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Vesicular assembly and thermo-responsive vesicle-to-micelle transition from an amphiphilic random copolymer†

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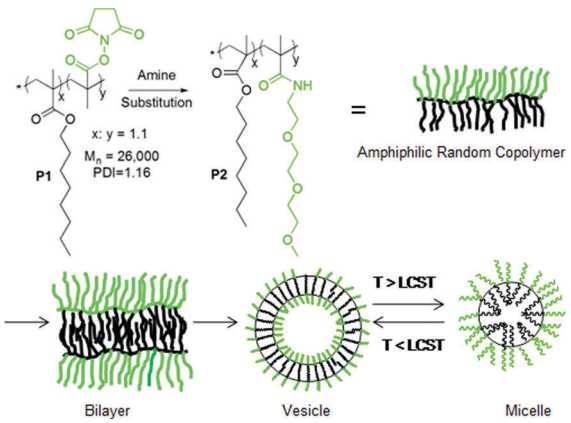
Vesicular assembly from a thermo-responsive amphiphilic random copolymer is reported. Vesicle-to-micelle transition above a critical morphology transition temperature (CMTT) resulted in selective triggered release of encapsulated hydrophilic guests over hydrophobic ones. The aggregation pattern of a control polymer indicated a defined role of the methacrylamide groups in the polymer backbone for such unprecedented self-assembly from a simple polymer.

Amphiphilic polymers are attractive candidates to be investigated as delivery vehicles for therapeutics owing to their ability to generate versatile nano-structured assemblies[1] with tunable container property as well as stimuli-sensitive release behavior.[2] In this context polymeric vesicles/polymersomes[3] are unique because they can encapsulate both hydrophobic and hydrophilic guest molecules. Till date polymersomes have been prepared largely from amphiphilic block copolymers[3,4] apart from few exceptions.[5] However to the best of our knowledge there is no report on random copolymer based polymersomes. Random copolymer based scaffolds are advantageous because they can be achieved in a single polymerization step unlike block copolymers. Herein we report unprecedented vesicular-assembly from a remarkably simple thermo-responsive amphiphilic random copolymer (**P2**, Scheme 1) and its vesicle-to-micelle transition above a critical temperature (CMTT) which is close to the lower critical solution temperature (LCST)[6] of the polymer.

Our primary objective was to prepare a reactive pre-polymer by random copolymerization of a hydrophobic monomer along with another highly reactive one. We envisaged that such a reactive copolymer will allow producing a library of amphiphilic random copolymers by post-polymerization substitution[7] of the reactive group by various hydrophilic moieties and thus will provide an opportunity to investigate the effect of structural variation on aggregation properties of the resulting amphiphilic polymers with exactly the same degree of polymerization and extent of randomness. In this endeavor we recognized *N*-hydroxy-succinimide methacrylate ester (NHSMA) as a suitable reactive monomer because (i) its controlled radical polymerization and quantitative post-polymerization modification by primary amines



Scheme 1 Synthesis route of **P2** and schematic of its self-assembly.

are established in the literature[8] and (ii) post-polymerization substitution with amines would generate bio-compatible, biodegradable and thermo-responsive amide functional groups in the polymer backbone. Thus we synthesized a random copolymer using NHSMA and *n*-octyl methacrylate monomers (1 : 1 feed ratio) using reversible addition–fragmentation transfer (RAFT) polymerization[9] to get the reactive parent polymer **P1** (Scheme 1).[10] This could be substituted by an amine-containing oligooxyethylene unit to get the desired amphiphilic copolymer **P2** (Scheme 1).[10] Quantitative replacement of functional group substitution was confirmed by ^{1}H NMR (Fig. S1, ESI†) and FT-IR (Fig. S2, ESI†) studies.[10]

P2 could be directly dissolved in H_2O by sonication (~5 min) at 10^{-3} M concentration. Its aggregation property was checked using a hydrophobic pyrene probe because the emission-intensity of the first (I_1) and third (I_3) vibronic peaks (inset in Fig. 1a) of pyrene are sensitive to the polarity of the environment.[11] The I_1/I_3 ratio of pyrene, encapsulated in aqueous solution of **P2**, gradually decreased with increasing polymer concentration (see Fig. S3, ESI†, for spectral variation) till 0.02 mM of **P2** and then remained almost invariant (Fig. 1a). The final I_1/I_3 value (1.12) indicates that the probe is located in a hydrophobic environment[5a] which must have been provided by aggregation of **P2** beyond a critical concentration (0.02 mM). To test the nature of the aggregate we carried out transmission electron microscopy (TEM) studies (Fig. 1b) which showed spherical aggregates (diameter in the range of 250–500 nm) with a darker thin wall and hollow inside suggesting vesicular assembly. Further

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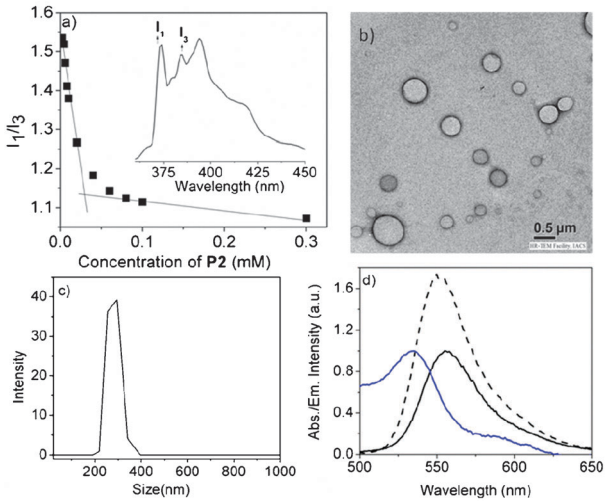

Fig. 1 (a) Variation of I_1/I_3 in the emission spectra of pyrene (fixed concentration = 10^{-6} M) encapsulated in **P2** solutions of varying concentration, inset: emission spectra of pyrene in H_2O in presence of 0.3 mM **P2**; (b) TEM images of aqueous solution of **P2** (0.1 mM); (c) size distribution of **P2** solution by DLS measurements; (d) intensity normalized absorption (blue) and emission (black) spectra of R6G encapsulated in 0.1 mM **P2** solution. The dashed-black line shows the concentration normalized emission spectra of R6G in polymer-free aqueous solution.

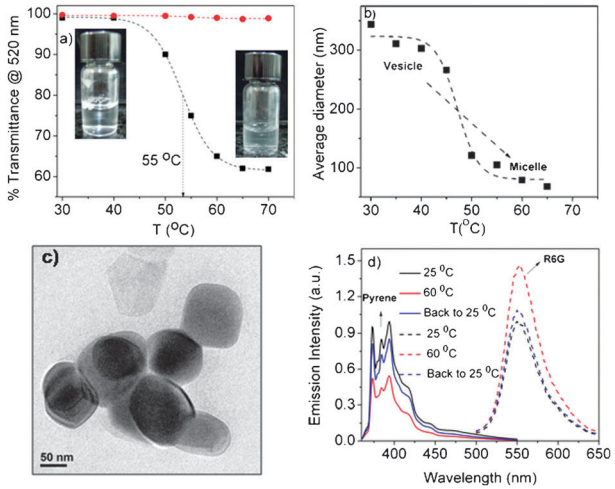

Fig. 2 (a) Variation of transmittance of aqueous **P2** solutions (black: 1 mM, red: 0.1 mM) as a function of temperature; picture in the left was taken at 25 °C and right was taken at 60 °C (top: 0.1 mM, bottom: 1 mM). (b) Size distribution of **P2** solution by DLS measurements at 60 °C. (c) TEM images of samples prepared from 0.1 mM **P2** solution at 60 °C. (d) Emission spectra of pyrene and R6G encapsulated in 0.1 mM **P2** solution at 25 °C (black), 60 °C (red) and after cooling back the hot solution to 25 °C (blue).

dynamic light scattering (DLS) studies revealed an average hydrodynamic diameter (D_H) in the range of 300 ± 50 nm (Fig. 1c). Few larger particles observed in TEM compared to DLS results can be attributed to flattening of the soft vesicular particles on the TEM grid while drying[4k] and/or fusion of smaller particles induced by a dehydrated hydrophilic corona. To further ascertain vesicular assembly, ability of the aggregates to encapsulate a hydrophilic guest was examined using the rhodamine 6G (R6G) dye. R6G was dissolved in an aqueous polymer solution and the mixture was subjected to dialysis (MWCO = 3000 kD) for 24 h to remove the non-encapsulated dye. Presence of prominent absorption (λ_{max} = 534 nm) and emission (λ_{em} = 557 nm) peaks due to the R6G chromophore in the dialyzed solution (Fig. 1d) confirmed guest encapsulation. Furthermore the emission intensity at 557 nm was ~40% reduced compared to that of the polymer free R6G solution of the same concentration (see ESI† for the procedure to estimate the concentration of the encapsulated R6G dye inside the vesicle). Such self-quenching nature of R6G has been previously observed for vesicular assembly[5a] and been attributed to the confinement effect.

To examine the LCST of **P2**, if it has any, we monitored the temperature effect on the %-transmittance (@520 nm where **P2** does not absorb) of an aqueous polymer solution (concentration = 1 mM) and noticed (Fig. 2a) that it remained almost invariant (~100%) till 40 °C and then sharply decreased to ~60% around 50–60 °C suggesting it to be the LCST of **P2**. However beyond the LCST instead of macroscopic precipitation the solution only became slightly turbid (insets in Fig. 2a). Intrigued by this observation, a relatively dilute solution (0.1 mM) was tested in which surprisingly no increase in turbidity was found till 70 °C either by cursory observation or variable-temperature transmittance study (Fig. 2a). To understand the implication of this on self-assembly we carried out variable-temperature DLS measurement (Fig. S4) with 0.1 mM aqueous polymer solution. It was noticed that with increasing temperature the particle size (~300 nm) remained almost invariant till 45 °C suggesting vesicular assembly but with further increase in temperature the particle size sharply decreased to ~70 nm and then again remained almost invariant (Fig. 2b). It is noteworthy that the CMTT for the observed change in particle size is close to the LCST of the polymer (Fig. 2a) which clearly indicates that the size reduction is actually related to the LCST of **P2**. TEM images (Fig. 2c) of the sample which was prepared by dipping the grid into a hot aqueous solution (60 °C) of **P2** revealed different morphology for the aggregates. Instead of hollow polymersomes (Fig. 1b), dark near-spherical particles were found with an average diameter in the range of 70–80 nm (which closely matches to high-temperature DLS results) suggesting micelle type aggregation. We then examined the effect of thermo-responsive vesicle-to-micelle transition[12] on the fate of the encapsulate guest molecules (Fig. 2d). The I_1/I_3 ratio of pyrene emission spectra was found to be 1.16 at 60 °C compared to 1.12 at 25 °C clearly suggesting that pyrene is still located in a hydrophobic environment even at elevated temperature. Contrastingly for hydrophilic R6G, going from 25 °C to 60 °C the emission intensity increased by ~1.5 times (Fig. 2d) suggesting release of the encapsulated guest from the confined water-pool inside the vesicle to bulk water. Note that for both dye molecules temperature-dependent spectral changes were completely reversible. Selective release of only hydrophilic guest molecules at elevated temperature can be attributed to the presence of only hydrophobic interior for micellar aggregates in sharp contrast to vesicles which have both hydrophobic walls as well as hydrophilic interior.

To rationalize the thermo-responsive vesicle-to-micelle transition near the LCST of **P2**, we propose in this case that above

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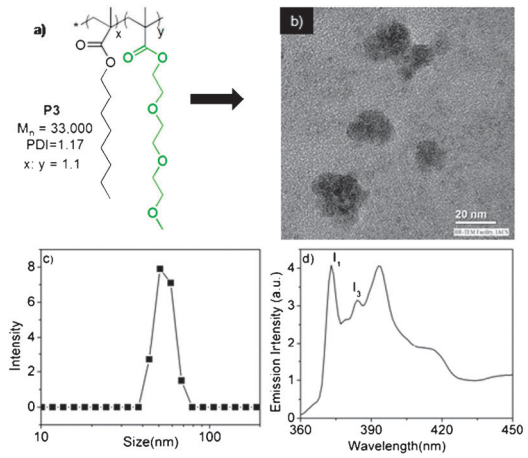

Fig. 3 (a) Structure of **P3**; (b) TEM images of aqueous solution of **P3** (0.1 mM); (c) intensity averaged size distribution of **P3** solution (0.1 mM) by DLS; (d) emission spectra of pyrene ($C = 10^{-6}$ M, $\lambda_{ex} = 337$ nm) encapsulated in **P3** aggregates (0.1 mM).

the LCST only the amide group becomes de-solvated but not the pendant oligooxyethylene segments and consequently the hydrophobic/hydrophilic balance is altered at elevated temperature resulting in a change in the aggregation pattern. To support this hypothesis a control polymer **P3** (Fig. 3a)[10] was studied in which the amide groups (as in the case of **P2**) were replaced by ester. TEM images (Fig. 3b) revealed the presence of near-spherical micelle-type aggregates[13] with an average diameter in the range of 30–40 nm which is in sharp contrast to vesicular assembly formed by **P2**. The hydrodynamic diameter (D_H) estimated by DLS studies (Fig. 3c) was found to be 30 ± 10 nm which is in good agreement with TEM results. Micelle formation was further supported by the ability of **P3** solution to encapsulate the hydrophobic guest pyrene ($I_1/I_3 = 1.26$) (Fig. 3d). However when this polymer was treated with the hydrophilic R6G dye following a similar procedure as described before for **P2**, no absorption band around 534 nm due to the R6G dye could be seen (Fig. S5, ESI†) in the dialyzed solution further ascertaining the absence of any vesicular assembly. Moreover aqueous solution of **P3** did not show any LCST. Thus based on these control experiments it can be concluded that the presence of the amide groups is indeed the key factor for unprecedented vesicle formation and thermo-responsive vesicle-to-micelle transition by **P2**.

We have demonstrated spontaneous vesicular assembly from a thermo-responsive amphiphilic random copolymer consisting of methacrylate-type hydrophobic and methacrylamide-type hydrophilic repeat units. Above a CMTT (which is close to the LCST of the polymer) the vesicular assembly was reversibly converted to micelle-type aggregates. Control experiments suggested that the amide groups in the polymer backbone are responsible for unprecedented stimuli-responsive behavior from a remarkably simple random copolymer. Currently we are engaged in chemical modification of the vesicular surface with appropriate ligand moieties and tuning the CMTT (preferably bringing it down to ~40 °C) by adjusting the hydrophobic/hydrophilic segments for taking this remarkably simple polymer to the biomedical domain as a targeted drug-delivery vehicle.[14]

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