Hierarchical self-assembled nanostructures of lactones derived thiobarbiturate homopolymers for the stimuli-responsive delivery applications

Piyali Mandal,[a] Diptendu Patra[a] and Raja Shunmugam*[a]

[*]Polymer Research Centre, Centre for Advanced Functional Materials,
Department of Chemical Sciences,
Indian Institute of Science Education and Research Kolkata.
Mohanpur-741246, Nadia, West Bengal, India
*[*]E-mail: **sraja@iiserkol.ac.in***

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Synthesis of Mono 1:

N-phenylmaleimide (5 gm) was taken in a double-necked round bottom flask and dissolved in dry DCM (10 ml) under nitrogen atmosphere. Then DEOM (5 ml) was added gradually into the solution. After that, the round-bottomed flask was kept in an ice bath to maintain the temperature between 0-5 ˚C. Then $SnCl_4$ (4.5 ml) was added dropwise to the mixture of the solution with continuous stirring. Then the reaction mixture was allowed to stir at room temperature for 24 h. Product was collected in the ethyl acetate layer and purified through column chromatography at 25% ethyl acetate/hexane mixture. The yield was 70%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.19(t, 6H), 4.18(q, 4H), 7.18(s, 1H), 7.32(d, 4H), 7.85(d, 2H); ^{13}C (500 MHz, DMSO-d_6): δ; MS (ESI) calculated for $C_{17}H_{17}NO_7$ (M^++Na) 370.01, observed 369.81 [Fig. S1(a)].

Synthesis of PCL-PCAP:

A known amount of caprolactone (1 gm) was weighed into a separate Schlenk flask, placed under nitrogen atmosphere, and dissolved in anhydrous THF (3 ml). DPP (20 mg) and mercaptoethanol (6 µl) were added to the reaction mixture as a catalyst and initiator respectively. The reaction was allowed to stir at room temperature for 24 h and it was quenched. White polymer was collected by precipitating in cold hexane. The yield was 67%. Advanced polymer chromatography was done in THF to determine the molecular weight. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.99(t, 4H), δ 2.23(t, 4H), δ 1.57(m, 6H), δ 1.31(t, 1H).

Synthesis of MAL-PCL-PCAP:

Mono 1 (0.1 gm) was taken in a round-bottomed flask under a nitrogen atmosphere and it was dissolved in dry THF (3 ml), then triethylamine (1 ml) was added to the solution and stirred for

30 minutes. After that **PCL-PCAP** was added to the mixture of solution and then it was allowed to stir at 50 ºC for 24 h. Product was collected by precipitating in cold hexane. The yield was 63%. Advanced polymer chromatography was done in THF to obtain the molecular weight. ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.81(d, 2H), δ 7.31(d, 2H), δ 7.15(s, 1H), δ 4.23(q, 4H; d, 2H), δ 4.01(t, 4H), δ 2.35(t, 2H), δ 1.67(t, 4H), δ 1.17(q, 6H; t, 5H)

Synthesis of TBA-PCL-PCAP:

50 mg of **MAL-PCL-PCAP** was dissolved in 5 ml of dry THF and freshly prepared sodium methoxide (50 mg) was dissolved in 20 ml dry methanol. Thiourea (50 mg) was added to the reaction mixture. The reaction mixture was stirred for 24 h at room temperature. The product was isolated by precipitating using excess THF. The yield was 60%. Advanced polymer chromatography was done in water. The molecular weight of the polymer was measured using dextran as standards. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.23(s, 2H), δ 7.80(d, 2H), δ 7.29(d, 2H), δ 7.15(s, 1H), δ 4.24(q, 4H; d, 2H), δ 4.0(t, 4H), δ 2.35(t, 2H), δ 1.66(t, 4H), δ 1.21(q, 6H; t, 5H).

Synthesis of PLA-PCAP, MAL-PLA-PCAP and TBA-PLA-PCAP:

PLA-PCAP, **MAL-PLA-PCAP** and **TBA-PLA-PCAP** were synthesized following the above procedures using lactide as monomer instead of caprolactone. **PLA-PCAP**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.0(m, 4H), δ 1.83(d, 6H), δ 1.76(m, 2H), δ 1.73(t, 1H). **MAL-PLA-PCAP**: ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.85(d, 2H), δ 7.37(d, 2H), δ 7.19(s, 1H), δ 5.51(m, 4H), δ 4.19(q, 4H; d, 2H), δ 1.52(d, 6H), δ 1.17(t, 3H), δ 1.15(t, 6H). **TBA-PLA-PCAP**: ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.25(s, 2H), δ 7.85(d, 2H), δ 7.37(d, 2H), δ 7.19(s, 1H), δ 5.51(m, 4H), δ 4.19(q, 4H; d, 2H), δ 1.52(d, 6H), δ 1.17(t, 3H), δ 1.15(t, 6H).

FT-IR spectroscopy:

FT-IR spectroscopy between **PCL-PCAP**, **MAL- PCL-PCAP** and **PLA-PCAP**, **MAL-PLA-PCAP** were done to confirm the click reaction by observing the –SH stretching frequency in both the cases. KBR technique was adopted for this experiment.

Self-assembly study:

Critical Aggregation Concentration (CAC) studies were done to investigate the self-assembly behaviour of both the polymers (**TBA- PCL-PCAP** and **TBA-PLA-PCAP**). From DLS we got to know about the size of the aggregates. TEM and cryo-TEM studies were done to investigate the morphology of the aggregates. In each case, polymers were dissolved in water and water solutions were taken for further experiment. For CAC, Nile red dye was used as a hydrophobic probe.

Dye encapsulation study:

Here, Nile red dye was taken into consideration and the solution was made in DCM with the concentration of 1 mg/ml. Three different concentrations (0.3 mg/ml, 0.5 mg/ml and 1 mg/ml) of both the polymers were prepared and 1 ml dye solution was added in each set of solutions and fluorescence intensities of DCM layer were measured before and after sonication.

Dye release study:

It was done in a water-octanol system. Dye loaded micelles were taken and added 1 ml of octanol to the solution and recorded fluorescence intensity with an interval of 45 minutes of the octanol layered solution.

Drug encapsulation study:

1 mg of Doxorubicin drug was taken and it was made neutral using excess triethylamine in DMSO solution. Polymeric micelles were prepared by dissolving the polymer into water (1 mg/ml). Encapsulation was done using dialysis method for 24 h with the micellar solution and neutral doxorubicin. Then the encapsulated micelles were lyophilized and dried.

Drug release study:

Drug release study was done using the doxorubicin-loaded micelles for both the polymers (**TBA-PCL-PCAP** and **TBA-PLA-PCAP**) following the same procedure in pH 5.5 solution with HCL-water and 7.4 with PBS buffer solution.

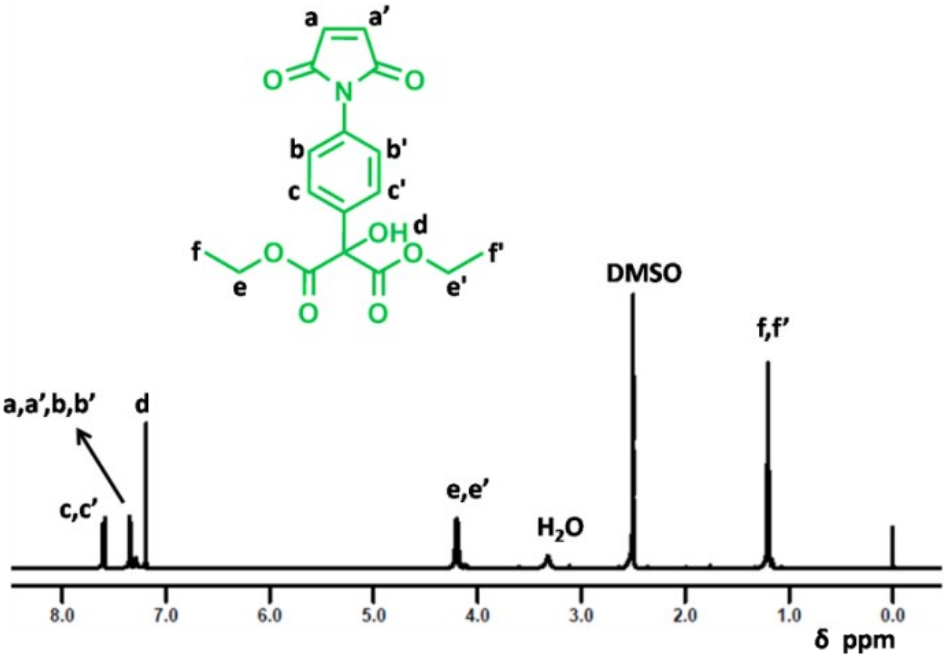

Fig. S1: ^{1}H NMR spectrum of Mono 1 in DMSO-d_6

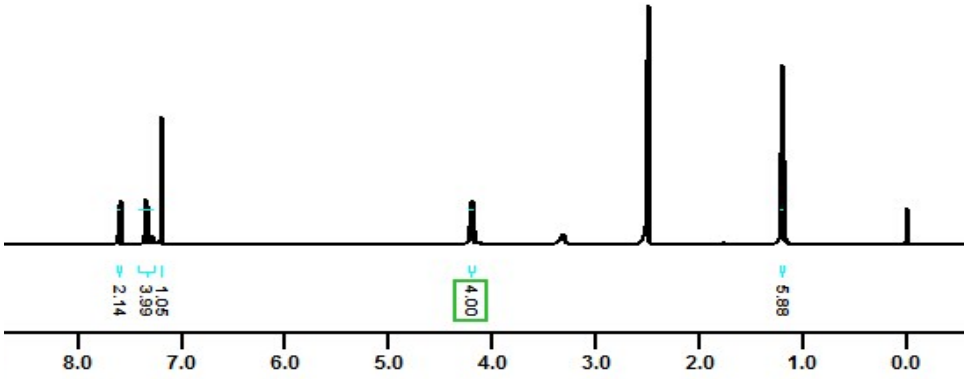

Fig. S2': ^{1}H NMR spectrum of Mono 1 (with integration value) in DMSO-d_6

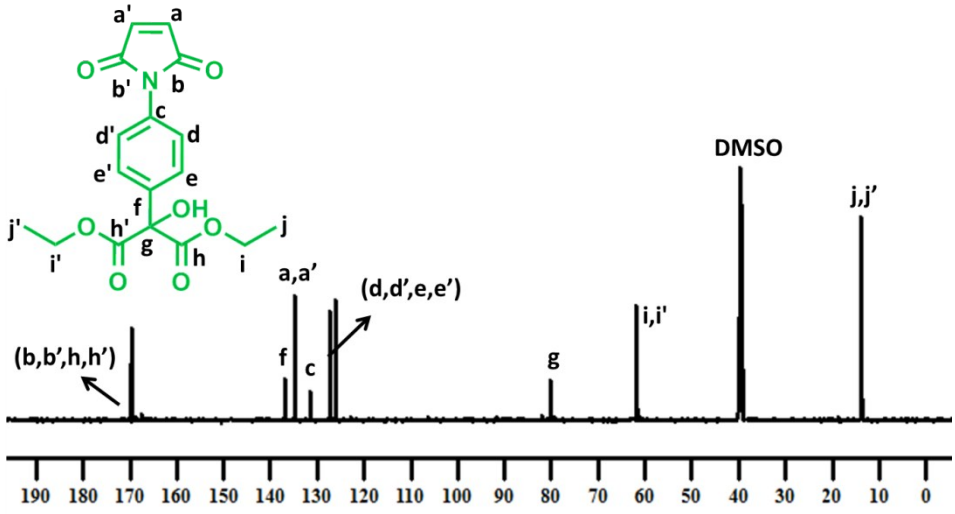

Fig. S3: ^{13}C NMR spectrum of Mono 1 in DMSO-d_6

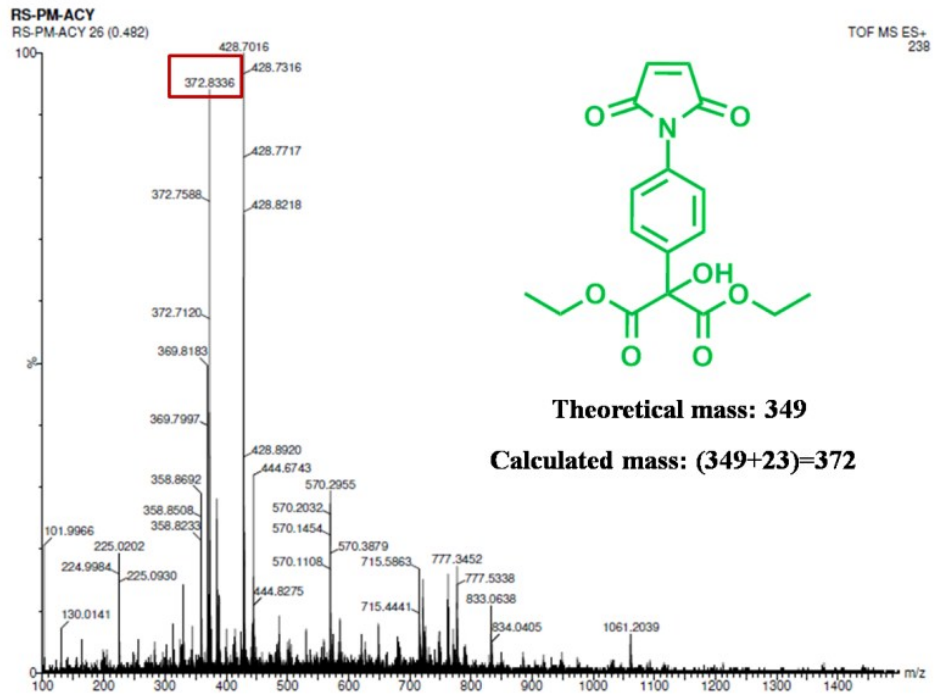

Fig. S4(a): Mass spectrum of Mono 1

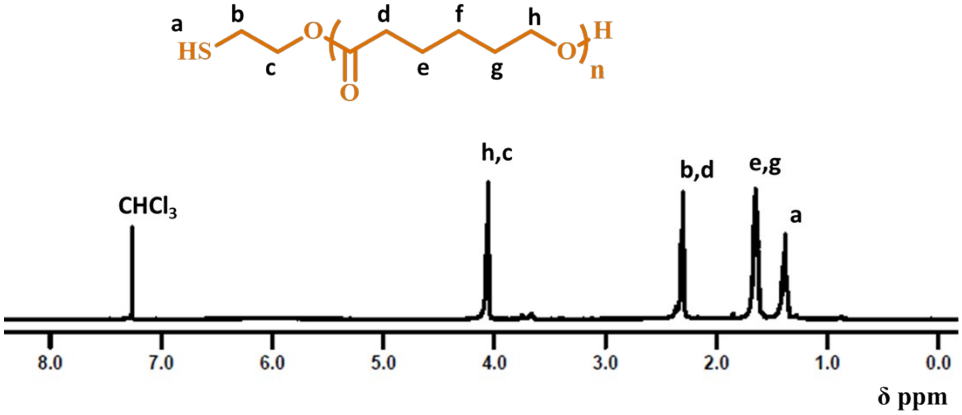

Fig. S5: ^{1}H NMR spectrum of PCL-PCAP in $CDCl_3$

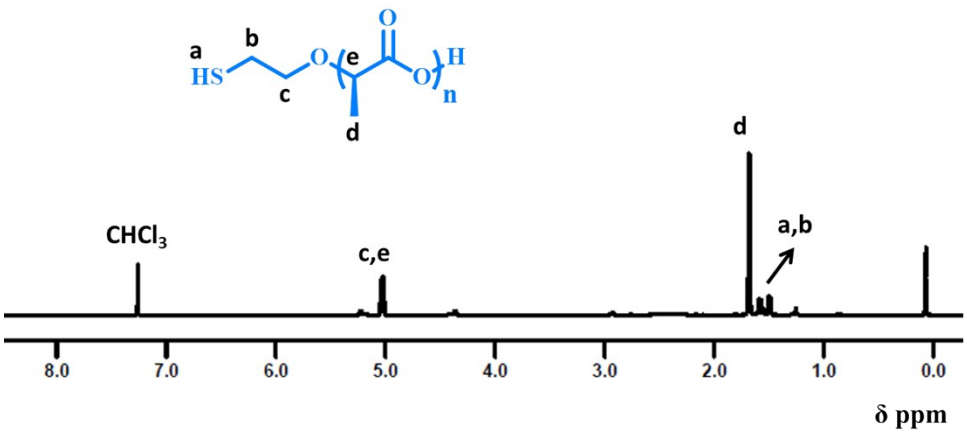

Fig. S6: ^{1}H NMR spectrum PLA-PCAP in $CDCl_3$

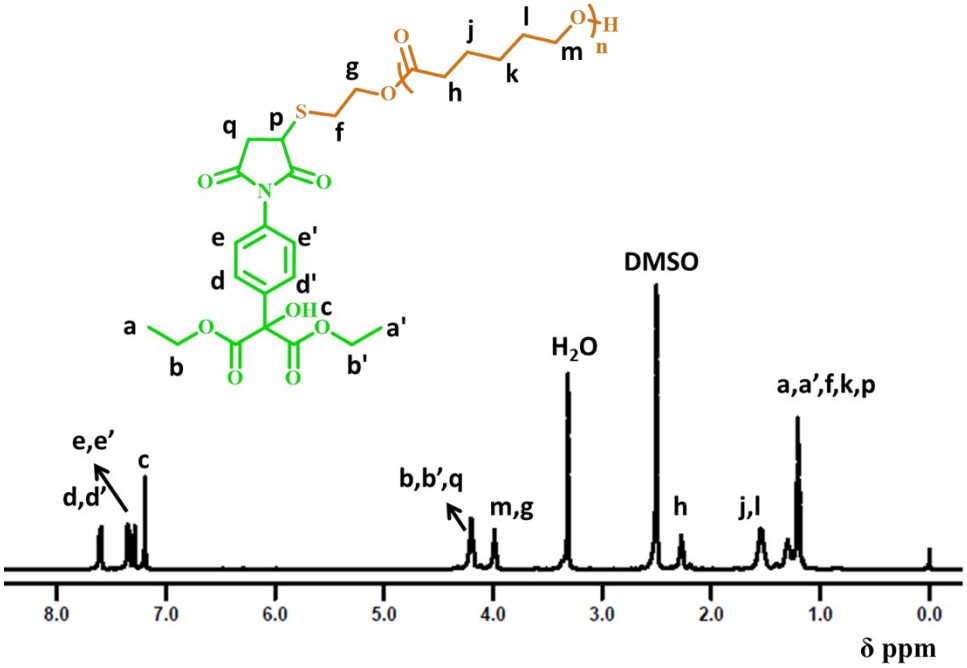

Fig. S7: ^{1}H NMR spectrum of MAL- PCL-PCAP in DMSO-d_6

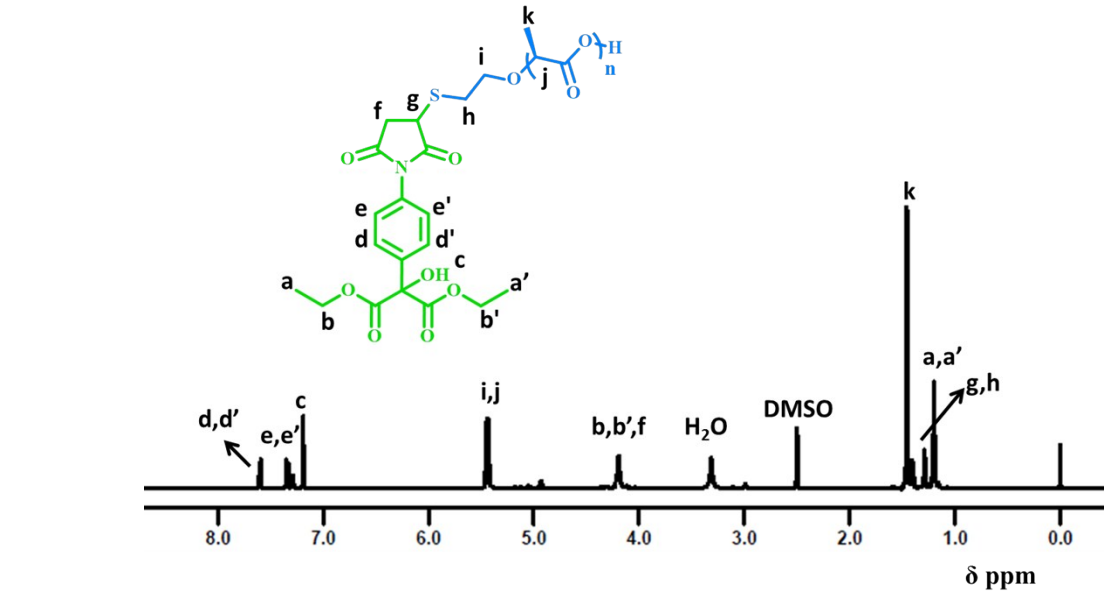

Fig. S8: ^{1}H NMR spectrum of MAL-PLA-PCAP in DMSO-d_6

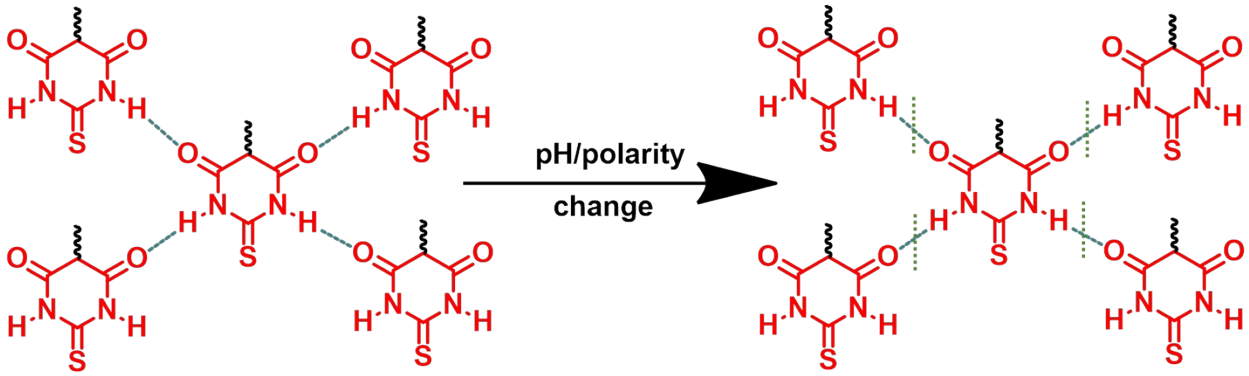

Fig. S9: Pictorial presentation of H-bonding of barbiturate moieties in water and breaking of that bonding with the variation of pH and polarity

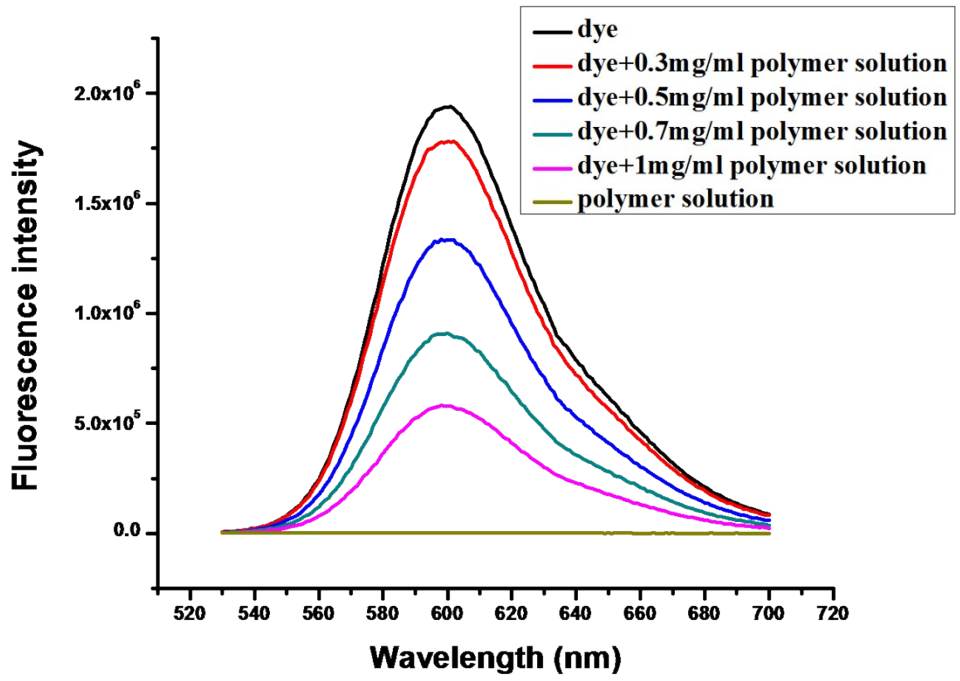

Fig. S10: Hydrophobic dye (Nile Red) encapsulation study of TBA- PCL-PCAP

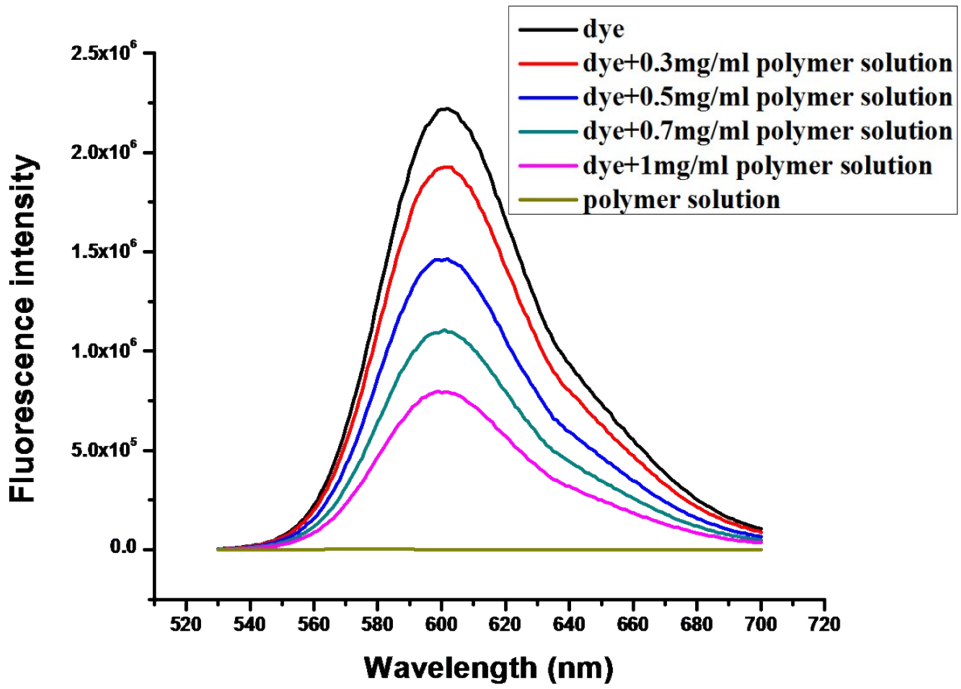

Fig. S11: Hydrophobic dye (Nile Red) encapsulation study of TBA-PLA-PCAP

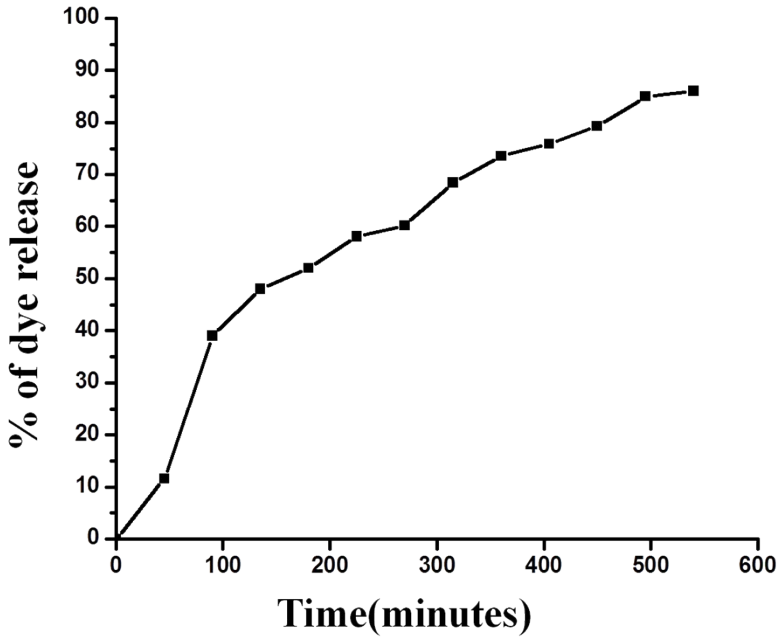

Fig. S12: Dye release study of TBA- PCL-PCAP in water-octanol system

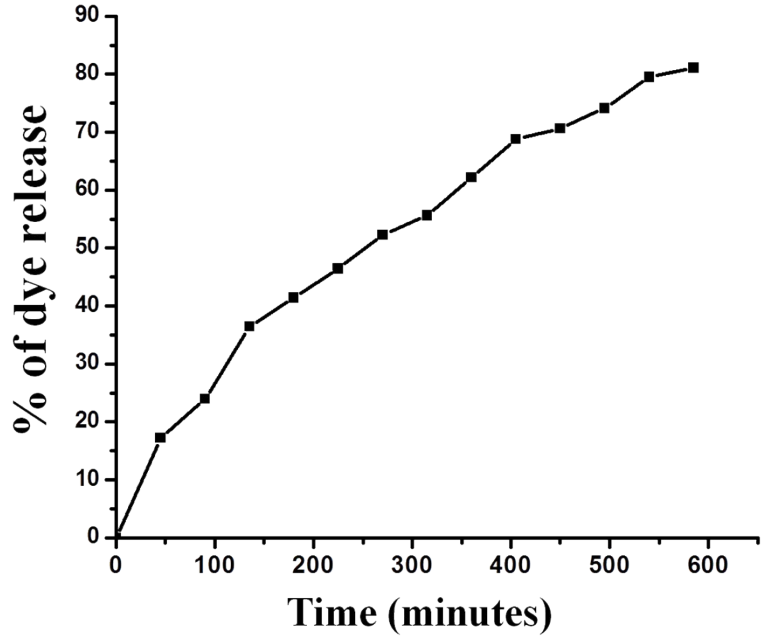

Fig. S13: Dye release study of TBA-PLA-PCAP in water-octanol system

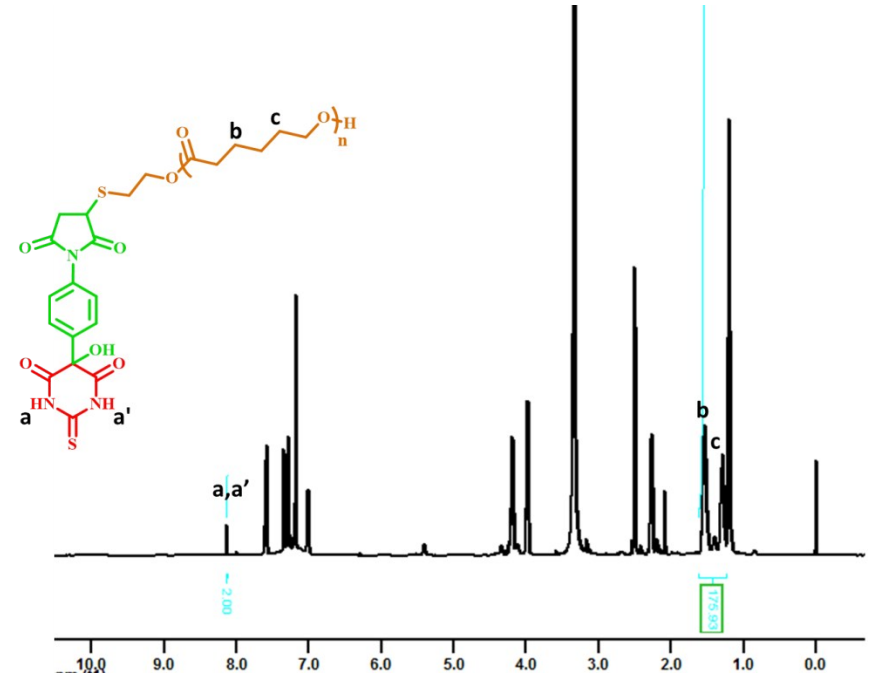

Fig. S14: ^{1}H NMR spectrum of TBA- PCL-PCAP in DMSO-d_6

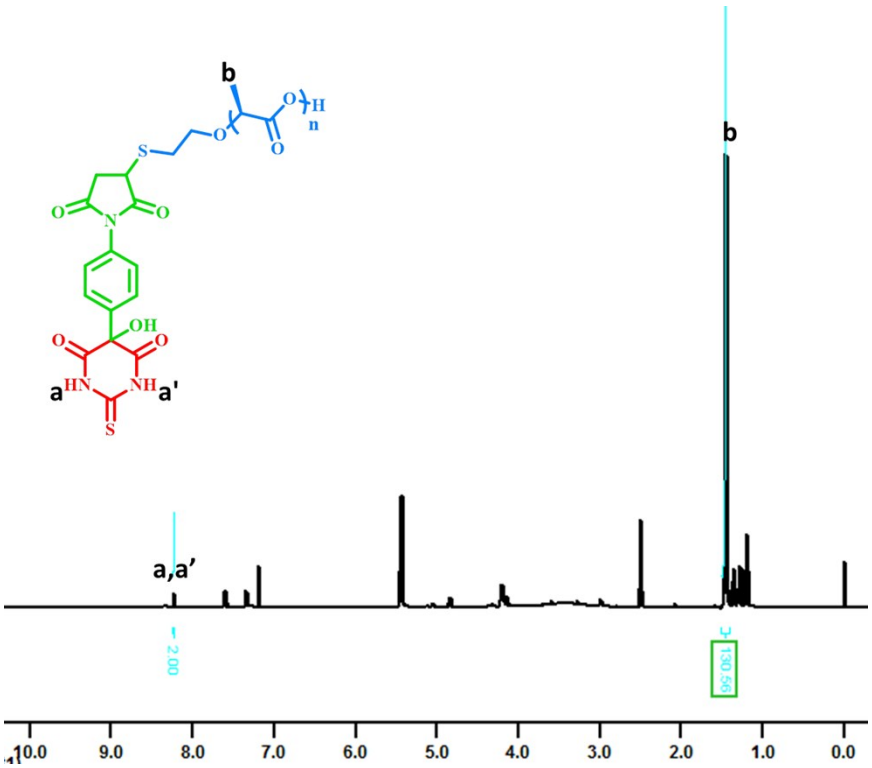

Fig. S15: ^{1}H NMR spectrum of TBA-PLA-PCAP in DMSO-d_6

The molecular weight of TBA- PCL-PCAP and of TBA-PLA-PCAP from APC and ^{1}H NMR spectroscopy

Sl. No.	Name of the polymer	Molecular Weight ^{APC}	Molecular Weight ^{NMR}
1	TBA- PCL-PCAP	4400	5000
2	TBA-PLA	4700	6200