Electronic Supplementary Material (ESI) for RSC Advances.


# PEG based random copolymer micelles as drug carriers: Effect of hydrophobe content on drug solubilization and cytotoxicity

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## 1. Synthesis of 2-(2-cyanopropyl)-dithiobenzoate (CPDB)

Chain transfer agent (CTA), 2-(2-cyanopropyl)-dithiobenzoate (CPDB) for RAFT polymerization was synthesized starting from benzyl chloride by using elemental sulphur, potassium ferricyanide ($K_3[Fe(CN)_6]$) and AIBN following the reported method.[1]

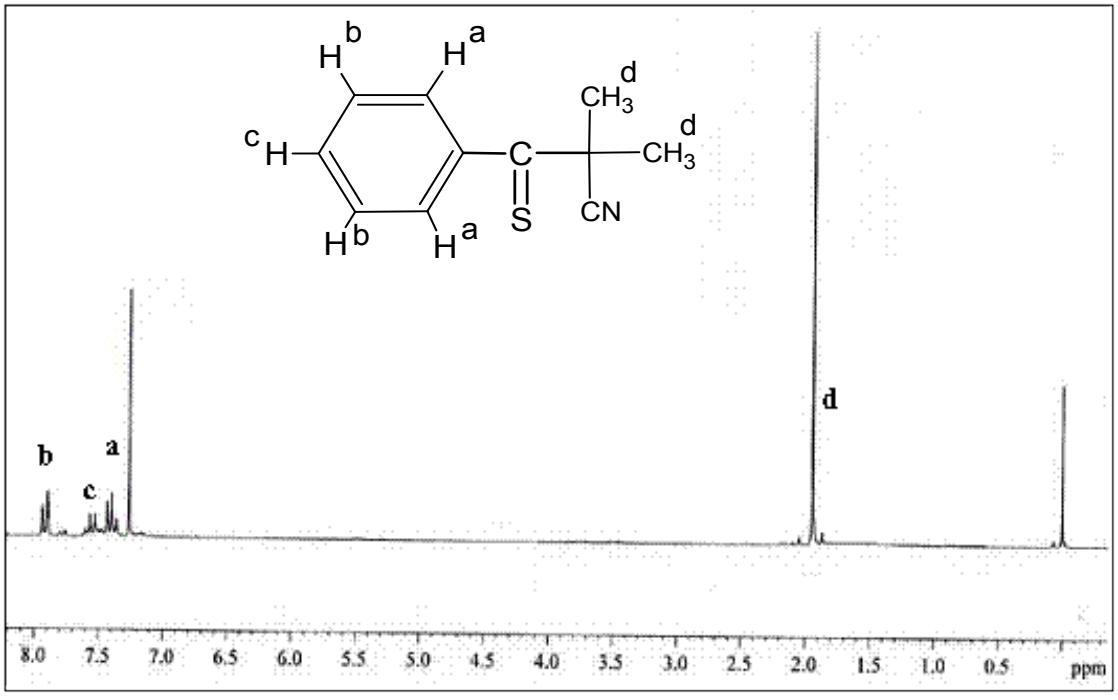
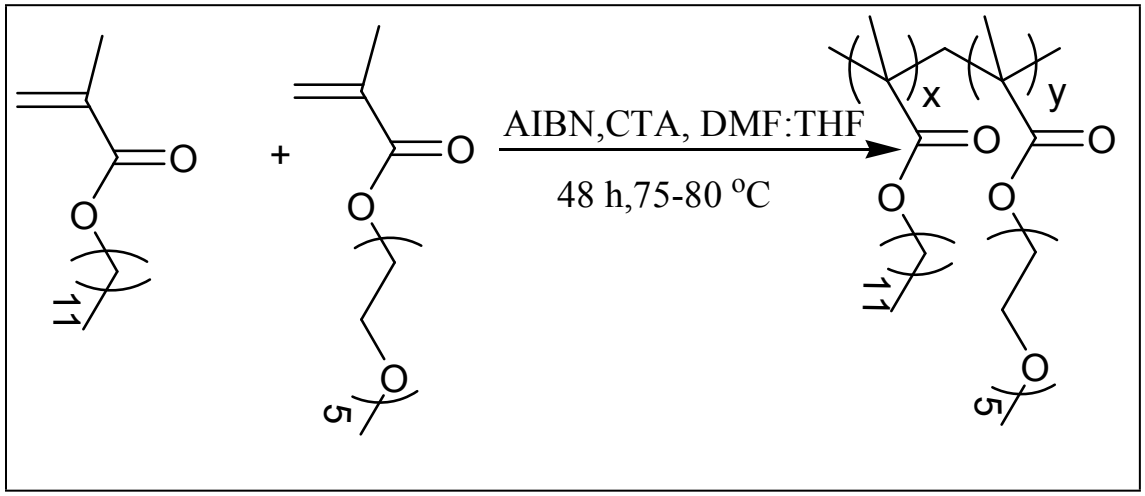



**Figure S1**. $^1$H NMR (400MHz) spectrum of CPDB in $CDCl_3$.

## 2. Synthesis of Polymers




**Scheme S1.** General synthetic scheme for poly[$DMA_x$-co-$mPEG_y$] copolymers.

## 3. NMR Spectrum of Polymers

**$P_{12}$**

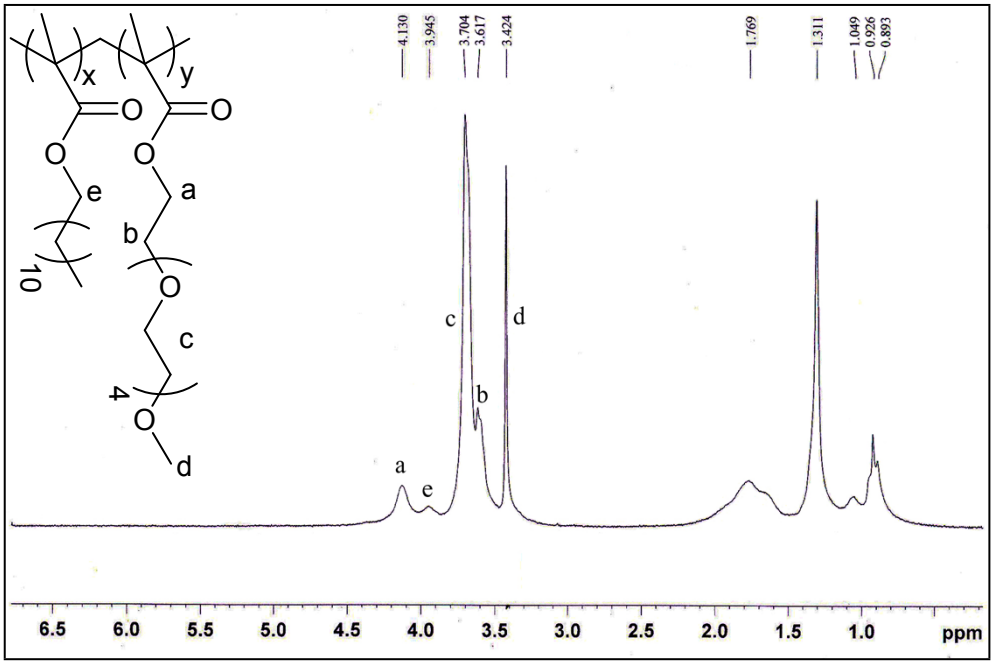



**Figure S2**. $^1H$ NMR (400MHz) spectrum of **$P_{12}$** in $CDCl_3$.

**$P_{14}$**

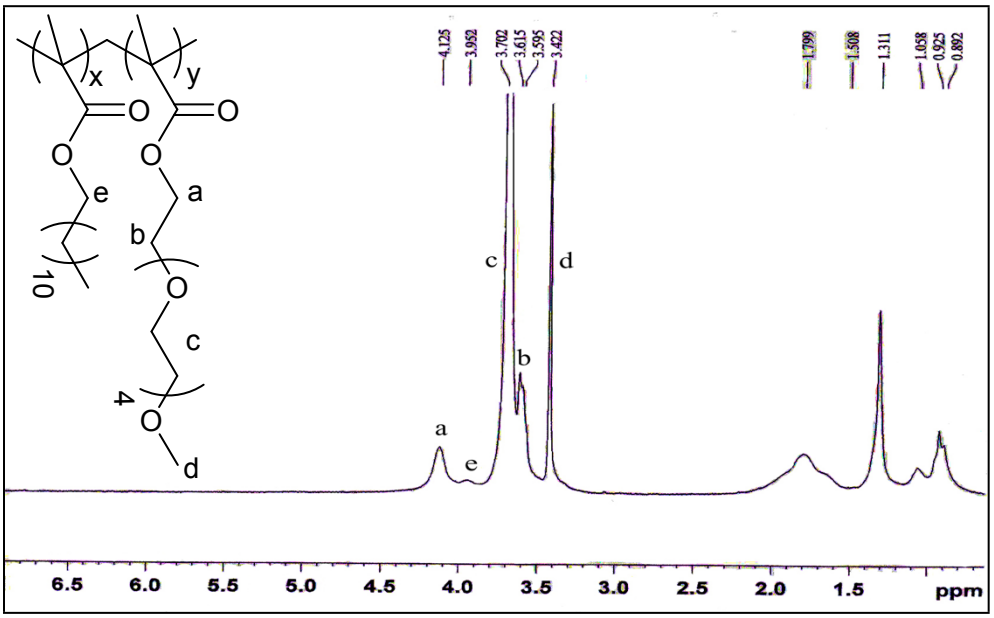



**Figure S3**. $^1$H NMR (400MHz) spectrum of **$P_{14}$** in $CDCl_3$.

**$P_{16}$**

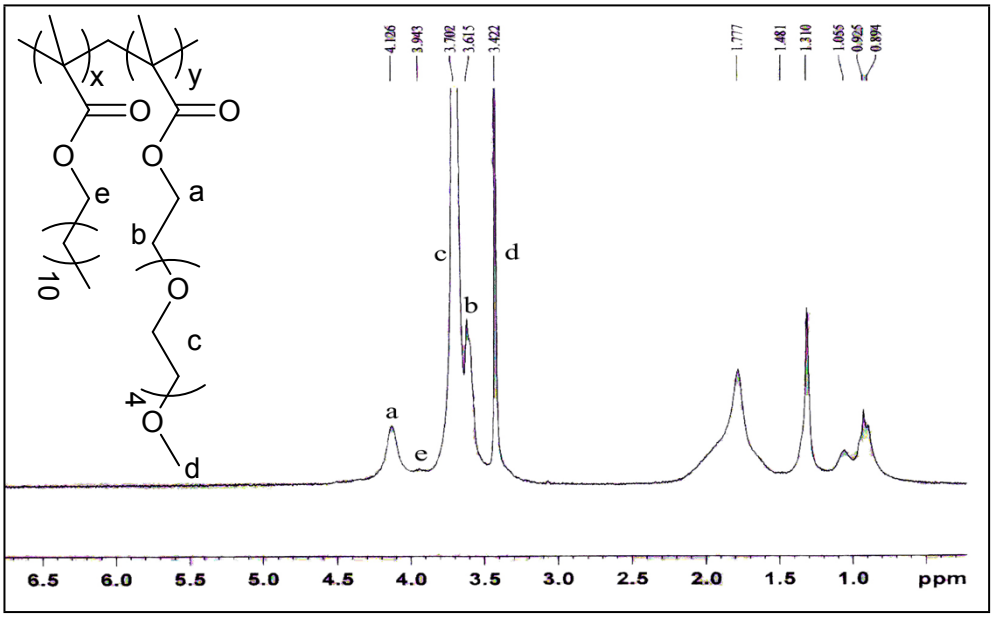



**Figure S4**. $^1$H NMR (400MHz) spectrum of **$P_{16}$** in $CDCl_3$.

## 4. GPC Result of the polymers

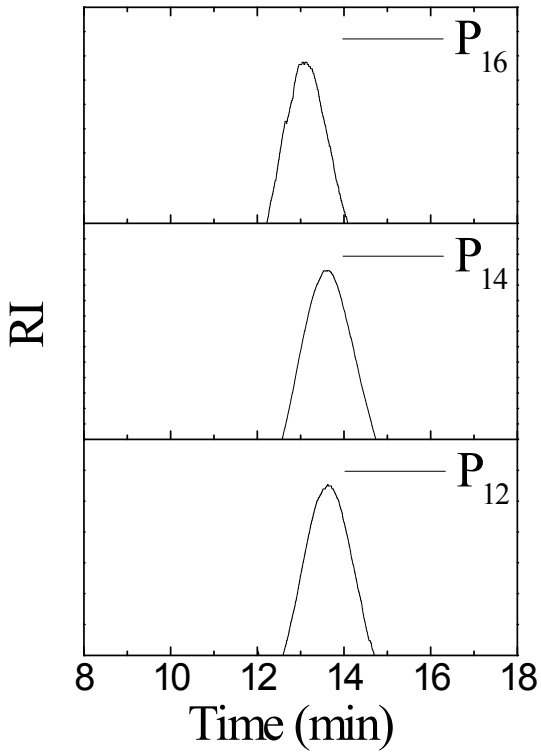



**Figure S5**. Plots of RI (refractive index) vs Time in GPC profile to determine molecular weight.

## 5. Shift of $\lambda_{max}$ of NPN probe

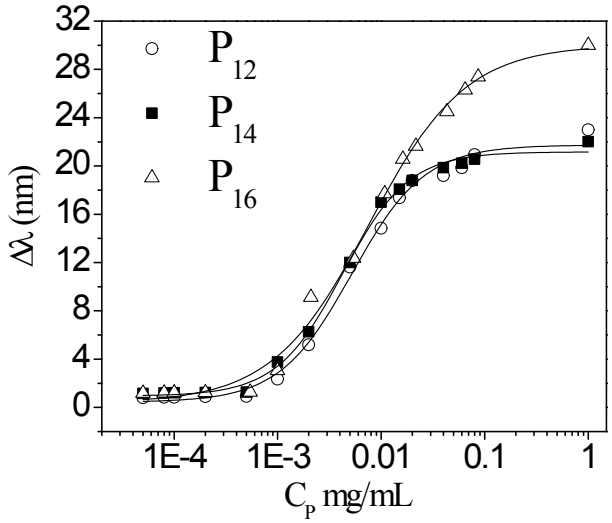



**Figure S6.** Plots of shift of emission maximum [$\Delta\lambda = \lambda_{max}$(water) - $\lambda_{max}$(polymer)] of NPN probe with increasing concentration ($C_p$, mg/mL) of the polymer solutions.

## 6. Hydrodynamic size distribution at different concentrations

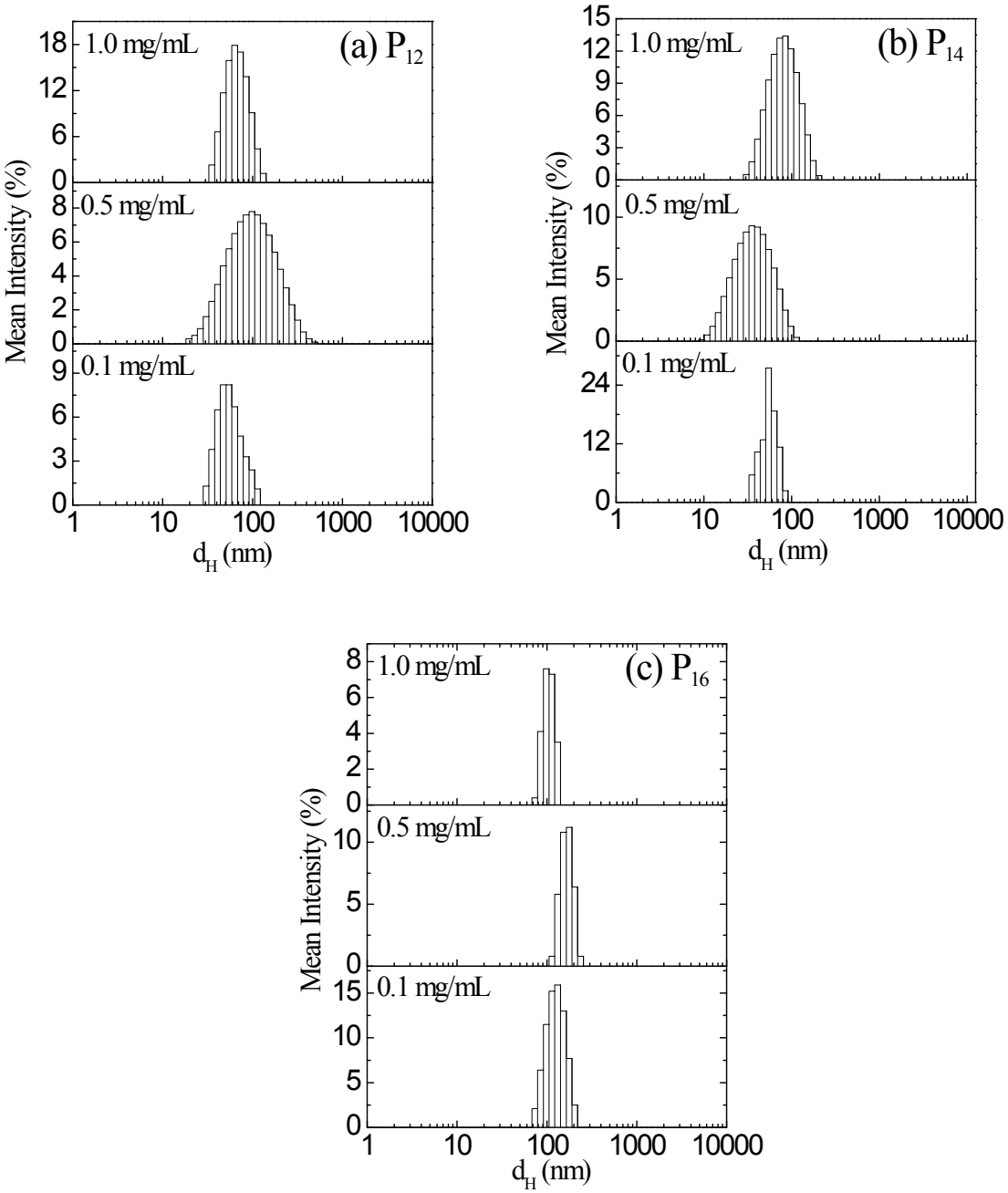



**Figure S7.** Hydrodynamic size (diameter, $d_H$) distribution profile at different concentrations (1.0, 0.5, and 0.1 mg/mL) of the PMs at 298 K.

## 7. Anisotropy measurement using DPH as fluorescent probe

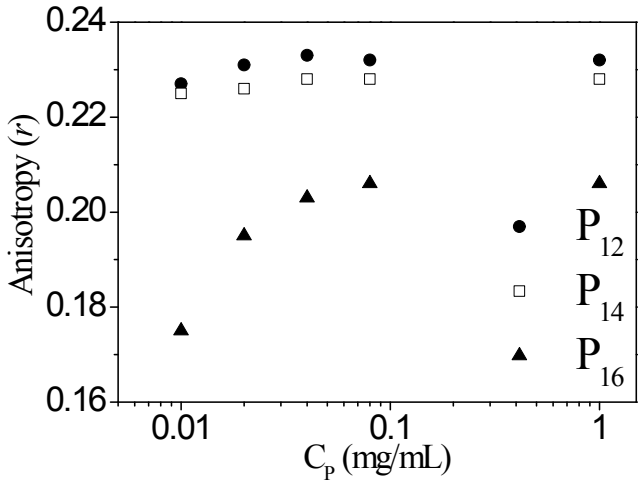



**Figure S8**. Plot of anisotropy (*r*) values at different polymer concentrations ($C_{p,}$ mg/mL) at 25 $^{o}$C.

**Table S1.** Microviscosity ($\eta_m$), and fluorescence lifetime ($\tau_f$) and fluorescence anisotropy (*r*) vales of DPH probe in 1.0 mg/mL polymer solutions.

| **HMPs** | ***r*** **(±0.001)** | **$\tau_f$** **(±0.1) ns** | **$\eta_m$ (mPa s)** |
|---|---|---|---|
| $P_{12}$ | 0.232 | 7.5 | 175 |
| $P_{14}$ | 0.228 | 6.3 | 141 |
| $P_{16}$ | 0.206 | 6.0 | 104 |

## 8. Camptothecin solubilization

The drug-loading content (%) and drug-entrapment efficiency (%) have been calculated by using the following equations-

Drug-loading content (%) = (Weight of the drug in the polymer/Weight of the polymer) x 100

Drug-entrapment efficiency (%) = (Weight of the drug in the polymer/Weight of the drug feed initially) x 100

**Table S2.** Drug-loading content (%) & drug-entrapment efficiency (%) for all the CPT encapsulated polymers ($C_P$ 1.0 mg/mL) in water at 298 K.

| Copolymers | Drug-loading content (%) | Drug entrapment efficiency (%) |
|---|---|---|
| $P_{12}$ | 2.0 | 40 |
| $P_{14}$ | 2.32 | 46.46 |
| $P_{16}$ | 2.96 | 59.3 |

## 9. Fluorescence spectra of Camptothecin (CPT)

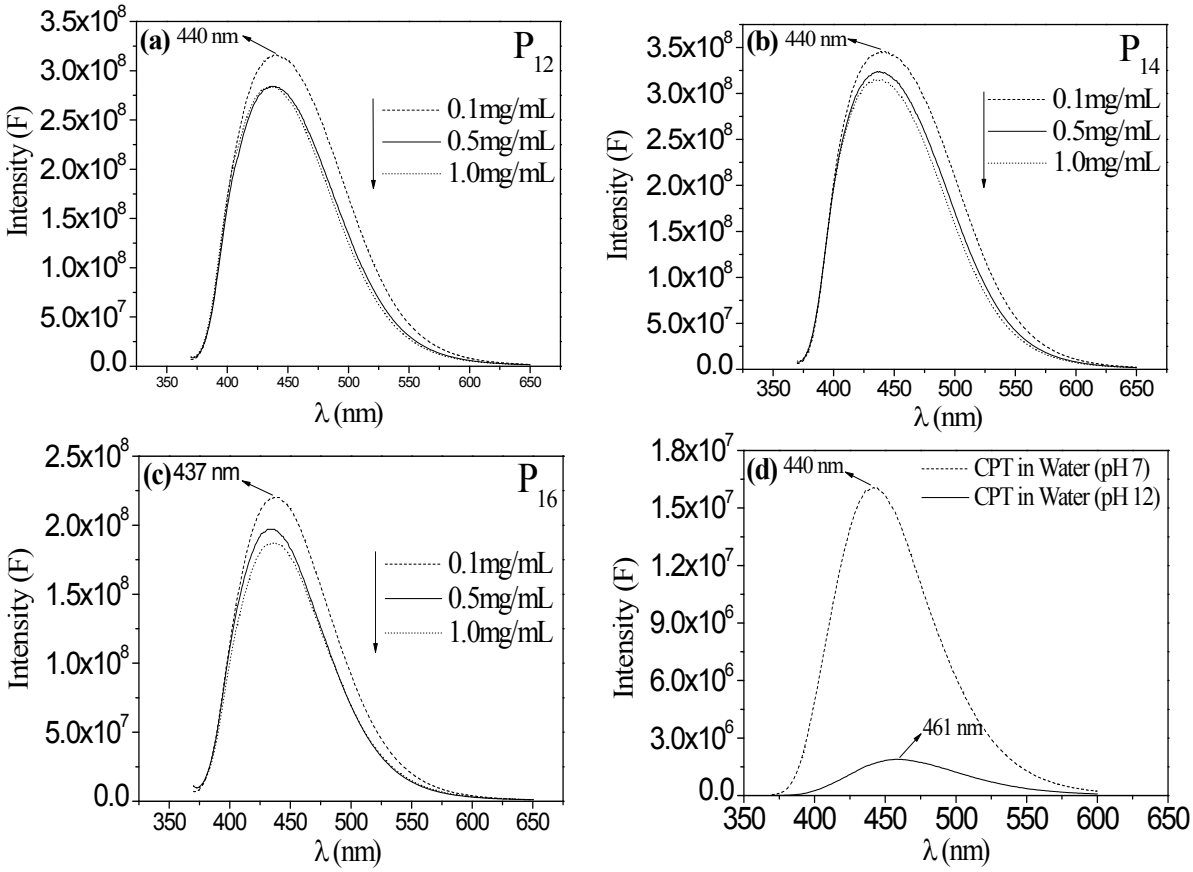



**Figure S9.** Fluorescence Spectra of CPT drug - (a, b, c) in presence of polymers and (d) at different pH.

**10. <u>Thermal stability of the PMs (at lower conc)</u>**

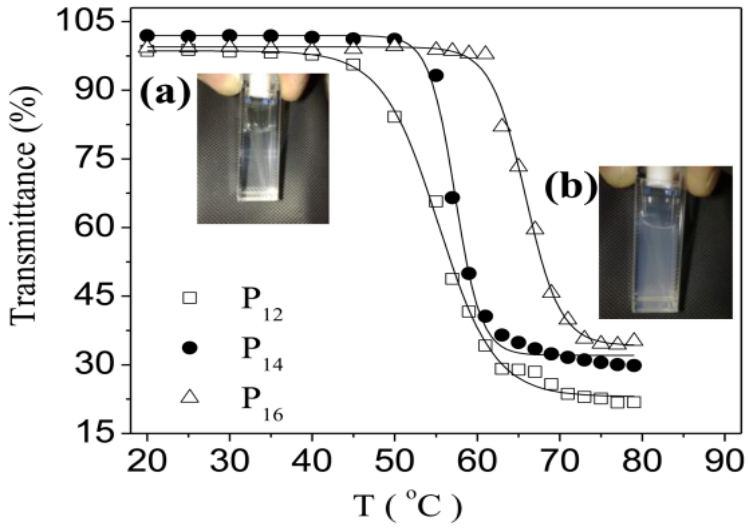



**Figure S10**. Plots of transmittance (%T) as a function of temperature (°C) for 0.5 mg/mL polymer solution in aqueous media. Inset: (a) & (b) are the photographs of the polymer solutions below and above the LCST respectively. At comparatively lower concentration (unlike at 1.0 mg/mL), all the polymer solutions show a very low but not exactly zero transmittance after LCST.

**11. DLS study at different temperature**

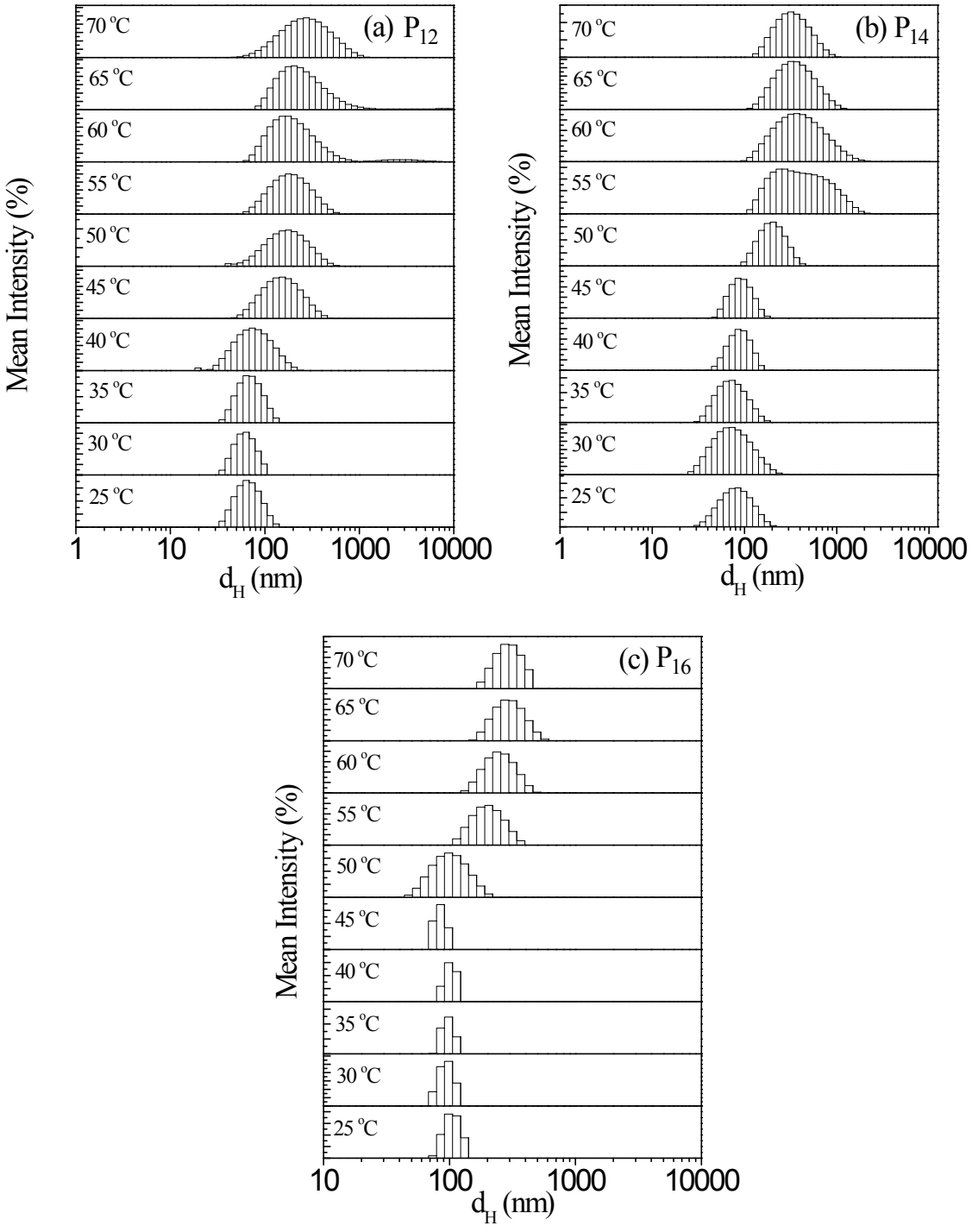



**Figure S11.** Plots of the hydrodynamic size distribution (diameter, $d_H$) at various temperature (25-70 °C) for all the polymers ($C_P$ 1.0 mg/mL) in aqueous media.

## 12. DLS of polymeric aggregates at different pH

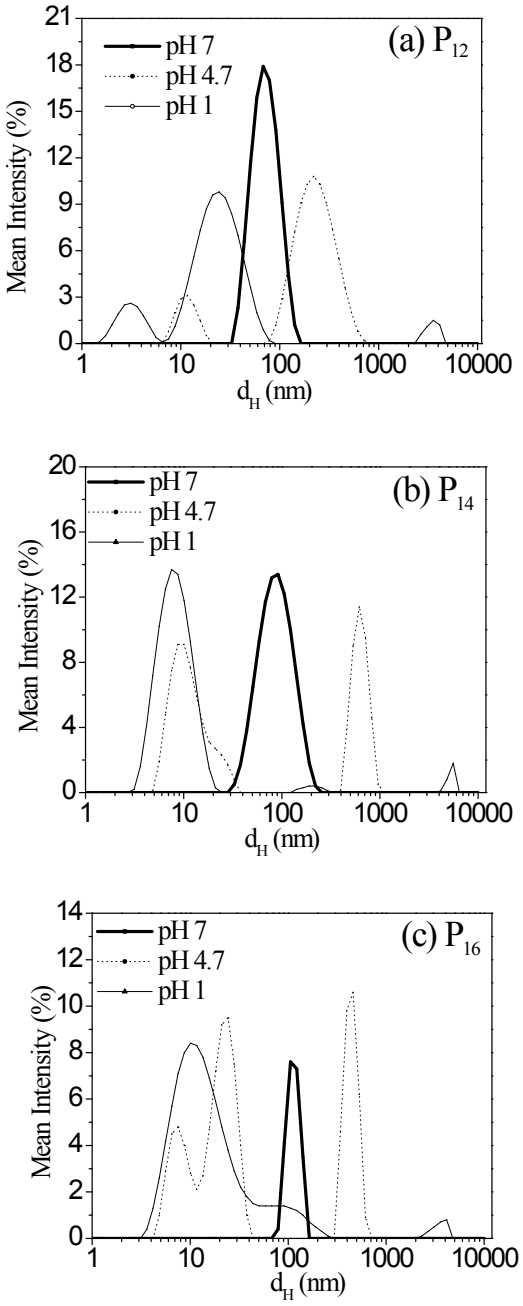



**Figure S12**. Hydrodynamic size (diameter, $d_H$) distribution profile of polymeric micelles at different pH (pH 7, 4.7 and 1) after 14 h of incubation. Concentration of the copolymer ($C_p$) was 1.0 mg/mL for each of the study.

**13. <u>In Vitro Cytotoxicity on L929 (normal cells)</u>**

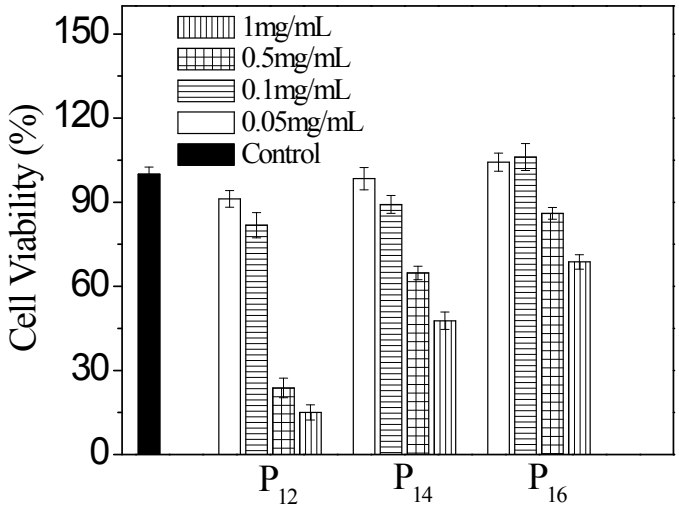



**Figure S13**. Cytotoxicity effects of the polymers on L929 cells. The cells were treated for 24 h with both the polymers at a concentration ranging from 0.05 to 1.0 mg/mL. Cell viability was measured by MTT assay and it was expressed as the percentage of growth with respect to untreated control cells. The data were presented as the mean ± SD.

**Reference**

[1] Perrier, S., Barner-Kowollik, C., Quinn, J. F., Vana, P., & Davis, T. P. Origin of inhibition effects in the reversible addition fragmentation chain transfer (RAFT) polymerization of methyl acrylate. *Macromolecules* **2002**, 35(22), 8300-8306.