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A library of layered amphiphilic hyperbranched copolymers capable of reversible self assembling from polymersomes to aggregates is developed by a combination strategy of "Strathclyde method" and click reaction. Owing to a wide difference in reactivity and polarity between the reacting monomers with respect to the reaction medium, almost a sequential and controlled "Strathclyde" polymerization happens (exhibit PDI<1.5 but high MW). In fact, sequential polymerization creates distinct layers of hydrophilic and hydrophobic groups in the copolymers. Further functionalization at the pendant groups induces an optimum balance in hydrophilic and hydrophobic layers which develops self assembling properties in the copolymers in response to pH of the medium. A thorough examination through various experiments (DLS, zeta potential, FESEM and rheology) confirms that an optimized extent of hyper branching and presence of pendant cetyltrimethyl ammnonium triazole ion pairs as well as carboxyl groups play pivotal roles in the formation and subsequent stabilization of the different macromolecular architectures of the copolymers in different ranges of medium pH. The presence of both hydrophilic and hydrophobic groups also creates layers along with an aqueous core in the copolymers at high medium pH for which both hydrophobic and hydrophilic molecules are encapsulated. However, retention of different guest molecules by the copolymers at low pH differs due to increase in hydrophobicity by polymer aggregation. In this work, the new type of hyperbranched copolymer as mentioned is unique to our knowledge and thus may appeal to many researchers in near future. In fact these hyperbranched copolymers are good promising tools for removal of organic wastes from water bodies.

INTRODUCTION

Architecturally unsymmetrical hyperbranched polymers (HBPs) have earned widespread recognition over symmetrical dendrimers owing to ease of synthesis, low cost of production with no special requirement for infrastructure for the latter type.¹ Akin to dendrimers, HBPs feature high solubility in range of solvents, low solution viscosities, high degree of functionality with more void spaces for encapsulation of molecules.^{1, 2} Further functionalization either at numerous chain ends or at pendant groups may induce responsive properties in HBPs against various environments.³ Till date most of the commercialized HBPs are synthesized by step growth polycondensation of AB_n ($n \ge 2$) type monomers wherein high degree of chain end functionality is intrinsically

obtained. Other viable routes to the generation of highly functionalized HBPs include SCVP, ROP, proton transfer polymerization and other living polymerization techniques.⁴⁻⁷ On the contrary, a much cheaper FRP using a balanced ratio of a branching agent (BA) and a chain transfer agent (CTA); usually a thiol ("Strathclyde method")⁸ is less popular in the synthesis of functionalized HBPs as the method yields HBPs with an uncontrolled DB and broad MWD. Despite the shortcomings, the "Strathclyde method" is still followed when hyper branching in vinyl polymers like those based on acrylates is desired on a larger scale. Supramolecular engineering of HBPs prepared by the "Strathclyde method" often proves to be challenging. Yet there are reports of synthesis of amphiphilic HBPs in presence of thiols where responsive pendant groups are present.⁹ Self assembling of such amphiphilic copolymers to vesicles, micelles or aggregates in response to external stimuli, is an important phenomenon which occurs due to differential interaction energy with the solvent surface or due to interaction with varied functional groups through chemical/ physical connectivity.¹⁰ We felt that among the different classes of stimuli responsive HBPs, pH responsive HBPs may prove to be useful in drug/ gene delivery to specific human body parts, glucose sensing, waste water treatment, as

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templates for nanoparticle synthesis etc. Weaver et al successfully developed amphiphilic HBP scaffolds of DEA and PEGMA in presence of EGDMA and DDT/ TG.¹¹ There is another work by the group of Weaver where they developed amphiphilic HBPs of MA and PEGMA with dodecane chain ends.¹² These HBPs assisted in reversible self assembling of highly stable emulsion droplets to kinetically trapped liquid structures (engineered emulsions: self supporting, monolithic emulsion assemblies) through hydrogen bonding at the droplet surface, at a low pH of the medium. To further explore the world of pH responsive HBPs, there is a need to envisage influence of the structure of HBPs on nature of self assemblies and their physical properties (stability, size, encapsulating efficiency etc) as compared to the conventional polymeric scaffolds. Often post polymer functionalization techniques are employed for functionalization of polymer pendant groups. Among the various approaches for post polymer functionalizations, Cu['] catalyzed Huisgen type 1,3-dipolar cycloaddition between azides and terminal alkynes to form stable heterocyclic linker: 1,4- disubstituted 1,2,3- triazole,¹³ commonly known as "click reaction" is considered unsurpassed owing to mild reaction conditions and stoichiometric balance.¹⁴ The beauty of click reactions lies in the fact that both azides and alkynes are inert to various solvents, molecular oxygen and common reaction conditions. In situ prepared Cu^I catalyst by reduction of Cu^{II} salts with NaAsc has gained enormous popularity in the catalysis of click reaction in terms of purity of the catalyst salt.¹⁵ Here we introduce a new strategy by combining the "Strathclyde method" for the synthesis and Cu"/ NaAsc catalyzed click chemistry for post polymer functionalization to develop reversible self assembling amphiphilic and pH responsive HBPs from propargyl acrylate (PA) and acrylic acid (AA) with high pH dependant efficiency for encapsulation of both hydrophobic and hydrophilic molecules; thereby making them potential candidates in treatment of waste water rich in organic wastes.

EXPERIMENTAL SECTION

Preparation of hyperbranched copolymer of PA and AA

In this article, the "Strathclyde method" as developed by the group of Sherrington was modified for the syntheses of

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branched copolymers of PA, AA and DVB (HBP1) using DDT as a CTA.⁸ In a typical case for the synthesis of HBP1_{50/50/2.5/2.5}, PA (10 m.mol), AA (10 m.mol), DVB (2.5 m.mol) and DDT (2.5 m.mol) were dissolved in DMF (10 ml), taken in a 50 ml long necked round-bottom flask. Then AIBN (1 mol% of total C=C moles) was added to the homogenized solution which was further kept under a blanket of N_2 atmosphere for 15 mins. The polymerization was carried out at 75°C for 24 hrs to ensure high degree of conversion as in the FRP of HBPs; the monomer conversion is generally poor. The entire polymerization process is depicted in Scheme 1. HBP1_{50/50/2.5/2.5} was precipitated from water, centrifuged and freeze dried. Further the dried polymer was precipitated from diethyl ether and dried under vacuum at 60°C for an overnight to yellow coloured sol. Yield of HBP1_{50/50/2.5/2.5} was measured by gravimetry and further characterized by FTIR (Fig. S1A, S.I) and ¹HNMR (Fig. S2, S.I) spectroscopic methods.

IR (KBr, cm⁻¹): Broad shoulder around 3500, v_{O-H} str; 3289, v_{C=CH} str; 2925 & 2845, v_{C-CH2} asym & sym str; 1730, v_{COOR} str; 1640, v_{COOH} str; 814, v_{Ar-p-CH=CH2} str. ¹HNMR (CD₃SOCD₃, 400 MHz, δ ppm): 12.379 (s, 1H, -COOH), 7.95 (d, 4H, Ar-H when DVB unit free at para position), 7.059 (d, 4H, Ar-H when DVB unit is crosslinked), 4.782-4.667 (t, 2H, Ar-CH=CH₂ of free DVB end), 4.222-4.178 (m, 2H, -CH₂ of polymer backbone), 3.434 (d, 1H, Ar-C(H)(CH₂-)(CH₂-)), 2.569-2.55 (d, 1H, -C=CH of propargyl moiety), 2.5 (d-DMSO), 2.462-2.422 (t, 2H,-^αCH₂ of DDT), 2.357-2.331 (t, 2H,-⁸CH₂ of DDT), 1.775 (d, 1H, Ar-CH=CH₂ of free DVB end), 1.611 (m, 1H, -SH of DDT), 1.597 (s, 1H, R₃-CH of DVB), 1.242 (m, 18H,-^Y(CH₂)₉ of DDT), 0.854 (m, 6H, -CH₃ of DDT).

The above mentioned procedure was followed for the syntheses of other compositions of HBP1 copolymers, varied in the ratio of BA to CTA as reported in Table 1.

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Table 1. Molecular characterization data of linear poly (PA)-co-poly (AA) and hyperbranched poly (PA)-co-poly (DVB)-co-poly (AA)

Sample	PA: AA (m.mol feed ratio)	DVB: DDT (m.mol feed ratio)	Yield (%) ^ª	$\overline{M_n}$ x 10 ⁵ / PDI [g mol ⁻¹ /-] ^b	[Ŋ] of P1/ HBP1 [dl g ⁻¹] ^c	α	pK _a of P1/ HBP1 ^d	D _h of P1/ HBP1 [nm] ^e	ξ of P1/ HBP1 [mV] ^e
P1 or P1 _{50/50/0/0}	50: 50	-	93.02	4.24/ 1.02	0.754	0.71	4.50	820.7	-50.1
HBP1 _{50/50/1.25/2.5}	50: 50	1.25: 2.5	83.10	3.52/ 1.07	0.398	0.67	4.58	397.3	-49.0
HBP1 _{50/50/2.5/2.5}	50: 50	2.5: 2.5	85.31	3.57/ 1.08	0.195	0.58	4.51	363.0	-49.3
HBP1 _{50/50/5.0/2.5}	50: 50	5.0: 2.5	79.10	2.66/ 1.20	0.097	0.63	4.62	128.0	-48.9

^{a)}Yields of polymers were measured by gravimetry; ^{b)}Determined from GPC analysis; ^{c)}Experimental data for polymers dissolved in DMF at a constant temperature of 30°C; determined from [Ŋ] value. Data were obtained by extrapolation after linear curve fitting on the original data points and value of α determined using Mark-Houwink equation ^{d)}Determined from change in ξ value; ^{e)}Experimental data for 0.5 w/v aqueous solutions of various compositions of P1/ HBP1 maintained at pH ~ 8.0. The following abbreviations denote $\overline{M_n}$ – Number average molecular weight, PDI- Poly dispersity index, α - Mark- Houwink parameter, [Ŋ]- Intrinsic viscosity, pK_a-Acid-base transition, D_h- Average particle size obtained from DLS and ξ - Zeta potential value. Notably owing to insolubility of XP1 or XP1_{50/50/2.5/0} in every known solvent, no experimental data are furnished in Table 1.

Functionalization of linear and hyperbranched copolymers of PA and AA using Cu^{II}/ NaAsc catalyzed click reaction

In a typical case, alkyne functionalized P1 or HBP1 (P1/ HBP1-C=CH, 200 mg where the concentration of alkyne group was 10 m.mol, as determined theoretically) and cetyltrimethylammonium azide (CTA-N₃) (3.2655 g, 10 m.mol) were suspended in t-BuOH/H₂O (1:2 v/v, 30 ml) mixture. CuSO₄.5H₂O (2.5 g, 10 m.mol) and sodium ascorbate (NaAsc) (1.98g, 10 m.mol) were then added. The resultant suspension was heated at 60°C for 48 hrs. The solid product was isolated by centrifugation, washed several times by successive centrifugation/ redispersion cycles with deionized water. The product was further purified by dialysis against EDTA solution (0.2 M) and subsequently against Milli Q to remove any entrapped Cu salt from the surface of the final polymer. Subsequently the product was freeze dried and grounded into fine green powder. HBP2 $_{50/50/2.5/2.5}$ was characterized by FTIR (Fig. S1B, S.I) and ¹HNMR (Fig. S6, S.I) spectroscopic methods.

IR (KBr, cm⁻¹): Sharp shoulder around 3400, v_{N-H} str; 2925 & 2845, v_{C-CH2} asym & sym str; 1730, v_{COOR} str; 1640, v_{COOH} str; 1261, v_{N-N} str of triazole.¹HNMR (CD₃SOCD₃, 400 MHz, δ ppm): 12.198 (s, 1H, -COOH), 7.976- 7.945 (m, 4H, Ar-H of DVB unit), 7.69 (s, 1H, Ar-H of triazole unit), 4.584- 4.372 (m, 2H, -CH₂ of polymer backbone), 3.324 (m, 2H, -^αCH₂N⁺ of CTA unit), 3.038 (s, 6H, head group protons of CTA unit), 2.505 (d-DMSO/ ^αCH₂ of DDT), 1.236 (s, 20H, main chain protons of CTA unit).



Scheme 1. Scheme for the synthesis of HBP1 and HBP2

RESULTS AND DISCUSSIONS

Branched poly (PA)-co-poly (DVB)-co-poly (AA); HBP1 copolymers with varying quantities of branching were prepared by FRP in the presence of AIBN and DDT in DMF at 75°C for 24 hrs (Scheme 1). Use of a balanced ratio of DDT and DVB in the presence of comonomers controlled the kinetic chain lengths of the polymers and thereby prevented premature crosslinking by introducing fewer branch points as compared to the crosslinked analogue. We prepared a linear copolymer (P1, follow S.I), a crosslinked analogue (XP1, follow S.I) and three other different sets of HBPs (HBP1s) by varying ratio of DVB to DDT as mentioned in Table 1. Each HBP1 were readily soluble in CH₃OH, DMF, DCM, (CH₃)₂CO, CHCl₃, Ph-CH₃, DMSO and alkaline H₂O which indicate that the copolymers were indeed branched but not crosslinked. Studies of FTIR (Fig. S1A, S.I) and 1 HNMR (Fig. S2, S.I) spectra of HBP1_{50/50/2.5/2.5} revealed the presence of some major groups like -C=CH (v: 3289 cm⁻¹ and δ : 2.569-2.55 ppm), -COOH (v: 1640 cm⁻¹ and δ : 12.379 ppm), aromatic part of the DVB (v: 814 cm⁻¹ and δ :

7.59/ 7.059 ppm) and DDT (δ: 1.611 ppm for SH and 1.242 ppm for $^{\gamma}(CH_2)_9$). Significant signals around δ : 4.782-4.667 ppm reflected the presence of methylene hydrogens of pendant unreacted vinyl groups derived from the DVB units in which only one vinyl group per DVB underwent polymerization. This definitely suggests that most of the DVB was incorporated as branching units in HBP1_{50/50/2.5/2.5} as the presence of unreacted vinyl comonomers was negligible in the copolymer (as the polymerization was continued for an extended time and also the polymer was thoroughly purified prior to characterization). Moreover, the signal at δ : 1.597 ppm attributed to tertiary hydrogens of the DVB unit which again indicates successful attachment of DVB to HBP1. Gravimetric yields of P1/ HBP1 (Table 1) decreased along introduction as well as increasing the concentration of DVB. GPC results (Table 1) further revealed that $\overline{M_n}$ of P1 was slightly higher than that of HBP1s, as in the former case higher monomer conversion to polymer (93.02%) was possible owing to the absence of DDT. In fact, on increasing the amount of branching in HBP1s (as expected from the increasing DVB feed), $\overline{M_n}$ decreased. Owing to the presence of DDT and increasing amount of DVB in a

polymerization system (which furnished a greater number of free radicals with different reactivity), even in the initial stage, the termination rate was greater than the propagation rate. Hence, both the gravimetric yield and $\overline{M_n}$ of HBP1s decreased with the increasing quantity of branching. Interestingly, unlike the conventional HBPs synthesized by FRP, MWD of HBP1s were quite narrow (PDI < 1.5) but $\overline{M_n}$ was also quite high in spite of faster termination. Often hyper branching in polymers broadens MWD.¹⁶ It may be possible that in our case during polymerization, the growing HBP1s might have self assembled to a core-shell architecture owing to a marked difference in polarity between the poly (PA)-DVB chains and the poly (AA) chains with respect to DMF wherein the poly (PA)-DVB chains resided inside the core of the assembly. The FESEM image (Fig. S7, S.I) of HBP1_{50/50/2.5/2.5} showed that the polymer chains were indeed arranged in a definite fashion (some polymer chains were more exposed towards the surrounding medium whereas other polymer chains were at the core). Such a core-shell morphology might have caused narrowing of the MWD of HBP1 in DMF as claimed in the work of Weaver et al.¹¹ Narrow PDI also suggests absence of any homopolymers in the system. Nevertheless, MWD of HBP1s slightly broadened (still PDI < 1.5) when the extent of branching increased. On the contrary, unlike the conventional "Strathclyde method" for the synthesis of HBPs, quite high conversion of monomers to HBP1s happened owing to the high reactivity ratio of both PA and AA monomers for which all HBP1s exhibited high $\overline{M_n}$. Often [n] is considered to predict hyper branching in polymers. Measured [n] (Table 1) of HBP1s were remarkably lower than P1. We know that [n] are inversely related to the compactness of random coils of a polymer. Thus lower [n] suggest that HBP1s were more compact than P1. Actually at infinite dilution, polymer-solvent interaction is less influenced by any undesirable polymer inter chain entanglements and overlapping which otherwise restricts mobility of the polymer chains in the solution phase.¹⁷ Thus, lower [ŋ] of HBP1s indicate that the individual polymer chains occupied smaller space (more globular structure) than P1 (extended structure) and thus experienced lesser friction to move in the solvent. α (Table 1) of HBP1s were also lower than P1. Even α decreased with the increasing amount of DVB in HBP1s which indicates that greater the extent of branching, greater was the compactness of the copolymer. Moreover, lower [ŋ] (irrespective of any soluble medium) means smaller D_h of a single polymer chain (for more coiled structure) which in turn obviously causes lower resultant D_h of the polymer.^{18, 19} DLS measurements (Table 1) revealed that in an alkaline medium, P1 was more extended (exhibited higher D_h) than HBP1s and with the increasing extent of branching, D_h decreased owing to more coiling of the polymer structures. We further analyzed the surface charge distribution on P1 and HBP1s in an alkaline medium. Due to the presence of COO⁻ groups in P1/ HBP1s, §

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values were highly negative at high pH. On increasing acidity of the medium, $\boldsymbol{\xi}$ gradually increased and became positive at a certain pH, which we consider as the acid- base transition point (pK_a) of the copolymer. pK_a of P1 and HBP1s as registered in Table 1 resembled pK_a of poly (AA). Hence, P1 and HBP1s were pH sensitive. However, none of them formed any structure in the aqueous phase in the entire range of medium pH. This was presumed as there was no sharp jump in D_h and ξ at the pK_a of the respective copolymers. This might be due to insufficient difference in polarity between the poly (PA)-DVB chains and the poly (AA) chains with respect to water at the acid-base transition point for inducing any self assembling property to the copolymers (however HBP1 formed a core-shell structure in DMF as mentioned above). Hence, in an attempt to develop self assembling properties into HBP1s in aqueous media, we further enhanced hydrophobicity of the poly (PA)-DVB chains by functionalization of alkyne moieties with CTAN₃. Post polymer functionalization was carried out by a cycloaddition reaction between P1/ HBP1s and CTAN₃, in the presence of equimolar ratio of Cu["] and NaAsc reductant in t-BuOH/ H₂O (1:2) medium at a temperature of 60°C for 48 hrs (Scheme 1). Samples P1_{50/50/0/0}, HBP1_{50/50/1.25/2.5}, HBP1_{50/50/2.5/2.5} and HBP1_{50/50/5.0/2.5} after functionalization were designated as P2_{50/50/0/0}, HBP2_{50/50/5.0/2.5} HBP2_{50/50/1.25/2.5}, HBP2_{50/50/2.5/2.5} and respectively. The intermediate CTAN₃ was synthesized from a nucleophilic substitution reaction between CTAB and NaN₃ in DMF (see the S.I for the synthesis protocol and characterization details). From ESI MS (Fig. S3, S.I), FTIR (Fig. S4, S.I) and ¹HNMR (Fig. S5, S.I) spectra of CTAN₃, successful attachment of N_3^- groups to $C_{19}H_{42}N^+$ (CTA) groups were confirmed. On introduction of a long alkyl chained triazole groups, P2 and HBP2s became insoluble in various organic solvents except DMSO and alkaline H₂O. FTIR spectrum (Fig. S1B, S.I) of HBP2_{50/50/2.5/2.5} revealed complete disappearance of peak at 3289 cm⁻¹ and 2128 cm⁻¹ which otherwise indicate absence of $-C \equiv CH$ and N₃ groups respectively. Rather the characteristic peak for triazole moiety at 1261 cm⁻¹ was observed in the FTIR spectrum. Again, the characteristic signals at δ : 3.324 ppm for -^{α}CH₂, 3.053 for head group (HG) and 1.243 ppm for main chain (MC) of CTA group, at δ : 7.976- 7.942/ 7.690 ppm for aromatic hydrogens of DVB and triazole moiety and at δ : 12.198 ppm for –COOH groups of poly (AA) chains were observed in the ¹HNMR spectrum (Fig. S6, S.I). Hence, all the structural characterization data of HBP2 envisage successful formation of poly (1-cetyltrimethyl ammonium-1,2,3- triazol- 4-yl) methacrylate)-co-poly (divinylbenzene)-copoly (acrylic acid)/ poly (CTA-Tz acrylate)-co-poly (DVB)-co-poly (AA).



Figure 1. A) Hydrodynamic diameter & B) Zeta potential of $P2_{50/50/0/0}$, HBP $2_{50/50/1.25/2.5}$, HBP $2_{50/50/2.5/2.5}$ & HBP $2_{50/50/2.5/2.5}$ & HBP $2_{50/50/2.5/2.5}$ & HBP $2_{50/50/2.5/2.5}$ with the set of the

To study the aqueous solution behavior of P2 and HBP2s, 0.5 mg ml-1of each of the copolymers was initially dissolved in 0.1% aqueous NaOH solution. DLS results showed that in an alkaline medium, D_h of P2 and HBP2s were in the range of 200-550 nm (Fig. 1A). On decreasing the medium pH of P2, D_h hardly changed below the pK_a. However, for all the HBP2s, D_h exhibited a sharp jump (almost by 4-4.5 times) at a particular pH of the medium which corresponds to pK_a of the respective copolymers. Even ξ of HBPs switched from ~ -30 mV to ~ +10 mV at the respective pK_a. From the combined results of DLS and zeta potential, we may infer that some aggregates might have formed at the pK_a of the respective copolymers. However, in case of P2, the aggregates formed at the pK_a were hardly stable and thus broke. Hence, D_h of P2 remained almost constant over the entire range of medium pH. On the contrary, HBP2s formed highly stable aggregates at their pK_a. Interestingly, when pH of the aqueous medium was gradually raised, D_h fell sharply at the pK_a of $HBP2_{50/50/2.5/2.5}$ and then became constant (a hysteresis loop was observed in Fig. S8, S.I). This indicates reversible nature of the hierarchical structures which formed through physical interactions.

To further establish that the hierarchical structures of the pH sensitive copolymers were stable only in case of HBP2s and not in P2, we compared the nature of the respective DLS graphs at

their pK_a. In Fig. S9 (S.I), it is vividly seen that PSD of HBP2_{50/50/2.5/2.5} was much narrower than that of P2 at the pK_a. Such narrow PSD is possible only when all the polymer particles are aggregated in a definite fashion with similar resultant particle size. This also suggests that a definite number of polymer particles might have formed the structures and kept them in suspensions (exhibited quite high + ξ below the pK_a which otherwise indicates high stability of the aqueous suspensions). Thus, in HBP2s, polymer particles aggregated to a definite size depending upon the difference in polarity between the poly (CTA-Tz acrylate)-DVB chains and the poly (AA) chains in the aqueous phase at the pK_a. Hence we may claim that hyper branching in copolymers induced a wide difference in polarity among the polymer chains with respect to an aqueous medium and stabilized any developing structure. The proposed fact was further supported by comparing the results of FESEM studies of P2 and HBP2s above and below the respective pK_a.



Figure 2. FESEM images of A) P2_{50/50/20} B) HBP2_{50/50/125/2.5} C) HBP2_{50/50/2.5/2.5} and D) HBP2_{50/50/2.5/2.5} above the pK_a of the respective copolymers (at pH ~ 8); scale bar of A) 200 nm, B, C, D) 100 nm

FESEM images (Fig. 2) clearly depicted that above the pK₂ of P2 and HBP2s, all the samples exhibited flower like morphology. Above the pK_a, carboxyl groups of the poly (AA) chains were negatively charged (consider Table 1 for ξ values) and hence made the resultant copolymers quite hydrophilic (or polar with respect to alkaline water); thus soluble in alkaline water. It is highly probable that at high pH, there might have been a competition between free OH⁻ groups (present in water) and Tz- groups (present in P2/ HBP2s) to exist as ion pairs with the positively charged hydrophilic HGs of CTA. Hence, CTA groups were probably more oriented towards the surrounding aqueous phase from the surface of the copolymers. These CTA groups thus appeared as petals of the flower like structures. Now that MCs of CTA of poly (CTA-Tz acrylate) chains being hydrophobic would definitely be attached to majority of the DVB segments which in turn formed a hydrophobic layer before being attached to the poly (AA) segments (as if a sequence was maintained in the random copolymer). Again, the poly (AA) chains being hydrophilic and highly negatively charged probably formed the inside part of the spherical

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flowers of HBP2s. There is a high chance that the inner hydrophilic segments of the poly (AA) chains might have encircled an aqueous pool. However, such a hypothetical aqueous pool at the heart of the copolymer could not exist in P2. This is best explained by the fact that the extent of hyper branching played a crucial role in affecting the morphology of the copolymers. Owing to the absence of branching in P2, the flower like morphology was much elongated like tuberoses (Fig. 2A). However, as the extent of hyper branching increased in HBP2s, the flower like morphology became more spherical and distinct due to increased stability (Fig. 2B-D). Thus, the presence of an aqueous phase at the core of a copolymer is more possible in the spherical structures (HBP2) rather than in the elongated structures (P2). Interestingly, the CTA petals also became thinner (yet did not disappear) with increasing hydrophobicity by the DVB units. We believe that with increasing hydrophobicity in HBP2s, CTA groups were less exposed to the surrounding water medium. We also observed that the flower like morphology of P2 and HBP2s remained intact until the respective pK_a transition points were reached.



Figure 3. FESEM images of A) P2_{50/50/10} B) HBP2_{50/50/1.25/2.5} C) HBP2_{50/50/2.5/2.5} and D) HBP2_{50/50/5.0/2.5} below the pK_a of the respective copolymers (at pH ~ 4); scale bar of A, C) 200 nm, B, D) 1 μ m

Finally, below the pK_a of the respective copolymers, we observed that the flower like structures transformed into aggregated particles (Fig. 3). Absence of DVB in P2 indeed did not stabilize the aggregates that formed below the pK_a (Fig. 3A clearly shows that polymer particles were shapeless). However, increasing the DVB content stabilized any aggregates that formed below the pK_a (Fig. 3B-D shows polymer particles with definite shape clustered in the aggregates). In fact, hyper branching in the copolymers not only helped in stabilization of the aggregates but also affected the morphology of polymer particles present in the aggregates. Increasing the extent of hyper branching in HBP2s changed the polymer particle shapes from nearly spherical to spherical and finally rods. Undoubtedly, below the pKa, the poly (AA) chains got protonated and caused hyper coiling of the polymer structures which is the characteristic property of poly (AA) at low pH.²⁰ However, as already observed from the DLS and zeta potential

results that the transition from extended polymer structures to polymer aggregates were abrupt (in case of HBP2s), we infer that protonation of the poly (AA) chains increased hydrophobicity of the resultant copolymers by sudden collapsing of the aqueous core. The conjecture established regarding the presence of a hypothetical aqueous core at high pH and then sudden collapsing of the same at low pH (in case of HBPs only) was supported later by encapsulation studies with two oppositely charged hydrophilic dyes.

P2 already contained hydrophobic segments. Introduction and increment of the DVB segments in the mentioned copolymer undoubtedly enhanced the degree of hydrophobicity. This variation in the hydrophobic environment of HBP2s with the changing pH of the aqueous medium was further analyzed by PL study using a hydrophobic probe (pyrene). Changes in relative intensities of the first and third vibrational bands (I1 at 373 nm and I₃ at 383 nm) of pyrene molecules are very useful in determining the extent of hydrophobicity of a hydrophobic material in an aqueous medium.¹¹ Owing to intrinsic hydrophobicity, some pyrene molecules got encapsulated within P2 and thus we observed a peak at 383 nm (I_3) in the normalized pyrene emission spectra and the intensity of the peak I₃ remained constant irrespective of the medium pH (Fig. 4A). However, we observed intriguing results as hyper branching was introduced into the copolymers. In case of HBP2_{50/50/1.25/2.5}, intensity of the I₃ peak increased dramatically as the medium pH decreased (Fig. 4B). But HBP2_{50/50/2.5/2.5} exhibited a sharp jump in the I_3 intensity at the pK_a of the copolymer (Fig. 4C). On the contrary, HBP2_{50/50/5.0/2.5} produced similar results as HBP2_{50/50/1.25/2.5}. In HBP2_{50/50/5.0/2.5}, higher DB might have reduced pyrene encapsulation to a certain extent at low pH due to rod like morphology of the copolymers (Fig. 4D). A rod definitely has a lower surface area than a sphere which reduces surface adsorption of any molecule. It is interesting to note that $\mathsf{HBP2}_{\mathsf{50/50/2.5/2.5}}$ showed maximum pyrene encapsulation at the pK_a and also the intensity of the I₃ (around 1.6) was much higher than the other HBP2s. This may indirectly substantiate that the polymer aggregates were most stable in case of $HBP2_{50/50/2.5/2.5}$ at the pK_a. However, with lowering of the medium pH, the polymer aggregates of $HBP2_{50/50/2.5/2.5}$ somehow broke and thus the intensity of $I_{\rm R}$ decreased slightly as some pyrene molecules escaped into the surrounding water medium. Even the vibronic intensities ratio (I_3/I_1) for different copolymers at varied solution pH supported our assumption (insets of Fig. 4). In our set up, I_3/I_1 for pyrene in water was calculated to be 0.51. $I_3/I_1 > 0.51$ was observed in P2 and HBP2s irrespective of the medium pH. This indicates all the copolymers were quite hydrophobic as pyrene resided in their hydrophobic environment and as I_3/I_1 increased with lowering of the solution pH, definitely hydrophobicity of the copolymers increased. However, HBP2_{50/50/2.5/2.5} displayed a very high I_3/I_1 (almost 1.76) at the pK_a. Such a high I_3/I_1 for pyrene encapsulation may justify maximum hydrophobicity of HBP2_{50/50/2.5/2.5} at the pK_a owing to maximum stability of the hydrophobic polymer aggregates.



Figure 4. Normalized emission spectra of pyrene encapsulated in A) P2_{50/50/125}, B) HBP2_{50/50/125/25}, C) HBP2_{50/50/2.5} & D) HBP2_{50/50/2.5} in aqueous media with varied solution pH; The inset in each image denotes plot of I₃/ I₁ as a function of solution pH



Figure 5. UV-Vis absorption spectra for encapsulation of A) CR & B) MB by $HBP2_{50/50/2.5/2.5}$ in aqueous media at different pH, measured after 5 mins of addition of the guest molecules

Often hydrophobic aggregates constitute an aqueous pool deep at the core especially when the copolymers are made up of polymer chains differing widely in polarity.²¹ In order to test whether any such aqueous pool reside inside HBP2s (as predicted from the FESEM studies), we monitored sequestration of hydrophilic guest molecules from the aqueous medium by HBP2_{50/50/2.5/2.5} with variation in the medium pH. Surprisingly HBP2_{50/50/2.5/2.5} sequestered both congo red (CR); negatively charged and methylene blue (MB); positively charged molecules at high pH (Fig. 5). Encapsulation proficiency of HBP2_{50/50/2.5/2.5} for CR was better than MB in the wide range of pH as CR was completely encapsulated (no peak was observed in the UV-Vis spectrum-Fig. 5A) and even CR molecules were retained after 24 hrs. On the other hand, MB was neither completely encapsulated nor retained fully inside HBP2_{50/50/2.5/2.5}. A good amount of MB molecules was released from $HBP2_{50/50/2.5/2.5}$ into the aqueous medium when the medium pH was reduced below the pK_a. This observation indicates that indeed at high pH there was an aqueous core surrounded by the hydrophilic poly (AA) chains which enabled sequestration of both CR and MB molecules. Now that, as pH of the medium dropped sufficiently, the aqueous core collapsed (as predicted from the FESEM studies) and the poly (AA) chains also got protonated which bound tightly with CR molecules but repelled MB molecules. On the contrary, P2 sequestered and retained CR molecules only at a very low pH.

P2 copolymer hardly encapsulated MB molecules (suggests lack of aqueous core). Thus, we surmise that only HBP2s were highly pH responsive and exhibited reversible transformation of polymersomes to aggregates at the pK_a. In fact, these polymeric architectural variations in HBP2s occurred due to change in balance between the hydrophobic and the hydrophilic segments of the copolymers. Our findings regarding encapsulation proficiency of HBP2_{50/50/2.5/2.5} for both hydrophobic and hydrophilic molecules are summarized in Scheme 2. In fact, HBP2s may find usefulness in removal of any type of organic wastes from treated or untreated industrial effluents. One of the greatest advantages of using HBP2s as water purifier is that they can sequester both hydrophobic (within 12 hrs) and hydrophilic (within 5 mins) wastes without any pre-concentration even at moderate solution pH.



Scheme 2. Scheme for encapsulation proficiency of ${\sf HBP2}_{{\sf S0/S0/2},{\sf 52/25}}$ in aqueous media for hydrophobic and hydrophilic molecules above and below the ${\sf pK}_a$

We further compared the rheological characteristics of P2 and HBP2s in aqueous media maintained at pH 8 (where the polymer chains were more extended as observed in the FESEM images, Fig 2) for better understanding of the role of hyper branching on the properties of the copolymers. HBP2s exhibited unique dynamic flow (complex viscosity) and mechanical properties (storage modulus G', loss modulus G' and damping factor tan δ) as compared to the linear analogue.

By applying empirical Cox Merz rule, we determined flow behaviour of the different copolymers in low angular frequency zone. Cox Merz rule as expressed in the Equation 1 states that frequency dependence of $|\eta^*|$ is analogous to the shear rate dependence of $\eta(\gamma')$.

$$\eta(\gamma') = \left| \eta^*(\omega) \right|_{\gamma'=\omega} \tag{1}$$

Shape of steady state shear viscosity curve as a function of shear rate (or else complex viscosity as a function of angular frequency, ω) helps in conjecturing flow behaviour of the viscoelastic materials (whether exhibits Newtonian or non Newtonian flow). In Fig. 6, it is seen that in all the HBP2s, $|\eta^*|$ was independent of ω (typical Newtonian flow) unlike in P2 where $|\eta^*|$ increased with lowering of ω . These unique dynamic flow characteristics of HBP2s resemble that of the conventional HBPs or dendrimers. Owing to much compact chain topology, HBP2s experienced lesser friction in a solvent and thus flowed like Newtonian fluids.



Figure 6. $|\eta^*|$ of solutions/ suspensions of P2 and HBP2s in aqueous solution at pH 8 as a function of ω from dynamic oscillation measurements at a temperature of 30°C

To compare the dynamic mechanical properties of P2 and HBP2s, we thoroughly studied the nature of rheology curves of aqueous solutions/ suspensions of the respective copolymers, maintained at pH 8. Multiple maxima on tan δ curve in a given ω range indicates multi phase distribution in a polymer matrix arising from the several branch points due to different levels of relaxation hierarchy.²² In our case, we observed that two maxima developed on the tan δ curve in case of HBP2_{50/50/1.25/2.5} (Fig. 7B) and that prominent multiple maxima appeared in the tan δ curve of HBP2_{50/50/2.5/2.5} (Fig. 7C). Hence, copolymers showed a complicated and hindered relaxation properties only when the extent of branching in the copolymers was medium. Both $P2_{50/50/0/0}$ and the highly branched HBP2_{50/50/5.0/2.5} revealed almost similar rheological behaviour (like both had almost zero G $\acute{}$ in low ω domain for which tan δ was abnormally very high and very poor

mechanical properties; also low value of G''). Both $HBP2_{50/50/1.25/2.5}$ and $HBP2_{50/50/2.5/2.5}$ showed good G' (i.e.; branching induced elasticity in the copolymers). On the other hand, excessive branching increased stiffness of the copolymers (showed reduction in G'). P2 displayed such low mechanical property in spite of very high $\overline{M_n}$. This may be due to the potential role of entanglements on the rheological properties of P2 and HBP2s. In low ω zone, if G'' dominates over G' then it suggests lack of chain entanglements.²³ G'' > G' was observed for $P2_{50/50/0/0}$ and $HBP2_{50/50/5.0/2.5}$ (Fig. 7A and D) in the scanned ω range. However, G^{''} < G['] was observed for HBP2_{50/50/1.25/2.5} and HBP2_{50/50/2.5/2.5}. To explain these results, we estimated the critical molecular weight for entanglement, M_e of the different copolymers. In our acquired results, no definite plateau for G' was observed in the rheology curves in the scanned angular ω zone for any copolymer. At the plateau region (which generally appears at high ω zone), G' is maximum (more elastic) and G'' is minimum (less viscous) for any polymer. Hence we employed an indirect method to estimate the plateau modulus in low ω zone, G_N by considering maximum G' corresponding to ω displaying minimum G''. Then we used the Equation 2 to calculate M_e of the respective copolymers.

$$M_e = \frac{\rho RT}{G_N} \tag{2}$$

(ignoring the 4/5 factor of Doi and Edwards); where ρ is the polymer density (Table S1, S.I), R is the universal gas constant and T is the absolute temperature. M_e for P2_{50/50/00}, HBP2_{50/50/1.25/2.5}, HBP2_{50/50/2.5/2.5} and HBP2_{50/50/5.0/2.5} were calculated to be 3.89×10^5 g mol⁻¹, 430.45 g mol⁻¹, 7.44 g mol⁻¹ and 5153.14 g mol⁻¹ respectively. Now if we consider the proposed model of Bersted and Anderson, we find a relationship between M_e and σ as expressed in the Equation 3.

$$\sigma \propto \sum_{i=1}^{\infty} \left(\frac{\rho N_0}{M_i} \right) \left[\left(\frac{M_i - M_T}{M_e} \right) \right] w_i \right]$$
(3)

where σ is the number of entanglements per molecule for molecules of molecular weight M_i (i.e.; entanglement density of polymer chains with molecular weight M_i), ρ being the polymer density, M_T the threshold molecular weight of polymer chains below which there is no contribution of polymer chains to the strength, Me the critical molecular weight between entanglements and w_i the weight fraction of i. Hence, $\sigma \propto 1/M_e$ for any viscoelastic material. From our estimated M_e of the linear and various hyperbranched copolymers, we may predict that σ is lower in P2_{50/50/0/0} and HBP2_{50/50/5.0/2.5} (however greater than P2_{50/50/0/0}). Other HBP2s exhibited very high σ (HBP2_{50/50/2.5/2.5} exhibited highest σ). Due to very high $\overline{M_n}$ of P2, each polymer chains were quite long and thus were less entangled. However, the reason for low σ value of HBP2_{50/50/5.0/2.5} is quite different. The very high quantity of the DVB (which is quite bulky) increased stiffness of $\mathsf{HBP2}_{\mathsf{50/50/5.0/2.5}}$ (as concluded from the poor modulus) and restricted polymer chain entanglements.

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Figure 7. Variation in rheological parameters; G´, G´' and tan δ with ω for aqueous solutions/ suspensions of A) P2_{50/50/00} B) HBP2_{50/50/1.25/2.5} C) HBP2_{50/50/2.5/2.5} and D) HBP2_{50/50/2.5/2.5} at pH 8 and a constant temperature of 30°C



Figure 8. Variation in rheological parameters; G', G'' and tan δ with ω for aqueous solutions/ suspensions of A) $P2_{50/50/0/0}$ and B) HBP2_{50/50/2.5/2.5} at pH 4 and a constant temperature of 30°C

Finally, rheological behaviours of P2_{50/50/0/0} and HBP2_{50/50/2.5/2.5} were further compared in aqueous solutions/ suspensions maintained at pH 4 (i.e.; below the pK_a of the respective copolymers where stable polymer aggregates were formed in case of the latter). High chain entanglements were retained in HBP2_{50/50/2.5/2.5} (Fig. 8B) which suggests that an optimized level of hyper branching (or rather chain entanglements) helped in stabilization of any polymer aggregates formed in the aqueous medium below the pK_a. Interestingly, elasticity of $\mathsf{HBP2}_{\mathsf{50/50/2.5/2.5}}$ decreased as the medium pH decreased due to transformation of morphology from flexible polymer chains to coarse polymer particles. This comparative rheological study again indirectly shows a crucial role of hyper branching in the development of definite polymer architecture and its subsequent stabilization with variation in pH of the aqueous medium in case of HBP2_{50/50/2.5/2.5}.

CONCLUDING REMARKS

In this work, we have successfully prepared amphiphilic and pH responsive HBPs capable of adapting two different macromolecular architectures (polymersomes and polymer aggregates) under two different ranges of medium pH which

also encouraged pH responsive encapsulation and retention of hydrophilic/ hydrophobic molecules. The reacting monomers were chosen in such a fashion with respect to the reaction medium that almost a sequential and controlled radical polymerization was possible which created distinct hydrophilic and hydrophobic layers in the copolymers. We observed through various experiments (DLS, zeta potential, FESEM, PL and rheology) that an optimized extent of hyper branching and presence of pendant cetyltrimethyl ammnonium triazole ion pairs as well as carboxyl groups are necessary for formation and stabilization of hierarchical structures of the copolymers and also for sufficient encapsulation/ retention of hydrophilic/ hydrophobic molecules in the aqueous media, maintained at various pH. This work basically highlights that adjusting an optimum balance between hydrophilic and hydrophobic content induces responsive properties in unresponsive polymers. Further the new hyperbranched copolymer that we synthesized has high commercial importance as it may be used in removal of both hydrophobic and hydrophilic organic wastes (even in large scale) from water bodies within reasonable time.

ABBREVIATIONS

S.I, supporting information; HBP, hyperbranched polymer; SCVP, self condensing vinyl polymerization; ROP, ring opening polymerization; FRP, free radical polymerization; DB, degree of branching; MWD, molecular weight distribution; DEA, 2-(diethylamino)ethyl methacrylate; PEGMA, poly (ethylene glycol) methacrylate; EGDMA, ethylene glycol dimethacrylate; DDT, dodecane thiol; TG, thioglycerol; MA, methacrylic acid; PA, propargyl acrylate; AA, acrylic acid; DVB, divinylbenzene; AIBN, azobisisobutyronitrile; BA, branching agent; CTA, chain transfer agent; m.mol, milli molar; CTA-N₃, cetyltrimethyl ammonium azide; NaAsc, sodium ascorbate; EDTA, ethylenediaminetetracetic acid; HG, head group; MC, main chains; CR, congo red; MB, methylene blue

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Hyperbranched copolymers self assembled from polymersomes to aggregates and encapsulated both hydrophilic/ hydrophobic molecules but with varied retention proficiency depending upon the medium pH.