POLYCAPROLACTONES BASED AMPHIPHILIC DIBLOCK COPOLYMERS

Thesis submitted towards the partial fulfillment of

BS-MS dual degree program

By

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CERTIFICATE

This is to certify that this dissertation entitled **"POLYCAPROLACTONES BASED AMPHIPHILIC DIBLOCK COPOLYMERS"** towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents original research carried out by **"Maitreyee Mhatre at IISER, Pune"** under the supervision during the academic year 2014-2015.

Date: 25/3/2015

Place: Pune.

Signature of the Supervisor

25/3/2015

DECLARATION

I hereby declare that the matter embodied in the report entitled **"POLYCAPROLACTONES BASED AMPHIPHILIC DIBLOCKCOPOLYMERS "** 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: 25/3/2015

Place: Pune

Signature

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I thank all my lab mates Smita, Pramod, Anantharaj, Narasimha, Rajendra, Bhagyashree, Sonashree, Nilesh, Mehak and Thameez for making the memories special in this last one year. This place has left a lasting impression on me. This has been undoubtedly the best environment I have studied in and the above people were responsible for it.

Last but not the least, I owe my deep sense of gratitude to my family and friends as they have been very supportive throughout my life during the good and hard times.

-Maitreyee

CONTENTS

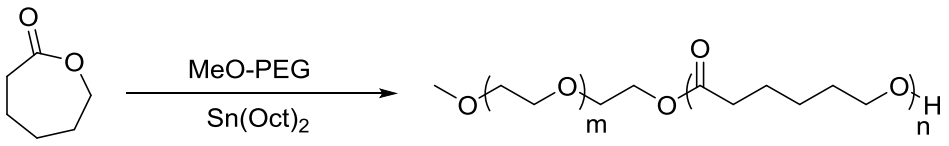
1. Abstract

In this thesis, we have successfully synthesized and characterized non-pegylated amphiphilic diblock copolymer based on caprolactone using "click chemistry". Poly (caprolactone) polymer is highly hydrophobic in nature. To maintain the balance of hydrophilicity and hydrophobicity in the polymer, new carboxylic substituted caprolactone monomer was synthesized. Ring opening polymerization (ROP) of new monomer carried out using propargyl alcohol as a imitator to produce alkyne end capped carboxylic substituted polycaprolactone (Prg-CPCL). On the other hand, ROP of caprolactone was performed using chloro ethoxy ethanol as initiator which gives chloro capped polycaprolactone (Cl-PCL). This chloro-capped PCL was converted to its azide capped PCL. Aforementioned alkyne and propargyl end capped polymers were covalently attached together to create diblock copolymers using click chemistry. All the intermediates of to produce monomer and all the above mentioned homo and diblock polymers were characterised by NMR, MALDI FT-IR and Gel permeation chromatography (GPC). The new diblock copolymer may be useful for drug delivery carrier for further pharmaceutical applications.

2. Introduction

Block copolymers are important classes of polymeric systems for both fundamental understanding of macromolecular self-assembly and also new applications in plastic industry and in biomedical devices. Block copolymers with appropriate hydrophilic and hydrophobic content were found to self-organize into micelles, vesicles and nano-particles for loading and delivering of anticancer drug molecules.[1,2] Various block copolymers such as polystyrene-*block*-methacrylic esters, polystyrene-*block*-mathacrylic acids were prepared by controlled radical polymerization (ATRP) or living anionic polymerization. In a indirect route, Individual hydrophilic and hydrophobic blocks were also connected through covalent bonds to make A-B, A-B-A, B-A-B and A-B-C blocks. Triblock copolymers such as PEG-PPG-PEG and PPG-PEG-PPG are very good examples for commercial available materials. Aliphatic polyesters based block copolymers were also produced by ring opening polymerization (ROP) of caprolactones or cycli-dilactides with suitable chain length PEG-alcohols as initiator.[3] In this process, PEG-*block*-PCL and PEG-*block*-PLA were made with precise length as well as hydrophilic/hydrophobic content. These diblock polymers were found to be degradable under intracellular environment to deliver the drug or genes.[4,5]

Polycaprolactone is one of the most studied polymers, for its biocompatible and bio degradable properties[6]. Many derivatives of this polymer were synthesized over years, to alter the properties of this polymer. Food and Drug Administration (FDA) has also approved the use of polycaprolactone as a drug delivery device. Polycaprolactone is synthesized by ROP of the caprolactone. Polycaprolactone is completely hydrophobic polymer, to balance the hydrophobicity for the desired self-assembly, caprolactone monomer can be substituted with hydrophilic functional groups or polycaprolactone can be copolymerised with hydrophilic molecules such as Polyethylene Glycol (PEG), as shown in scheme 1.



Scheme 1 : Synthesis of PEG-PCL diblock by ROP.

Problems associated with this route are (i) lack reactive sites, (ii) non stimuli responsiveness, and (iii) sometimes solubility. To tackle these difficulties, scientist came up with the idea of functionalization either of PCL back bone. Polycaprolactone properties can be varied by attaching different types of functional groups. The ring opening polymerisation can be initiated using desired functional group,[7] which becomes end group of polymer.[8] But the effect of this functional end group on entire polymer can be very less when compared to its molecular weight (Mn), therefore caprolactone monomer itself can be substituted with desired functional group and can be copolymerized with polycaprolactone.

There are few reports known on this concept: Functionalities like hydroxyl, alkyl, azide, and benzyl or α- cholesteryl groups grafted polycaprolactones were reported elsewhere. Lately, Stefan et.al. summarised all the functional caprolactone monomer which produces the functional polycaprolactone in one review (see fig 1). Most of them are again non-stimuli responsive drug delivery vehicles, so the existed problems not yet solved.[9]

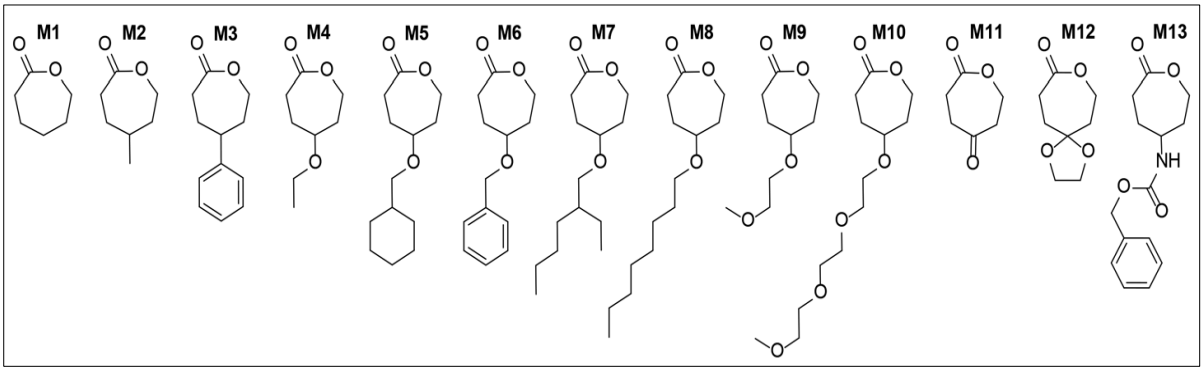


Figure 1 : Functional caprolactone monomer .

Recently, from our group, Bapurao et. al. established the route for synthesis of PEG-*b*-$CPCL_{100}$, the carboxylic polycaproctone with good yield.[10] The newly developed carboxylic acid substituted block copolymers were found to be pH responsive. These diblocks were found to be self-assembled as vesicular structures in water and PBS. Anticancer drugs camptothecin and anti-inflammatory drug Ibuprofen were loaded in these vesicular assemblies. The drug loaded vesicles were

very stable in the stomach (pH < 2.0) and selectively ruptured at pH = 7.4 to deliver the drugs to small intestine.

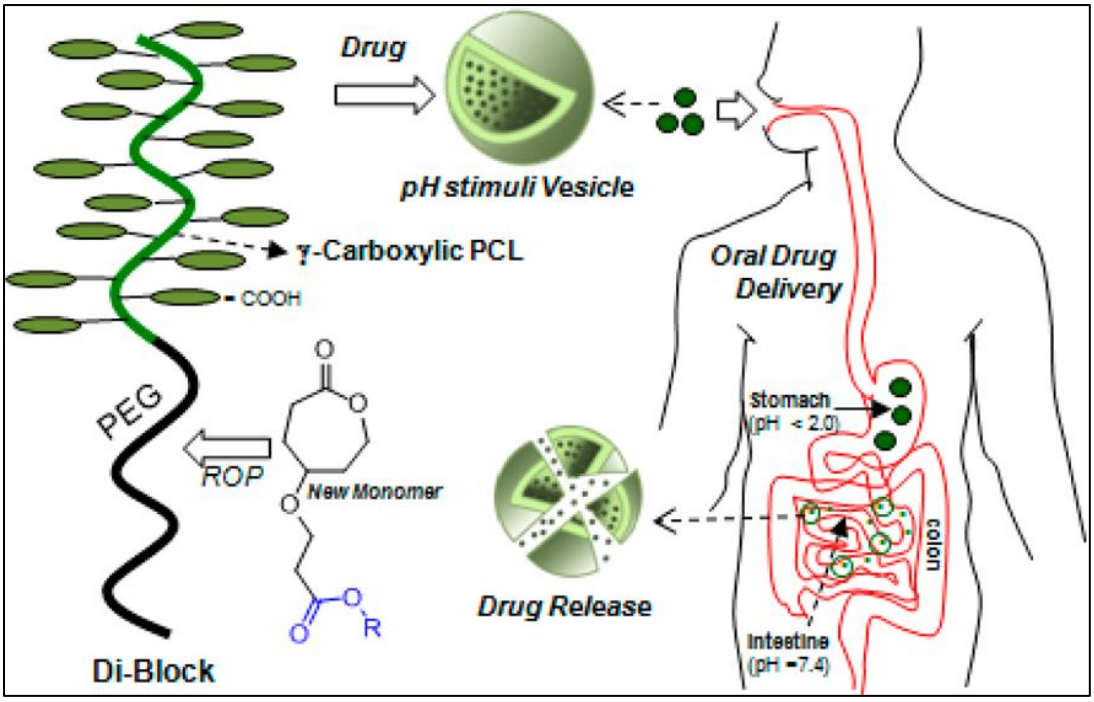


Figure 2 . Synthesis of PH responsive vesicles for oral drug delivery.

Literature of drug delivery describes that the PEGylation of any self-assembly protects it from attacks of various proteins and increases the half-life of nanoparticles in blood plasma. But, very recently the concept of "**Relieving of PEGylation**" emerged in drug delivery area.[11]This explains that PEG chain plays a tremendous role in transportation of drugs to targeted sites, but it also slower the process of delivery. Depending on this concept very few reports were published.[12]

In this work we have established chemistry, where we have prepared non-pegylated diblocks using click chemistry as robust tool , by synthesizing two different homopolymers i.e. PCL and BPCL and clicked them to form diblock copolymers. These diblock copolymers are highly hydrophobic, and further deprotection of butyl group provide the highly water soluble non pegylated diblocks. Copolymerisation of PCL with CPCL maintains the hydrophilic and hydrophobic balance for the desired self-assembly (see figure 3). Ring opening polymerization (ROP) of ε-caprolactone method is generally used for synthesis of polycaprolactone. Molecular weight can be controlled using ROP method, it gives low polydispersity index (PDI) and well-

defined structure of polymer can also be achieved. Through ROP method, it is possible to have desired end group on polymer and also copolymerization is possible using different monomers by varying initiators and catalyst. Functional end group of the polymer is from the initiator by which ROP is initiated; by varying this initiator, catalyst and termination reaction the functional groups of polycaprolactone can be varied.

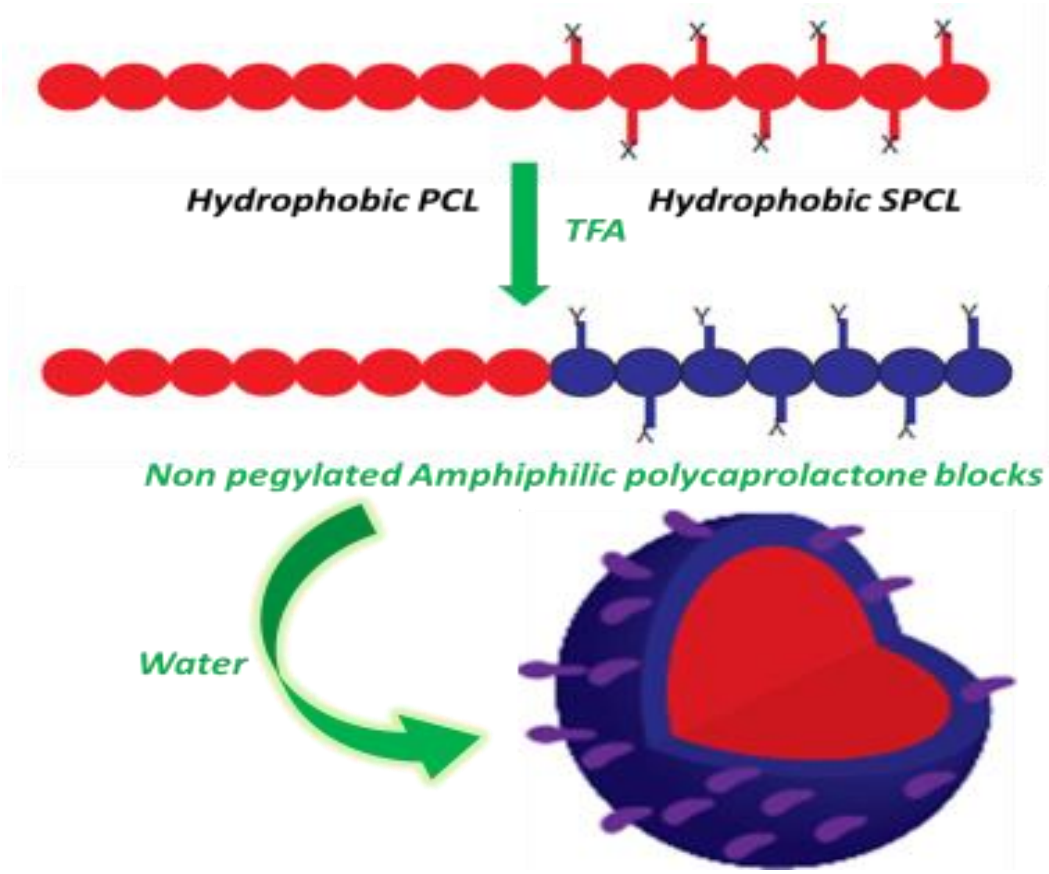


Figure 3: Synthesis and self-assembly of amphiphilic polycaprolactone block.

3. **Aim of the work:**

The aim of this project is to synthesize and characterise non-Pegylated diblock of hydrophilic and hydrophobic polycaprolactone by "Click Chemistry", as drug delivery vehicle , which is completely biocompatible and biodegradable and can form micelle or vesicular self-assembly in water. As we have discussed above, polycaprolactone synthesized by ring opening polymerization of caprolactone contains only hydrophobic group in the polymer backbone. Hence to acquire desired self-assembly we need to maintain hydrophilic and hydrophobic balance in polymer backbone. This can be done either by substituting caprolactone with hydrophilic functional group or by copolymerizing polycaprolactone by hydrophilic polymer *e.g*. Polyethylene Glycol (PEG). To achieve complete biodegradable diblock co polymers, we have synthesised carboxylic functionalised caprolactone monomer through multiple organic steps . This monomer was then polymerised under controlled ROP using propargyl alcohol as initiator to form carboxylic substituted polycaprolatone (CPCL) . On the other hand caprolactone was also polymerised using chloro ethoxy ethanol as initiator to form polycaprolactone (PCL) . After this diblock copolymer was synthesized using both carboxylic substituted poly(caprolactone) (CPCL) and polycaprolactone (PCL) through **"Click Chemistry" .**

3. MATERIALS AND METHODS

Materials :

1,4-Cyclohexane diol, potassium t-butoxide, t-butyl acrylate, pyridinium chlorochromate(PCC), metachloroperbenzoicacid (m-CPBA), molecular sieves ($4A^0$), tin(II) 2-ethylhexanoate (Sn(Oct)2), ε-caprolactone , 2-(2-Chloroethoxy)ethanol , sodium azide(NaN_3), potassium Iodide (KI), propargyl alcohol , copper bromide (CuBr), N,N,N′,N′,N″-pentamethyldiethylenetriamine (PMDETA) were purchased from sigma-Aldrich chemicals. Solvents like dichloromethane (DCM) ,tetrahydrofuran (THF) , trifluoroacedic acid (TFA) , were purchased locally and distilled and were kept under inert condition prior to use . THF was dried using sodium and benzophinone prior to click reaction.

Measurements :

^{1}H NMR was recorded using 400-MHz JEOL NMR spectrophotometer . All NMR spectra were recorded in CDCl3 containing TMS as internal standard . Mass of polymers were determined by using Applied Biosystems 4800 PLUS MALDI TOF/TOF analyzer . The polymer samples were dissolved in HPLC grade Tetrahydrofuran (THF) at 10mg/ml . Dihydroxy benzoic acid (DHB) was used as matrix. The matrix solution was prepared by dissolving 10mg in 1mL methanol (or 30% ACN). To aid sample ionization , the MALDI target was prespotted with 2mg/mL NaI in methanol and was allowed to air dry . A 1-2 µL aliquot of the polymer/matrix mixture was deposited on top of NaI and air dried .FT-IR spectra for was recorded using Thermo Scientific Nicolet 6700 FTIR spectrometer, potassium bromide(KBr) pallets were prepared for each sample from powdered dry KBr. Mass of small intermediate precursors was determined using HRMS . Gel permeation chromatographic (GPC) analysis was performed using Viscotek VE 1122 pump , Viscotek VE 3580 RI detector and Viscotek VE 3210 UV/Vis detector in tetrahydrofuran (THF) using polystyrene as standards .

Synthesis of Monomer (Carboxylic substituted caprolactone):

Synthesis of tert-butyl 3-((4-hydroxycyclohexyl)oxy) propionate (1) :

1,4 Cyclohexane diol (10 g , 86.20mmol) was dissolved in 150 mL THF under inert conditions at RT . To this , catalytic amount of potassium tert butoxide was added . Then tert-butyl acrylate (8.83 g , 68.96 mmol) was dissolved in 25 mL of THF and it was added slowly to the reaction mixture . The reaction was kept at 80^0c for 30

h refluxing under nitrogen atmosphere. After completion of the reaction THF was evaporated and product was dissolved in DCM. As reactant is in insoluble in DCM it precipitates and can be removed by filtration. The product was purified by column chromatography in ethyl acetate and hexane (25 % PE / EA). Yield = 43%. ^{1}H NMR (400 MHz , $CDCl_3$) δ ppm : 3.65 (m, 3H, O-CH_2-and O-CH), 3.39-3.25(m, 1H , HO-CH) , 2.43 (t, 2H , -CH_2CO-), 1.96-1.81 (m, 4H, OCH$(CH_2)_2$) , 1.64 and 1.32 (m, 4H, CO$(CH_2)_2$),1.45(s,9H,-C$(CH_3)_3$). FT-IR(cm^{-1}): 3420,2974,2936,2865,1725,1457,1395, 1367, 1254, 1150, 1108 and 1035

Synthesis of 4-((5, 5 - dimethyl - 3- oxohexyl) oxy) cyclohexanone (2): Compound 1 (7 g , 28.6 mmol) was dissolved in dry DCM under inert conditions and stirred under RT . Then PCC (9.27 g , 43.03 mmol) was added slowly followed by the addition of molecular sieves ($4A^0$) . the reaction was kept for 6 h at RT . After completion of reaction , reaction mixture was filtered , filtrate was evaporated under reduced pressure . Product was purified by column chromatography. (20% PE/EA) . Yield = 82.6 %. ^{1}H NMR (400 MHz , $CDCl_3$) δ ppm : 3.74 (m, 3H ,-OCH) , 2.57 (t, 2H, -CH_2-CO) , 2.50 (m, 4H, -CH_2-) , 2.26 (m, 2H , -CH_2-) , 2.24 (m, 2H, CH_2), 1.98 (m, 2H, -CH_2) and 1.44 (s, 9H , -C$(CH_3)_3$) . FT-IR (cm^{-1}) : 2970, 2870, 2362, 1715, 1455, 1420, 1390, 1363, 1300, 1250, 1215 and 1105 .

Synthesis of tert-butyl 3 ((7-oxooxepan-4-yl) oxy) propionate : Compound 2 (5.5 g , 22.72mmol) was dissolved in 60 mL DCM . Then sodium bicarbonate (3.82 g, 45.45 mmol) was added into the mixture followed by m-CPBA (7.83 g, 45.45 mmol). The reaction was kept under inert conditions at RT for 12 h. After completion of reaction the product was stirred into saturated mixture of NaS_2O_3 and $NaHCO_3$ in water and ethyl acetate. The product was soluble in ethyl acetate and was removed from water by separating funnel.The compound was purified using column chromatography (25% PE/EA) . Yield = 93.35 %.^{1}H NMR (400 MHz , $CDCl_3$) δ ppm : 4.46(dd ,1H, COOCH), 4.02 (dd, 1H, COOH), 3.66 (m, 4H, OCH_2, OCH, COCH), 2.95(t, 1H, COCH) , 2.46 (t, 2H, $COCH_2$), 2.03 1.79 (m, 4H, OCH-$(CH_2)_2$), 1.44 (s, 9H, -C$(CH_3)_3$) FT-IR (cm^{-1}) : 2920, 1720, 1450, 1390, 1360, 1250, 1150, 1100, 1050 HRMS (ESI+) : m/z [M+Na^+] calcd. for $C_{13}H_{22}O_4$ [M^+] :281.31; found :281.13.

Ring Opening polymerization (ROP) Caprolactone: Caprolactone was dried by azeotropic distillation from toluene under vacuum. For 50 ($[M_0]/[I_0]$ = 50) repeating

units of polycaprolactone , 2-(2-Chloroethoxy)ethanol (10.81 mg ,0.087mmol) was taken as initiator in clean , flame dried schlenk tube followed by Caprolactone (500mg , 4.38 mmol) and finally as catalyst stannus octoate ,$Sn(Oct)_2$ (5.41mg ,0.044mmol) was added in the schlenk tube . The mixture was kept at high vacuum for 30-40 min. to remove all the moisture. Then schlenk tube was kept in preheated oil bath at 110^0C for 24 hours with constant stirring. After completion of reaction the product was dissolved in minimum (~2ml) of distilled DCM and then was precipitated in MeOH for purification. Purification of polymer was done at least done twice to obtain highly purified polymer. Polymer precipitated as white solid. Characterisation was done by NMR and end group was confirmed by MALDI. By varying monomer to initiator ratio ($[M_0]/[I_0]$) different number of repeating units (n) were obtained. ^{1}H NMR (400 MHz , $CDCl_3$) for 50 repeating units δ ppm : 4.24 (t, 2H , $-CH_2$-OCO), 4.06 (t, 100H ,$-CH_2$-O) , 3.73(t, 4H, $-CH_2$-O),3.63(t , 2H, Cl-CH_2), 2.30-2.25 (t, 100H, OCO-CH_2), 1.64-1.50 (m, 200H,$-CH_2-CH_2$-) , 1.38 (m, 100H, $-CH_2$-) .

Synthesis of azide capped polycaprolactone: Polycaprolactone (500 mg, 0.396 mmol, n =25) was dissolved in DMF at RT. Then Sodium azide (134.5 mg, 1.98 mmol) was added into the reaction mixture followed by catalytic amount of KI was added in the mixture. The reaction was kept at 60^0c for 48 h under reflux and nitrogen atmosphere. After completion of reaction DMF was evaporated under high vacuum and the product was dissolved in minimum amount of DCM and then it was precipitated in MeOH and was filtered out. Characterisation was done by NMR and end group was confirmed by MALDI. ^{1}H NMR (400 MHz , $CDCl_3$) for 50 repeating units δ ppm : 4.24 (t, 2H , $-CH_2$-OCO), 4.06 (t, 100H ,$-CH_2$-O) , 3.73(t, 4H, $-CH_2$-O), 3.30(t , 2H, Cl-CH_2), 2.30-2.25 (t, 100H, OCO-CH_2), 1.64-1.50 (m, 200H,$-CH_2-CH_2$-) , 1.38 (m, 100H, $-CH_2$-) .

Synthesis of propargyl end capped substituted polycaprolactone: For 50 repeating units of substituted polycaprolactone, Propargyl alcohol (2.17 mg , 0.038mmol) ; was taken in clean, dry schlenk tube followed by substituted caprolactone compound 3. (500mg, 1.94 mmol) and finally stannous octate, $Sn(Oct)_2$ (7.85mg , 0.0194 mmol) was added . The mixture was kept at high vacuum for 30-40 min. to remove all the moisture. Then reaction was kept at 110^0C for 24 hours. After completion of reaction the product was dissolved in minimum (~2ml) of DCM and then was precipited in Hexane. Purification of polymer was done at least twice to

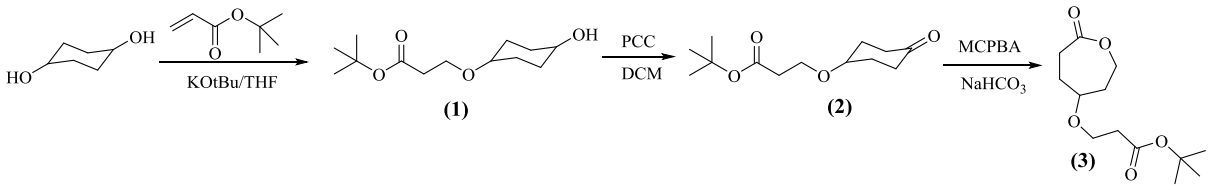
obtain highly purified polymer. Characterisation was done by NMR and end group was confirmed by MALDI. By varying monomer to initiator ratio (M_0 /I_0) different numbers of repeating units (n) were obtained. ^{1}H NMR (400 MHz , $CDCl_3$) for 50 repeating units δ ppm : 4.68 (m, 2H , C_2H-CH_2-OCO), 4.14(s, 100H, -OCH_2-) , 3.65 (s , 100H, -CH_2-O) , 3.44 (s, 50H, $(CH_2)_2$-CH-O) , 2.55-2.37(m , 200H , -CH_2-CO) , 1.86-1.78 (m, 200H , -CH_2-) , (s , 450H, $OC(CH_3)_3$).

Synthesis of diblock through "Click chemistry":

For synthesizing diblock of 50 repeating units azide capped polycaprolactone (N_3-PCL_{50}) and 50 repeating units of propargyl alcohol initiated carboxylic substituted polycaprolactone (Prg-$CPCL_{50}$) , azide capped polycaprolactone (N_3-PCL_{50}) (200mg , 0.034mmol) and propargyl alcohol initiated carboxylic substituted polycaprolactone (Prg-$CPCL_{50}$) (479.33 mg ,0.037mmol) were weighed in clean flame dried schlenk tube . Then tube was sealed using rubber septa . Both the polymers were dissolved in 6mL of Dry THF. After dissolving the polymers additional 4ml of Dry THF was added to the schlenk tube. The mixture was then deoxygenated using one freeze-pump-thaw cycle followed by addition of PMDETA (6.41mg, 7.72µL, 0.037mmol). Two freeze-pump-thaw cycles were then performed and then Copper Bromide (CuBr) (5.30mg, 0.037mmol) added in presence of nitrogen. Again two cycles of freeze-pump-thaw were performed. The reaction was kept at 60^0C for 72h. Diblock polymers like N_3-PCL_{50}-Prg-$CPCL_{20}$, N_3-PCL_{50}-Prg-$CPCL_{75,}$ $_{N3}$-PCL_{25}-Prg-$CPCL_{50}$ were synthesized using same procedure. All the final compounds were then passed through neutral alumina twice to remove copper. The products were washed thrice with Ether to remove the reactants and to get a pure compound. Characterization was done using NMR, FT-IR and Gel permeation chromatography (GPC).

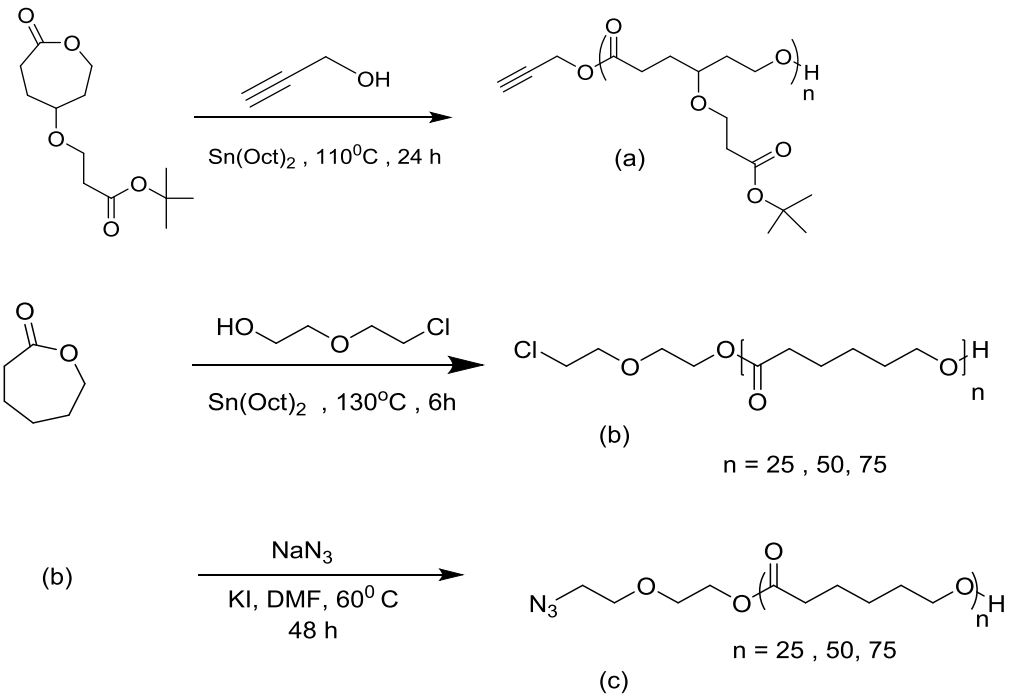
5 . Results and Discussion.

Synthesis of carboxylic substituted monomer is described in the experimental section. This monomer was synthesized using multiple organic steps by using commercially available compounds. The monomer was prepared starting from 1,4-Cyclohexanediol through multi-step synthesis to produce compound 1 in high yield. Then to convert the hydroxyl group into cyclohexanone, the compound 1 was oxidized with PCC forming compound 2. The compound 2 was then converted into final carboxylic substituted caprolactone monomer (compound 3) through veliger oxidation. this reaction proceeds in high yield .synthesis is showed in scheme 1.



Scheme-1 : synthesis of carboxylic substituted caprolactone monomer .

Synthesis of chloride capped caprolactone was done using ROP of Caprolactone. The ROP was initiated using Chloro ethoxy ethanol as initiator and $Sn(Oct)_2$ as catalyst. Mole ratio of $Sn(Oct)_2$ and chloro ethoxy ethanol were kept as 1:2. This procedure allowed to generate the Sn-OR for the ROP initiation. Monomer to initiator ratio was varied $[M_0]/[I_0]$ = 25, 50, 75 to produce 25, 50, 75 repeating units of polymer All the chloride capped poly(caprolactone) polymers were then converted in azide using NaN_3 , in order to perform click reaction between PCL and CPCL polymer. KI was used catalyst and DMF was used as solvent for all the reactions (See scheme 2).Thus, a series of azide capped polycaprolactone (N_3-PCL_x) were produced , where x is repeating units of caprolactone . As chloride is more electronegative than azide, there is a clear shift in NMR peak (d) from 3.63 to 3.30 corresponding to CH_2 proton near to chloride azide .(See fig 4)



scheme-2: Synthesis of Cl-PCL through ROP and Chloro to azide end group conversion. Synthesis of Prg-CPCL.

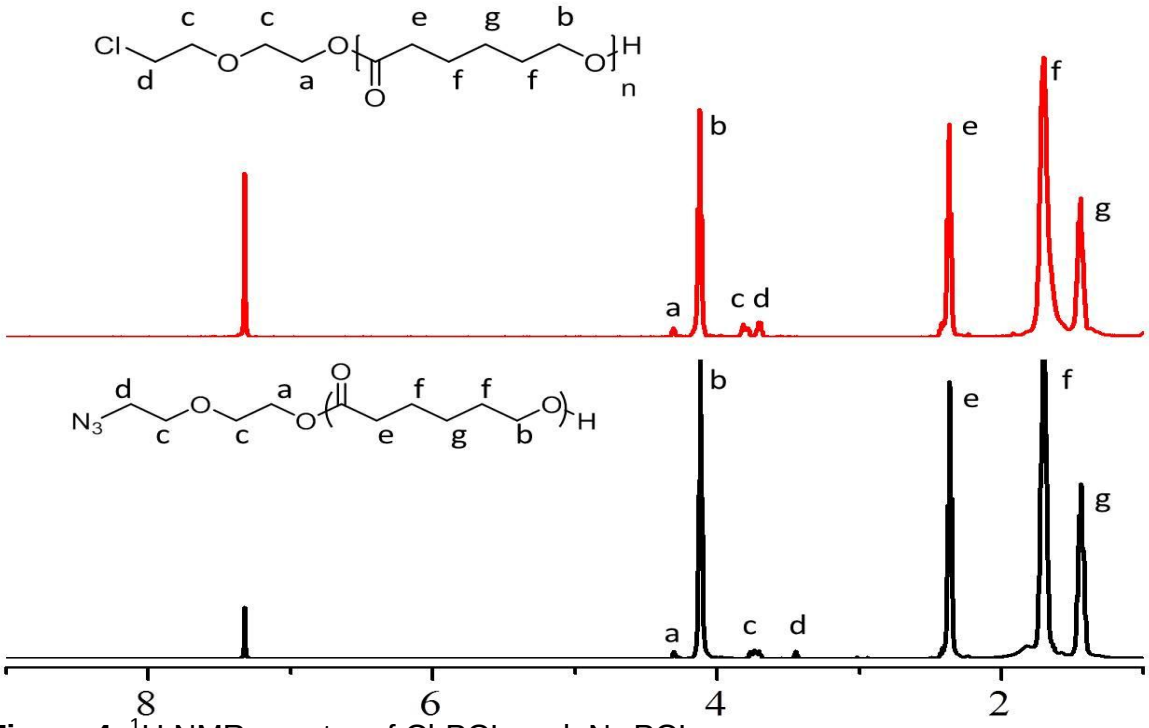


Figure 4 :^{1}H NMR spectra of Cl-PCL and N_3-PCL

A MALDI spectrum is a powerful tool for the determination of the end groups of polymers. From the MALDI Spectra shown in fig 5 , we can say that conversion of chloride end group of PCL to azide end group is successfully done , as the peak (*e.g.* n_{10}) for azide is shifted towards higher mass when compared to peak (n_{10}) of chloride capped polymers .

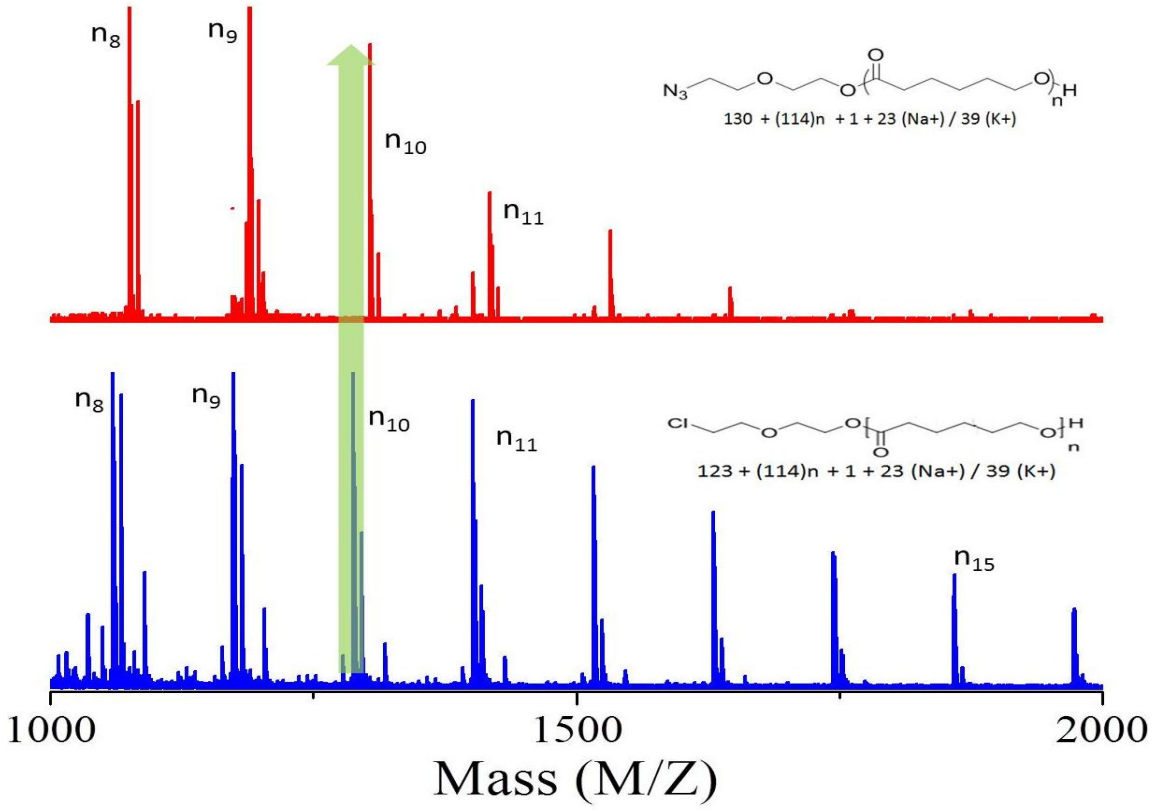


Figure 5 :MALDI spectra of Cl-PCL and N_3-PCL

The ^1H spectra for all polymers of N_3-PCL series are shown in figure 6, where quantification of caprolactone repeating units in N_3-PCL polymers were done by comparing the -C**H$_2$**OCO- proton in the Chloro ethoxy ethanol (initiator) to one of the proton (*e.g.*-CH_2O) in polymer. When intensity of -C**H$_2$**OCO- proton peak at 4.55ppm was kept constant , the intensity of proton (*e.g.* -CH_2O) peaks corresponding to polymer increases with increasing number of repeating unit of caprolactone .

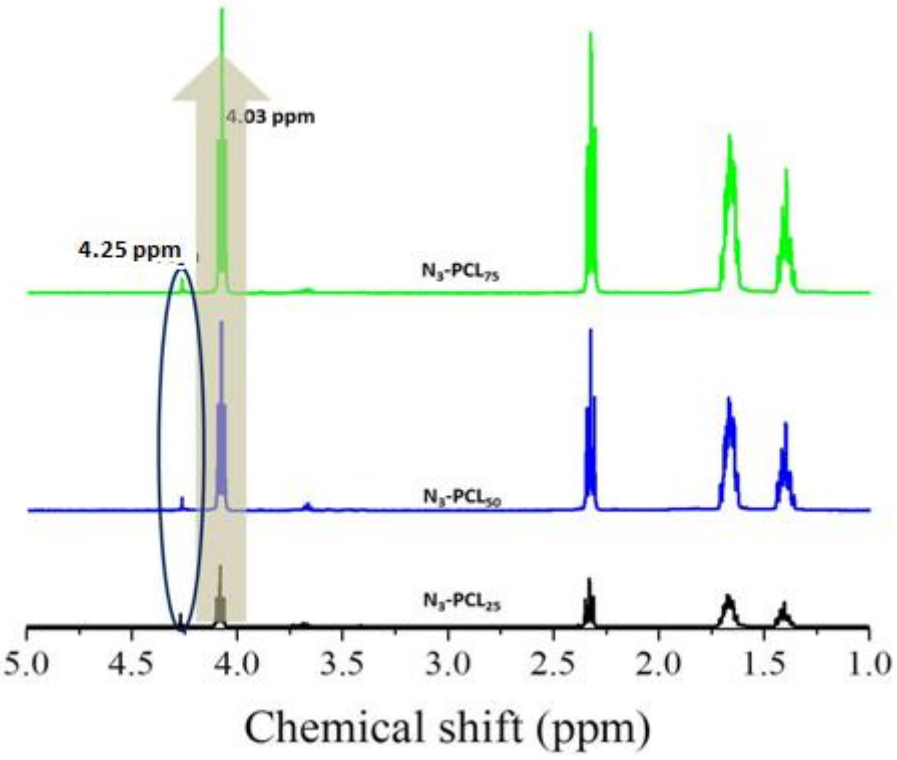
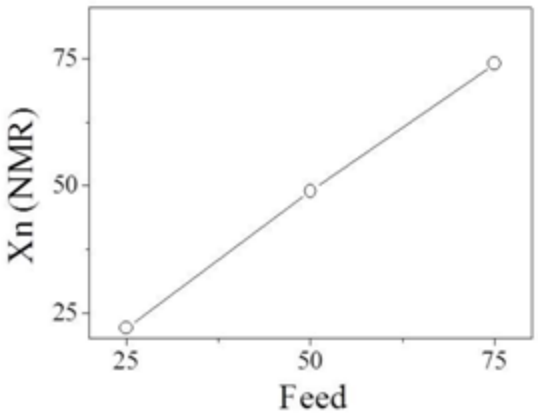


Figure 6 : 1H NMR for N3-PCL series .

The plot of feed vs substituted repeating units(from NMR) is shown in figure 7 . The linear plot shows that molecular weight and repeating units of the polymers is controlled.



Feed	Repeating units (NMR)
25	21
50	49
75	74

Table 1: Values repeating of feed and from NMR for N3-PCL series

Figure 7: plot of feed vs. substituting repeating units (NMR).

The molecular weight of the polymers was determined by GPC (Gel permeation chromatography) using polystyrene standard in THF as eluent. From GPC chromatograms all N_3-PCL series polymer (N_3-PCL_x ; x = 25, 50, 75) showed monomodal distribution and confirmed that formation of high molecular weight polymers (see figure 8).

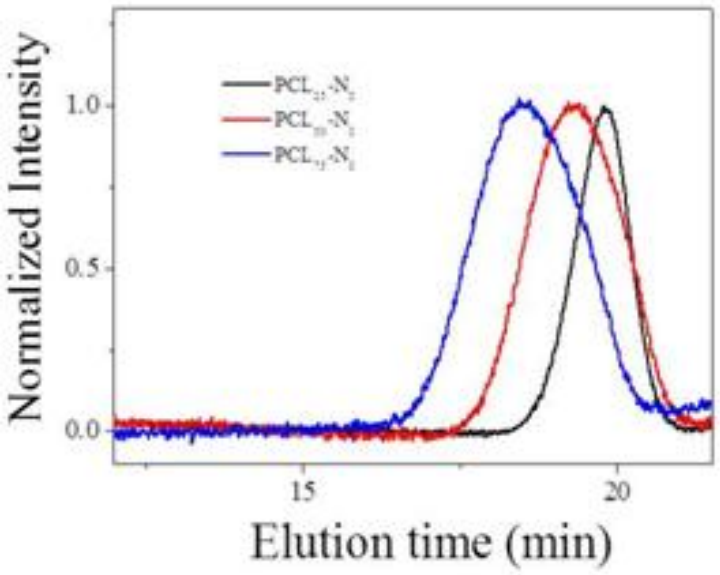


Figure 8 : GPC chromatogram for N3-PCL polymer series .

Propargyl capped carboxylic substituted polycaprolactone (Prg-CPCL):

Synthesis of chloride capped caprolactone was done using ROP of carboxylic substituted caprolactone monomer **(3)**. The ROP was initiated using propargyl alcohol as initiator and $Sn(Oct)_2$ as catalyst (see scheme 3). Mole ratio of $Sn(Oct)_2$ and propargyl alcohol were kept as 1:2 for the ROP initiation. Monomer to initiator ratio was varied $[M_0]/[I_0]$ = 25, 50, 75 to produce 25, 50, 75 repeating units of polymer ^{1}HNMR of Propargyl capped carboxylic substituted polycaprolactone (Prg-CPCL) series is shown in figure 9 .

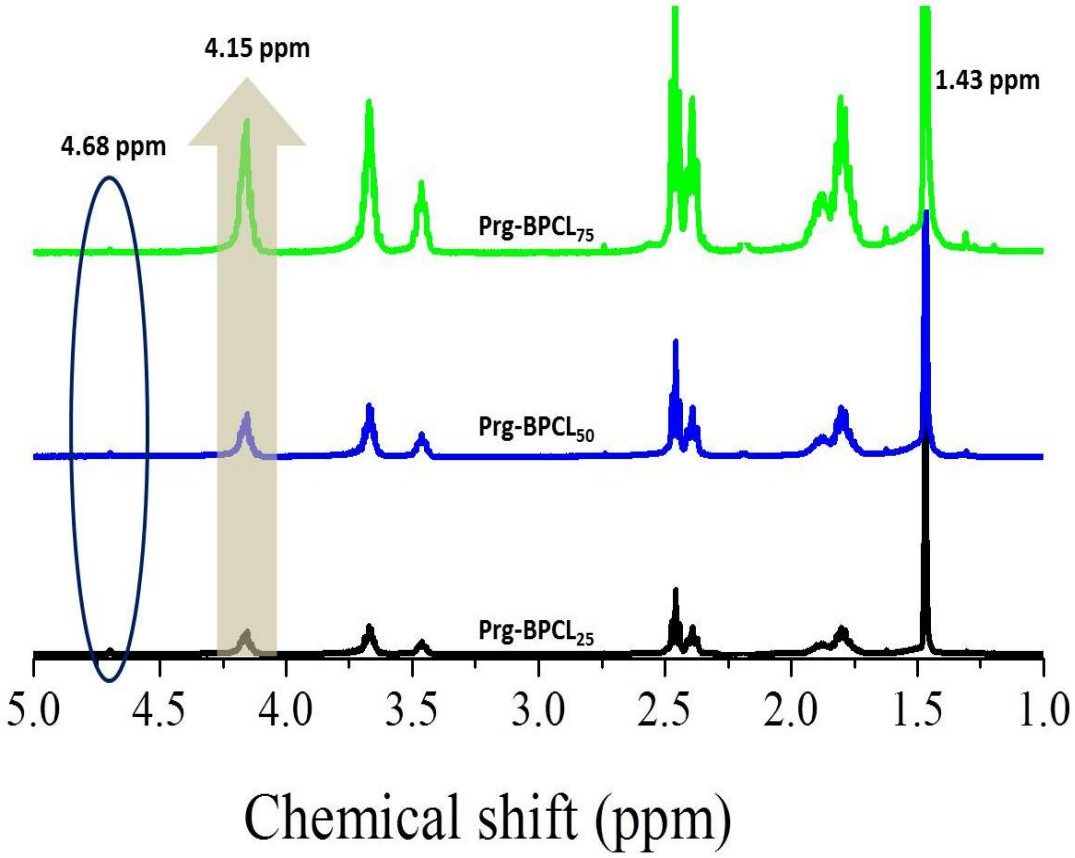
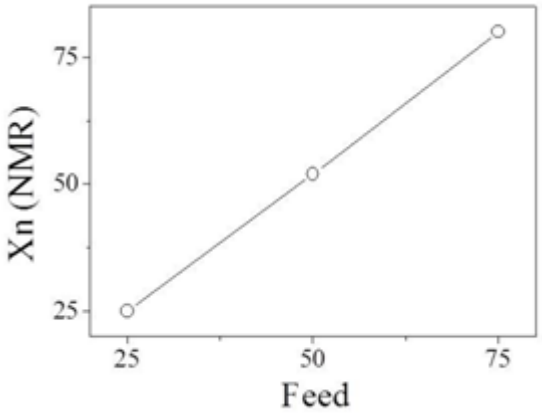


Figure 9 : 1H NMR of Prg-CPCL Series

The ^{1}H spectra for all polymers of Prg-CPCL series are shown in figure 9, where quantification of carboxylic substituted caprolactone monomer**(3)** repeating units in Prg-CPCL polymers were done by comparing the -C-CH_2-O proton in the propargyl alcohol (initiator) to one of the proton (*e.g.*O-CH_2-) in polymer . When intensity of -C-CH_2-O proton peak at 4.68 ppm was kept constant , the intensity of proton (*e.g.* -O-CH_2-) peaks corresponding to polymer increases with increasing number of repeating unit of caprolactone . The plot of feed vs substituted repeating units (NMR) is shown in figure 10. The linear plot shows that molecular weight and repeating units of the polymers is controlled.



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Feed	Repeating units (NMR)
25	25
50	50
75	80

Table 2 : Values repeating of feed and from NMR for Prg-CPCL Series

Figure 10: plot of feed vs substituting repeating units (NMR).

The molecular weight of the polymers was determined by GPC (Gel permeation chromatography) in THF as eluent. From GPC chromatograms all N_3-PCL series polymer (N_3-PCL_x ; x = 25, 50, 75) showed monomodal distribution and confirmed that formation of high molecular weight polymers (see figure 11). Molecular weights(Mn) calculated by NMR and GPC and Polydispersity index (PDI) for both the series(N3-PCL and Prg-CPCL) are shown in Table 3.

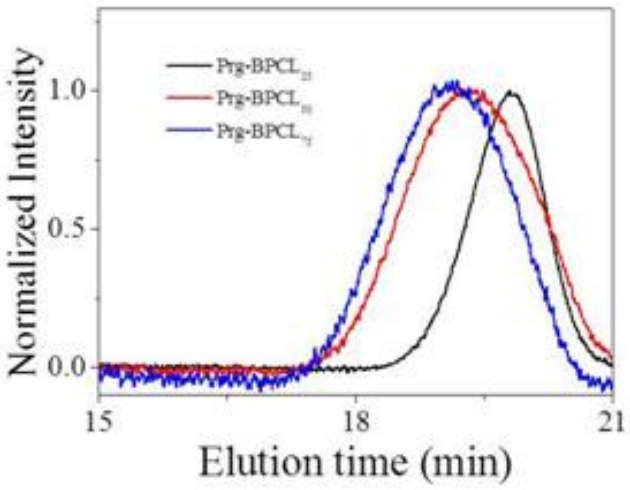


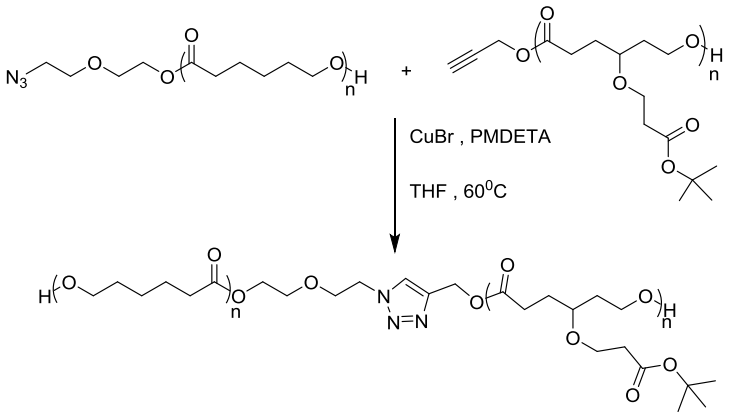
Figure 11 : GPC chromatogram for Prg-CPCL polymer series .

Sample	Feed (M/I)	n(NMR)	Mn (GPC)	Mn (NMR)	M_w (GPC)	M_w (GPC)
CCL_{25}-Prg	25	25	3,000	6,505	4,000	1.55
CCL_{50}- Prg	50	52	3,400	13,471	4,300	1.25
CCL_{75}-Prg	75	80	3,600	20,695	4,700	1.3
PCL_{25}-N_3	25	21	8,100	2,524	10,200	1.26
PCL_{50}- N_3	50	56	10,200	6,514	17,200	1.68
PCL_{75}-N_3	75	74	14,000	8,566	21,900	1.56
PCL_{100}-N_3	100	105	22,100	12,100	37,100	1.67

Table 3 : Molecular weights (Mn) and polydispersity index (PDI) by NMR, GPC.

Characterization of Diblock copolymer synthesized by "Click Chemistry":

Final diblock copolymer is synthesized using "click chemistry" is purely based on caprolactone. Two homopolymers1, N_3-PCL having azide as end group and 2. Prg-CPCL having propargyl as end group were synthesized by ROP using $Sn(Oct)_2$ as catalyst . Upon clicking of these polymers, azide and propargyl alcohol end of the two polymers comes together to form aromatic triazole ring and the diblock copolymer is synthesized (See scheme-4) .



Scheme 4 : "Click Chemistry" of two homopolymers (N3-PCL and Prg-CPCL) .

Here, we have successfully synthesized PCL_{50}-$CPCL_{25}$ and PCL_{25}-$CPCL_{50}$.

The most challenging and difficult problem was to optimise the conditions for the "Click Chemistry", which took most of the time for this project . Recently, this problem was solved and "Click Chemistry" was optimised for other polymers.

The key is, copper (I) should be at oxidation state (I) and it should not be converted to copper (II), for the entire duration of the reaction (72h). If copper (I) is getting converted to copper (II), the colour of reaction will change from bottle green to electric blue and reaction will not proceed. To achieve this , reaction was performed in clean , flame dried schlenk tube which was properly sealed using rubber septa , THF was dried using sodium and benzophenone prior to reaction and all the reactant were added in presence of nitrogen . Also, reacting groups are the end groups of the polymer which make it further hard to proceed the reaction, hence mixture should be dilute for the end groups to react. To give more energy for the formation of triazole from azide, reaction was heated at 60^0 C. Copper was removed by passing the product through neutral alumina. The formation of diblock was confirmed by appearance of triazol proton (1H) peak at aromatic region in the NMR. Unfortunately, peaks of both the polymers are overlapping so we cannot integrate the values. But the positions of peaks for both the polymers are appearing on the same position as they were in homopolymer .

^{1}H NMR 400Hz spectra was recorded for the diblock (see figure 12). The aromatic peak of proton a at δ 7.58 shows the formation of azide linkage between two polymers. Although most of the peaks of PCL and CPCL are overlapping we can see there is a peak at specific δ ppm value for each proton of both the polymers. Unfortunately it is impossible to integrate number of protons for the both polymers.

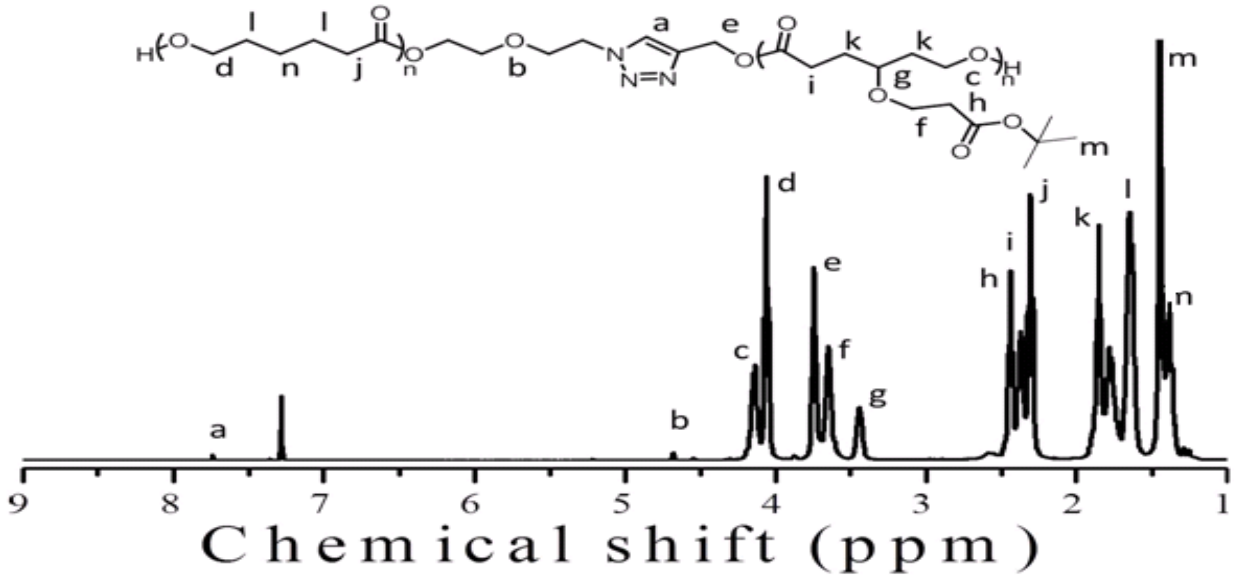


Figure-12: 1H NMR of PCL-CPCL diblock copolymer

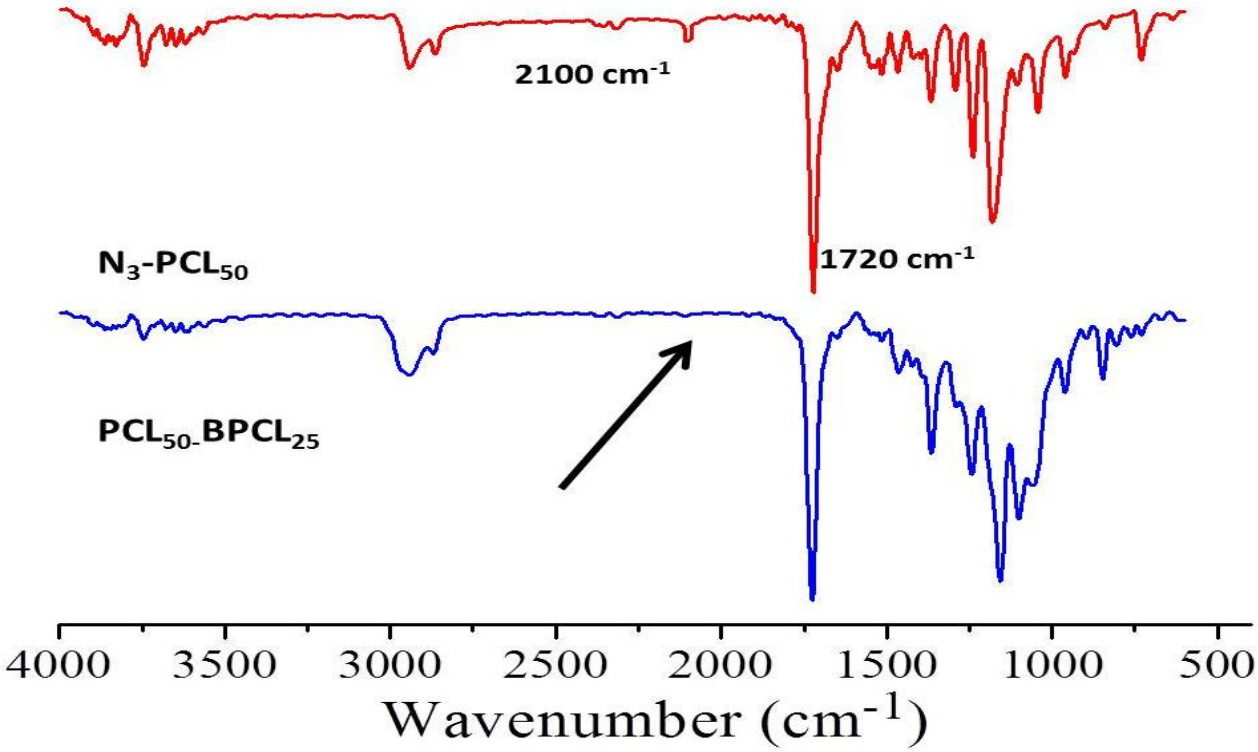


Figure 13 : FT-IR spectra of N3-PCL and PCL-CPCL diblock copolymer.

FT-IR is a powerful technique to verify the fictional groups. Potassium bromide (KBr) pallets were prepared for each sample from powdered dry KBr. In the FT-IR spectra of N_3-PCL the azide peak at 2100cm^{-1} stretching frequency can be seen very clearly. This peak's intensity is very less as azide (N_3) functional group is the end group of the polymer. Whereas, the intensity peak at stretching frequency of C=O at 1720 cm-1 is very high as this functional group is at monomer. (See figure 13).

Characterization of Diblock copolymer using GPC:

Here, two GPC chromatograms have shown for of diblock copolymer PCL_{50}-$CPCL_{20}$ and PCL_{25}-$CPCL_{50,}$. Peak of the diblock seems to be significantly shifted compared to N_3-PCL and Prg-CPCL polymer (See figure14) . Which means that molecular weight of the product has drastically increased by the click reaction and two polymer are clicked together successfully. Monomodal distribution of the peaks shows that the diblock is pure and has no reactant left after washing with ether.

Molecular weight for both N_3-PCL and Prg-CPCL homopolymers and diblock

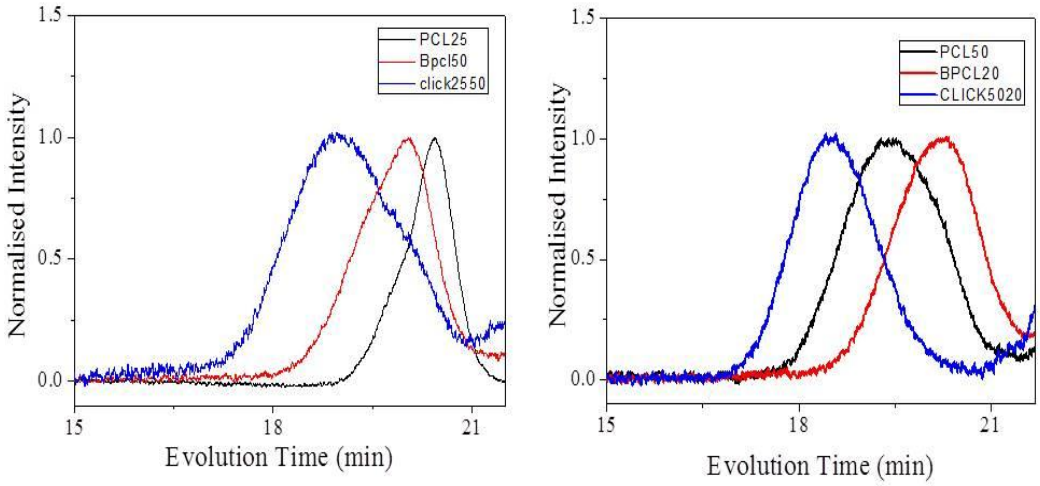


Figure-14 : of PCL_{25}-$CPCL_{50}$, PCL_{50}-$CPCL_{20}$

Sample	Feed (M/I)	M_n (GPC)	M_w (GPC)	M_w /M_n
PCL_{25}-N_3	**25**	**4,300**	**6,200**	**1.30**
CCL_{50}-Prg	**50**	**7,600**	**9,000**	**1.56**
Click	**1:1.1**	**12,500**	**20,600**	**1.20**
PCL_{50}-N_3	**50**	**5,600**	**7,300**	**1.20**
CCL_{20}-Prg	**20**	**9,200**	**12,000**	**1.25**
Click	**1:1.1**	**23,000**	**30,000**	**1.50**

Table 4: Molecular weights of homo and diblock polymers determined using GPC.

Aforementioned results clearly indicate that we are able to produce non-pegylated polymers, which are purely made by polycaprolactone blocks. These polymers are highly hydrophobic in nature as their components are caprolactone and carboxylic substituted caprolactone. To achieve the amphiphilicity in these polymer t-Bu groups has to be deprotected using TFA (trifluoro acetic acid). This gives water soluble polymers which can self-assemble to form micelles, where hydrophobic drugs can be loaded and delivered to the tumour tissues.

6. Conclusion.

In this project, we have successfully synthesized and characterized diblock copolymer which is purely based on caprolactone for the first time, which is not available in the literature. Carboxylic functionalized caprolactone monomer was designed from commercial starting materials such as 1,4-cyclohexane diol and polymerized under ROP. Characterization of this monomer was done using NMR, FT-IR and HRMS. Polycaprolactone (PCL) was produced by ROP of caprolactone by using chloro ethoxy ethanol as initiator and $Sn(Oct)_2$ as catalyst . The chloro end group was then converted into azide by using sodium azide (NaN_3) and potassium iodide (KI) as catalyst, resulting homopolymer N_3-PCL. Carboxylic substituted polycaprolactone (CPCL) was produced by ROP of carboxylic functionalized caprolactone monomer using propargyl alcohol as initiator and $Sn(Oct)_2$ as catalyst , resulting homopolymer Prg-CPCL. Synthesis of diblock copolymer was achieved by the click chemistry of both N_3-PCL Prg-CPCL homopolymer, using copper bromide (CuBr) as catalyst and PMDETA as ligand.

7. Future Work

- To achieve the hydrophilicity in diblock copolymers we have to deprotect the t-Bu groups to their carboxylic analogues.
- We have to study the self-assembly of this diblock copolymer in water.
- We also have to study drug encapsulation and release capacity of this self-assembly in Simulated Gastrointestinal Fluid (SGF) and Simulated Intestinal Fluid(SIF).

8. **References:**

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