CHAPTER 4

Synthesis and Characterization of DOX-loaded Amphiphilic Poly(*N*-vinyl pyrrolidone) (PNVP)-*b*-PDLLA-*b*-PNVP Triblock Copolymers and Its *In Vitro* Drug Release Study

4.1 Introduction

Syntheses of well-defined linear and 4 arm star amphiphilic diblock copolymers of D, L- lactide (DLLA) and NVP *via* combination of the controlled ring opening polymerization (ROP) of DLLA and the controlled xanthate-mediated RAFT polymerization of NVP are described in **Chapters 2** and **3**, respectively. [Ramesh *et al*. (2012), (2015)] In this chapter, I am interested in the synthesis of well-defined amphiphilic PNVP-*b*-PDLLA-*b*-PNVP triblock copolymers. Kang *et al*. (2004) have reported synthesis of PVP-*b*-PDLLA-*b*-PVP by conventional free radical polymerization of NVP in the presence of PDLLA dithiol chain transfer agent. The resultant polymers were of broad molecular weight distribution and self-assembled in aqueous solution. They also reported the loading of two hydrophobic drugs indomethacin and pacilitaxel in such polymeric micelles by dialysis method. To the best of our knowledge, there is no report about the synthesis of well-defined amphiphilic PNVP-*b*- PDLLA-*b*-PNVP triblock copolymers. In this chapter, I have reported the synthesis of well-controlled amphiphilic triblock copolymers PVP-*b*-PDLLA-*b*-PVP via the combination of ROP of DLLA and xanthate-mediated RAFT polymerization of NVP. At first, HO-PDLLA-OH is synthesized by ROP using ethylene glycol as initiator. [**Scheme 4.1**] The –OH end-groups are then converted to the corresponding –Br end groups (Br-PDLLA-Br) through a reaction with 2-bromopropionyl bromide. Then, this –Br end-group is converted to the corresponding *O*-ethyl xanthate end group X-PDLLA-X through an ionic substitution reaction with potassium *O*-ethyl xanthate. After that, the controlled/living radical polymerization of NVP is performed to synthesized well-defined amphiphilic PNVP-*b*-PDLLA-*b*-PNVP triblock copolymers using the macro chain transfer agent X-PDLLA-X. The resultant

polymers have been characterized by ^{1}H NMR and GPC studies. Further, the self-assembly behavior of the resultant amphiphilic block copolymers is studied using ^{1}H NMR, fluorescence spectroscopy, transmission electron microscopy and light scattering study. DOX-loaded polymeric micelles of $PNVP_{51}$-*b*-$PDLLA_{48}$-*b*-$PNVP_{51}$ amphiphilic triblock copolymers are prepared and characterized by TEM and DLS. Moreover, we have studied antimicrobial activity of the DOX-loaded polymeric micelles.

4.2 Experimental Section

4.2.1 Materials

Ethylene glycol (S. D. Fine, Mumbai, India, 99%) was dried over CaO and distilled under reduced pressure. Doxorubicin (Adriamycin) was purchased from Selleckchem, USA. Other reagents are used as mentioned in the **2.2.1 section** of **Chapter 2.**

4.2.2. General Methods.

Measurement procedures of NMR, GPC, TEM, Fluorescence, UV visible and DLS are same as reported earlier in **Chapter 3.**

4.2.3 Synthesis of Dihydroxyl-terminated Poly(D,L-lactide) (HO-$PDLLA_{48}$-OH) [run1, Table 4.1]

Dihydroxyl-terminated poly(D, L-lactide) (HO-$PDLLA_{48}$-OH) was synthesized through ROP of D,L-lactide using ethylene glycol as initiator and $Sn(Oct)_2$ as the catalyst. 5.0 g (3.46×10^{-2} mol) of D, L-lactide was placed in a 100 mL Schlenk tube, heated at 80 ^oC for 4 h under vacuum and dried. After cooling to RT, 0.092 mL (0.10 g, 1.65×10^{-3} mol) of ethylene glycol was added to the flask. Then, the reaction mixture was purged with nitrogen for 30 min. The Schelnk tube was then tightly closed, and heated to 150

^{o}C and then 20 μL (0.025 g, 6.2 × 10^{-2} mmol, 0.5% (w/w) ratio of lactide) stannous octoate was injected into the reactor vessel. The reaction was continued at 150 ^{o}C for 15 h. The polymerization process was stopped by freezing the reaction mixture with liquid N_2. The crude product was dissolved in 10 mL THF and precipitated from 200 mL hexane. The precipitated polymer was collected by centrifugation. The precipitated polymer was again dissolved in THF and precipitated from hexane twice and finally dried under vacuum at room temperature for 24 h. Gravimetric yield (%) = 3.9 g (93.5%.)

^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.65-1.70 (m, $6H_c$), 1.52-1.60 (d, $3H_e$), 5.1-5.3 (m, $2H_b$), 4.2-4.4 (t, $4H_a$+ $2H_d$)

M_n(NMR) = 3,400 g mol^{-1}, M_n(GPC) = 5200 g mol^{-1}, M_w/M_n = 1.32.

4.2.4 Synthesis of Dibromo-terminated PDLLA [Br(CH_3)CHCO-PDLLA_{48}-COCH (CH_3) Br] (Br-PDLLA_{48}-Br) [run 2, Table 4.1]

In a dried and nitrogen purged 250 mL round-bottom flask, 3.5 g [1.018 × 10^{-3} mol, calculated on the basis of molecular weight (3,400 g/mol) obtained from ^{1}H NMR] HO-PDLLA_{48}-OH was dissolved in a mixture of 25 mL of dry THF and 0.8 mL (5.089 × 10^{-3} mol) triethylamine under stirring in nitrogen atmosphere and cooled in an ice bath. 0.43 mL (4.07 × 10^{-3} mol) 2-bromopropionyl bromide was added drop by drop to the above-mentioned reaction mixture under stirring. Then, the reaction was continued for 72 h under stirring at RT. The precipitated byproduct Et_3N.HBr was removed by filtration and filtrate was evaporated to dryness. The residue was dissolved in dichloromethane and washed thoroughly with 5 % (w/v) aqueous sodium bicarbonate solution (4 × 250 mL). The organic layer was further washed with water (4 × 300 mL) and then dried over anhydrous Na_2SO_4, and filtered. The filtrate was evaporated and

dried under vacuum at room temperature. The residue was dissolved in THF and precipitated from hexane and then dried under vacuum at room temperature for 24 h. Gravimetric yield (%) = 2.8 g (80%).

^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.54-1.58 (m, $3H_c$), 1.83-188 (d, $3H_g$), 4.2-4.5 (m, $2H_f$ + 4 H_a), 5.1-5.3 (m, $2H_b$),

M_n(NMR) = 3,800 g mol^{-1}, M_n(GPC) = 5700 g mol^{-1}, M_w/M_n = 1.31.

4.2.5 Synthesis of Dixanthate-terminated PDLLA [$C_2H_5O(S)CS(CH_3)CHCO$-$PDLLA_{48}$-$C(O)CH(CH_3)SC(S)OC_2H_5]_2$ (X-$PDLLA_{48}$-X) [run 3, Table 4.1]

In a dried and nitrogen purged 250 mL round-bottom flask, 2.5 g (7.26×10^{-4} mol) Br-$PDLLA_{48}$-Br and 0.7 g (4.36×10^{-3} mol) potassium *O*-ethyl xanthate were dried and degassed by three freeze-pump-thaw cycles. In another well dried and nitrogen purged 50 mL round-bottom flask, 6.5 mL (7.70×10^{-2} mol) pyridine was dissolved in 30 mL CH_2Cl_2 by stirring under nitrogen atmosphere. This solution was added to the previous reaction mixture during stirring under nitrogen. The reaction mixture was stirred for 36 h at room temperature and diluted with 100 mL CH_2Cl_2. The solution was washed consecutively with saturated NH_4Cl solution (4 × 50 mL), saturated $NaHCO_3$ solution (4 × 50 mL), and water (4 × 100 mL). The organic layer was dried over anhydrous $MgSO_4$ and filtered. The filtrate was dried under vacuum at RT for 48 h. The residue was dissolved in THF and precipitated from hexanes followed by drying under vacuum at room temperature for 24 h. Gravimetric yield (%) = 2.1 g (84%).

^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.35-1.70 (m, 6Hc+6Hg+$6H_i$), 4.55-4.7 (q, $4H_h$), 4.3-4.5 (m, $4H_a$ +$2H_f$), 5.1-5.2 (m, $2H_b$).

M_n (NMR) = 4,200 g mol^{-1}, M_n (GPC) = 6900 g mol^{-1}, M_w/M_n = 1.33.

4.2.6 Typical Synthesis of the ABA type Double Hydrophilic Amphiphilic Tri Block Copolymer $PNVP_{23}$-*b*-$PDLLA_{48}$-*b*-$PNVP_{23}$ (run 1, Table 4.2)

In a dried and nitrogen purged 30 mL Schlenk tube, 0.1 g [2.36×10^{-5} mol, calculated on the basis of molecular weight (4,200 g mol^{-1}) obtained from ^1H NMR] (X-$PDLLA_{48}$-X) was dissolved in 1 mL THF. To it, 0.25 mL (0.261 g, 2.36×10^{-3} mol) NVP and 1.93 mg (1.18×10^{-5} mol) AIBN are added. A homogeneous solution was obtained after stirring and degassed under nitrogen for 30 min. The Schlenk tube was then immersed in an oil bath preheated at 80 ^oC for 24 h. The reaction was stopped by freezing the reaction mixture with liquid nitrogen. A small portion of the polymerization mixture was used to determine the monomer conversion by ^1H NMR. The rest part of the polymerization mixture was dissolved in 5 mL THF, precipitated from 200 mL hexane, and dried under vacuum at RT for 24 h. Observed gravimetric yield (%) = 0.232 g (50.5%)

^1H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.2-1.8 (m, $6H_c+4H_j+6H_g+6H_i$), 1.8-2.2 (m, $4H_p$), 2.2-2.5 (m, $4H_q$), 3.0-3.5 (m, $4H_p$), 3.5-4.0 (m, $2H_l$), 5.2 (m, $2H_b$).

M_n(NMR) = 9800 g mol^{-1}, M_n(GPC) = 12100 g mol^{-1}, M_w/M_n= 1.35.

4.2.7 Drug Loading and Release Study

30 mg of $PNVP_{51}$-*b*-$PDLLA_{48}$-*b*-$PNVP_{51}$ tri-block copolymer (**run 2, Table 4.2**) was dissolved in 2 mL of DMF, and 6 mg (0.01 mmol) of DOX·HCl with 4.6 μL (3.3 mg, 0.033 mmol) TEA (3 mol eq. to DOX·HCl) were added into the polymer solution. The mixture was stirred at room temperature for 24 h. Final mixture was then dialyzed using a dialysis membrane [molecular weight cut off (MWCO) = 3500 g mol^{-1}] against distilled water which was renewed every 3 h during the course of initial 12 h, next every 6 h to remove the unloaded drug for 24 h. After dialysis, dialyzed drug-loaded

micellar solution was filtered and concentrated to 3.0 mL and lyophilized. Lyophilized drug-loaded micelle was then dissolved in DMF and analyzed by UV absorbance at 485 nm, using a standard calibration curve experimentally obtained with DOX /DMF solutions. Drug loading content (DLC) and drug loading efficiency (DLE) were calculated according to the following formula:

Drug loading content (DLC) (wt. %) = weight of loaded drug /weight of polymer) ×100%

Drug loading efficiency (DLE) (wt. %) = weight of loaded drug /weight in feed) ×100%

DOX loaded polymer sample (5 mg) was dissolve in 2.0 mL phosphate buffer solution (PBS) (pH = 7.4) solutions and transferred into dialysis tubing (molecular weight cut off (MWCO) = 3500 g mol^{-1}). The tubing was placed into 20 mL PBS solution. The system was stirred at 37^{o}C. At predetermined interval, 2.0 mL PBS solution was taken out and volume of solution was held constant by adding 2 mL PBS solution after each sampling. The amount of DOX released from drug-loaded micelles at any interval was measured by UV spectroscopy at 485 nm.

4.3 Results and Discussion

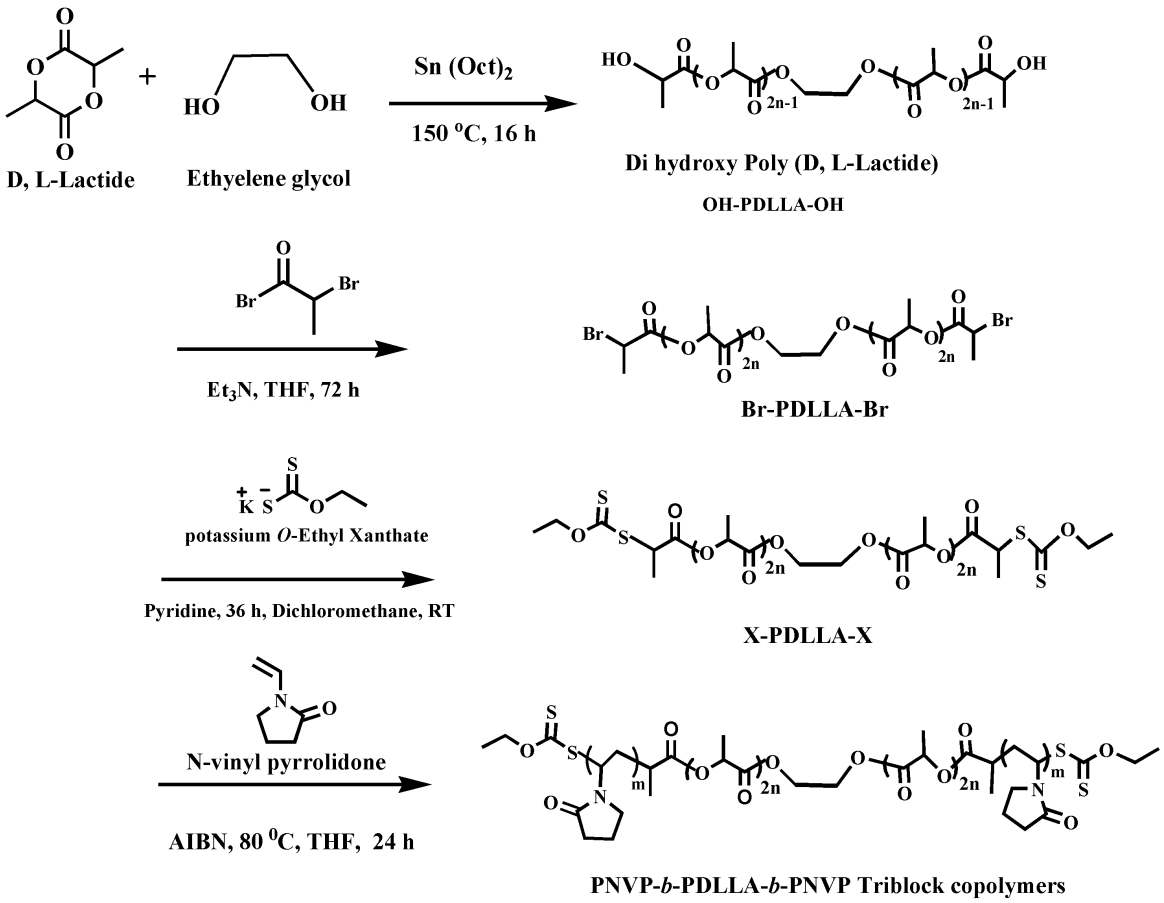
4.3.1 Synthesis of PNVP-*b*-PDLLA-*b*-PNVP Amphiphilic Triblock copolymers

Amphiphilic triblock copolymers with poly(D,L-lactide) as a hydrophobic core and poly(*N*-vinylpyrrolidone) as a hydrophilic shell were synthesized *via* combination of ROP and xanthate-mediated RAFT polymerization as shown in [**Scheme 4.1**].

Table 4.1 Synthesis of X-PDLLA-X macro-chaintransfer agent.

Run	Sample	Yield (%) [d]	Conv(%)[e] (nmr.)	M_n[e](NMR) g mol^{-1}	M_n[f](GPC) g mol^{-1}	PDI [f] (GPC)	Coments
1	OH-PDLLA$_{48}$-OH [a]	94	96	3400	5200	1.32	Unimodal
2	Br-PDLLA$_{48}$-Br [b]	80	100	3800	5700	1.31	Unimodal
3	X-PDLLA$_{48}$-X [c]	84	100	4200	6900	1.33	Unimodal

[a] Bulk polymerization using (D,L-lactide) and Sn(oct)$_2$ in the presence of ethyelen glycol at 150 ^{o}C for 16 h;
[b] Using HO-PDLLA-OH:triethylamine: 2-bromopropionylbromide: 1:5:4 in THF at RT for 72 h;
[c] Using Br-PDLLA-Br : potassium *O*-ethyl xanthate : pyridine : 1 : 6 : 106 in dichloromethane (DCM) at room temperature for 36 h
[d] Determined by gravimetrical
[e] Determined by ^{1}H NMR
[f] Determined by GPC (DMF, 0.5 mL/min, 40 ^{o}C) calibrated against PMMA standards.



Scheme 4.1. Synthesis of PNVP-*b*-PDLLA-*b*-PNVP Triblock copolymer via ROP and xanthate mediated RAFT polymerization methods.

In the first step, HO-PDLLA-OH has been synthesized in bulk with 94% yield via ROP of DLLA at 150 ^{o}C using ethylene glycol as initiator and $Sn(Oct)_2$ as catalyst (**run 1**, **Table 4.1**). Its formation is confirmed from ^{1}H NMR [**Figure 4.1(A)**]. GPC chromatogram [**Figure 4.2(a)]** depicts unimodal nature with M_n (GPC) = 5,400 g mol^{-1} and PDI = 1.32. The HO-PDLLA-OH was then converted to the corresponding Br-PDLLA-Br on reaction with 2-bromopropionyl bromide in the presence of triethyl amine [**run 2, Table 4.1**] and is confirmed from ^{1}H NMR [**Figure 4.1(B)**] by the revelation of the characteristic methine '*f*' and methyl '*g*' protons of 2-bromopropionyl end group at 4.72 and 1.80 ppm, respectively. GPC chromatogram [**Figure 4.2(a)**] shows unimodal nature with M_n(GPC) and PDI of 5,700 g mol^{-1} and 1.31, respectively.

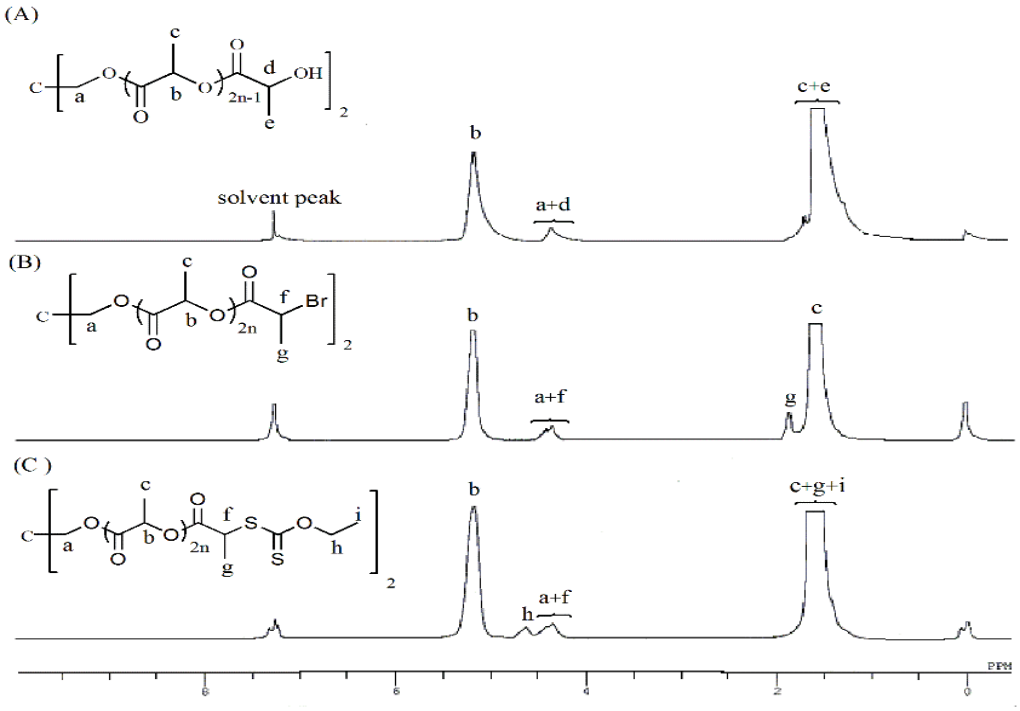
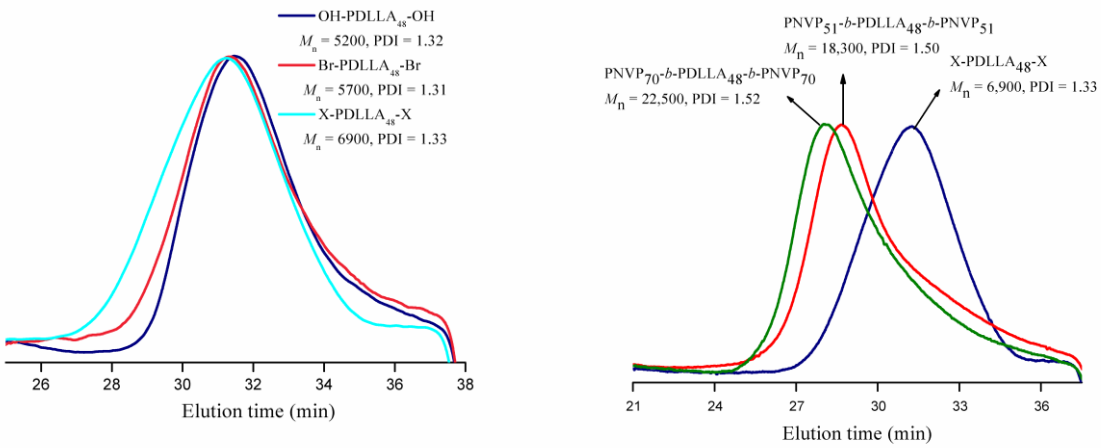


Figure 4.1. ^{1}H NMR Spectra (**A**) HO-PDLLA-OH, (**B**) Br-PDLLA-Br and (**C**) X-PDLLA-X in $CDCl_3$.

Br-PDLLA-Br was then end-functionalized using potassium-*O*-ethyl xanthate to yield X-PDLLA-X (**run 3**, **Table 4.1**) and was confirmed by ^{1}H NMR [**Figure 4.1(C)**] with the appearance of new peak characteristic of the methylene '*h*' protons of the xanthate end group at 4.6 ppm. GPC chromatogram [**Figure 4.2(a)**] confirms unimodal nature with M_n(GPC) of 6, 900 g mol^{-1}and PDI of 1.33.



(a) **(b)**

Figure 4.2 (a) Gel permeation chromatograms of HO-PDLLA$_{48}$-OH (**run 1**), Br-PDLLA$_{48}$-Br (**run 2**) and X-PDLLA$_{48}$-X (**run 3**) (**Table 4.1**). **(b)** Gel Permeation Chromatograms of macro-chain transfer agent X-PDLLA$_{48}$-X and the resulted block copolymers PNVP$_{51}$-*b*-PDLLA$_{48}$-*b*-PNVP$_{51}$ (**run 2**) and PNVP$_{70}$-b-PDLLA$_{48}$-*b*-PNVP$_{70}$ (**run 3**) (**Table 4.2**).

X-PDLLA$_{48}$-X was used as a macro-chain transfer agent for the xanthate mediated RAFT polymerization of NVP in THF using [X-PDLLA$_{48}$-X]: [AIBN] = 1: 0.5 at 80 ^{o}C for 24 h. The results of the synthesis and characterization of PNVP-*b*-PDLLA$_{48}$-*b*-PNVP block copolymers from the corresponding X-PDLLA$_{48}$-X polymers

are shown in **Table 4.2**. **Runs 1**, **2** and **3** correspond to 100, 200, and 300 equivalents of NVP monomer loading with respect to X-PDLLA$_{42}$-X macro-chain transfer agent, respectively. Molecular weight and PNVP content of the resulted block copolymers are increased with the increase in the monomer loading as expected [**Table 4.2** and **Figure 4.2(b)**].

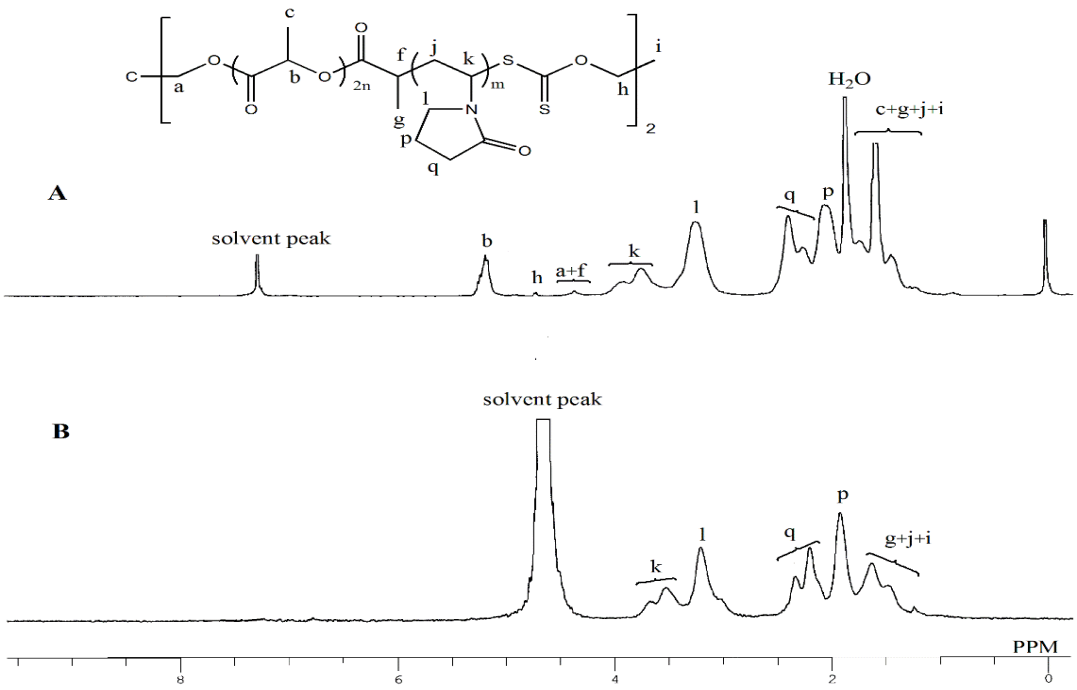


Figure 4.3 ^{1}H NMR spectra of PNVP$_{23}$-*b*-PDLLA$_{48}$-*b*-PNVP$_{23}$ triblock copolymer in (**A**) *d*-chloroform, and (**B**) D_2O at room temperature.

The typical ^{1}H NMR spectrum (in $CDCl_3$) [**Figure 4.3(A)**] of the block copolymer, prepared in **run 1** (**Table 4.2**), revealed, in addition to the characteristic peaks of the PDLLA block, the presence of the characteristic peaks of the PNVP backbone methine proton '*k*' at ~ 3.5-4.0 ppm, the methylene protons '*l*', '*q*', and '*p*' of the pyrrolidone ring at ~ 3.0-3.5, 2.2-2.5, and 1.8-2.2 ppm, respectively, apart from methylene protons '*j*' of the PNVP block overlapped between ~ 1.2-1.8 ppm range with

methyl protons '*c*' of PDLLA block. All triblock copolymers were completely soluble in water. All these results indicate the successful occurrence of hetero chain-extension.

Table 4.2 Characteristic Data of PNVP-*b*-PDLLA-*b*-PNVP Block Copolymer[a]

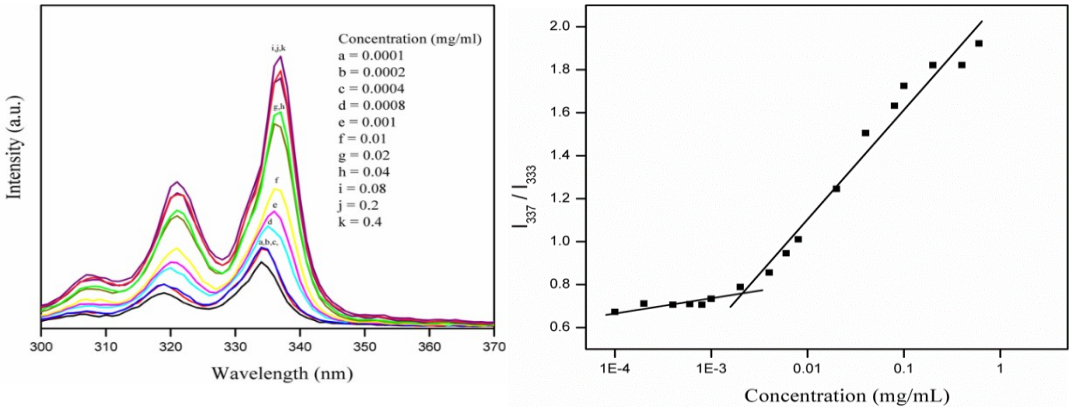
Run	Block copolymer	NVP[b] (equi.)	Convn.[c] (NMR)	M_n[d] (theor.)	M_n[e] (NMR)	M_n/PDI[f] (GPC)	X_{NVP}[g] (GPC)	X_{NVP}[g] (NMR)	CMC[h] (mg/L)
1	$PNVP_{23}$-*b*-$PDLLA_{48}$-*b*-$PNVP_{23}$	100	38	8,500	9,800	12,000/1.35	0.51	0.57	2.10
2	$PNVP_{51}$-*b*-$PDLLA_{48}$-*b*-$PNVP_{51}$	200	40	13,100	15,500	18,300/1.50	0.62	0.73	4.02
3	$PNVP_{70}$-*b*-$PDLLA_{48}$-*b*-$PNVP_{70}$	300	42	18,400	22,100	22,500/1.52	0.69	0.79	6.30

[a] Using 0.5 equivalent AIBN with respect to X-$PDLLA_{48}$-X macroinitiator in THF at 80 °C for 24 h.
[b] With respect to X-$PDLLA_{48}$-X macroinitiator.
[c] Conversion was determined by using ^{1}H NMR comparing the peak area of the residual vinylic sigments of the NVP monomer at ~7.0 - 7.1ppm (1H) with that of the methylene proton of the PNVP block of the polymer at 3.0-3.5 ppm.
[d] M_n(theor) = ^{1}H NMR mol. wt. of X-PDLLA-X + $([NVP]_o/[X\text{-}PDLLA\text{-}X]_o$ x fraction conversion of NVP(NMR) x mol. wt. of NVP).
[e] Determined from ^{1}H NMR by compairing the peak area of the methylene protons of PDLLA block at ~5.2 ppm with that of the methylene proton of PNVP block at ~3.0 - 3.4 ppm.
[f] Determined by GPC(DMF, 0.5 mL/min, 40 °C) calibrated against PMMA standards.
[g] X_{PNVP} = mol-fraction of PNVP.
[h] CMC value determined by fluorescence spectrometer.

4.3.2 Self-assembly of Amphiphilic PNVP-*b*-PDLLA-*b*-PNVP Triblock Copolymers in Water

The ^{1}H NMR spectrum of $PNVP_{23}$-*b*-$PDLLA_{48}$-*b*-$PNVP_{23}$ triblock copolymer in D_2O is shown in **Figure 4.3(B)**. Here, the peaks attributed to PDLLA are suppressed in comparison with the ^{1}H NMR spectrum obtained in *d*-chloroform [**Figure 4.3(A)**]. This observation indicates the possible formation of micellar aggregates in aqueous solution with PDLLA block as the core and PNVP block as the shell. In order to study the critical micellar concentration (*cmc*) of such triblock copolymers in water, fluorescence spectroscopy is used with pyrene as the probe. Typical fluorescence excitation spectra (300 - 360 nm) of pyrene (6×10^{-7} M) at different $PNVP_{23}$-*b*-$PDLLA_{48}$-*b*-$PNVP_{23}$ [**run 1**, **Table 4.2**] concentrations recorded at an emission wavelength of 394 nm are shown in **Figure 4.4(a)**. **Figure 4.4(b)** shows the corresponding plot of the I_{337}/I_{333} intensity

ratio (from fluorescence measurements) *vs.* the log of the $PNVP_{23}$-*b*-$PDLLA_{48}$-*b*-$PNVP_{23}$ block copolymer concentration (mg/mL) in water. The observed *cmc*s of the block copolymers $PNVP_{23}$-*b*-$PDLLA_{48}$-*b*-$PNVP_{23}$, $PNVP_{51}$-*b*-$PDLLA_{48}$-*b*-$PNVP_{51}$ and $PNVP_{70}$-*b*-$PDLLA_{48}$-*b*-$PNVP_{70}$ are ~ 2.10×10^{-3}, 4.02×10^{-3}, and 6.30×10^{-3} mg/mL, respectively [**Table 4.2**].



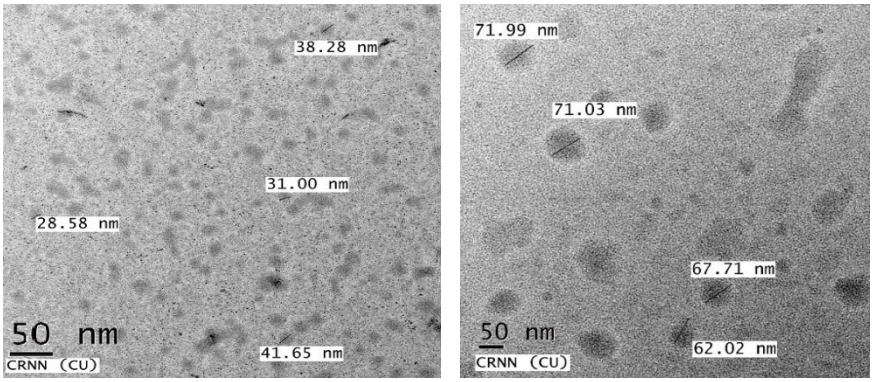
(a) **(b)**

Figure 4.4(a) Fluorescence excitation spectra (monitored at λ_{em} = 394 nm)of pyrene (6×10^{-7} M) in the presence of increasing concentration (mg/mL) of $PNVP_{23}$-*b*-$PDLLA_{48}$-*b*-$PNVP_{23}$ triblock copolymer (**run 1**, **Table 4.2**) solution in water and **(b)** the corresponding semilogarithmic plot of the fluorescence excitation intensity ratio (I_{337}/I_{333}) of pyrene *vs.* the concentration of polymer.

These values indicate that the *cmc* value of such amphiphilic block copolymers increases with the increase in the chain length of PNVP block. Similar type of results is also reported in the literature. [Mishra *et al*. (2011), Mishra *et al*. (2013)].

From the TEM image [**Figure 4.5(a)**] the average diameter of $PNVP_{51}$-*b*-$PDLLA_{48}$-*b*-$PNVP_{51}$ micelles is observed at 34.8 nm. But the DLS measurement [**Figure 4.6**] revealed that the average hydrodynamic diameter and the PDI of $PNVP_{51}$-*b*-$PDLLA_{48}$-*b*-$PNVP_{51}$ micelle are 75.3 nm and 0.423, respectively. The observed

smaller size of the micelle in TEM measurement is probably arising from the dehydration and shrinkage of the micelles during drying in the TEM measurement.



(a) **(b)**

Figure 4.5. The TEM image of (**a**) blank micelles and (**b**) with DOX loaded micelles of $PNVP_{51}$-*b*-$PDLLA_{48}$-*b*-$PNVP_{51}$ amphiphilic block copolymer.

4.3.3 DOX Loading and *In Vitro* Release

The DOX-loaded polymeric micelles were prepared using dialysis method. The amount of DOX encapsulated into polymeric micelle of $PNVP_{51}$-*b*-$PDLLA_{48}$-*b*-$PNVP_{51}$ was calculated from the absorbance of DOX at 485 nm in its UV spectrum on comparison with the corresponding calibration curve. The drug loading content is 7.1 % and drug loading efficiency is 37.5 %. TEM result [**Figure 4.5(b)**] shows that the average diameter of the DOX-loaded micelles is 68.2 nm where as DLS result [**Figure 4.6**] reveals that hydrodynamic diameter (R_h) and PDI of the same are 150 nm, and 0.176, respectively. Both TEM and DLS results show that the size of the drug-loaded micelles is larger than that of blank micelles. All these results confirm the successful loading of DOX in the $PNVP_{51}$-*b*-$PDLLA_{48}$-*b*-$PNVP_{51}$ micelles.

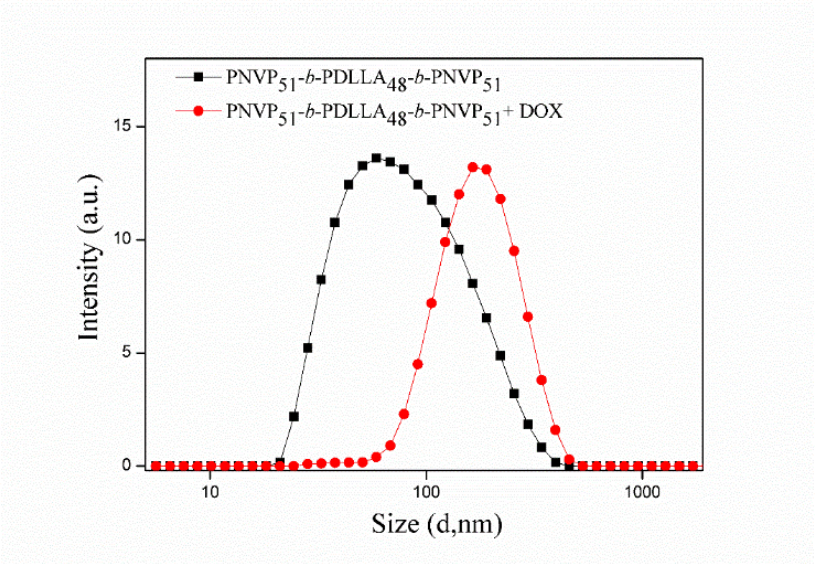


Figure 4.6 Plot of scattering intensity vs. the effective hydrodynamic diameter of without and with DOX loaded micelles of $PNVP_{51}$-*b*-$PDLLA_{48}$-*b*-$PNVP_{51}$ block copolymer at 0.1 mg/mL concentration in water at 90° scattering angle.

In vitro release study were carried out from DOX-loaded micelles of $PNVP_{51}$-*b*-$PDLLA_{48}$-*b*-$PNVP_{51}$ at 37 ^{o}C in pH=7.4 PBS solution. **Figure 4.7** shows that sustained DOX drug release behavior up to 36 h with a maximum of 15.60 % release.

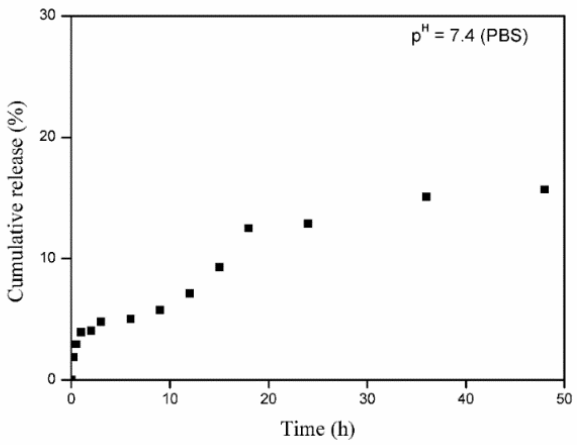


Figure 4.7 Percentage of cumulative release of DOX from DOX-loaded micelles of $PNVP_{51}$-*b*-$PDLLA_{48}$-*b*-$PNVP_{51}$ block copolymer in pH = 7.4 PBS at 37 ^{o}C temperature.

4.4 Conclusions

In summary, synthesis of amphihilic poly(*N*-Vinylpyrrolidone)-*b*-poly(D,L-lactide)-*b*-poly(*N*-Vinylpyrrolidone) ABA type tri-block copolymers *via* the combination of ROP and xanthate-mediated RAFT polymerization. The resultant polymers were characterized by ^{1}H NMR and GPC. The critical micellar concentrations of these tri-block copolymers were determined by fluorescence spectroscopy using pyrene as probe. This block copolymers form polymeric micelles in water as confirmed by TEM, DLS and supported by ^{1}HNMR. DOX could be efficiently loaded into the micelles with a loading efficiency is 37.5%. DOX-loaded $PNVP_{51}$-$PDLLA_{48}$-$PNVP_{51}$ block copolymer showed a sustained release up to 36 h.