

**Supporting Information**

**Sequential amphiphilic and pH responsive hyperbranched copolymer: influence of hyper branching/ pendant groups on reversible self assembling from polymersomes to aggregates and usefulness in waste water treatment**

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## **Materials**

Propargyl acrylate (PA, 98%), divinylbenzene (DVB, technical grade: 80% contains 1000 ppm p-tert-butylcatechol as an inhibitor) and dodecane thiol (DDT,  $\geq 98\%$ ) were purchased from Sigma Aldrich, India. Acrylic acid (AA, 99%), azobisisobutyronitrile (AIBN,  $\geq 98\%$ ), cetyltrimethyl ammonium bromide (CTAB), sodium azide ( $\text{NaN}_3$ ), blue vitriol ( $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ ), L-ascorbic acid, sodium hydroxide pellets (NaOH), ethylenediaminetetracetic acid (EDTA), methylene blue (MB, dye content  $\approx 95\%$ ), congo red (CR, dye content  $\approx 95\%$ ), dimethyl formamide (DMF,  $\geq 99.8\%$ ), diethyl ether, dichloromethane (DCM) and Whatman filter paper no. 40 were received from Merck, India. Initiator AIBN was purified by dissolving in hot methanol and subsequent recrystallization. PA was distilled at reduced pressure to remove any self polymerized masses and stored under refrigeration until use. DVB and AA were passed through cold basic alumina columns to remove inhibitor prior to use. Milli Q water was used in the nucleophilic substitution reaction, for the purification of polymers and for encapsulation studies. All other reagents were used without further purification. Solvents used in the organic syntheses and for the sample purification were of standard laboratory reagent grades.

### **Preparation of linear and crosslinked copolymer of PA and AA**

For synthesis of poly (PA)-co-poly (AA), typically in a 50 ml long necked round-bottom flask, PA (10 m.mol) and AA (10 m.mol) were dissolved in DMF (10 ml). Then AIBN (1 mol% of total C=C moles) was added to the homogenized solution and the resultant mixture was purged with  $\text{N}_2$  for 15 minutes. The polymerization was carried out in a sealed flask at  $75^\circ\text{C}$  for 24 hrs. The polymer ( $\text{P}_{150/50/0/0}$ ) was recovered by precipitation from water and subsequently centrifuged at 4000 r.p.m for 10 mins. Finally  $\text{P}_{150/50/0/0}$  was freeze dried. The dried polymer was further

purified by precipitation from diethyl ether and dried under vacuum at 60°C for an overnight. Yield of P1<sub>50/50/0/0</sub> was measured by gravimetry. In a similar fashion, crosslinked (PA)-co-poly (DVB)-co-poly (AA) (XP1<sub>50/50/2.5/0</sub>) was synthesized in the presence of DVB (2.5 m.mol) which underwent gelation within 2 hrs of the reaction. The gelled polymer was isolated and purified by sequential precipitation/ centrifugation/ re-precipitation from water and diethyl ether.

Texture of P1<sub>50/50/0/0</sub>- yellow sol

Texture of XP1<sub>50/50/2.5/0</sub>- yellow colored gel

### **Procedure for azidation of CTAB**

Typically, CTAB (20 m.mol) was suspended in DMF (25 ml) with NaN<sub>3</sub> (60 m.mol) and heated at 100°C for 24 hrs. The solution turned orange after a stipulated period of time indicating formation of the respective azide. The unreacted masses were removed by filtration through a Whatman filter paper no. 40. To ease the process of drying, the orange solution in DMF was further precipitated from diethyl ether, filtered and dried under vacuum at 40°C for an overnight. The final azide compound was characterized by ESI Mass (Fig. S3), FTIR (Fig. S4) and <sup>1</sup>HNMR (Fig. S5) spectroscopic methods.

MS ESI (35 eV); m/z: 284 & 285, [C<sub>19</sub>H<sub>42</sub>N<sup>+</sup> & C<sub>19</sub>H<sub>42</sub>NH<sup>+</sup>]; IR (KBr, cm<sup>-1</sup>): 2915 & 2845, ν<sub>C-CH<sub>2</sub></sub> asym & sym str; 2099, ν<sub>C-N<sub>3</sub></sub> str; <sup>1</sup>HNMR (CD<sub>3</sub>SOCD<sub>3</sub>, 400 MHz, δ ppm): 3.301- 3.258 (m, 2H, -<sup>α</sup>CH<sub>2</sub>N<sup>+</sup>), 3.053 (s, 6H, head group/HG protons), 1.705-1.646 (m, 2H, -<sup>β</sup>CH<sub>2</sub>), 1.3 (m, 6H, -<sup>γ</sup>CH<sub>2</sub>), 1.243 (s, 20H, main chain/ MC protons/ DMSO), 0.874- 0.84 (t, 3H, -<sup>ω</sup>CH<sub>2</sub>).

### **Procedure for preparation of sodium ascorbate (NaAsc)**

An aqueous solution of L- ascorbic acid was neutralized with equimolar amount of NaOH and the solid product was isolated by freeze drying.

### **Procedure for pH dependant pyrene encapsulation study**

For pH dependant aggregation study, typically the as synthesized and purified P2 or HBP2s (required amount) were suspended in 2 ml of Milli Q water maintained at various pH. The resultant polymeric suspensions were kept undisturbed for 12 hrs. Finally the aggregation was monitored by DLS, zeta potential, FESEM and fluorescence spectroscopy (using pyrene probe).

In order to study the effect of the medium pH on the encapsulation of pyrene molecules by P2<sub>50/50/0/0</sub>, HBP2<sub>50/50/1.25/2.5</sub>, HBP2<sub>50/50/2.5/2.5</sub> and HBP2<sub>50/50/5.0/2.5</sub>, the following procedure was followed. Initially a pyrene stock solution (2 ml, 10<sup>-3</sup> (M)) was prepared in DCM from which 20 µl of the same stock solution was transferred separately to 32 thoroughly cleaned glass vials. After evaporation of DCM from the vials, suspensions constituting 5 ml of 0.5 w/v % of P2<sub>50/50/0/0</sub>, HBP2<sub>50/50/1.25/2.5</sub>, HBP2<sub>50/50/2.5/2.5</sub> and HBP2<sub>50/50/5.0/2.5</sub> maintained at pH ~ 8, ~7, ~6, ~5, ~4.5, ~3 and ~2 were each transferred to the vials containing dried pyrene. The resultant suspensions were left undisturbed for 12 hrs prior to acquisition of emission spectra.

### **Procedure for pH dependant hydrophilic dye encapsulation study**

For determination of chemical structure of HBP2s, two different hydrophilic dyes (positive and negative) were chosen. In a typical process, CR dye stock (20 mM) was prepared in 1 ml of Milli Q water from which 200 µl aliquots of the dye solution were taken in 4 vials each. In the meantime, UV-Vis absorption spectrum of the neat dye was recorded by diluting stock solution of CR by addition of 3 ml of water to one of the vial. 3 ml of the aqueous suspension of HBP2<sub>50/50/2.5/2.5</sub> (0.5 w/v %), each maintained at pH ~ 4, ~ 6 and ~ 8 were then added to the other 3 vials separately containing 20 µl of stock dyes. The final mixtures were allowed to stand for 5 minutes. Finally UV-Vis spectra of the dye loaded HBP2<sub>50/50/2.5/2.5</sub> were recorded. Relative change in absorption intensity of the UV-Vis spectra gave an indication of the hydrophilic dye

encapsulation efficiency of HBP2<sub>50/50/2.5/2.5</sub> in different ranges of solution pH. Dye uptake study with MB dye was also repeated in a similar fashion as that of CR uptake study.

### Procedure for determination of density of P2 and HBP2

For determination of density of P2<sub>50/50/0/0</sub>, HBP2<sub>50/50/1.25/2.5</sub>, HBP2<sub>50/50/2.5/2.5</sub> and HBP2<sub>50/50/5.0/2.5</sub>, we followed the floatation method as all the polymers exhibited powdered textures. In a typical process, at first 25 ml of water was taken in a measuring cylinder. Then exactly 1 gram of the copolymer was added to the water medium. The copolymer formed an aqueous suspension which settled on prolonged standing. At this moment, a liquid having density higher than water (e.g.; glycerol) was added dropwise to the aqueous suspension until the copolymer particles just floated over the water. The final mixture was stirred well and the density of the copolymer was determined using a specific gravity bottle. The following Equation S1 was used to calculate density of the respective copolymers.

$$\rho_{polymer} = \left\{ \frac{(W_3 - W_1)}{(W_2 - W_1)} \times \rho_{H_2O} \right\} \quad (S1)$$

where,  $\rho_{polymer}$  is the density of P2/ HBP2,  $W_1$  is the weight of empty specific gravity bottle,  $W_2$  is the weight of specific gravity bottle + water,  $W_3$  is the weight of specific gravity bottle + solution (after filtration of the copolymers) and  $\rho_{H_2O}$  is the density of water at 30°C (0.99576 g ml<sup>-1</sup>).

Density,  $G_N$  and  $M_e$  of different copolymers are registered in Table S1.

**Table S1.** Estimated  $\rho$ ,  $G_N$  and  $\sigma$  of P2/ HBP2s

Sample	$\rho_{polymer}$ (g ml <sup>-1</sup> ) <sup>a</sup>	$G_N$ (Pa) <sup>b</sup>	$M_e$ (g mol <sup>-1</sup> )
P2 <sub>50/50/0/0</sub>	0.997	0.00634	389611.87
HBP2 <sub>50/50/1.25/2.5</sub>	0.999	5.75	430.45
HBP2 <sub>50/50/2.5/2.5</sub>	1.019	339	7.44
HBP2 <sub>50/50/5.0/2.5</sub>	1.015	0.488	5153.14

<sup>a</sup>)Determined from floatation method; <sup>b</sup>)Determined from rheology curves in the low angular frequency zone; temperature maintained at 30°C

## Characterization

**NMR study.**  $^1\text{H}$ NMR spectra of CTA- $\text{N}_3$ , HBP $_{50/50/2.5/2.5}$  and HBP $_{250/50/2.5/2.5}$  were acquired in  $\text{CD}_3\text{SOCD}_3$  using a Bruker DPX-400 spectrometer operating at 400 MHz.

**ESI Mass Spectrometry.** The ESI mass spectrum of the as synthesized CTA- $\text{N}_3$  was recorded in a quadrupole time-of-flight (Q-TOF) Micro YA263 mass spectrometer.

**FTIR Spectroscopy.** The FTIR spectra of CTA- $\text{N}_3$ , HBP $_{50/50/2.5/2.5}$  and HBP $_{250/50/2.5/2.5}$  were recorded by mixing with KBr in a 1: 120 (w/w) ratio, in transmission mode on a Shimadzu IR affinity- 18000 FTIR spectrometer.

**GPC study.** The number average molecular weight ( $\overline{M}_n$ ) and the poly dispersity index (PDI) of P $_{150/50/0/0}$ , HBP $_{150/50/1.25/2.5}$ , HBP $_{150/50/2.5/2.5}$  and HBP $_{150/50/5.0/2.5}$  were determined by size exclusion chromatography using a WATERS 1515 isocratic HPLC pump connected to two WATERS styragel HR-3 and HR-4 columns (4.6 x 300 mm) and a WATERS 2414 refractive index detector at a temperature of 30°C. HPLC grade DMF was used as the mobile phase with a flow rate of 1 ml min $^{-1}$ . The columns were calibrated against standard poly (styrene) samples of different molecular weights.

**Viscosity measurement.** Intrinsic viscosities of P $_{150/50/0/0}$ , HBP $_{150/50/1.25/2.5}$ , HBP $_{150/50/2.5/2.5}$  and HBP $_{150/50/5.0/2.5}$  were determined from the flow times, recorded using an Ubbelohde viscometer (size 1, number 424 and calibrated as per ASTM D 445) in DMF at a constant temperature of 30°C.

**DLS & Zeta potential measurement.** DLS and zeta potential studies of 0.5 w/v % aqueous solution/ suspension of P $_{150/50/0/0}$ , HBP $_{150/50/1.25/2.5}$ , HBP $_{150/50/2.5/2.5}$  and HBP $_{150/50/5.0/2.5}$  at a pH ~8 and that of P $_{250/50/0/0}$ , HBP $_{250/50/1.25/2.5}$ , HBP $_{250/50/2.5/2.5}$  and HBP $_{250/50/5.0/2.5}$  at different pH (~2, ~3,

~4, ~4.5, ~5, ~6, ~7 and ~8) were performed on a Zeta Sizer NANO Series (Malvern, USA) using a HeNe gas laser operating at a wavelength of 632.8 nm at 25°C.

**PL study.** Steady state fluorescence spectra of pyrene encapsulated in P2<sub>50/50/0/0</sub>, HBP2<sub>50/50/1.25/2.5</sub>, HBP2<sub>50/50/2.5/2.5</sub> and HBP2<sub>50/50/5.0/2.5</sub> were recorded on Shimadzu RF 5301 spectrofluorometer in water upon excitation at a wavelength of 337 nm using a slit width of 2 nm.

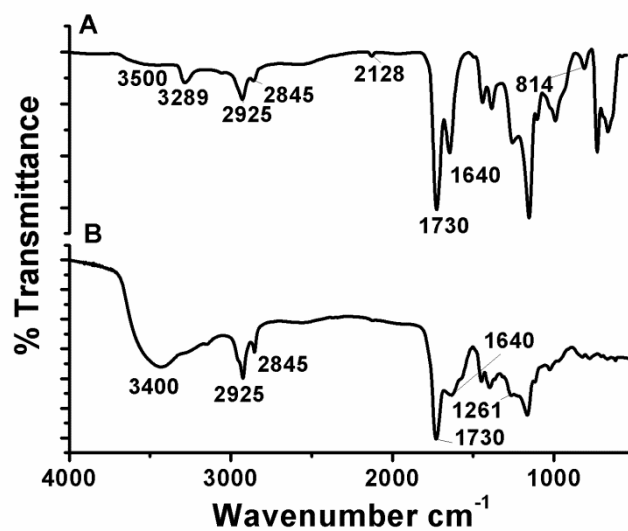
**UV-Vis characterization.** The exact structure of HBP2<sub>50/50/2.5/2.5</sub> was predicted from CR/ MB sequestration studies on a UV-Vis spectrophotometer (CECIL CE7200, 7000 series, UK). All UV-Vis spectra were recorded against air at 25°C from 200 nm to 800 nm using a quartz cuvette of 1 cm optical path length.

**Rheology study.** The linear viscoelastic behavior of the aqueous solutions/ suspensions of P2<sub>50/50/0/0</sub>, HBP2<sub>50/50/1.25/2.5</sub>, HBP2<sub>50/50/2.5/2.5</sub> and HBP2<sub>50/50/5.0/2.5</sub>, was studied using an Anton Paar Rheometer, Physica (MCR-102) on an oscillatory shear mode. We employed cone (1°, 25 mm diameter) and plate for determination of dynamic oscillatory behavior of alkaline (pH 8) and acidic (pH 4) solutions/ suspensions of various copolymers. The angular frequency scan was done between 0.01 and 1 rad s<sup>-1</sup> at a constant temperature of 30°C.

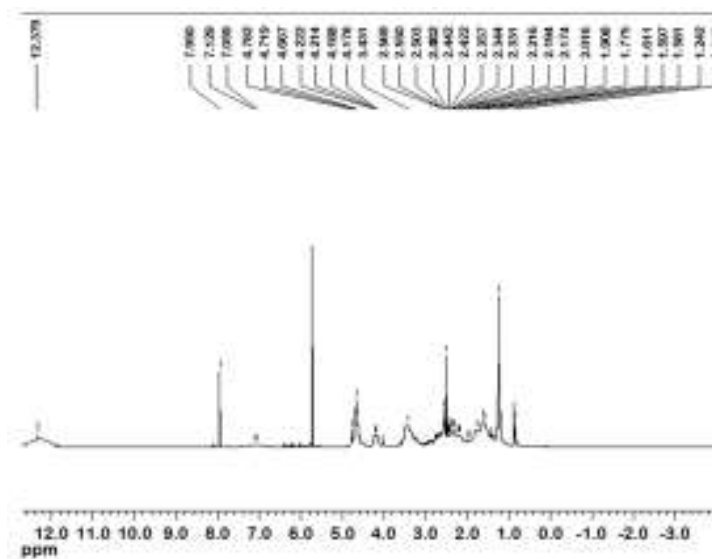
**FESEM & EDX studies.** Thoroughly dried P2<sub>50/50/0/0</sub>, HBP2<sub>50/50/1.25/2.5</sub>, HBP2<sub>50/50/2.5/2.5</sub> and HBP2<sub>50/50/5.0/2.5</sub> samples were suspended in water at pH ~ 4 and ~ 8. After prolonged sonication of the aqueous suspensions, they were drop casted onto cover slips and dried under vacuum for an overnight. Each sample loaded cover slips were then adhered to a metal stub using copper tapes and sputter coated with gold to minimize charging before acquiring images. FESEM images were captured by placing the samples under a ZEISS JSM-6700F electron microscope operating at an accelerating voltage of 15 kV. EDX mapping of dried HBP2<sub>50/50/2.5/2.5</sub> was done

using the EDX attachment to the FESEM equipment in order to analyze its exact elemental composition.

### Additional Figures

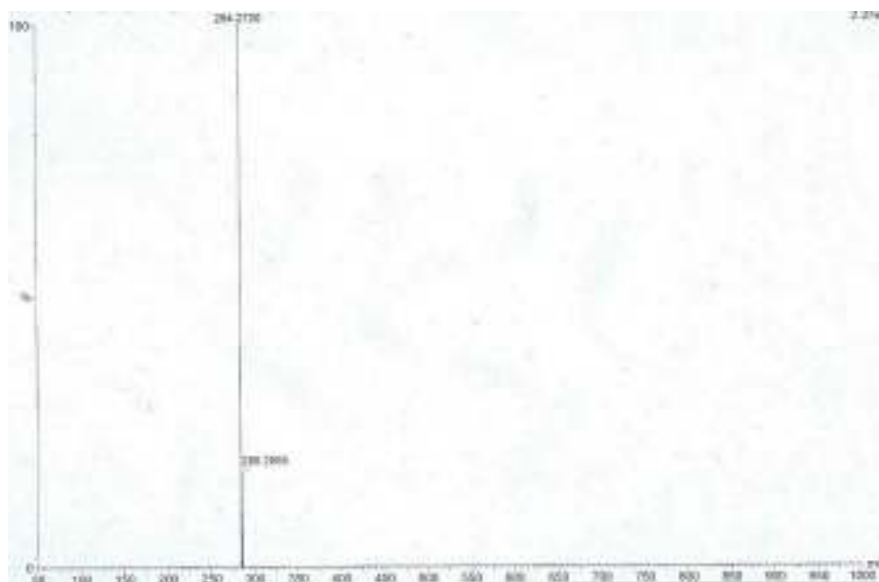


**Figure S1.** The FTIR spectra of A) HBP1<sub>50/50/2.5/2.5</sub> and B) HBP2<sub>50/50/2.5/2.5</sub>

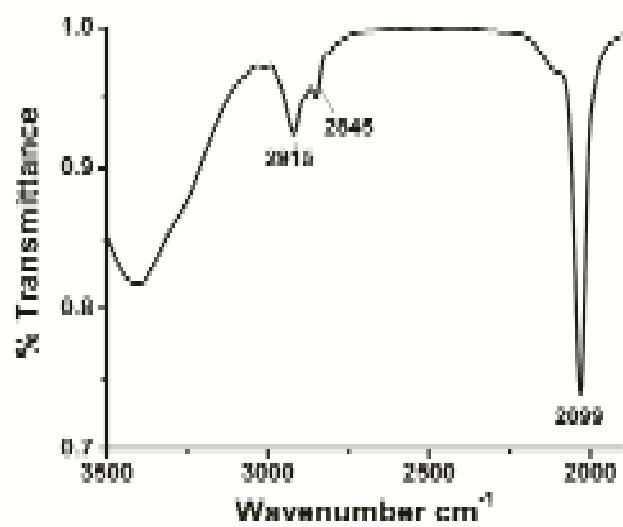


**Figure S2.** The <sup>1</sup>H NMR spectrum of HBP1<sub>50/50/2.5/2.5</sub> in DMSO

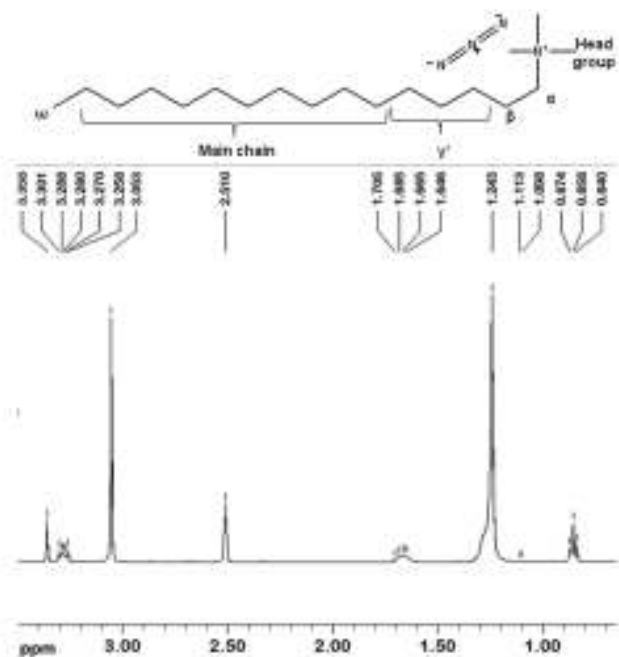




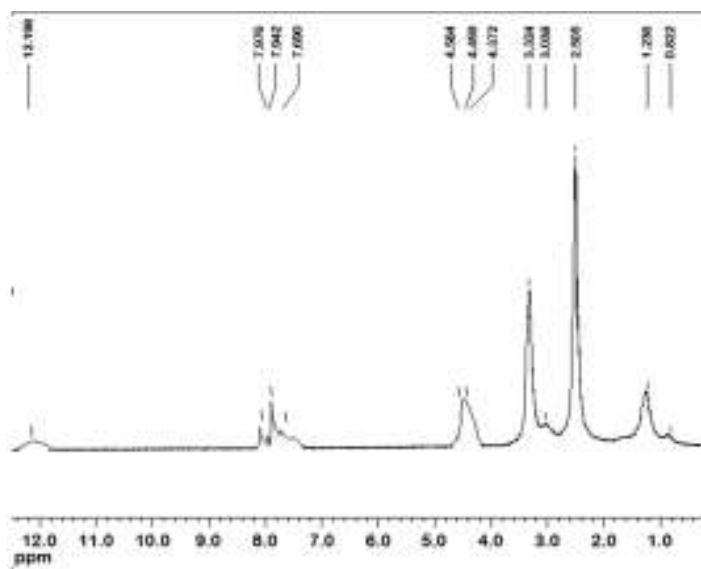
**Figure S3.** The ESI Mass spectrum of CTAN<sub>3</sub>



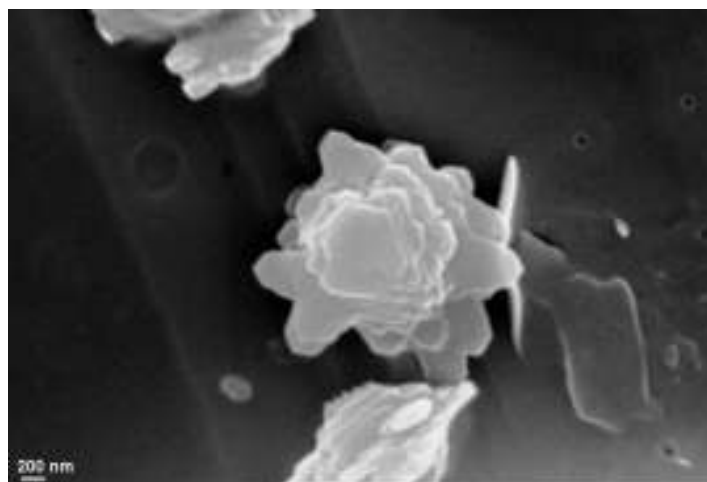
**Figure S4.** The FTIR spectrum of CTAN<sub>3</sub>



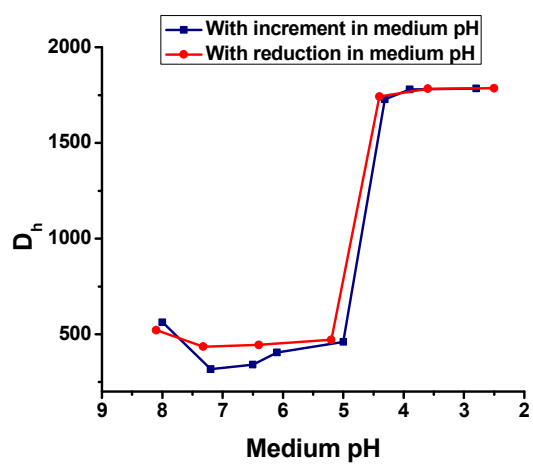
**Figure S5.** The  $^1\text{H NMR}$  spectrum of CTAN<sub>3</sub> in DMSO



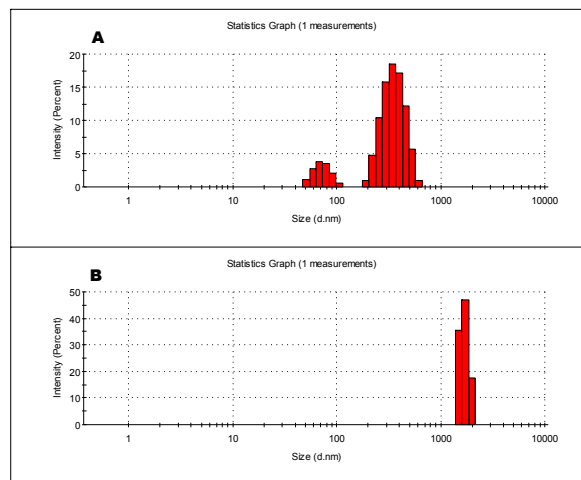
**Figure S6.** The  $^1\text{H NMR}$  spectrum of HBP<sub>250/50/2.5/2.5</sub> in DMSO



**Figure S7.** The FESEM image of HBP1<sub>50/50/2.5/2.5</sub> in DMF



**Figure S8.** Hysteresis loop obtained in  $D_h$  value of HBP2<sub>50/50/2.5/2.5</sub> with reversible change in pH of the aqueous medium



**Figure S9.** Comparison of particle size distribution of A) P2<sub>50/50/0/0</sub> and B) HBP2<sub>50/50/2.5/2.5</sub> at the pK<sub>a</sub> as obtained from the DLS measurement