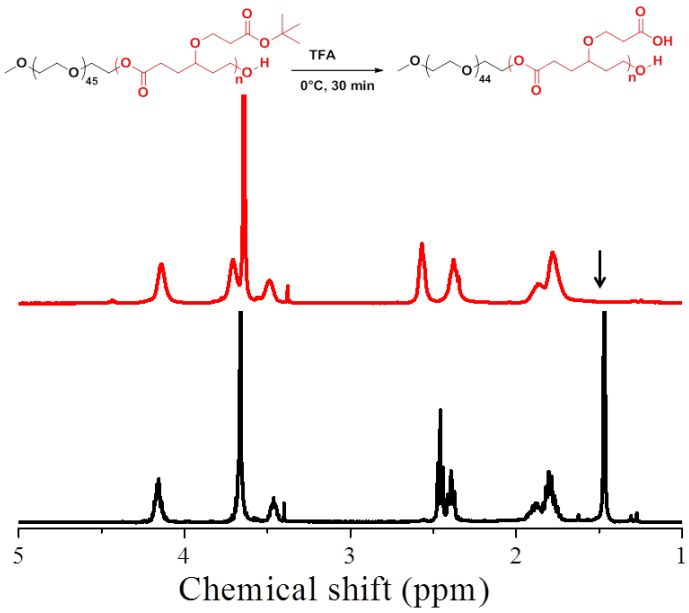



*Figure 3.6.$^{1}$H-NMR spectrum for protected and deprotected polymers (missing t-butyl peak)*

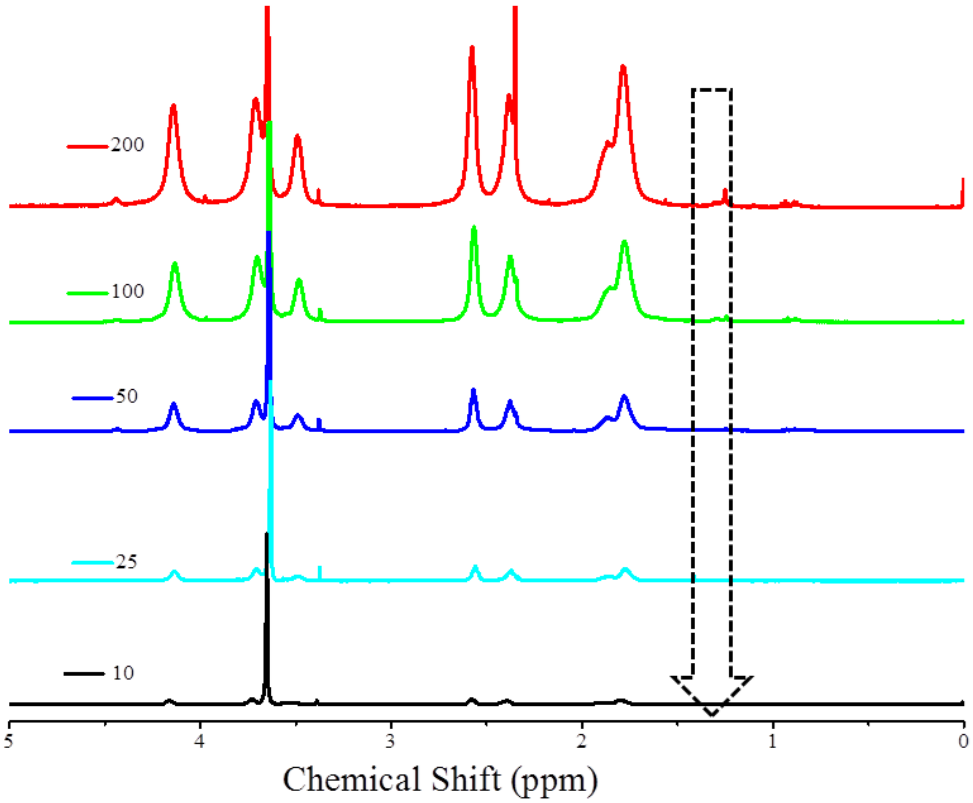



*Figure 3.7. Stacked $^{1}$H-NMR plot for deprotected polymers*

The aqua complex of cisplatin was prepared by the reaction of cisplatin with silver nitrate followed by filtration of silver chloride salt. The aquated cisplatin (1.1 eq) was then reacted with the sodium salt of the polymer (10 mg, 1eq) in water for 24 h at room temperature (Fig. 3.8). The solution was filtered through a 0.45 µm syringe filter and dialyzed against water for 48 h to remove unreacted cisplatin. Lyophilization of this solution gave brown colored cisplatin stitched polymer. The polymers $PEG_{2000}$-b-$CPCL_x$ with x= 10, 20, 50, 100 and 200 were chemically conjugated to cisplatin. The polymer-drug conjugates were then subjected to IR analysis. (Fig 3.9) The IR peak at 1728 $cm^{-1}$ corresponding to CO stretching frequency in the deprotected polymer disappeared to reveal a new peak at 1593 $cm^{-1}$. This new peak corresponds to Pt-O-C=O stretching frequency in the metal carboxylate nanoparticle.

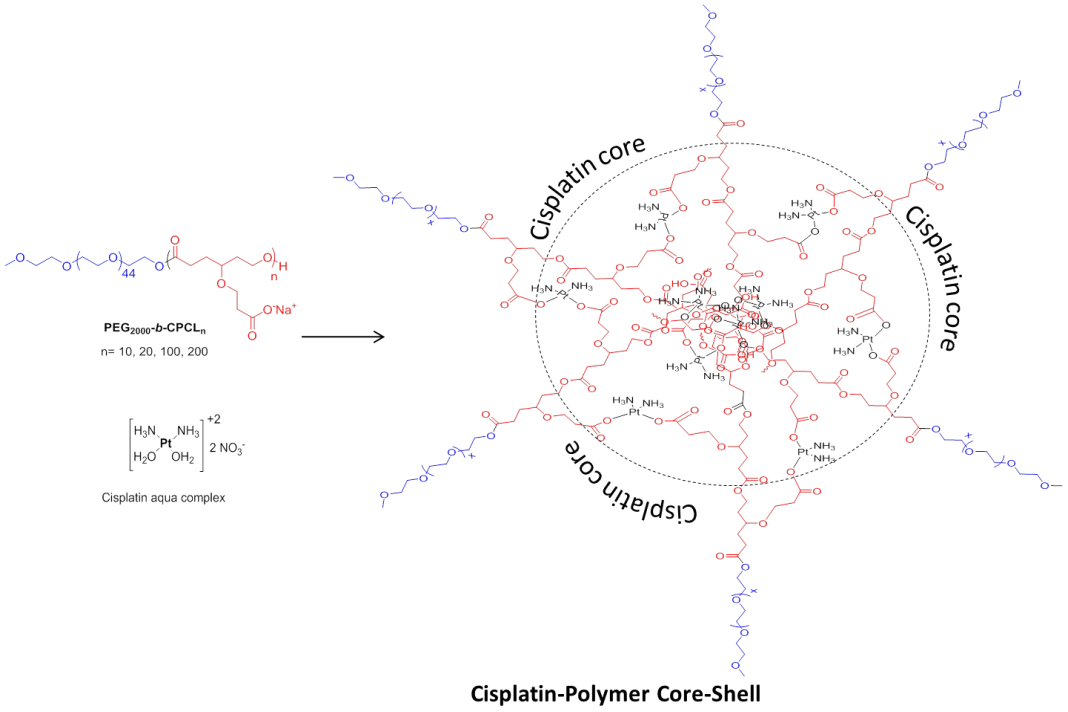



*Figure 3.8.Cisplatin conjugation to the polymers*

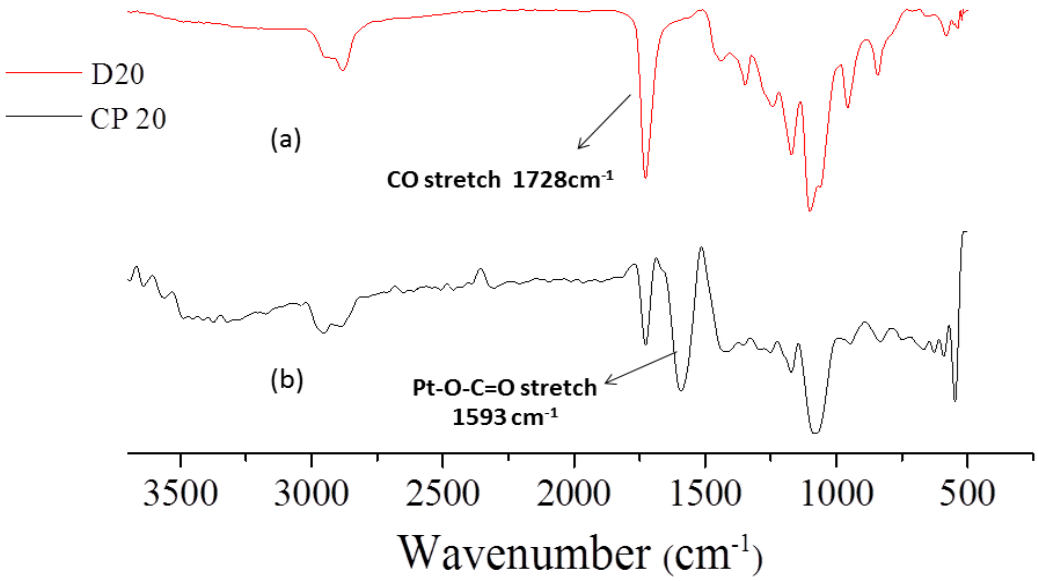



*Figure 3.9. FT-IR spectrum of (a) $PEG_{2000}$-b-$CPCL_{20}$ and (b) cisplatin stitched polymer*

## 3.2 Thermal Analysis

The thermal properties of the $PEG_{2000}$-b-$BPCL_x$ were studied by thermo gravimetric analysis (TGA) and by Differential scanning calorimetry (DSC). The TGA profiles show that the polymers were stable up to 200 °C ($T_d$) (Fig 3.10-a). To study the nature of the polymer, whether crystalline or amorphous, they were subjected to DSC analysis. The DSC thermogram revealed that the polymer with 10, 25 and 50 repeating units are semi-crystalline in nature while the polymers with 100 and 200 repeating units are amorphous. With the increase in the number of substituted caprolactone units, the nature of the polymer changes from semi-crystalline to amorphous, possibly due to the presence of tert-butyl group that disturbs the packing of the polymer chains. Additionally, there is an associated cold crystallization peak with $PEG_{2000}$-b-$BPCL_x$ for x= 25, 50. Cold crystallization is a phenomenon where the chains do not align completely when cooling; therefore upon heating, these polymer chains begin to melt yielding a peak in addition to the crystallization peak. (Fig. 3.10-b) The compiled DSC and TGA data is tabulated in Table 3.2. A plot of enthalpy verses number of repeating units shows that the enthalpy decreases with the increase in number of repeating units of caprolactone. (Fig. 3.11)

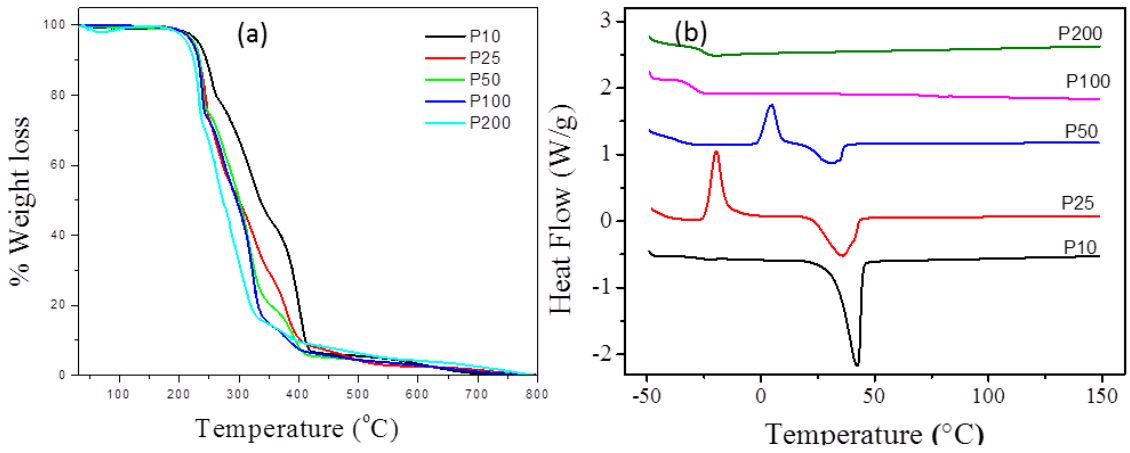



*Figure 3.10. (a)TGA profile and (b) DSC thermogram for nascent polymer*

*Table 3.2. Compilation of TGA and DSC data*

| Polymer | TGA | DSC | | | | |
|---|---|---|---|---|---|---|
| | $T_d$ (°C) | $T_c$ (°C) | $T_m$ (°C) | $\Delta H_c$(J/g) | $\Delta H_m$(J/g) | $T_g$ (°C) |
| $PEG_{2000}$-b-$BPCL_{10}$ | 210 | -28.42 | 42.39 | 16.73 | 57.7 | -27.67 |
| $PEG_{2000}$-b-$BPCL_{25}$ | 200 | - | 36.03 | - | 30.40 | - |
| $PEG_{2000}$-b-$BPCL_{50}$ | 200 | - | 31.350 | - | 14.05 | -36.21 |
| $PEG_{2000}$-b-$BPCL_{100}$ | 195 | - | - | - | - | -28.69 |
| $PEG_{2000}$-b-$BPCL_{200}$ | 200 | - | - | - | - | -25.17 |

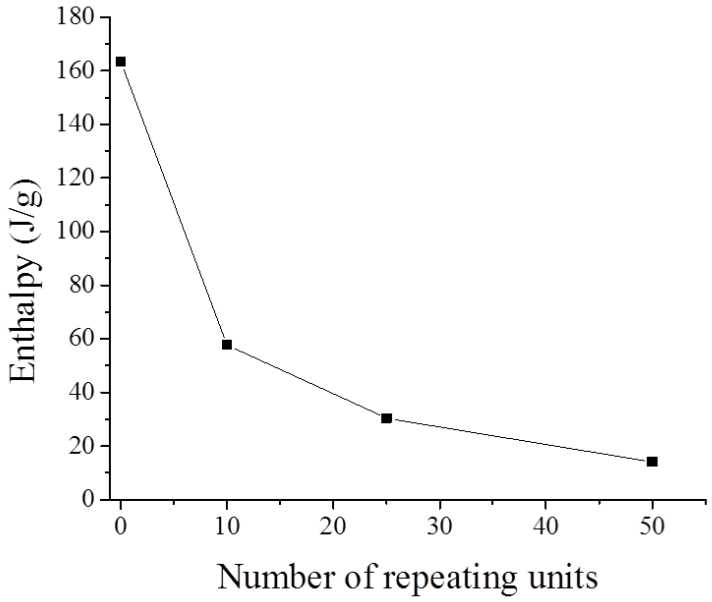



*Figure 3.11. Plot of enthalpy for different polymers*

Thermo gravimetric Analysis (TGA) for cisplatin alone and polymer-cisplatin drug conjugate was done to estimate the drug loading content of the polymers. The TGA data for the polymer-drug conjugate and the drug alone is shown in Fig. 3.12. The TGA profile of cisplatin alone shows 60% weight loss below 400 °C due to the loss of its four coordinating ligands ($NH_3$ and $Cl^-$). Increasing temperature beyond 400 °C does not cause any weight loss and the TGA profile remains flat until 800 °C. The polymer- drug conjugates on the other hand show stepwise degradation and the TGA profile can be divided into three major regions. The first step in the weight loss is due to the detachment of ligands attached to platinum; the second due to the degradation of the polymer; the third step is due to the presence of residual Platinum.

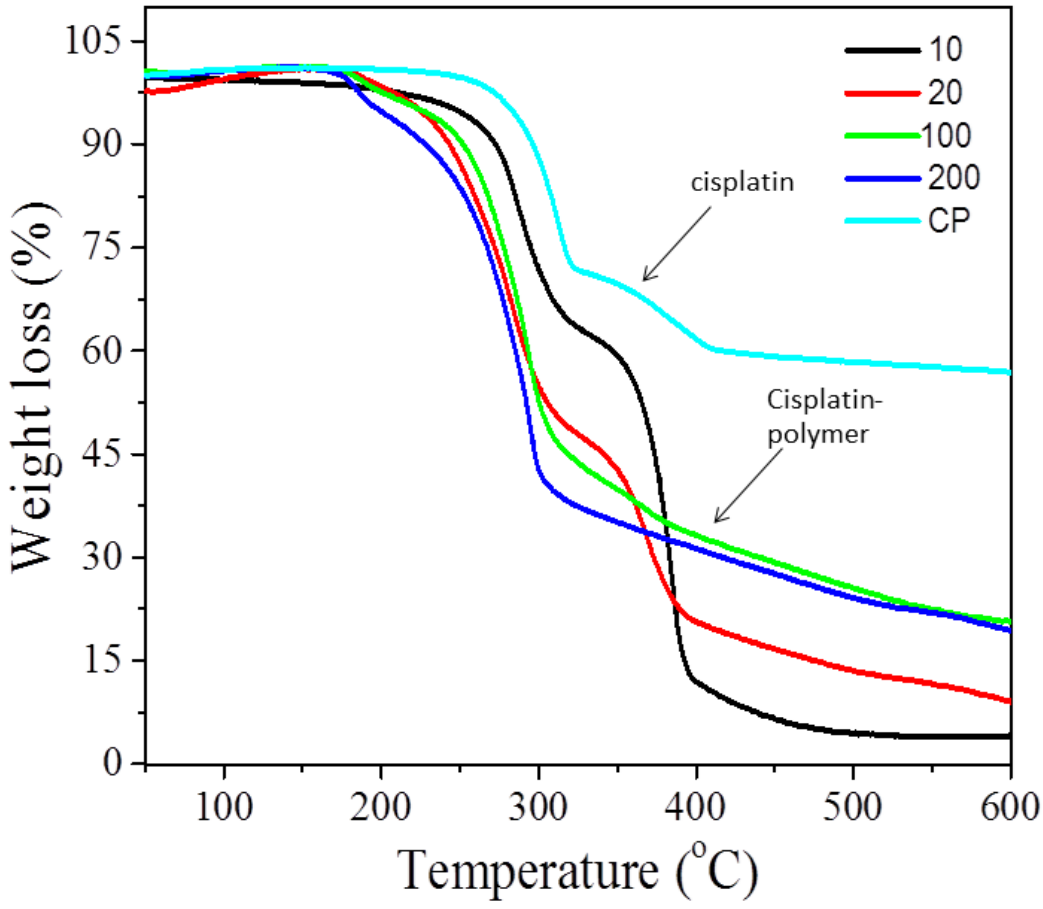



*Figure 3.12. TGA profile for cisplatin stitched polymers*

The TGA profile for the polymers $PEG_{2000}$-b-$CPCL_x$ with x= 10, 20, 50, 100 and 200 is shown in Fig.3.9. The % weight loss is the maximum for the polymer with 10 caprolactone units and is the least for polymer with 200 caprolactone units with $PEG_{2000}$-b-$CPCL_{20}$ and $PEG_{2000}$-b-$CPCL_{100}$ profile in between the two extremes. The differences in the TGA profile can be explained by examining the % content of platinum in these polymer-drug conjugates. The $PEG_{2000}$-b-$CPCL_{10}$ with 96% weight loss contains only 4% platinum while $PEG_{2000}$-b-$CPCL_{200}$ undergoes 80% weight loss with 20% platinum content. The polymer $PEG_{2000}$-b-$CPCL_{20}$ suffers a weight loss of 91% with platinum content of 9%. However, not much difference is seen in the platinum content for the polymer $PEG_{2000}$-b-$CPCL_{200}$ and$PEG_{2000}$-b-$CPCL_{100}$. Therefore, it can be inferred from the plots that cisplatin content in the polymer increases with the increase in the number of substituted caprolactone units (or with the increase in number of carboxylic units available for chemical conjugation to cisplatin). An interesting observation, however, is that cisplatin content does not linearly increase with the increase in the number of carboxylic units. (Fig. 3.13)

The drug loading content (DLC) and drug conjugation efficiency (DCE) for the polymers were calculated using the following equations.

$$\mathbf{DCE = \{mPt_{exp}/m\ Pt_{theo}\} * 100\ \%}$$

$$\mathbf{= \{(W_{Pt}/\ M_{Pt}\ )\ /\ (W_{acid}\ /2\ M_{acid})\} * 100\ \%}$$

Where $mPt_{exp}$ is the experimental amount of platinum, $mPt_{theo}$ is the experimental molar amount of platinum, $W_{Pt}$ is the weight percent of platinum from TGA, $M_{Pt}$ is the molar mass of platinum, $W_{acid}$ is the weight percent of the acid from TGA and $M_{acid}$ is the molar mass of the acid repeating unit.

**DLC= {initial feed* DCE/ [amount of polymer + (initial drug feed *DCE)]}*100**

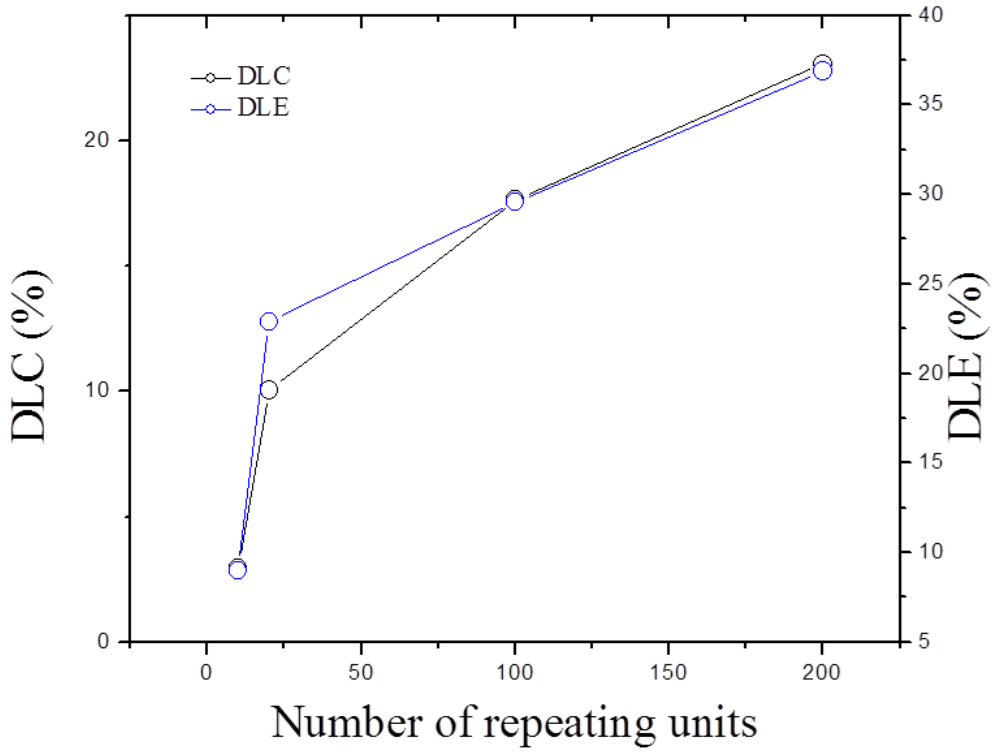



*Figure 3.13 DLC and DLE from TGA*

3.3 Self-assembly Studies in water: Studying their sizes and shape

The polymers (1mg/ml) upon chemical conjugation with cisplatin were dispersed in water and were subjected to dynamic light scattering (DLS) analysis. The DLS study of the polymer showed mono-modal distribution. (Fig. 3.14) Moreover, the sizes of the particles were seen to increase with the increase in the number of caprolactone units. The size of $PEG_{2000}$-b-$CPCL_{10}$ was found to be 122 nm while that of $PEG_{2000}$-b-$CPCL_{200}$ was found to be 255 nm. The sizes of these particles were also confirmed by FESEM analysis and the sizes from DLS seem to corroborate with that obtained from FESEM studies. FESEM images showed spherical morphology of the polymer-drug conjugates (Fig.3.15.). The spherical nature of the particle is accounted for by the core-shell assembly of the polymer drug-conjugate. The core is formed by the carboxylic unit on the caprolactone chemically cross-linked by cisplatin stitching while the hydrophilic polyethylene glycol forms the shell of the nanoparticle.

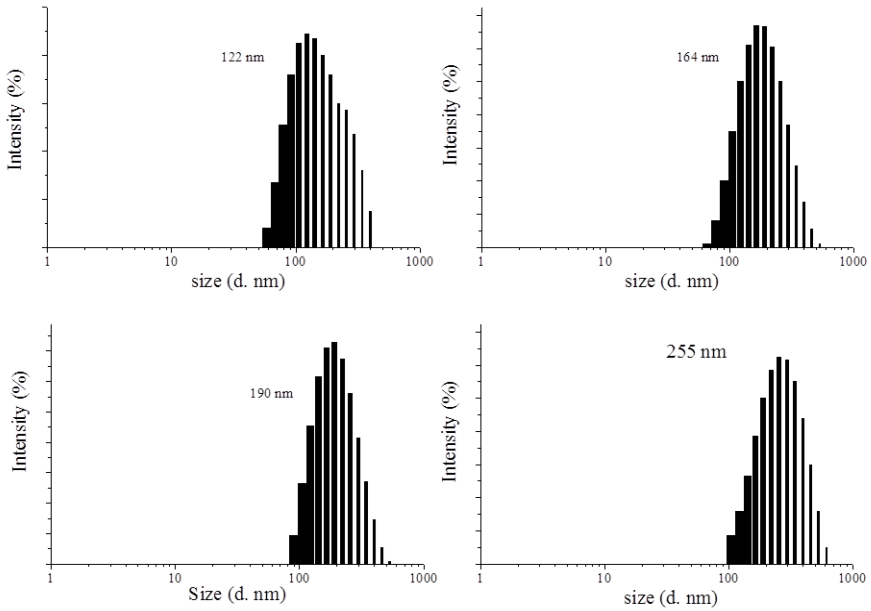



*Figure 3.14. Sizes of self assembled particles using DLS*

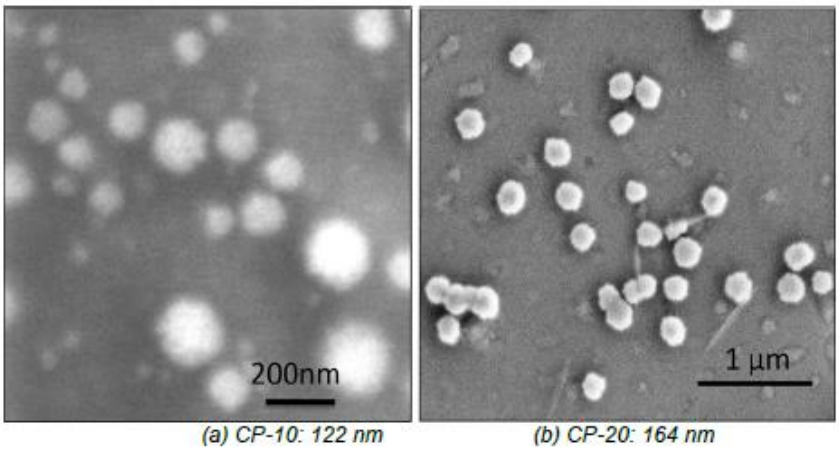



*Figure 3.15. Sizes of self assembled particles using FESEM*

## 3.4 Ortho-Phenylene diamine Assay (OPD) for estimation of DLC and DLE

100µl of cisplatin-polymer conjugate in water (1mg/ml) was taken in a vial and 3 mL of OPD in DMF (1.2mg/ml) was added to this. The solution was then heated at 100 °C for 2 hours. The amount of cisplatin in the sample was then measured by absorption spectroscopy using UV-Vis spectrophotometer. The amine of OPD bonds with platinum and absorbs at 706 nm, hence absorption at 706 nm was monitored for each of these polymer. (Fig.3.16-a.) Using the molar extinction coefficient as 24310 L $mol^{-1}$ $cm^{-1}$, the values for DLC and DLE were estimated using the equations stated above. Both DLC and DLE were found to increase with the increase in the number of carboxylic units. (Fig. 3.16-b.)

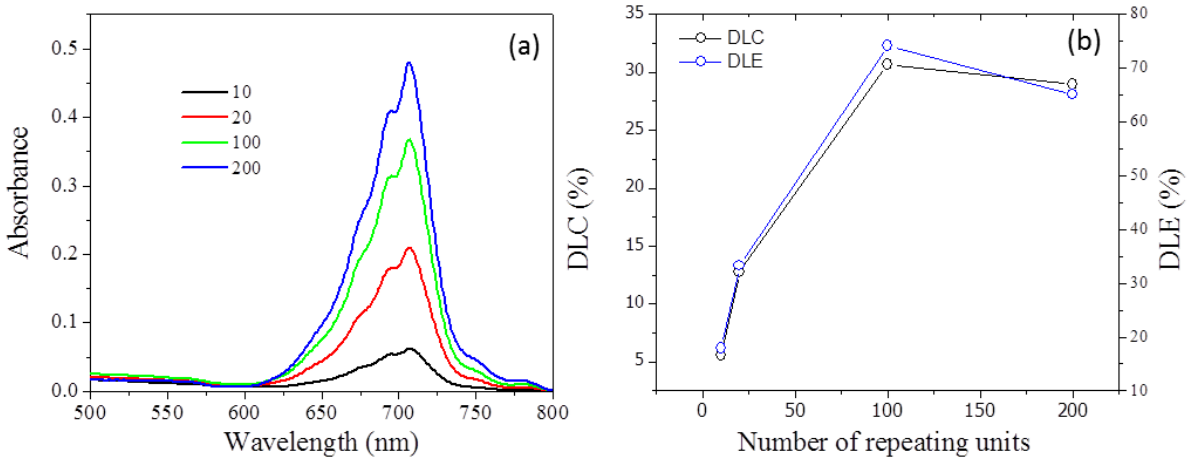



*Figure 3.16.(a) UV spectrum for OPD assay for cisplatin-polymer conjugate (b)DLC and DLE for different polymers by Absorbance*

## 3.5 In-vitro Studies in PBS solution

To study the release of cisplatin from the nanoparticle, the studies were carried out in PBS solution at room temperature. The phosphate ions in the PBS can in principle de-chelate cisplatin from the polymer hence their effect on cisplatin-drug conjugate was studied. For this purpose, $PEG_{2000}$-b-$CPCL_{20}$ and $PEG_{2000}$-b-$CPCL_{100}$ were subjected to release studies under PBS as buffer. The polymer containing cisplatin were dialyzed against PBS at room temperature for 48 h and aliquots of PBS were collected at regular

time intervals. The aliquots containing cisplatin were then quantified using OPD assay. (Fig.3.17.)

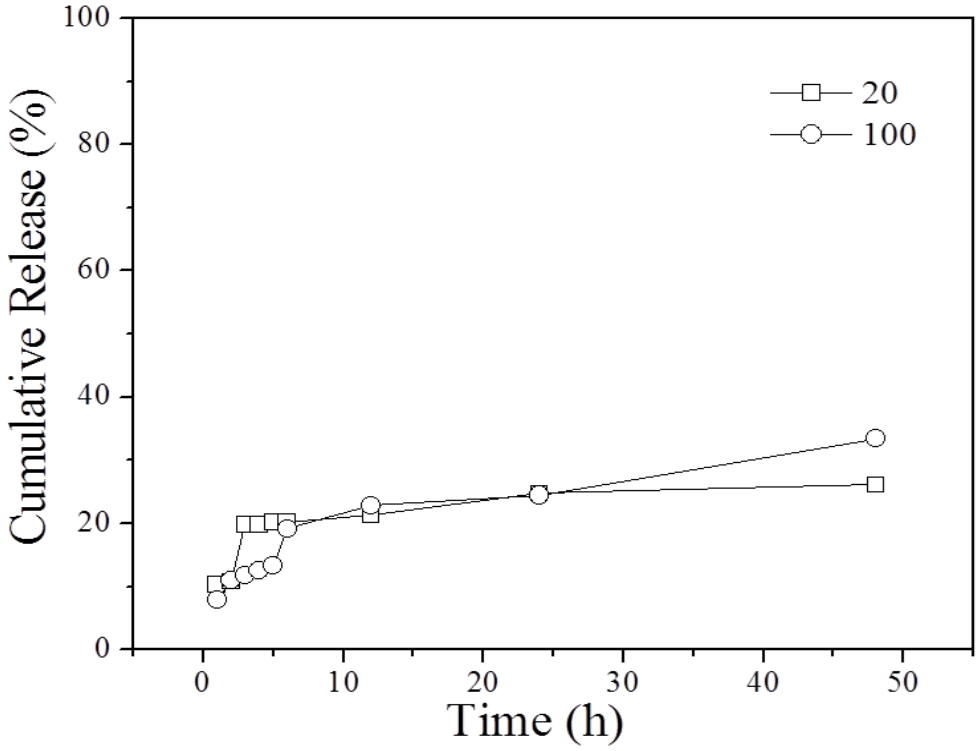



*Figure3.17.Cumulative release of cisplatin from nanoparticle in PBS*

From the figure, it can be seen that $PEG_{2000}$-b-$CPCL_{100}$ leaches about 35% cisplatin while for that of $PEG_{2000}$-b-$CPCL_{20,}$ the cumulative release is about 20%. One possible explanation for this could be that PEG forms a thicker shell around the small core of polymer with 20 repeating units and only allows minimal leaching out of cisplatin whereas more amount of cisplatin leached out of$PEG_{2000}$-b-$CPCL_{100}$ owing to a thinner shell of PEG.

3.6 Encapsulation Study with Doxorubicin

The same polymeric micelles were examined for drug loading capacity by physical encapsulation. Doxorubicin hydrochloride was neutralized by using triethylamine to yield doxorubicin. The polymer solution in dimethylsulfoxide (DMSO) was treated with doxorubicin, followed by addition of water. The resulting solution was then transferred to a dialysis bag and dialysed against large volume of water for 48 h. The particles

obtained were characterized by using dynamic light scattering (DLS) technique. (Fig 3.18)

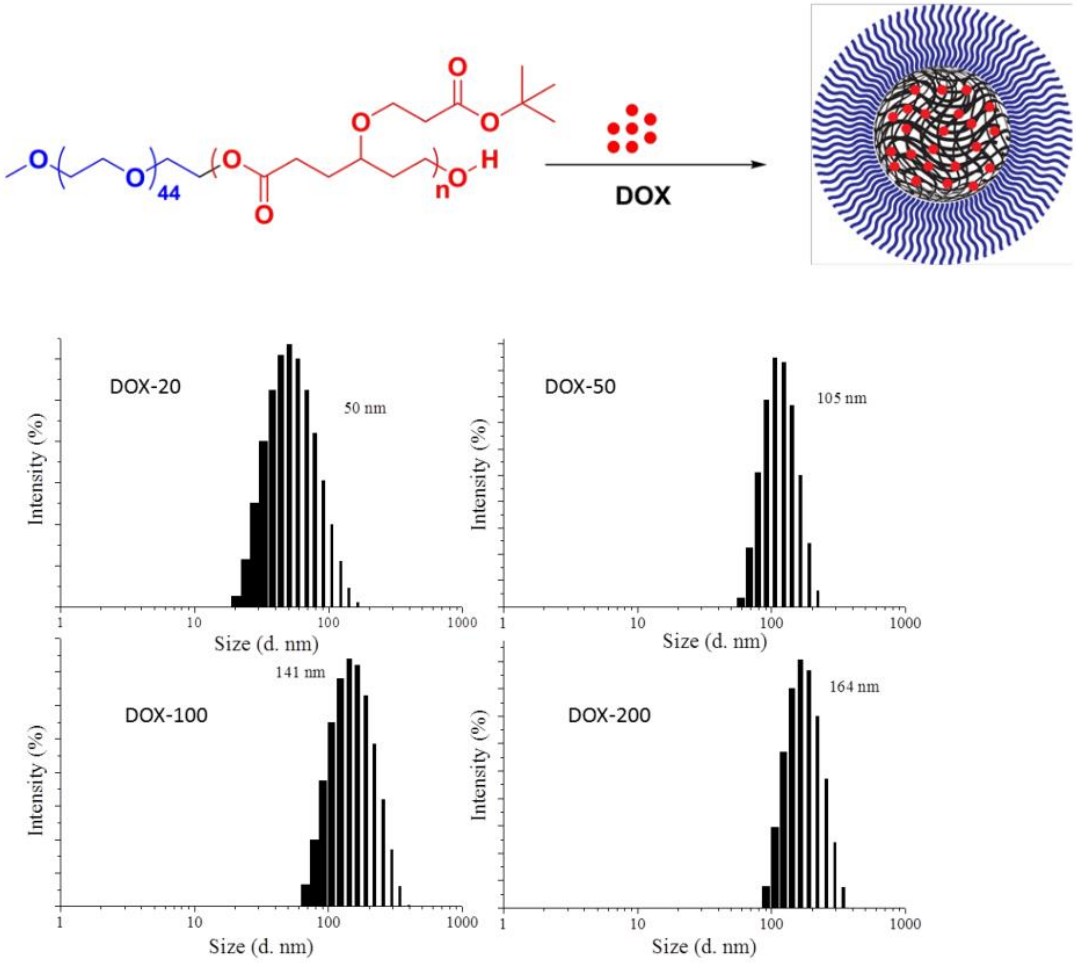



*Figure3.18. Self-assembly of DOX encapsulated nanoparticle*

The amount of doxorubicin drug encapsulated in the nanoparticle was measured by Absorption spectroscopy (Fig 3.19). Further, drug loading content (DLC) and drug loading efficiency (DLE) was estimated using the absorbance values obtained. (Fig. 3.20)

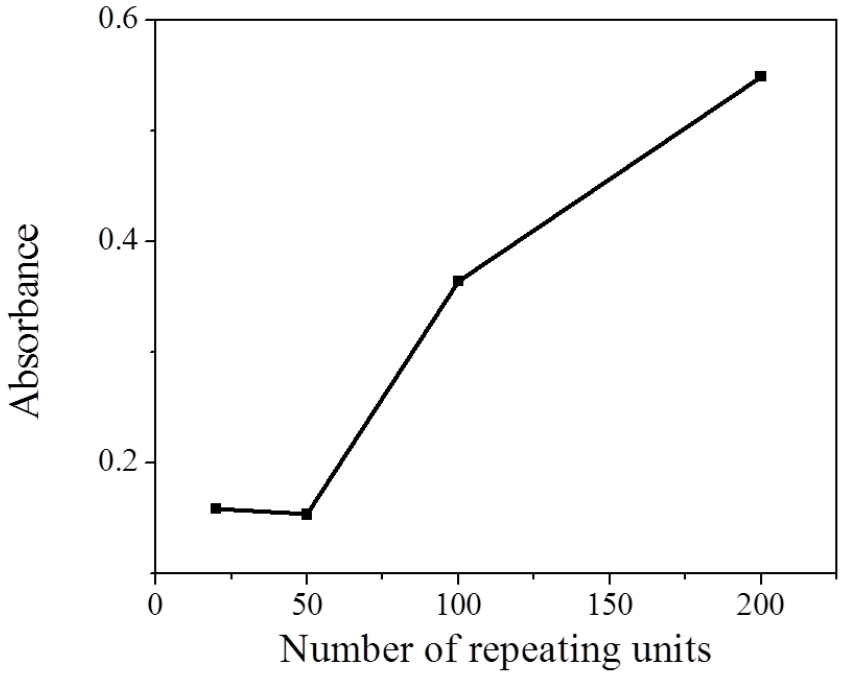



*Figure3.19. Absorbance of Doxorubicin in various nanoparticles*

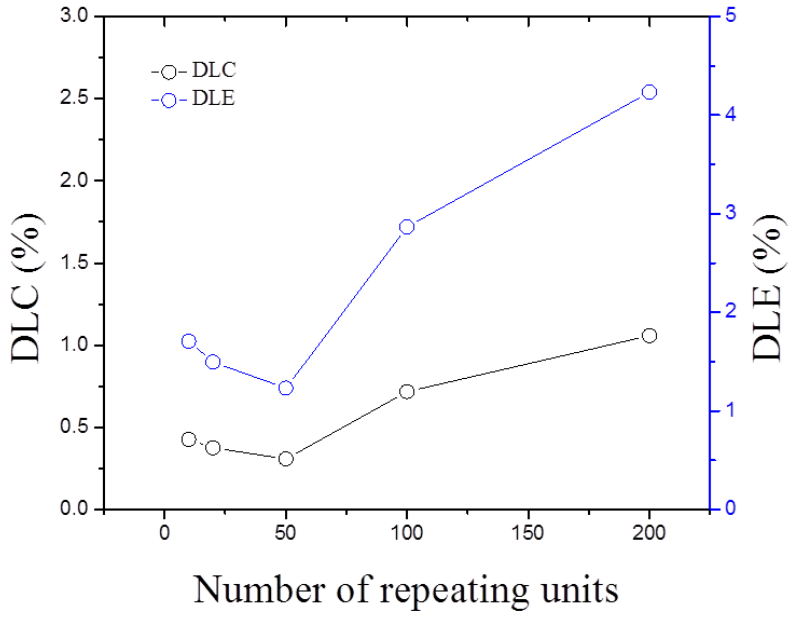



*Figure3.20. DLC and DLE for Doxorubicin loaded nanoparticles*

An immediate importance of loading doxorubicin into these diblock micelles is their applications in dual drug delivery. Combination therapy is another such field where multiple drugs are loaded for treatment of platinum resistant cancer cells. This provides a motive to load doxorubicin into pre-synthesized cisplatin polymer nanoparticle. We can infer from these experiments that the cisplatin stitched core-shell nanoparticle can be loaded with doxorubicin as well providing us with a platform for dual drug delivery to cancer cells.

The core-shell particles synthesized remain to be tested in cell lines. The cytotoxicity of polymer alone, cisplatin and cisplatin stitched core- shell particles to normal cells and cancer cells will be studied in the subsequent weeks. It is hypothesized that these particles will be better able to protect cisplatin from GSH toxification in the cancer cells aiding in better therapeutic efficacy of the drug. Following this, these cellular uptakes of these particles will also be checked by confocal microscopy. The data obtained from these experiments will be a part of thesis presentation.

## CONCLUSION

In conclusion, we have synthesized amphiphilic di-block copolymers from biocompatible monomers namely γ-substituted caprolactone and polyethylene glycols. The polymer, $PEG_{2000}$-b-$CPCL_x$, was synthesized by melt route ring opening polymerization of carboxylic substituted caprolactone by using polyethylene glycol monomethyl ether ($M_w$ 2000 g/mol) as the initiator. Various polymers were synthesized by varying the number of substituted caprolactone units while keeping the number of ethylene glycol units constant. These polymers were characterized by $^{1}$H-NMR, $^{13}$C-NMR and Gel Permeation Chromatography (GPC) techniques. The thermal properties of the polymer were also studied by carrying out Thermo gravimetric analysis and differential Scanning Calorimeter. The nascent polymers were stable up to 200 °C beyond which they undergo thermal decomposition. The DSC thermogram distinctly establish the semi-crystalline nature of $PEG_{2000}$-b-$CPCL_x$for x= 10, 20 and 50. The crystalline nature of the polymer is lost as the number of substituted caprolactone repeating units in the polymer increases. Consequently, the polymers $PEG_{2000}$-b-$CPCL_x$for x= 100 and 200 are amorphous in nature. Upon characterization of these polymers, the carboxylates in the polymer were stitched with the anti-cancer drug, cisplatin. The assembly of cisplatin stitched polymers was done by dynamic light scattering (DLS) and Field Emission Scanning Electron Microscopy (FESEM). The sizes of the particles are found to increase with the increase in number of caprolactone units. The DLS sizes of the polymer-drug conjugate are in good accordance with the sizes of particles obtained by FESEM images. Further, the amount of drug chemically conjugated to the polymers (DLC) and the efficiency with which the polymer takes up these particles (DLE) were estimated by thermo gravimetric analysis and by Orthophenylenediamine assay (OPD Assay). It was found that the drug loading capacity of the polymers increase with increase in number of caprolactone units. However, not a very significant increase in DLC is seen when moving from 100 to 200 repeating units. The particles were found to be stable in water. The in-vitro release studies of the particles were studied in PBS solution. Following this, these nanoparticles will be examined for the cytotoxicity to normal and cancer cells. A detailed study on the cellular uptake of the nanoparticles will also be carried out.

## REFERENCES


1. Uhrich, K. E.; Cannizzaro, S. M.; Langer, R. S.; Shakesheff, K. M. Polymeric Systems for Controlled Drug Release. *Chem. Rev.* **1999**, 99**,** 3181–3198.

2. Haag, R.; Kratz, F. Polymer therapeutics: Concepts and applications. *Angew. Chem. Int. Ed.* **2006**, 45, 1198–1215.

3. Piotrowska, U.; Sobczak, M. Enzymatic polymerization of cyclic monomers in ionic liquids as a prospective synthesis method for polyesters used in drug delivery systems. *Molecules.* **2015**, 20, 1–23.

4. Behera, B. K.; Mohapatra, R.; Sahoo, S. K. Novel Functionalized Polymers in Drug Delivery : A Brief Review. *J. Curr. Pharma Res.* **2014**, 4, 1201–1210.

5. Trollsås, M.; Lee, V. Y.; Mecerreyes, D.; Löwenhielm, P.; Möller, M.; Miller, R. D., James, L. Hedrick Hydrophilic aliphatic polyesters: design, synthesis, and ring-opening polymerization of functional cyclic esters. *Macromolecules.* **2000**, 33, 4619–4627.

6. Labet, M.; Thielemans, W. Synthesis of polycaprolactone: a review. *Chem. Soc. Rev.* **2009**, 38, 3484-3504.

7. Rainbolt, E. a.; Washington, K. E.; Biewer, M. C.; Stefan, M. C. Recent developments in micellar drug carriers featuring substituted poly(ε-caprolactone)s. *Polym. Chem.* **2015**, 6, 2369–2381.

8. Senevirathne, S. A.; Washington, K. E.; Biewer, M. C.; Stefan, M. C.; Washington, K. E. PEG based anti-cancer drug conjugated prodrug micelles for the delivery of anti-cancer agents. *J. Mater. Chem. B.* **2015**, 4, 360–370.

9. Surnar, B.; Jayakannan, M. Stimuli-responsive poly(caprolactone) vesicles for dual drug delivery under the gastrointestinal tract. *Biomacromolecules.* **2013**, 14, 4377–4387.

10. Taylor, M. J.; Tanna, S.; Sahota, T. In vivo study of a polymeric glucose-sensitive insulin delivery system using a rat model. *J. Pharm. Sci.* **2010**, 99, 4215–4227.

11. Klein, A. V.; Hambley, T. W. Platinum drug distribution in cancer cells and tumors. *Chem. Rev.* **2009**, 109, 4911–4920.

12. Florea, A.-M.; Büsselberg, D. Cisplatin as an anti-tumor drug: cellular mechanisms of activity, drug resistance and induced side effects. *Cancers (Basel).* **2011**, 3, 1351–71.

13. Oberoi, H. S.; Nukolova, N. V.; Kabanov, A. V.; Bronich, T. K. Nanocarriers for delivery of platinum anticancer drugs. *Adv. Drug Deliv. Rev.* **2013**, 65, 1667–1685.

14. Surnar, B.; Subash, P. P.; Jayakannan, M. Biodegradable Block Copolymer Scaffolds for Loading and Delivering Cisplatin Anticancer Drug. *Z. Anorg. Allg. Chem.* **2014**, 640, 1119–1126.

15. Surnar, B.; Sharma, K.; Jayakannan, M. Core–shell polymer nanoparticles for prevention of GSH drug detoxification and cisplatin delivery to breast cancer cells. *Nanoscale*, **2015**, 7, 17964–17979.