Photocleavable and Tunable Thermoresponsive Amphiphilic Random Copolymer: Self-Assembly into Micelles, Dye Encapsulation, and Triggered Release

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ABSTRACT: A double-responsive amphiphilic random copolymer (P(OEtOxA)-*ran*-PNBA) composed of thermoresponsive poly(oligo(2-ethyl-2-oxazoline)acrylate) (P(OEtOxA)) segments and photocleavable poly(2-nitrobenzyl acrylate) (PNBA) segments is synthesized via combination of cationic ring-opening polymerization (CROP) and reversible addition-fragmentation chain transfer (RAFT) polymerization techniques. The P(OEtOxA)-*ran*-PNBA copolymer exhibits lower critical solution (LCST)-type soluble-to-turbid phase transition in water with tunable cloud point (T_{cp}) with respect to chain length of P(OEtOxA) segments by UV irradiation transforms amphiphilic P(OEtOxA)-*ran*-PNBA to fully hydrophilic P(OEtOxA)-*ran*-poly(acrylic acid) resulting in the appreciable increase of T_{cp} of copolymer in

INTRODUCTION In recent years, design and synthesis of stimuli-responsive copolymers have gained immense research interests because of their wide range of applications including controlled delivery of drug and genes, tissue engineering, catalysis, biosensors and bioimaging etc.^{1–7} These responsive polymers are "smart" and "intelligent" because of their inherent active functionalities capable of exhibiting sharp responses against some minor changes in the external stimuli such as temperature,^{8–11} light,^{12,13} pH,^{14,15} ionic strength,¹⁶ CO₂,¹⁷ glucose¹⁸ etc. Indeed, amphiphilic responsive copolymers can self-assemble into various nanostructured macromolecular architectures (e.g., micelles, vesicles, etc.) in solution that can spontaneously disassemble or undergo some changes in their morphology upon change in their local environment.8,19-21 Thus, these "smart" copolymer systems are particularly advantageous for encapsulation of drugs and genes and their stimuli directed delivery at the desired site.²²

Till date, the responsive behaviors of amphiphilic random/ block copolymers in solution are studied with respect to single stimulus.^{23–27} However, the presence of two or more aqueous solution. Owing to the amphiphilic nature, the P(OEtOxA)-*ran*-PNBA copolymer molecules self-assemble into well-dispersed spherical micelles in water. There is a disruption of the copolymer micelles with UV light irradiation as well as shrinkage of micellar size with increasing temperature above the LCST of copolymer in solution. Finally, the encapsulation of hydrophobic guest molecule (nile red) into P(OEtOxA)-*ran*-PNBA copolymer micelles and thermo- and photo-triggered release of nile red are demonstrated. © 2017 Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. **2017**, *00*, 000–000

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different stimuli-responsive segments/blocks in one copolymer chain provides more opportunities to fine tune their solution properties as well as their self-aggregated morphologies in multiple ways.^{28,29} Thus, the development of multistimuli responsive copolymers are challenging and key to prepare advanced materials for sophisticated applications. It is obvious that temperature- and light-sensitive polymeric materials are very much attractive because of easy accessibility of these two physical stimuli and can be operated from outside the system without any additional reagents. Thermoresponsive polymers have been studied intensely for last few decades because of their unique characteristic feature of showing either soluble-to-insoluble or insoluble-to-soluble phase transitions in solution above the lower critical solution temperature (LCST) or upper critical solution temperature (UCST), respectively.^{8,30-34} On the other hand, photoresponsive polymers are also equally attracted attention as their properties can be tuned with respect to time and space with shining light on it.^{12,13,35,36} Thus, we can tune many different properties of such copolymers in solution in more sophisticated and controlled way by changing both its temperature

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and shining light on it. Poly[N-isopropylacrylamide-co-N-(4phenylazophenyl)acrylamide] (PNIPAAm-co-PAzoAm) was the first example of light- and thermo-responsive copolymer whose LCST-type phase transition temperature (T_{cp}) in water increased from 21 to 27 °C due to the trans-cis photoisomerization of azobenzene moieties upon irradiation with UV light as reported by Irie et al.³⁷ After Irie's pioneering work, the synthesis of many different dual thermo- and lightresponsive anphiphilic block copolymers have been reported where the solution properties and their aggregation state can be altered by tuning these two stimuli.^{9,29,38-40} Recently, Yuan et al. synthesized a UV light breakable and tunable thermoresponsive amphiphilic poly(2-nitrobenzyl methacrylate)-block-poly(2-(2-methoxyethoxy)ethylmethacrylate-co-oligo(ethyleneglycol) methacrylate) (PNBM-b-P(MEO₂MA-co-OEGMA)) block copolymer (BCP) by ATRP.³⁹ They also studied the self-assembly of this BCP into micelles and their disruption in water by UV light irradiation followed by their reassembly into micelles upon heating above its LCST. These BCPs are well studied in terms of their responsiveness towards light and temperature and are good candidates to be assembled into varieties of polymeric nanostructures. However, the synthesis of such dual/multi-responsive BCPs generally requires multistep sequential controlled polymerization techniques or postpolymerization modifications such as grafting, substitution, or "click" reactions and consequently it is time-consuming and tedious.^{41,42} On the other hand, the synthesis of random copolymer is relatively easy and less time-consuming, as they can be achieved simply by onestep copolymerization of two different functional monomers. Accordingly, Wang et al. recently reported the synthesis of a multiresponsive amphiphilic poly(2-nitrobenzyl methacrylate)-co-poly(dimethylaminoethyl methacrylate) (PNBM-co-PDMAEMA) random copolymer consisting of light-sensitive PNBM and acid-/thermo-responsive PDMAEMA segments.⁴³ The self-assembly of this random copolymer into micelles and its morphology tuning by varying light intensity, temperature, and pH are also studied. Recently, in an excellent review, Thayumanavan et al. summarized the self-assembly behaviors of different multistimuli responsive amphiphilic random copolymers.²⁰

Furthermore, the above mentioned dual/multi responsive amphiphilic random/block copolymer systems mostly contain PNIPAAm, PDMAEMA, PEG or P(OEGMA) as the hydrophilic thermoresponsive segments and they are biocompatible and hence can be used in biomedical applications.^{44,45} However, the nonbiodegradability of P(OEGMA) or PEG make it unsuitable for biological applications.^{46,47} In this context, polymer of oligo(2-ethyl-2-oxazoline) has recently been identified as a potential alternative for P(OEGMA) as stealth polymers. The nonionic and biodegradable as well as biocompatible nature of poly(2-ethyl-2-oxazoline)⁴⁸ make the polymer of oligo(2-ethyl-2-oxazoline) as a suitable candidate for biomedical applications. Being low DP, oligo(2-ethyl-2-oxazoline) do not exhibit any thermoresponsive transition in water.⁴⁸ However, very recently, Schubert et al. showed that its homopolymer, poly(oligo(2-ethyl-2-oxazoline)methacrylate) P(OEtOxMA) as well as

its random copolymer, P(OEtOxMA)-*co*-PMMA exhibit LCSTtype phase transition in water.⁴⁹ However, there is still many aspects to explore in terms of designing of new dual- or multiresponsive block/random copolymers best on this temperature sensitive polymer segment containing oligo(2-ethyl-2oxazoline). Thus, the synthesis of dual photo- and thermoresponsive random copolymer containing the thermosensitive oligo(2-ethyl-2-oxazoline) segments would really be interesting as its cloud point would be tunable with respect to the segments length as well as by tuning the light irradiation time. Furthermore, there has not been any report of study of selfassembly of this type of amphiphilic random copolymer into different micellar morphologies along with subsequent encapsulation of guest molecules and their photo/temperature-triggered release.

Thus, in this direction, we design and synthesize poly(oligo(2-ethyl-2-oxazoline)acrylate)-random-poly(2-nitrobenzyl acrylate) (P(OEtOxA)-ran-PNBA) copolymer showing simultaneous responsiveness in water toward both light and temperature. This copolymer is synthesized by combination of cationic ring-opening polymerization (CROP) and reversible addition-fragmentation chain transfer (RAFT) polymerization techniques involving first the synthesis of oligo(2-ethyl-2oxazoline)-based acrylic macromonomer (OEtOxA) with varying molecular weights by microwave assisted CROP followed by direct end-capping of living oligo(2-ethyl-2-oxazoline) chains with acrylic acid. The copolymerization of OEtOxA macromonomer with 2-nitrobenzyl acrylate via RAFT polymerization technique afford the amphiphilic random P(OEtOxA)-ran-PNBA copolymer. Indeed, the P(OEtOxA)-ran-PNBA exhibits LCST-type cloud point (T_{cp}) , which can easily be tuned by applying UV light irradiation in water. It is observed that the P(OEtOxA)-ran-PNBA self-assemble into spherical micelles in water. It is also shown that, upon UV irradiation, the micellar structure of P(OEtOxA)-ran-PNBA disrupts due to the photocleavage of NBE groups of PNBA segments. Additionally, above LCST, there is shrinkage of the micelles into smaller size. Finally, as a proof of concept, it is observed that P(OEtOxA)-ran-PNBA copolymer micelles can encapsulate model hydrophobic guest molecule nile red (NR) whose released by applying heat or UV light is also studied.

EXPERIMENTAL

Materials

2-Ethyl-2-oxazoline (EtOx) (99%; Aldrich) was purified by distillation under reduced pressure over calcium hydride and stored under argon atmosphere prior to use. Methyl triflate (MeOTf; 98%), 2-nitrobenzyl alcohol (NBA; 97%), and the chain transfer agent (CTA), cyanomethyl methyl(phenyl)carbamodithioate (CMPC, 98%) were purchased from Aldrich and used without further purification. Azobisisobutyronitrile (AIBN) (98%; Avra, India) was recrystallized from hexane. Acrylic acid (98.5%; Merck, India) and acryloyl chloride (97%; Merck, India) was dried over potassium hydroxide and distilled under argon atmosphere prior to use

TABLE 1 Polymerization conditions and molecular weight characterization data of OEtOxA macromonomers, P(OEtOxA) homopolymer, and different P(OEtOxA)-*ran*-PNBA random copolymers

Polymer Sample	Monomer (<i>M</i>)/ Initiator (<i>I</i>)	[<i>M</i>] _o /[<i>I</i>] _o	<i>M</i> _{n,} (Theo.) (Da)	<i>M</i> n, (SEC) (Da)	<i>M_{n,}</i> (MALDI) (Da)	Ð (SEC)	PNBA Content (wt %) (¹ H NMR)
^a OEtOxA ₈₀₀	EtOx/MeOTf	9	823	1,100	802	1.21	-
^a OEtOxA ₄₀₀	EtOx/MeOTf	5	410	-	392	-	_
P(OEtOxA ₄₀₀)	OEtOxA ₄₀₀ /CTA	50:1	8,400	6,900	-	1.46	_
^b P(OEtOxA ₄₀₀)- <i>ran</i> - PNBA (P1)	OEtOxA ₄₀₀ /NBA/CTA	50:20:1	8,030	6,500	-	1.58	50
^b P(OEtOxA ₈₀₀)- <i>ran</i> - PNBA (P2)	OEtOxA ₈₀₀ /NBA/CTA	50:15:1	11,500	7,056	-	1.32	40

 a CROP for OEtOxA macromonomers: Solvent = ACN; [EtOx] = 4 mol $L^{-1};$ Temperature = 140 °C; Time = 1 min under Microwave irradiation. Yield \sim 80%.

in the reaction. THF was purified by distillation from metallic sodium with benzophenone. Acetonitrile (ACN) was distilled over calcium hydride and collected in a dry RB flask containing molecular sieves (3 Å) prior to use. All the aqueous solutions were prepared from Milli-Q (18.2 M Ω) water.

Synthesis of Acrylate End-Functional Oligo(2-Ethyl-2-Oxazoline) (OEtOxA) Macromonomer

The macromonomer, OEtOxA₄₀₀, was synthesized by microwave assisted CROP following our and the protocol of Weber et al. after certain modifications.^{50,51} Typically, microwave vials (10 mL) were first heated at 150 °C for overnight to remove any traces of moisture and cooled down to room temperature under an argon atmosphere before use. Dried vial (10 mL) was then charged with EtOx (1 mL, 9.83 mmol), acetonitrile (1.5 mL), and an initiator, MeOTf (223 μ L, 1.966 mmol). The EtOx monomer concentration was 4 M with a initial monomer-to-initiator $([M]_0/[I]_0)$ feed ratio of 5. The vial was sealed with a proper cap and then placed in the microwave synthesizer and the reaction was continued for 1 min at 140 °C. The mixture was cooled to ambient temperature using an air compressor. Acrylic acid (202 μ L, 2.949 mmol) was then added in 1.5-fold excess amount with respect to initiator via a degassed syringe through the septum of the capped microwave vial containing living oligo(2ethyl-2-oxazoline) (OEtOx) chains. Afterward, 2-fold excess of Et₃N (548 μ L, 3.932 mmol) with respect to initiator was added into the OEtOx solution via degassed syringe. Finally, end-capping reaction was performed by heating the solution at 80 °C in an oil bath under stirring with a bar magnet for 12 h. Acetonitrile was removed in a rotary evaporator and the remaining viscous liquid was dissolved in chloroform. The organic part was then extracted three times with saturated aqueous sodium hydrogen carbonate (NaHCO₃) solution and twice with saturated brine (NaCl) solution. The organic phase was collected by a separating funnel and dried over anhydrous sodium sulphate (Na₂SO₄) and filtered. The resultant white viscous oligomeric macromonomer was isolated under reduced pressure using a rotary evaporator and

^b RAFT for P(OEtOxA₄₀₀) homopolymer and P(OEtOxA)-*ran*-PNBA copolymers: Solvent = THF; [OEtOxA] = 0.5 mol L⁻¹; [CTA]/[AIBN] = 1:0.25; Temperature = 72 °C; Time = 18 h. Yield ~40%.

was stored under argon atmosphere at -4 °C in a refrigerator. Yield: 78%. Another macromonomer, OEtOxA₈₀₀ was also prepared by varying the initial monomer-to-initiator feed ratio as described in Table 1.

FTIR (KBr pellet, cm^{-1}): 1640 (C=0 str, amide), 1725 (C=0 str, ester of acrylate) (Supporting Information Fig. S1).

¹H NMR (500 MHz, CDCl₃), δ (ppm): 6.35 (d, 1H, =CH₂), 6.05 (m, 1H, -CH=CH₂), 5.82 (d, 1H, =CH₂), 4.23 (m, 2H, -CH₂-O-CO-), 3.40 (b, -N--CH₂--), 3.0 (b, 3H, -N--CH₃), 2.25 (b, 2H, -N--CO--CH₂--), 1.07 (b, 3H, -N--CO--CH₂--CH₃) (Supporting Information Figs. S2 and S3 for ¹H and ¹³C NMR, respectively).

Homopolymerization of OEtOxA400 Macromonomer

Typically, 450 mg (1.125 mmol) of OEtOxA400 and 600 µL of dry THF were taken in 10 mL long neck round bottom flask and purged with nitrogen gas for 5 min. Subsequently, 6.2 mg (0.0225 mmol) of CMPC CTA and 1.01 mg (5.6 \times 10^{-3} mmol) of AIBN were separately taken in 400 μ L of dry THF, and this mixture was injected into the reaction mixture via a degassed syringe. Nitrogen purging was continued for further 45 min with constant magnetic stirring to remove any traces of oxygen. Finally, the mixture was sealed with a silicone rubber septum and placed in a preheated oil bath under stirring condition at 70 °C. The ratio of [Macromonomer]:[CTA]:[AIBN] was 50:1:0.25. After 24 h, the polymerization was quenched by quickly cooling the flask under tap water. The obtained polymer solution was diluted with slight excess of THF and precipitated into cold diethyl ether. The precipitated P(OEtOxA400) was collected and dried in a vacuum oven at 25 °C. The polymer was dissolved in water and purified by dialysis against double distilled water for 2 days using a membrane with a molecular weight cutoff of 2000 g mol⁻¹ and was isolated by evaporating the water in a lyophilizer. The resulting light pink colored powdery polymer was stored in vacuum desiccator at room temperature. Yield: 43%.



¹H NMR (500 MHz, CDCl₃), δ (ppm): 4.13 (b, 2H, -CH₂-O-CO-), 3.48 (b, -N-CH₂-), 3.02 (b, 3H, -N-CH₃), 2.35 (b, 2H, -N-CO-CH₂-), 1.12 (b, 3H, -N-CH₂-CH₃).

Synthesis of 2-Nitrobenzyl Acrylate (NBA) Monomer

NBA monomer was prepared by following our earlier reported reaction protocol.⁵⁰ Typically, 2-nitrobenzyl alcohol (3.8 g, 25 mmol) was dissolved in 20 mL of dry THF in a two-necked RB flask fitted with a nitrogen gas balloon and rubber septum. Et₃N (3.5 mL, 25 mmol) was then added via syringe to the above mixture and stirred magnetically at 0 °C for 10 min. Afterward, acryloyl chloride (2 mL, 24.72 mmol) was added dropwise via a syringe with constant magnetic stirring at 0 °C over a period of 30 min. The reaction mixture was then allowed to come at room temperature and further stirred magnetically for overnight. THF was evaporated by a rotary evaporator, and the crude reaction mixture was dissolved in 50 mL of ethyl acetate. The resultant reaction mixture was purified by washing three times with water (3 \times 40 mL) and one time with brine (1 \times 40 mL) solution. The organic phase was collected in a separating funnel and dried over anhydrous Na₂SO₄ and filtered, and then the solvent was removed in a rotary evaporator. The yellow-brown crude product was purified by silica gel column chromatography with 6:1 hexane/ethyl acetate affording a yellow viscous liquid. Yield: 95%.

¹H NMR (500 MHz, CDCl₃), δ (ppm): 8.15 (d, 1H, <u>H</u>Ar), 7.65 (m, 2H, <u>H</u>Ar), 7.5 (t, 1H, <u>H</u>Ar), 5.95 (d, 1H, <u>=CH₂</u>), 6.5 (d, 1H, <u>=CH₂</u>), 6.25 (m, <u>1H</u>, <u>-CH</u>=CH₂), 5.62 (s, 2H, <u>-O-CH₂-Ar</u>) (Supporting Information Figs. S2 and S4 for ¹H and ¹³C NMR, respectively).

MS (ESI) (35 eV): m/z (%): 230 (100) [M + Na⁺] (Supporting Information Fig. S5).

Synthesis of Poly(Oligo(2-Ethyl-2-Oxazoline)Acrylate)-*Ran*-Poly(2-Nitrobenzyl Acrylate) [P(OEtOxA)-*Ran*-PNBA] Copolymer

The random copolymerization of OEtOxA and NBA monomer was carried using the similar procedure as used for the homopolymerization of the OEtOxA macromonomer by RAFT technique. In a representative case, 600 mg (1.5 mmol) of $OEtOxA_{400}$ and 800 μ L of dry THF were taken in 10-mL long neck RB flask and was purged with N₂ gas for 5 min and sealed with a septum. CMPC CTA (8.3 mg; 0.03 mmol) and AIBN (1.23 mg; 7.5 X 10^{-3} mmol) were separately taken in 400 µL of dry THF, and the mixture was consequently added into the reaction mixture via degassed syringe. Subsequently, 125 mg (0.6 mmol) of NBA was injected into the reaction mixture via degassed syringe. Finally, the entire reaction mixture was bubbled with N2 for another 45 min, sealed with a silicone rubber septum and stirred magnetically in a preheated oil bath at 70 °C for 24 h. The molar ratio of reactants was as follows: [OEtOxA₄₀₀]:[NBA]:[CTA]:[AIBN] = 50:20:1:0.25. Another copolymer (P2) from macromonomer OEtOxA₈₀₀ and NBA monomer with the molar ratio of [OEtOxA₈₀₀]:[NBA]: [CTA]:[AIBN] = 50:15:1:0.25 (Table 1) was also prepared using similar procedure as described above. The polymerization quenching, copolymer isolation and its purification procedures were similar to that used for homopolymerization. The final random copolymer was light yellow in color and sticky solid in nature. The compositions of the copolymers were determined from integration of the appropriate signals in the ¹H NMR spectrum of the purified product, which will be discussed later in Results and Discussion section.

FTIR (KBr pellet, cm^{-1}): 1644 (C=O str, amide), 1740 (C=O str, ester), 1528 (C=C str, Aromatic ring) (Supporting Information Fig. S1).

¹H NMR (500 MHz, CDCl₃), δ (ppm): 7.88–8.12 (b, HAr), 7.35–7.75 (b, HAr), 5.35 (b, 2H, $-O-CH_2-Ar$), 4.09 (b, 2H, $-CH_2-O-CO-$), 3.47 (b, $-N-CH_2-$), 3.02 (b, 3H, $-N-CH_3$), 2.35 (b, 2H, $-N-CO-CH_2-$), 1.01 (b, 3H, $-N-CO-CH_2-CH_3$), 1.38–2.04 ($-CH_2$ backbone) (Supporting Information Figs. S2 and S6 for ¹H and ¹³C NMR, respectively).

Photocleavage of P(OEtOxA)-Ran-PNBA Copolymer by UV Light Irradiation

For photocleavage, typically, the P(OEtOxA₄₀₀)-*ram*-PNBA (**P1**) copolymer solution in DI H₂O (0.04 mg mL⁻¹) was taken in a quartz tube. It was then placed in a photoreactor (Luzchem-ICH2, Canada) equipped with a set of UV lamps ($\lambda = 350$ nm, Intensity = 190 lx) and was irradiated for different times. An aliquot (~2 mL) was then withdrawn from the tube at different time intervals, and its UV-vis spectra were recorded for monitoring the extent of photodegradation of copolymer. The photocleavage of P(OEtOxA₈₀₀)-*ram*-PNBA (**P2**) copolymer was also carried out using similar procedure at a concentration of 0.04 mg mL⁻¹.

Determination of Cloud Point of Copolymer in Water before and after UV Irradiation

The cloud point (T_{cp}) of the aqueous solution of P(OEtOxA)ran-PNBA with varying compositions and at different concentration were determined by monitoring the % transmittance (% *T*) at $\lambda = 498$ nm on a UV-vis spectrophotometer equipped with a temperature controller. In a typical procedure, 0.2 wt % of aqueous solution of **P1** was first filtered through membrane filter (pore size 0.45 μ m). The resultant transparent solution was then taken in a quartz cuvette and placed in a UV-vis spectrophotometer. T_{cp} was then determined by recording the % *T* changes at 498 nm in the temperature window of 20-90 °C with increasing/decreasing the temperature at 2 °C min⁻¹ after equilibration for 2 min at the experimental temperature. T_{cp} is actually the temperature at which the solution transmittance is about 50% to its original value in the first heating ramp.

 $T_{\rm cp}$ of P(OEtOxA)-*ran*-PNBA copolymers after UV light irradiation was also determined by turbidimetry. In this case, a quartz cuvette containing 0.2 wt % aqueous solution of a copolymer was placed in a photoreactor and was irradiated with a UV light of $\lambda = 350$ nm, Intensity = 190 lx for 60 min at 20 °C. The color of the copolymer solution was turned to slightly yellow form colorless after UV irradiation indicating its photocleavage. The cuvette containing this light-yellow colored transparent solution was then placed in a UV-vis spectrophotometer and $T_{\rm cp}$ was also measured by a similar procedure as mentioned above.

Self-Aggregation and Photoinduced Disaggregation of P(OEtOxA)-*ran*-PNBA Copolymers

For self-assembly, **P1** and **P2** copolymers were first dissolved in DI water with an initial concentration of 0.30 mg mL^{-1} . The solution was then kept undisturbed for 5 h at room temperature for allowing the aggregation of the copolymer molecules to form nanostructures. The aggregation process was monitored by DLS, FESEM as well as by transmission electron microscopy (TEM).

For disaggregation study, after 5 h of aggregation of **P1** solution, an aliquot (~2 mL) was taken in quartz tube and placed in a photoreactor for exposing with UV light ($\lambda = 350$ nm) for 90 min. After that, 1 drop of exposed solution of **P1** was casted on a glass slide/TEM grid and examined via FESEM and TEM respectively for checking photodegradation of copolymer nanostructures that may formed in the aggregation process.

Dye Encapsulation by P(OEtOxA)-*Ran*-PNBA Copolymer Micelles and Photo- and Temperature-Induced Release Study

Nile red (NR) was chosen as the model hydrophobic dye for its encapsulation by the micelles of P(OEtOxA)-ran-PNBA copolymer in water. Typically, **P1** copolymer (0.30 mg mL⁻¹) dissolved in 2 mL of water and was mixed with 150 µL of NR solution in acetone (1.11 \times 10⁻⁴ mg mL⁻¹). Acetone was then removed by heating the solution for some time. The resultant aqueous solution was then kept undisturbed for overnight at 20 °C to allow micellization of P1 as well as encapsulation of NR into their hydrophobic core. Afterwards, excess NR was filtered and separated through centrifuge. Finally, one part of the NR-encapsulated aqueous P1 micellar solution (\sim 1mL) was irradiated with UV light of $\lambda = 365$ nm and the NR release was monitored through time-dependent fluorescence spectroscopy. Another part $(\sim 1 \text{ mL})$ of the NR-encapsulated P1 micellar solution was used to study the temperature-triggered NR release using fluorescence spectroscopy.

Characterization

Microwave Polymerization

CROP was carried out by taking EtOx monomer and MeOTf initiator in capped dried reaction vials in an Anton Paar research grade Microwave Reactor (Model: Monowave 300).

FTIR Spectroscopy

FTIR spectra of two macromonomers and copolymers were recorded from KBr pellets, prepared by mixing the respective dried samples with dried KBr in 1:100 (w/w) ratios in a Perkin Elmer FTIR Spectrum-400 spectrometer.



ESI Mass Spectrometry

The ESI mass spectrum of NBA monomer was recorded from acetonitrile solution using quadrupole time-of-flight (QTOF) Micro YA263 (Waters) mass spectrometer.

NMR Spectroscopy

¹H NMR and ¹³C NMR spectra of OEtOxA macromonomers, P(OEtOxA) homopolymer, NBA monomer, and P(OEtOxA)ran-PNBA copolymers were recorded in CDCl₃ solvent using a Bruker DPX 500 MHz spectrometer.

SEC Measurement

Molecular weight (M_n) and dispersities (\mathcal{D}) of OEtOxA macromonomer, P(OEtOxA) homopolymer and P(OEtOxA)-*ran*-PNBA copolymers were measured by size exclusion chromatography (SEC) using a Waters 1515 isocratic HPLC pump connected to three Waters Styragel HR1 ($M_n = 100-5000$), HR3 ($M_n = 500-30,000$), and HR4 ($M_n = 5000-5,00,000$) columns and a Waters 2414 refractive index detector at room temperature (25 °C). THF was used as eluent with a flow rate of 1 mL min⁻¹, and narrow polystyrene standards were used for calibrating the GPC. Waters Breeze Software was used for molecular weight analysis.

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry

The molecular weights and end group analysis of OEtOxA macromonomers were performed by matrix-assisted laser desorption/ionization time-of-flight mass (MALDI-TOF-MS) technique in a Bruker instrument equipped with a 337 nm nitrogen laser. 2,5-Dihydroxybenzoic acid (DHB, 20 mg mL⁻¹ in THF) was used as the matrix in the presence of NaI (4 mg mL⁻¹ in acetone) as counterion. The concentration of the OEtOxA macromonomer was 1.5 mg mL⁻¹ in chloroform. For measurement, final samples were prepared by mixing 15 μ L of OEtOxA solution, 20 μ L of matrix solution, and 10 μ L of counterion solution. The sample mixture (sample/matrix/ counterion) solution was then mixed homogeneously, spotted on the sample plate, and allowed to dry at room temperature. The M_n of OEtOxA macromonomer was recorded in the linear mode.

UV-vis Spectroscopy

UV-vis spectroscopic measurements were carried out on a Hewlett-Packard 8453 diode array spectrophotometer equipped with a Peltier temperature controller.

Dynamic Light Scattering

The dynamic light scattering (DLS) experiments were carried out in Malvern Instrument (model ZEN 3690 Zetasizer Nano ZS 90, Version 7.03) attached with a He-Ne gas laser (632.8 nm). Typically, for DLS analysis, 0.30 mg of **P1** and **P2** copolymer was dissolved in 1 mL of DI water and kept undisturbed 5 h at room temperature to instigate micellization. Then the solution was filtered through a membrane filter paper (pore diameter = 0.45 μ m). DLS measurement of the resultant filtered solution was then carried out at temperatures of 20 and 65 °C.

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Field Emission Scanning Electron Microscopy

For field emission scanning electron microscopy (FESEM) analysis, aqueous copolymer solutions (concentration = 0.30 mg mL⁻¹, before and after UV light irradiation) were drop casted on a small pieces of thoroughly washed glass slide and allowed the film to dry overnight in ambient condition. The glass slides were then placed on a copper tape supported on a metal stub and sputter coated with platinum to make it conducting. The coated samples were then placed in a ZEISS field emission scanning electron microscope and were imaged at an accelerating voltage of 15 kV.

Transmission Electron Microscopy

Aqueous **P1** and **P2** sample solutions (0.3 mg mL⁻¹) were drop casted onto carbon-coated cupper grids and let the samples dried completely. The images were then recorded on a JEOL JEM-1400 Plus electron microscope operated at an accelerating voltage of 120 kV.

Photoluminescence Spectroscopy

A FluoroMax-3 spectrophotometer from Horiba JobinYvon was used to record the emission spectra of NR and NR-encapsulated **P1** copolymer micellar solution in aqueous medium (0.32 mg mL⁻¹). The excitation wavelength was 550 nm (3 \times 3 slit).

RESULTS AND DISCUSSION

Synthesis of Poly(Oligo(2-Ethyl-2-Oxazoline)Acrylate)-*Ran*-Poly(2-Nitrobenzyl Acrylate) [P(OEtOxA)-*Ran*-PNBA] Copolymer

It has been reported that the aqueous solution of poly(2-ethyl-2-oxazoline) (PEtOx) exhibited LCST-type phase transition in the range of 61-64 °C depending on its molecular weights (20-500 kDa).⁴⁸ However, in this case, it was observed that oligo(2-ethyl-2-oxazoline)s (OEtOx)s of very low molar masses (800-1000 Da) did not show any such transition in water in the measurable temperature ranges of 10-90 °C. But, one can expect that the polymer of OEtOx monomer [P(OEtOx)] would show LCST-type thermoresponsiveness if it can be polymerized by introducing a vinyl group at one of its end. On the other hand, it is known that poly(2-nitrobenzyl acrylate) (PNBA) is a photoresponsive hydrophobic polymer and can easily be converted into hydrophilic poly(acrylic acid) (PAA) upon irradiation with UV light.⁵² Thus, it is expected that the random copolymerization of OEtOx macromomer and NBA monomer of varying feed compositions would certainly result in a series of copolymers with tunable responsiveness towards both temperature and light. This prompted us to synthesize various P(OEtOxA)ran-PNBA copolymers using different types of polymerization techniques as shown in Scheme 1. First step of the synthesis is the preparation of hydrophilic OEtOxA macromonomer where a direct end capping method was used for the functionalization of oligo(2-ethyl-2-oxazoline) with a acrylate group.⁵¹ To do this, microwave-assisted CROP of EtOx was performed using MeOTf as the initiator in acetonitrile at 140 °C (Step 1 of Scheme 1).^{53,54} The resultant living OEtOx

chain containing a oxazolinium species at the one end was in-situ terminated by direct addition of acrylic acid and triethyl amine into the reaction medium (Scheme 1). Two well-defined OEtOx macromonomers of varying molecular weights $(M_n s)$ (OEtOxA₈₀₀ and OEtOxA₄₀₀) were prepared by varying the initial monomer-to-initiator feed ratios (Table 1). FTIR spectrum (Supporting Information Fig. S1) of OEtOxA₈₀₀ revealed two intense bands at 1640 and 1725 cm⁻¹ due to the >C=O stretching vibrations of the amide moiety of EtOx unit and the acrylate ester functionality of the macromonomer respectively. ¹H NMR spectra of OEtOxA₈₀₀ showed signals at 3.40 and 4.23 ppm (Supporting Information Fig. S2A) were the characteristic of methylene protons of -N-CH₂and -CH2-O-CO- moieties of backbone along with three distinct signals at 6.35, 6.05, and 5.82 ppm corresponding to the vinyl protons of OEtOxA moiety. The ¹³C NMR spectrum (Supporting Information Fig. S3) of OEtOxA₈₀₀ further showed two clearly distinguishable signals for ester and amide carbonyl carbon at 165.8 and 174.7 ppm, respectively. ¹³C NMR (Supporting Information Fig. S3) of OEtOxA₈₀₀ also showed the signals at 128.2 and 131.7 ppm corresponding to the vinyl carbons. These results confirmed the successful polymerization of EtOx and attachment of acrylate functionality to generate OEtOxA macromonomer.

In the second step, NBA monomer was synthesized by the reaction of 2-nitrobenzyl alcohol with acryloyl chloride in dry THF (Step II of Scheme 1) following our earlier reported protocol.⁵⁰ The successful synthesis of NBA monomer was established from the appearance of three distinct signals at 5.95, 6.25, and 6.5 ppm as well as a signal at 5.62 ppm in the ¹H NMR spectrum corresponding to the vinyl protons and benzylic protons of the NBA moiety (Supporting Information Fig. S2B). Also, the ¹³C NMR spectrum of NBA monomer showed signals at 165.58 ppm for carbonyl carbon of ester and at 63.09 ppm for benzylic carbon (Supporting Information Fig. S4). The base peak in the ESI-MS spectrum (Supporting Information Fig. S5) at m/z 230.02 exactly corresponded to the mass of NBA monomer with a sodium cation. All these data indicated the successful synthesis of NBA.

Finally, the copolymerization of OEtOxA macromonomer (OEtOxA400 or OEtOxA800) with NBA was carried out by RAFT technique using CMPA as CTA and a pinch of AIBN as the initiator in THF. This process produced two random copolymers, P(OEtOxA400)-ran-PNBA and P(OEtOxA800)-ran-PNBA, which were designated as P1 and P2 respectively (Table 1). FTIR spectrum (Supporting Information Fig. S1) of **P2** copolymer showed characteristic bands at 1644 cm⁻¹ for >C=O stretching of amide moiety of the P(OEtOxA) segment and at 1740 cm⁻¹ for >C=O stretching of ester functionality present in P(OEtOxA) or PNBA unit. It also showed a band at 1528 cm⁻¹ due to the C=C stretching vibration of aromatic ring of PNBA moiety. These results clearly indicated the presence of both the P(OEtOxA) and PNBA segments in the copolymer. Furthermore, ¹H NMR spectrum (Supporting Information Fig. S2C) of P2 revealed clear signals at 5.35 ppm (board) for benzylic $-CH_2$ protons, 7.35-7.75 and



SCHEME 1 Synthetic pathway for P(OEtOxA)-*ran*-PNBA copolymer.

7.88–8.12 ppm for aromatic protons of phenyl groups of the PNBA moiety, 3.47 ppm (board) for the $-N-CH_2-$ protons and 4.09 ppm for the methylene protons of $-CH_2-O-CO-$ units of P(OEtOxA) backbone with all the other expected signals confirming the presence of both P(OEtOxA) and PNBA segments in the final copolymer. The ¹³C NMR spectrum (Supporting Information Fig. S6) of **P2** also showed clear signals at 125, 129, 131, 134, and 147 ppm for the aromatic carbons and at 63.57 ppm for the benzylic carbon of NBA moiety along with the signals at 26.71 ppm for the $-N-CH_2-CH_3$ carbon of P(OEtOxA) moiety. All these data clearly revealed the successful synthesis of P(OEtOxA)-*ran*-NBA copolymer.

The OEtOxA macromonomer samples were also analyzed by MALDI-TOF-MS to ascertain its end group as well as its M_n . MALDI-TOF spectrum of OEtOxA₈₀₀ showed a series of peaks set apart by 99 Da, which is exactly the same as the molar mass of the repeating unit of EtOx (Fig. 1). Among these, the indicative peaks at m/z = 705.825, 804.851, and 904.395 corresponding to the highest intensity molecular ions could

be fitted to OEtOxA containing an acrylate functionality in one end and a methyl group in the other end along with 6, 7, and 8 numbers of EtOx monomer units, respectively, as described in Figure 1. It should also mention that each species is ionized by sodium cation as sodium iodide was used as a salt for MALDI-TOF-MS experiment. Similar results were also obtained for OEtOxA₄₀₀ macromonomer (Supporting Information Fig. S7). These results confirmed the presence of a polymerizable acrylate group at the end of OEtOx chain. The molecular weights (M_n s) and dispersities (Ds) of OEtOxA₈₀₀ and OEtOxA₄₀₀ macromonomers were determined from the analyses of MALDI spectra using "Polytools" software and were found to be 804 and 392 Da with Ds of 1.03 and 1.31, respectively (Table 1). Note that we were unable to measure M_n and D of final copolymers by this technique.

We further measured $M_{\rm n}$ s and \mathcal{D} s of OEtOxA macromonomers and copolymers (**P1** and **P2**) by SEC technique. SEC trace (Fig. 2) of OEtOxA₈₀₀ revealed a unimodal curve with moderately narrow \mathcal{D} of 1.21 suggesting the controlled nature of CROP of EtOx monomer (Table 1). The $M_{\rm n}$ of



FIGURE 1 MALDI-TOF MS spectrum of OEtOxA₈₀₀ macromonomer and end group analysis.

OEtOxA₈₀₀ was measured to be 1100 Da, which was close to that (802 Da) measured from MALDI-TOF experiment (Table 1). This value was also very close to that ($M_{n,NMR} = 790.3$ Da) obtained from ¹H NMR analysis of OEtOxA₈₀₀. But, we were unable to measure M_n and D of OEtOxA₄₀₀ macromonomer from SEC because of very low M_n .

Furthermore, the SEC trace of P2 copolymer was also unimodal in nature and there was a shifting of the chromatogram towards lower retention time compared to that of OEtOxA₈₀₀ macromonomer (Fig. 2). More precisely, SEC trace of P2 copolymer was shifted to higher molar mass (equivalent to a molar mass of 6.05 kDa) compared to that of OEtOxA₈₀₀ after copolymerization with NBA, which clearly revealed the formation of P(OEtOxA₈₀₀)-ran-PNBA copolymer. As can be seen from Supporting Information Figure S8, the SEC trace of **P1** was also unimodal in nature. M_{n} s and Ds of **P1** and **P2** copolymers were calculated from SEC analysis and were found to be 6.5 kDa and 1.58 for P1 and 7.05 kDa and 1.32 for P2, respectively (Table 1). SEC measurement of the copolymers using polystyrene standards yielded slightly lower molecular weight compare to the theoretically calculated value presumably due to the interaction between NBA chromophore containing copolymers and column materials.⁵⁵ The moderately high D values and broadening of SEC traces of P1 and P2 prepared by RAFT copolymerization can be ascribed to the interference of NBA monomer, which is a radical scavenger during polymerization.⁵⁶

Furthermore, the PNBA content in these two copolymers **(P1, P2)** was estimated from their corresponding ¹H NMR spectrum (Supporting Information Fig. S2C). P(OEtOxA₈₀₀)-*ran*-PNBA **(P2)** copolymer exhibited the characteristic proton

resonance peak at $\delta(e)$ 1.19 ppm corresponds to the methyl protons (—N—CO—CH₂—C<u>H₃</u>) of P(OEtOxA) and the peak at $\delta(f)$ 5.36 ppm corresponds to the benzyl protons (—O—C<u>H₂-Ar</u>) of PNBA. PNBA content of the copolymers was then determined from the ratio of integrated intensities of these well-resolved signals and was found to be 40 wt % for **P2** respectively (Table 1). Whereas that for P1 was 50 wt % (Table 1).

Thermoresponsiveness of Copolymers in Water

As mentioned above, PEtOx with high degree of polymerization (DP) exhibited LCST-type soluble-to-insoluble phase transition in the range of 61–64 °C in water.^{48,57} Further, Schubert et al. also demonstrated the existence of LCST-type phase transition in the aqueous solution of poly(oligo(2-ethyl-2-oxazoline)methacrylate) (POEtOxMA) containing very short hydrophilic PEtOx side chains and hydrophobic polymethacrylate backbone.⁴⁹ This is because upon increasing temperature, the aqueous solution of PEtOx or PEtOx containing polymers became turbid because of their molecular association into larger aggregates by hydrophobic noncovalent interaction among the pendent ethyl groups. In this case, it should be noted that P(OEtOxA)-ran-PNBA copolymers were highly soluble in water and giving transparent solutions even though they contained hydrophobic PNBA segment. Therefore, the study of their thermoresponsive behavior in water would be highly interesting. As expected, the transparent aqueous solution of P1 (0.2 wt %) became turbid upon heating and transparency reappeared upon cooling, indicating the presence of LCST-type phase transition as can be seen from the photographs shown in Scheme 2.



FIGURE 2 SEC traces of OEtOxA $_{800}$ macromonomer and P(OEtOxA $_{800}$)-*ran*-PNBA (P2) copolymer.

When the temperature of this solution was increased, the transmittance gradually decreased from 100 to 0% with a T_{cp} at 30 °C (50% transmittance) and the process was fully reversible without any hysteresis (Fig. 3). It is apparent that the appearance of LCST in aqueous copolymer solution is solely because of P(OEtOxA) segment even if hydrophobic PNBA segment is covalently attached. Thus, to investigate the effect of hydrophobic PNBA segment in the LCST property of this copolymer, we measured the T_{cp} of P(OEtOxA₄₀₀) homopolymer having M_n value almost close to that of **P1** copolymer in water (Table 1 and Supporting Information Fig. S8). The $T_{\rm cp}$ of 0.2 wt % of P(OEtOxA₄₀₀) was found to be 61 °C (Fig. 3) as measured from heating and cooling cycle without any hysteresis. Whereas the T_{cp} of 0.2 wt % of **P1** copolymer in water was 30 °C (Fig. 3). Therefore, it is very clear that the presence of 50 wt % of hydrophobic PNBA in the **P1** resulted in decrease of the T_{cp} of P(OEtOxA₄₀₀) by more than 30 °C. Such lowering of LCST of a copolymer simply by incorporation of hydrophobic moiety was also reported for poly(ethoxytri(ethylene glycol) acrylateco-o-nitrobenzyl acrylate) (PTEGEA-co-PNBA) copolymer by Zhao and coworkers.⁵⁸ They showed a decrease of the T_{cp} of a copolymer by 15 °C compared to that of PTEGEA segment upon incorporation of 13 mol % of hydrophobic PNBA segment. The thermoresponsiveness of P2 copolymer in water was described later in this section.

It is known that the LCST/UCST-type $T_{\rm cp}$ of a polymer solution strongly depends on its concentration.^{49,59} Therefore, we further measured $T_{\rm cp}$ s of aqueous P(OEtOxA₄₀₀)-ran-

PNBA (**P1**) solution at its different concentration, which clearly revealed that $T_{\rm cp}$ have a strong dependency with concentration [Fig. 4(A)]. $T_{\rm cp}$ of **P1** was found to decreased initially from 42 to 30 °C with increasing concentration from 0.05 to 0.2 wt % as can be seen from Figure 4(A) because of the enhancement of intermolecular aggregation between polymer chains [Fig. 4(A)].⁶⁰ The measured $T_{\rm cp}$ s was then plotted against concentration to calculate the actual LCST of the copolymer in water was found to be 30.1 °C at minimum copolymer concentration of 0.292 wt % [Fig. 4(B)].

Dual Photo- and Thermo-Responsiveness of Copolymer in Water

As mentioned above, the PNBA segment of the copolymer contains a photoresponsive *o*-nitrobenzyl ester (NBE) moiety, which can be cleaved by UV light irradiation. 50,52 Upon UV light $(\lambda = 350 \text{ nm})$ irradiation, the benzylic C-O bond of NBE moiety breaks down to produce o-nitrosobenzaldehyde and a carboxylic acid as shown in Scheme 2.61 Thus, the photo-responsiveness of P(OEtOxA)-ran-PNBA (P1) copolymer in aqueous solution (0.04 mg mL⁻¹) was studied by irradiating with UV light for different intervals of time and the corresponding UV-vis spectra of were recorded for monitoring the photocleavage PNBA. It showed a clear absorption band at 280 nm corresponding to the o-nitrobenzyl ester (NBE) moiety of PNBA segment of copolymer before irradiation (Supporting Information Fig. S9). The intensity of this peak ($\lambda_{max} = 280$ nm) indeed decreased with increasing UV irradiation time due to cleavage of NBE moiety and reached a minimum after 40 min (Supporting Information Fig. S9). While an additional band was appeared at around 320 nm due to the formation of 2-nitrosobenzaldehyde in solution (Supporting Information Fig. S9). This process was accompanied by a color change of the solution of P1 copolymer from colorless to light yellow (photographs in Scheme 2). Consequently, the hydrophobic PNBA segment converted into hydrophilic poly(acrylic acid) (PAA), which resulted in the transformation of amphiphilic (P(OEtOxA)-ran-PNBA) to fully hydrophilic (P(OEtOxA)-ran-PAA) copolymer (Scheme 2).

Therefore it would be more interesting to investigate the LCST behavior of the resultant (P(OEtOxA)-ran-PAA copolymer in water. To investigate, aqueous P1 solution (0.2 wt %) was first irradiated with UV light for 60 min and $T_{\rm cp}$ of this exposed solution was measured by turbidimetry. The transparent light yellow solution obtained after UV light irradiation became turbid upon heating, and transparency reappeared upon cooling, indicating that the exposed solution also exhibited LCST-type phase transition (photographs in Scheme 2). As mentioned above, before UV light irradiation, T_{cp} of **P1** copolymer in water was 30 °C, which was increased to 39 °C after UV irradiation (Fig. 3). Also, it was observed that the $T_{\rm cp}$ of 0.05 wt % P1 solution increased from 44 (before UV irradiation) to 58 °C (exposed with UV irradiation) (Supporting Information Fig. S10). In the case of **P2** copolymer (0.2 wt %) in water, the $T_{\rm cp}$ was 46 °C before UV irradiation, which was also increased to 61 °C after UV irradiation (Fig. 5). Therefore, the first conclusion was that the cloud point of P2 was increased by 16 °C (from 30 to 46 °C)

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SCHEME 2 Photocleavage of P(OEtOxA)-*ran*-PNBA copolymer **(A)**; Photographs of P(OEtOxA₄₀₀)-*ran*-PNBA **(P1)** in water (0.2 wt %) before UV irradiation and after UV irradiation indicating the LCST-type soluble-to-insoluble transition **(B)**. [Color figure can be viewed at wileyonlinelibrary.com]

compared to that of P1 due to the increase of length of thermoresponsive P(OEtOxA) segments from P1 to P2. Furthermore it should be noted that $T_{\rm cp}$ values of UV light irradiated copolymers' solution was about 10 °C higher than that of the nonirradiated solution. Such increase of $T_{\rm cp}$ values can be explained in the following way. The photocleavage of NBE moiety of PNBA segment of P(OEtOxA)-ran-PNBA copolymer resulting in the transformation of hydrophobic PNBA segments into hydrophilic PAA segments (Scheme 2). Thus, the irreversible photocleavage of NBE moieties of PNBA increases the overall hydrophilicity of the P(OEtOxA)-ran-PNBA copolymer due to its transformation into P(OEtOxA)-ran-PAA copolymer and consequently, increases the cloud point by more than 10 °C. Thus, we can easily tune the $T_{\rm cp}$ of copolymer solution over a large window of temperature ranging from 28 to 60 °C not only by varying concentration but also simply by irradiating with UV light, making these copolymer highly appropriate for varieties of potential applications.

Self-Aggregation of P(OEtOxA)-*Ran*-PNBA Copolymer into Micelles and Their UV- and Temperature-Induced Disaggregation

It is very common that amphiphilic block copolymers selfassemble into micelles when they are dissolved in a selective solvent where two polymer blocks have differences in their solubility.⁶² However, such micelle formations due to difference in solubility of two polymer segments of an amphiphilic random copolymers are comparatively less reported.^{20,43} This prompted us to investigate the self-aggregation of P(OEtOxA)-ran-PNBA copolymer into micelles as P(OEtOxA) segment is soluble in water, whereas PNBA segment is not. The aqueous **P1** copolymer solution (0.3 mg mL⁻¹) was kept undisturbed for some time to allow its self-aggregation and the solution was examined by FESEM at 20 °C, which is below the LCST of P1 copolymer. The FESEM image [Fig. 6(A)] of aqueous P1 copolymer solution showed the formation of dispersed spherical nanostructures with an average diameter of 71 nm. These spherical nanosized particles [Fig. 6(A)] are nothing but the micelles formed by the selfaggregation of amphiphilic P1 copolymer in water. The formation of micelles was also confirmed from TEM study. The aqueous P1 and P2 copolymer solution ($\sim 0.30 \text{ mg mL}^{-1}$) were examined through TEM at 20 °C and images were taken, which clearly showed the formation of nanostructured spherical micelles with an average diameters of 90 and 80 nm for P1 [Fig. 6(B)] and P2 (Supporting Information Fig. S11), respectively. We further examined both aqueous **P1** and **P2** copolymer solution (0.3 mg mL⁻¹) by DLS at 20



FIGURE 3 % transmittances at wavelength of 500 nm of 0.2 wt % aqueous solution of P(OEtOxA₄₀₀) homopolymer and 0.2 wt % aqueous solution of P(OEtOxA₄₀₀)-*ran*-PNBA **(P1)** copolymer before UV irradiation and after UV light irradiation. The transmittances were measured with a UV-vis spectrometer. [Color figure can be viewed at wileyonlinelibrary.com]

°C to provide further evidence of self-aggregation of copolymer molecules into micelles. The DLS data clearly indicated the presence of particles with average hydrodynamic diameters (D_h s) of 165 and 191 nm for **P1** and **P2**, respectively (Fig. 7). The formation of such particles can be ascribed to the self-assembly of P(OEtOxA)-*ran*-PNBA copolymer into micelles (Scheme 3) as established through FESEM and TEM mentioned above [Fig. 6(A,B)]. As expected, the D_h of the micelles measured by DLS was always higher than that of the same particles measured by FESEM and TEM. Thus, DLS, FESEM, and TEM results clearly confirmed the formation of spherical micelles due to the self-aggregation of P(OEtOxA)*ran*-PNBA molecules in water as schematically shown in Scheme 3. The self-aggregation phenomenon of amphiphilic random copolymer into micelles has already been reported by quite few research groups.^{20,43} In this case, the micelle formation can be attributed to the difference in polarities of P(OEtOxA) and PNBA segments with respect to H₂O, where hydrophobic PNBA segments remain inside the core of the micelle and the hydrophilic P(OEtOxA) segments stretches toward the solvent front forming the corona (Scheme 3).

Thus, to investigate the effect of UV light irradiation on the self-assembly of the copolymer P(OEtOxA)-ran-PNBA in water, aqueous P1 micellar solution was irradiated with UV light for 45 min. The snapshot images evinced that the colorless micellar solution of P1 converted to a light yellow solution after UV light irradiation, suggesting some definite changes [Fig. 6(A,C)]. As mentioned above, before irradiation, P1 copolymer self-assembled into micelles with an average diameter of 71 nm (FESEM) in H₂O [Fig. 6(A)]. However, FESEM image of the aqueous P1 micellar solution after UV light irradiation did not show any particles (micelles); rather, a continuous polymer film was observed [Fig. 6(C)]. This is mainly because of the fact that upon light irradiation, PNBA segments of P(OEtOxA)-ran-PNBA copolymer converted hydrophilic PAA segments (Scheme 2), which resulted in the formation of fully hydrophilic P(OEtOxA)-ran-PAA from amphiphilic P(OEtOxA)-ran-PNBA copolymer. This transformation accompanied with the change of hydrophilic-lipophilic balance (HLB) of copolymer molecule, leading to the simple disruption of micellar assemblies as schematically shown in Scheme 3.



FIGURE 4 % transmittance curves for P(OEtOxA₄₀₀)-*ran*-PNBA (**P1**) copolymer with varying concentration in deionized water (heating rate 2 °C min⁻¹) (A); LCST-type phase diagram of binary mixture of P(OEtOxA₄₀₀)-*ran*-PNBA (**P1**) copolymer and H₂O (**B**). [Color figure can be viewed at wileyonlinelibrary.com]

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FIGURE 5 Variation of % transmittance (at $\lambda = 500$ nm) of 0.2 wt % aqueous solution of P(OEtOxA₈₀₀)-*ran*-PNBA **(P2)** before UV light irradiation and after 1 h of UV irradiation ($\lambda = 365$ nm) with temperature. [Color figure can be viewed at wileyonlinelibrary.com]

Furthermore, P(OEtOxA)-ran-PNBA copolymer exhibited LCST-type thermoresponsiveness in water as mentioned above. Therefore, one would expect some kind of change in the micellar structures of this copolymer above its cloud point before UV irradiation. To check this, aqueous micellar solution of **P1** copolymer (0.3 mg mL⁻¹) was heated above LCST (60 °C) and was examined by DLS. The $D_{\rm h}$ of the P1 micelles was found to be 140 nm with a PDI = 0.104 at 60 °C, which is lower than that of the same micelles (165 nm with a PDI = 0.250) when the measurement was carried out at 20 °C (Fig. 7). D_h value of P2 copolymer micelles was 166 nm with PDI of 0.156 at 60 °C, which is also lower than that (190 nm with a PDI of 0.219) same micelles measured at 25 °C (Fig. 7). We reason that at low temperature, P(OEtOxA) segments present in the corona of copolymer micelles remain in the extended coil form because of the hydrogen bonding interaction with water molecules. Upon increasing the temperature above LCST, hydrophilic P(OEtOxA) corona becomes hydrophobic and collapsed over micellar core resulting in the shrinkage of the micellar size (Scheme 3).^{40,43,63} Shrinkage of micellar size at above LCST was also observed from TEM study, which showed spherical particles with average diameter of 45 nm for P1 copolymer at 65 °C [Fig. 6(D)], comparatively lower than that (~90 and 71 nm) measured by TEM [Fig. 6(B)] and FESEM [Fig. 6(A)],



FIGURE 6 FESEM image and photograph of P(OEtOxA)₄₀₀-*ran*-PNBA (P1) micellar solution in H₂O before UV irradiation (A); TEM image of P(OEtOxA)₄₀₀-*ran*-PNBA (P1) micellar solution in H₂O before UV irradiation (B); FESEM image and photograph of the aqueous P1 micellar solution after UV light irradiation for 45 min (C); TEM image of the P1 micellar solution at 65 °C (D). ([P1] in H₂O = 0.3 mg mL⁻¹). [Color figure can be viewed at wileyonlinelibrary.com]



FIGURE 7 DLS data of $P(OEtOxA_{400})$ -*ran*-PNBA **(P1)** and $P(OEtOxA_{800})$ -*ran*-PNBA **(P2)** copolymers in H₂O (0.30 mg mL⁻¹) at two different temperatures. [Color figure can be viewed at wileyonlinelibrary.com]

respectively at 20 °C. Thus, above the $T_{\rm cp}$ (60 °C), hydrophobicity of the micellar corona increases resulting in the shrinkage of micelle (Scheme 3) as established from FESEM and TEM and DLS analysis.

Encapsulation of Dye and Its Photo- and Temperature-Induced Release

A model hydrophobic dye, nile red (NR) was used to study the encapsulation of guest molecules inside the hydrophobic core of P(OEtOxA)-ran-PNBA copolymer micelles and their photo- and temperature-induced release. NR is mostly insoluble in water; therefore, it does not exhibit any fluorescence. However, the intensity dramatically enhances if the dye resides in a hydrophobic environment, for example inside of a hydrophobic micellar core. In this case, the spectrum [Fig. 8(A)] of NR-encapsulated P1 micelle (for encapsulation protocol, see Experimental section) revealed a clear intense peak at around 630 nm ($\lambda_{ex} = 550$ nm), while there was no observable fluorescence peak of an aqueous solution of NR prepared under identical condition in the absence of P1 micelle [Fig. 8(A)]. This result indicated the encapsulation of NR inside P1 micelles in aqueous solution. Afterwards, NRencapsulated aqueous P1 micelle was subjected to UV light irradiation for NR release. Figure 8(B) showed that the intensity of emission at $\lambda_{max} = 630$ nm for NR present inside the micellar core decreased with increasing UV irradiation time (0-30 min). As can be seen clearly, the emission intensity of NR was decreased dramatically to almost zero upon light irradiation for only 10 min [Fig. 8(B)]. Actually, NRloaded P1 micelles were disrupted upon UV irradiation due to the photocleavage of NBE moieties of PNBA segments; consequently there was a release of loaded NR from hydrophobic micellar core to water medium outside of the micelles (Scheme 3). However, NR is not soluble in water and therefore it would not display any fluorescence. Hence, the emission intensity of NR decreased with increasing UV irradiation time which in turns confirmed the phototriggered release of NR (Scheme 3).



SCHEME 3 Schematic representation of P(OEtOxA)-*ran*-PNBA copolymer self-aggregation into micelles and nile red (NR) encapsulation followed by its temperature- and photo-induced release. [Color figure can be viewed at wileyonlinelibrary.com]



FIGURE 8 Emission spectra of NR-encapsulated P(OEtOxA₄₀₀)-*ran*-PNBA (**P1**) copolymer micelles and neat NR in absence of **P1** copolymer in water (**A**); Emission spectra of remaining NR inside the P(OEtOxA₄₀₀)-*ran*-PNBA (**P1**) copolymer micellar core in water after UV light irradiation for different times (**B**) and at different temperatures (**C**). [Color figure can be viewed at wileyonline-library.com]

The emission intensity of NR resides in the micellar core also recorded with increasing temperatures from 20 to 60 °C [Fig. 8(C)]. The data clearly showed that the emission intensity of NR decreased gradually with increasing temperature [Fig. 8(C)]. The reason for such decrease of NR intensity is the transformation of P(OEtOxA) corona of micelles from hydrophilic to hydrophobic nature resulting in the collapse of corona over micellar core and ultimately some disruption of micellar assembly upon increasing temperature. This leads to the release of encapsulated NR from micellar core into aqueous medium. However, the emission intensity was minimum at 60 °C, that is, just above the LCST of the copolymer [Fig. 8(C)]. Afterwards it remained constant and no further reduction of emission intensity of NR was observed [Fig. 8(C)] upon increasing of temperature indicating that some of the loaded NR was still remain encapsulated in the collapsed micelles (Scheme 3). These results further proved that the micelles were shrunk at above cloud point. Thus, one can

easily control the release of encapsulated guest molecules from aqueous P(OEtOxA)-*ran*-PNBA copolymer micellar solution simply by altering the temperature and UV light irradiation time.

CONCLUSIONS

In summary, we have designed and synthesized doublyresponsive amphiphilic random P(OEtOxA)-*ran*-PNBA copolymer with light-sensitive PNBA segments and thermoresponsive P(OEtOxA) segments via the combination of microwave-assisted CROP and RAFT polymerization techniques. Indeed, P(OEtOxA)-*ran*-PNBA copolymer exhibited tunable LCST-type phase transition with respect to copolymer compositions. Upon UV light irradiation, T_{cp} of the copolymer increased due to the photocleavage of PNBA segments resulting in the formation of more hydrophilic P(OEtOxA)-*ran*-PAA compared to that of P(OEtOxA)-*ran*-PNBA copolymer.



P(OEtOxA)-*ran*-PNBA copolymer indeed self-aggregated into well-dispersed spherical micelles in H_2O at 20 °C. Upon UV light irradiation, the amphiphilic P(OEtOxA)-*ran*-PNBA converted into fully hydrophilic P(OEtOxA)-*ran*-PAA, causing the disruption of micelles in water. Additionally, above LCST, the hydrophilic P(OEtOxA) segments of corona are converted into hydrophobic segments and it collapsed over micellar core, resulting in the shrinkage of the micelles into smaller size. Finally, as a proof concept, NR was successfully encapsulated into the P(OEtOxA)-*ran*-PNBA micellar core, and its light- and temperature-induced release was clearly demonstrated. This multiresponsive copolymer assembly can be potentially employed in highly important areas such as controlled release of drugs and dyes, biosensors and nanoreactors.

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