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Efficient Route to Synthesize Biocompatible Delivery System with Its Morphological Changes Upon Solvent Variation

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Supporting Information

Experimental Procedure:

1. Materials:

All reagents N-Phenylmaleimide, Mercaptoethanol, Methacrylic acid, Diethyl oxomalonate (DEOM), Tin chloride (IV) ($SnCl_4$), Thiourea, Sodium, Triethylamine (Et_3N), 1-Ethyl-3-(3-dimethylamino propyl) carbodiimide (EDC-HCl), 4-dimethyl aminopyridine (DMAP), 2-Ethylbromoisobutyrate (2-EBiB), N, N, N', N", N", Pentamethylethylenetriamine (PMDETA), Copper (I) Bromide (CuBr), Doxorubicin, ethanol (EtOH) and dry dimethylformamide (dry DMF) was purchased from Sigma Aldrich. The solvents tetrahydrofuran (THF), dichloromethane (DCM), and methanol (MeOH) were dried over calcium hydride (CaH_2) and sodium metal used for reactions. The sodium methoxide (NaOMe) was freshly prepared from methanol and Na metal. All deuterated solvents like chloroform-d ($CDCl_3$), dimethyl sulphoxide-d (DMSO-d_6), deuterium Oxide (D_2O) were purchased from Cambridge Isotope Laboratories.

2. Characterization:

Nuclear Magnetic Resonance (NMR): The ^{1}H and ^{13}C NMR spectroscopy were carried out on a Bruker 500 MHz spectrometer using $CDCl_3$, DMSO-d_6 and D_2O as solvents. ^{1}H and ^{13}C NMR spectra were calibrated to tetramethylsilane as an internal standard (δ: 0.00).

Advanced Polymer Chromatography (APC): Molecular weights and PDIs were measured by Waters ACQUITY Advanced Polymer Chromatography. The instrument contains an HPLC pump, an ACQUITY® refractive index (RI) detector, ACQUITY APC™ XT 2002.5 µM (4.6 × 7.5 mm) column in THF and water at 45 ºC at 0.25 mL/min flow rate. Polymethylmethacrylate (PMMA) was used to calibrate the instrument.

Fluorometry: Fluorescence emission spectra were recorded on a Fluorescence spectrometer (Horiba Jobin Yon, Fluromax-3, Xe-150 W, 250-900 nm).

Dynamic Light Scattering (DLS): Particle size of QDs were measured by dynamic light scattering (DLS), using a Horiba Zetasizer Nano equipped with a 4.0 mW HeNe laser operating at λ = 633 nm. All samples were measured in aqueous as well as methanol at room temperature and a scattering angle of 173°.

Scanning Electron Microscopy (SEM): High-resolution SEM was performed on a Zeiss Microscope; SUPRA 55VP-Field Emission Scanning Electron Microscope. High-performance variable pressure FE-SEM with patented GEMINI column technology. Schottky type field emitter system, single condenser with crossover-free beam path. Resolution: 1.0 nm at 15 kV; 1.6 nm at 1 kV high vacuum mode; 2.0 nm at 30kV at variable pressure mode.

Transmission Electron Microscopy (TEM): Transmission electron microscopy (TEM) was performed on a JEOL 200 CX microscope. TEM grids were purchased from Ted Pella, Inc. and consisted of 3-4 nm amorphous carbon film supported on a 400-mesh copper grid. High-resolution Cryo-TEM was performed on an FEI instrument using Tecnai 20 using a 626 GATAN cryo-holder.

3. Synthesis:

Synthesis of MALME:

First, mercaptoethanol (1.35 gm) was dissolved using DCM (5 ml) in a round-bottomed flask and then slowly added triethylamine (0.1 gm). This mixture was allowed to stir at room temperature for 30 minutes. Next N-phenyl maleimide (1 gm) was added into that reaction mixture. Finally, it was allowed to stir for another 24 h at room temperature. The product was collected in the DCM layer. ^{1}H NMR (500 MHz, DMSO-d_6): δ_{ppm} 7.50 (d, 2H), 7.44 (t, 1H), 7.28 (t, 2H), 4.91 (s, 1H), 4.19 (t, 1H), 3.60 (t, 2H), 3.40 (d, 1H), 2.90 (d, 1H), 2.80 (t, 2H); ^{13}C NMR (500 MHz, DMSO-d_6): δ_{ppm} 176.49, 174.80, 132.81, 129.41, 128.88, 127.49, 61.10, 36.85 and 33.88.

Synthesis of MALMEMA:

Methacrylic acid (1 gm) was dissolved in DCM (5 ml), then EDC.HCl (2.67 gm) was added into that and allowed to stir for 45 minutes. Then MALME (3 gm) in DCM (5 ml) was added into that followed by DMAP (catalytic amount) and the system was allowed to stir for another 24 h at room temperature. Product was collected in DCM layer and purified through column chromatography using ethyl acetate/hexane mixture. ^{1}H NMR (500 MHz, DMSO-d_6): δ_{ppm} 7.50 (d, 2H), 7.44 (t, 1H), 7.28 (t, 2H), 6.06 (s, 1H), 5.70 (s, 1H), 4.19 (t, 1H), 3.60 (t, 2H), 3.40 (d, 1H), 2.90 (d, 1H), 2.80 (t, 2H), 1.96 (s, 3H); ^{13}C NMR (500 MHz, DMSO-d_6): δ_{ppm} 176.43, 174.57, 166.84, 136.15, 132.74, 129.39, 128.90, 127.45, 126.54, 63. 75, 36.62, 30.23 and 18.44.

Synthesis of PMMA-MAL:

CuBr (I) (0.00125 gm) and the ligand PMDETA (0.002 ml) were dissolved in dry DMF (2 ml). Next, the monomer MALMEMA (0.250 gm) was added into that mixture followed by the addition of initiator 2-EBiB (0.00125 gm). The reaction mixture was allowed to stir overnight at a temperature of 80 ºC. Next, the reaction mixture was diluted with THF and precipitated in

cold MeOH. The precipitate was filtered off and dried under a vacuum. ^{1}H NMR (500 MHz, $CDCl_3$): δ_{ppm} 7.3 to 7.5 (aromatic protons), 4.20, 3.96, 3.29, 3.00, 2.66, 1.96, 1.65.

Synthesis of PMMA-OXOMAL:

PMMA-MAL (1 gm) was dissolved in dry DCM (5 ml) under a nitrogen atmosphere. Then DEOM (1 ml) was added gradually into the solution. After that, the round-bottomed flask was kept in an ice bath to maintain the temperature between 0-5 ˚C. Then $SnCl_4$ (0.9 ml) was added dropwise to the mixture of the solution with continuous stirring. Finally, the reaction mixture was allowed to stir at room temperature for 24 h. The product was collected by precipitation in cold MeOH. Advanced polymer chromatography was done in THF to obtain the molecular weight. ^{1}H NMR (500 MHz, DMSO-d_6): δ_{ppm} 7.3 to 7.5 (aromatic protons), 4.15, 3.11, 2.98, 2.74, 1.84, 1.2.

Synthesis of PMMA-TBA:

50 mg of **PMMA-OXOMAL** was dissolved in 5 ml of dry THF and freshly prepared sodium methoxide (50 mg) was dissolved in 20 ml dry methanol. Thiourea (50 mg) was added to the reaction mixture. The reaction mixture was stirred for 24 h at room temperature. The product was isolated by precipitating using excess THF. ^{1}H NMR (500 MHz, DMSO-d_6): δ_{ppm} 8.03, 7.44, 7.29, 4.23, 3.96, 3.30, 2.90, 2.65, 1.98, 1.28.

4. Self-assembly study:

Critical Aggregation Concentration (CAC) studies were done to investigate the self-assembly behaviour of the polymer. From DLS we got to know about the size of the aggregates. TEM and cryo-TEM studies were done to investigate the morphology of the aggregates. The polymer was dissolved in water (1 mg/ml) and water solution was taken for further experiment. For CAC, Nile red dye was used as a hydrophobic probe.

5. Dye encapsulation study:

Here, Nile red dye was taken into consideration and the solution was made in DCM with the concentration of 1 mg/ml. Three different concentrations (0.3 mg/ml, 0.5 mg/ml, 0.7 mg/ml, and 1 mg/ml) of the polymer were prepared and 1 ml dye solution was added in each set of solutions, and fluorescence intensities of the DCM layer were measured before and after sonication.

6. Dye release study:

It was done in a water-octanol system. Dye-loaded aggregates were taken and added 1 ml of octanol to the solution and recorded fluorescence intensity with a certain interval of time the octanol layered solution.

7. Drug encapsulation study:

1 mg of Doxorubicin drug was taken and it was made neutral using excess triethylamine in DMSO solution. Polymeric aggregates were prepared by dissolving the polymer into water (1 mg/ml). Encapsulation was done using the dialysis method for 24 h with the aggregate solution and neutral doxorubicin. Then the encapsulated nano-aggregates were lyophilized and dried.

8. Drug release study:

A drug release study was done using the doxorubicin-loaded polymers following the same procedure in pH 5.5 solution with HCL-water and 7.4 with PBS buffer solution.

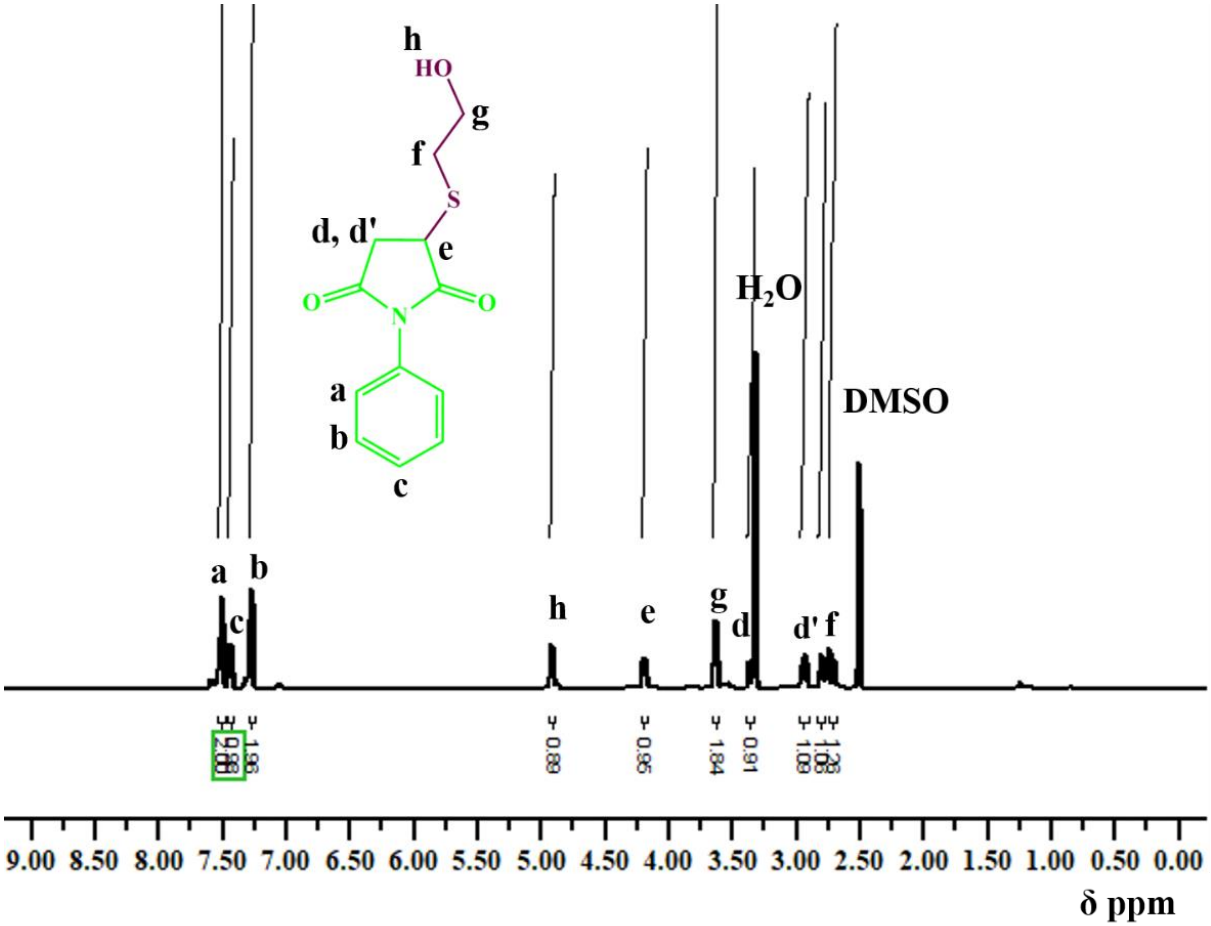


Figure S1a ^{1}H NMR spectrum of MALME in DMSO-d_6

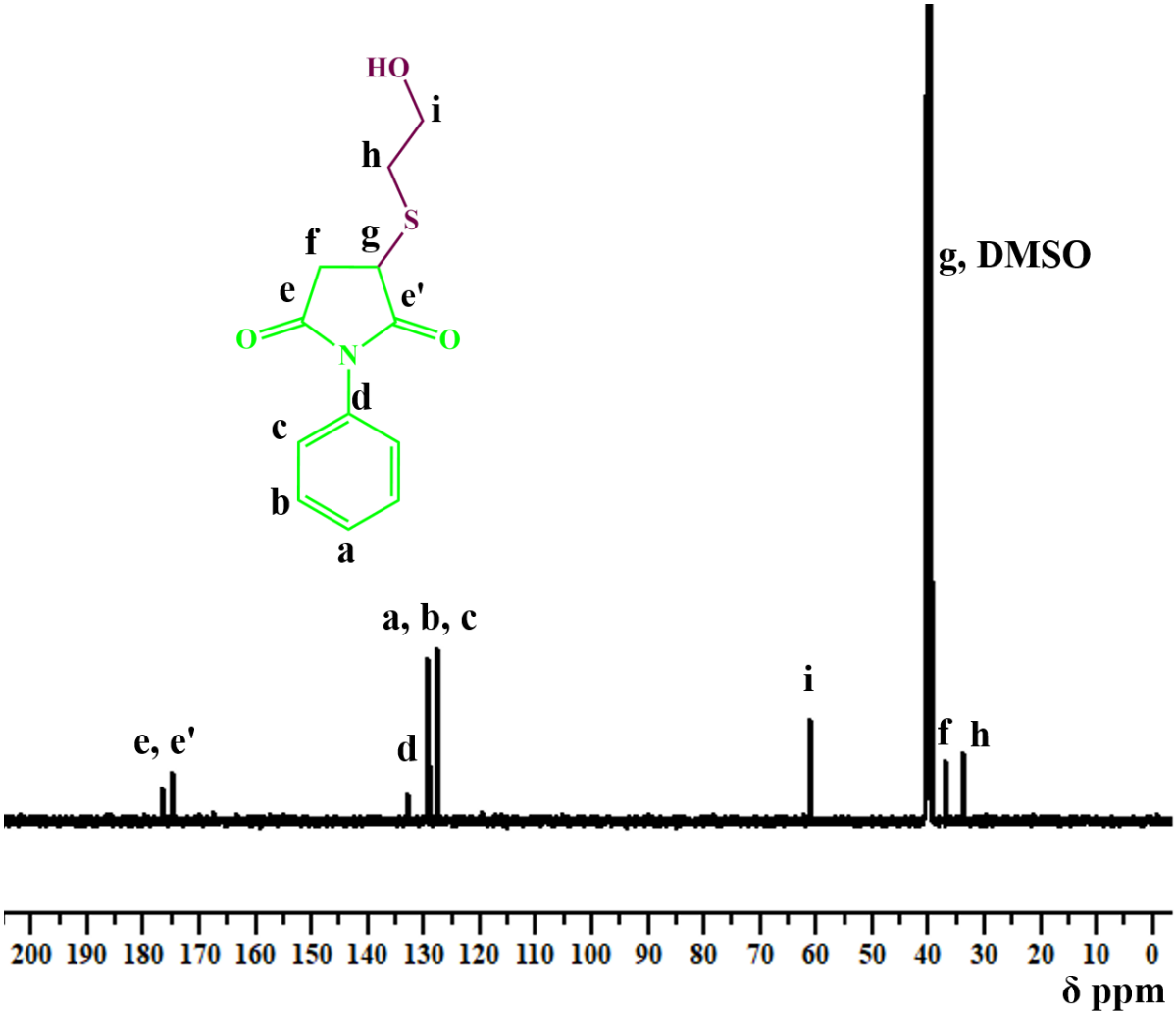


Figure S1b ^{13}C NMR spectrum of MALME in DMSO-d_6

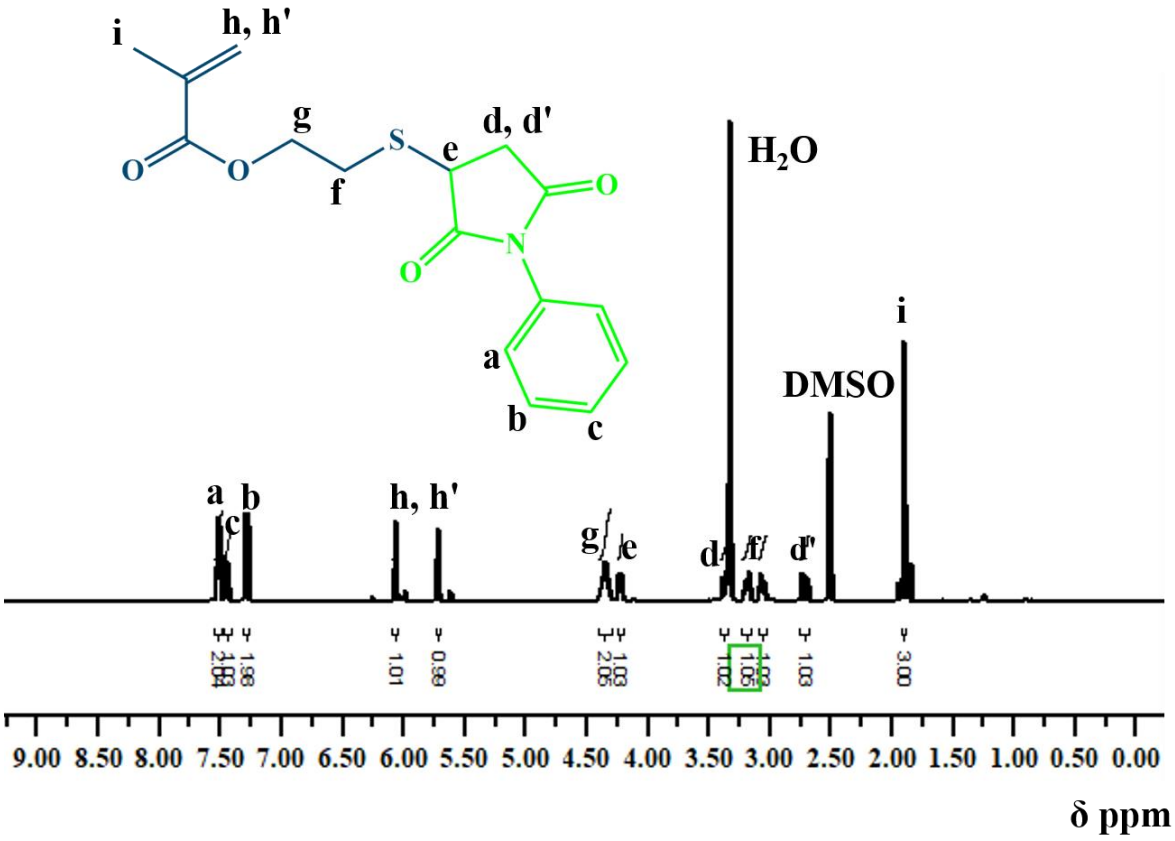


Figure S2a ^{1}H NMR spectrum of MALMEMA in DMSO-d$_6$

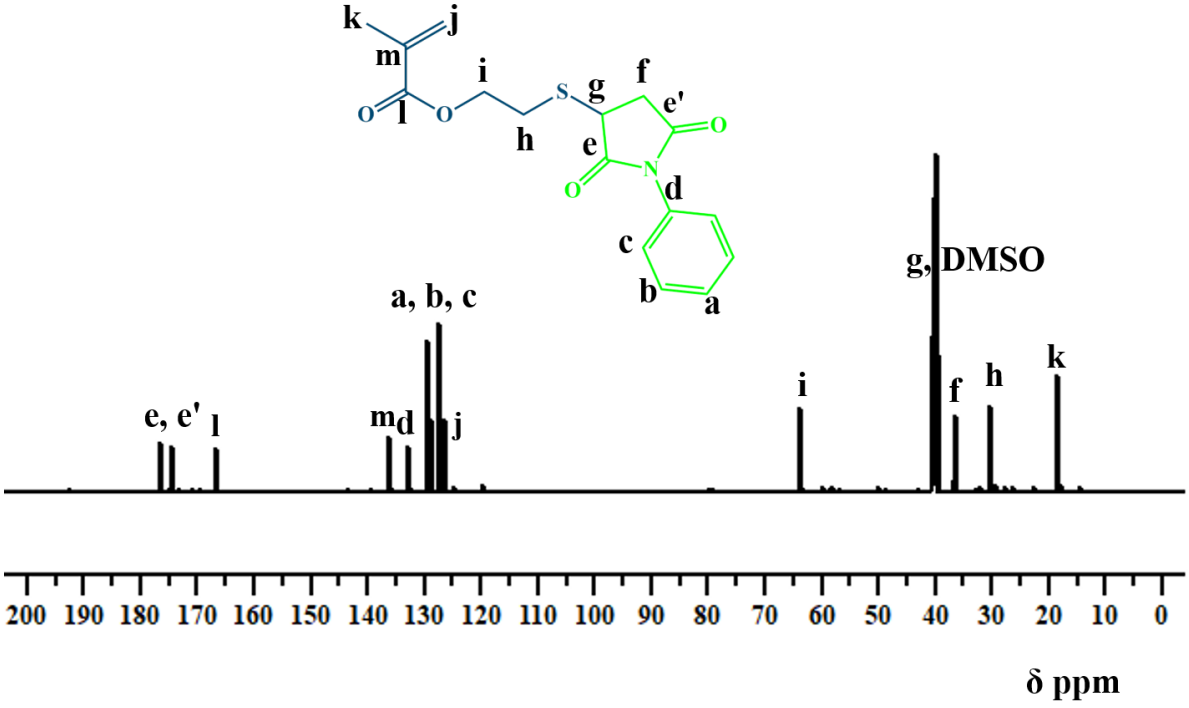


Figure S2b ^{13}C NMR spectrum of MALMEMA in DMSO-d$_6$

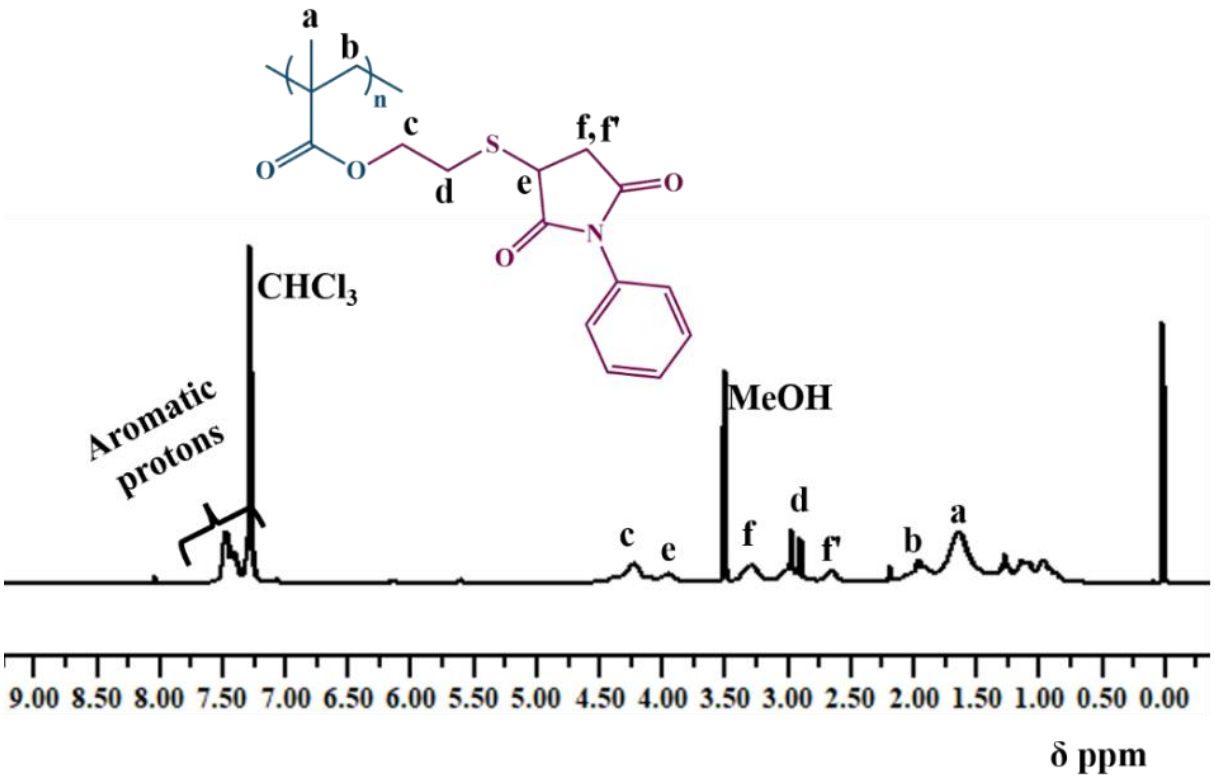


Figure S3 ^{1}H NMR spectrum of PMMA-MAL in $CDCl_3$

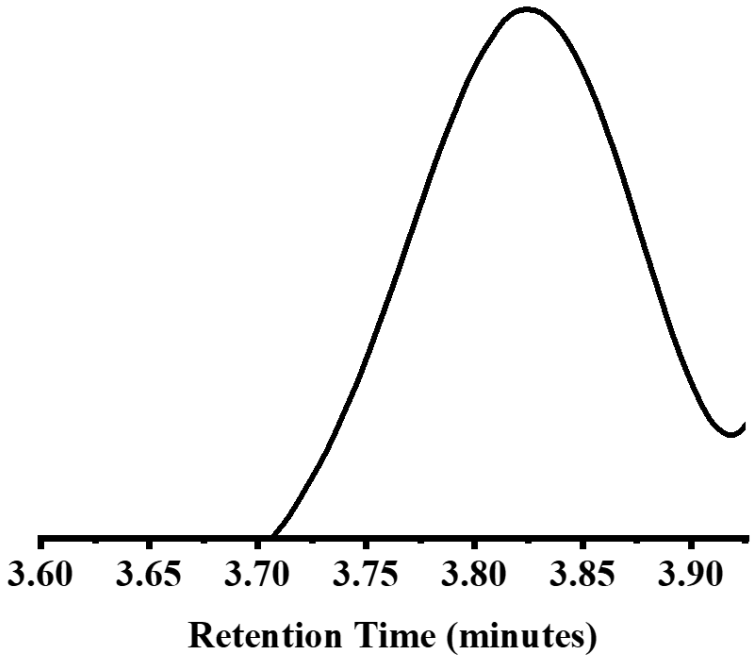


Figure S4 APC trace of PMMA-MAL in THF

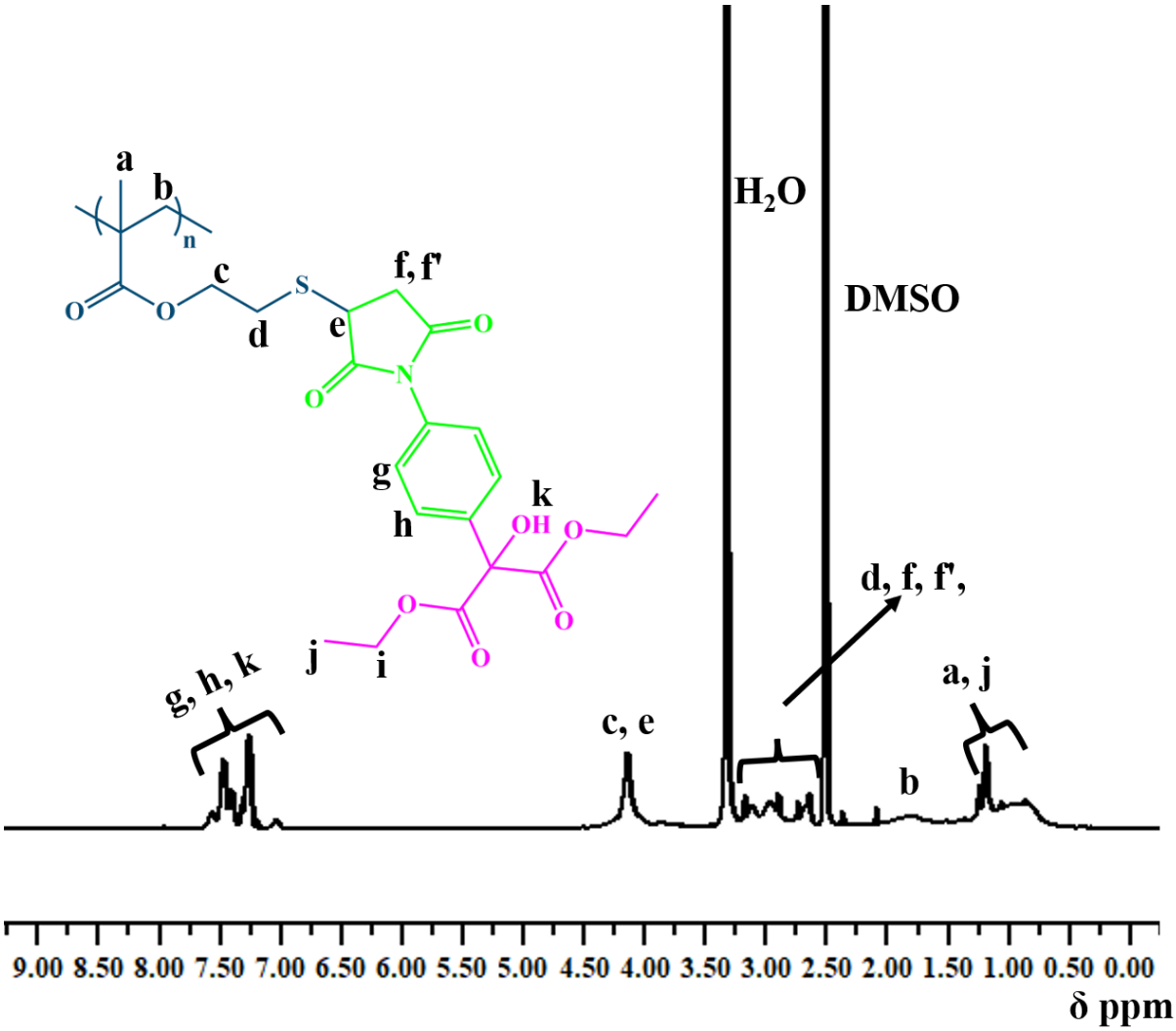


Figure S5 ^{1}H NMR spectrum of PMMA-OXOMAL in DMSO-d$_6$

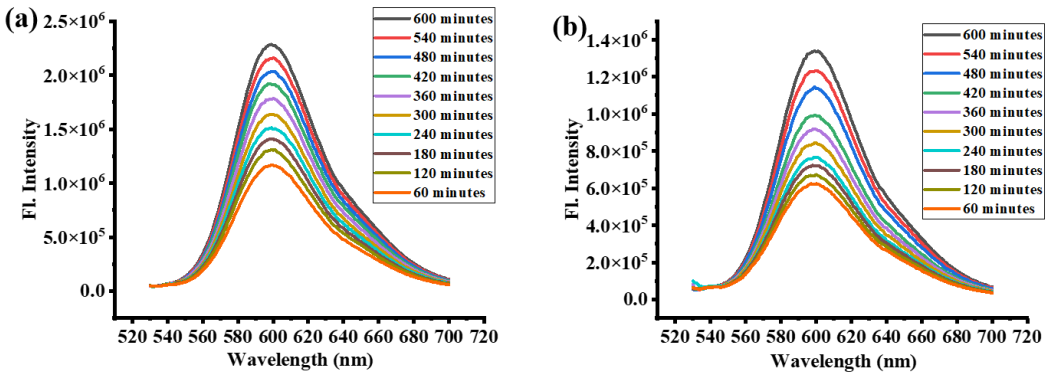


Figure S6 Dye release studies (titration curves) with PMMA-TBA (a: in octanol; b: at pH 5.5)

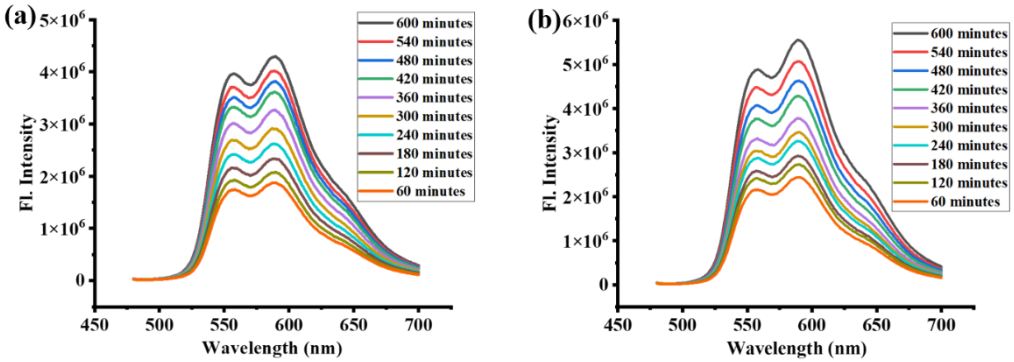
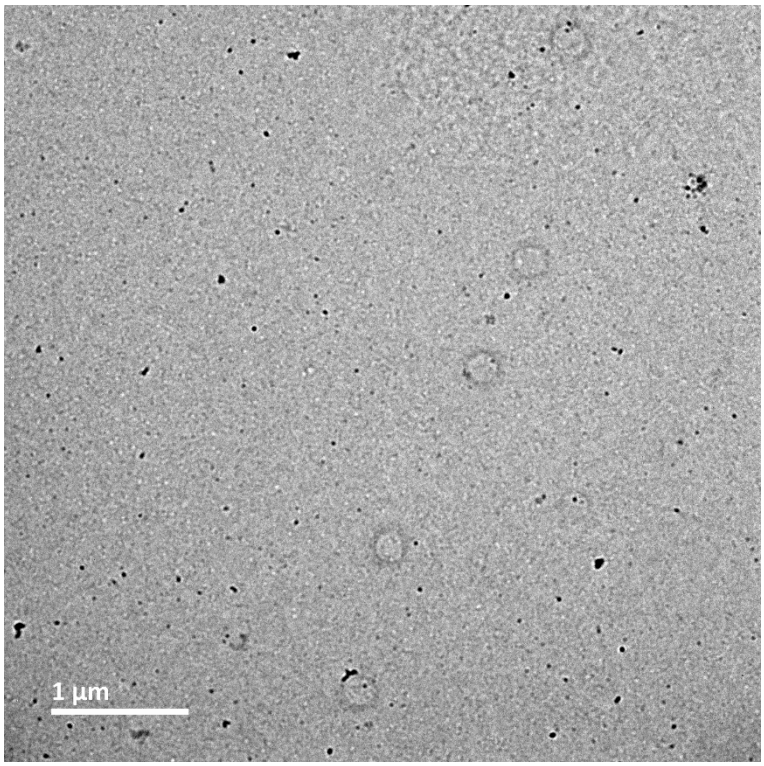
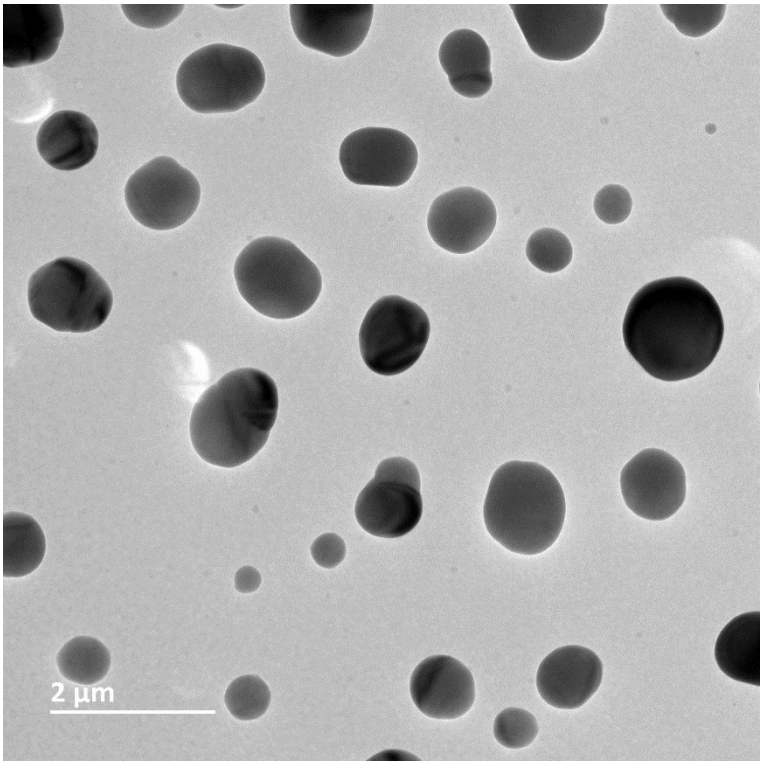


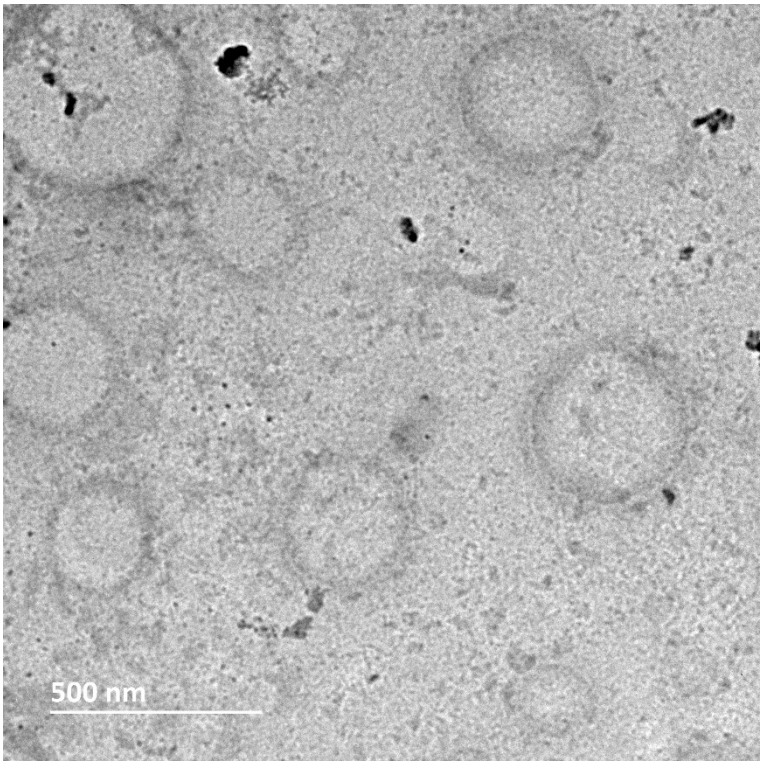
Figure S7 Drug release studies (titration curves) with PMMA-TBA (a: in octanol; b: at pH 5.5)



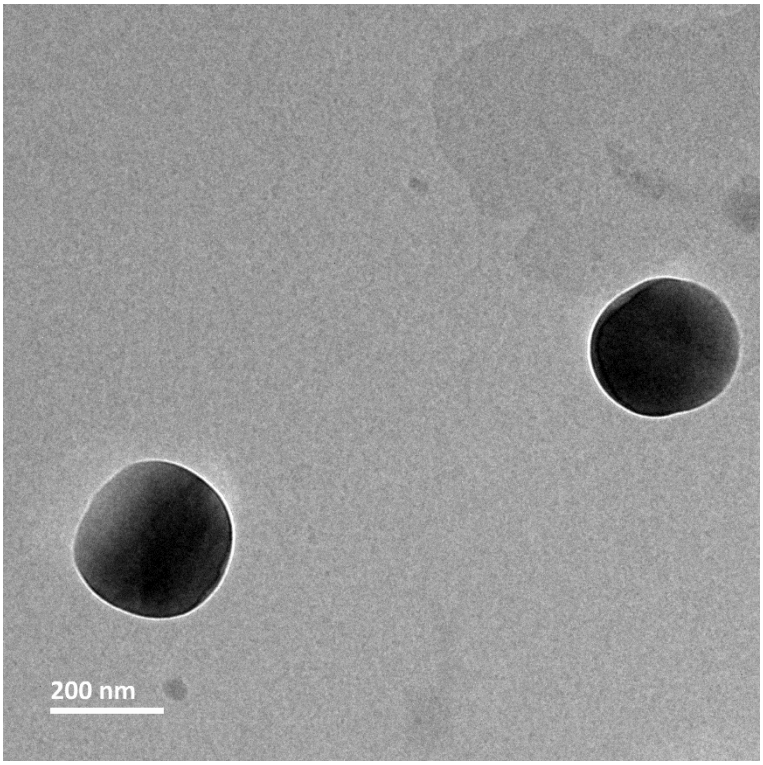
TEM image of PMMA-TBA in water



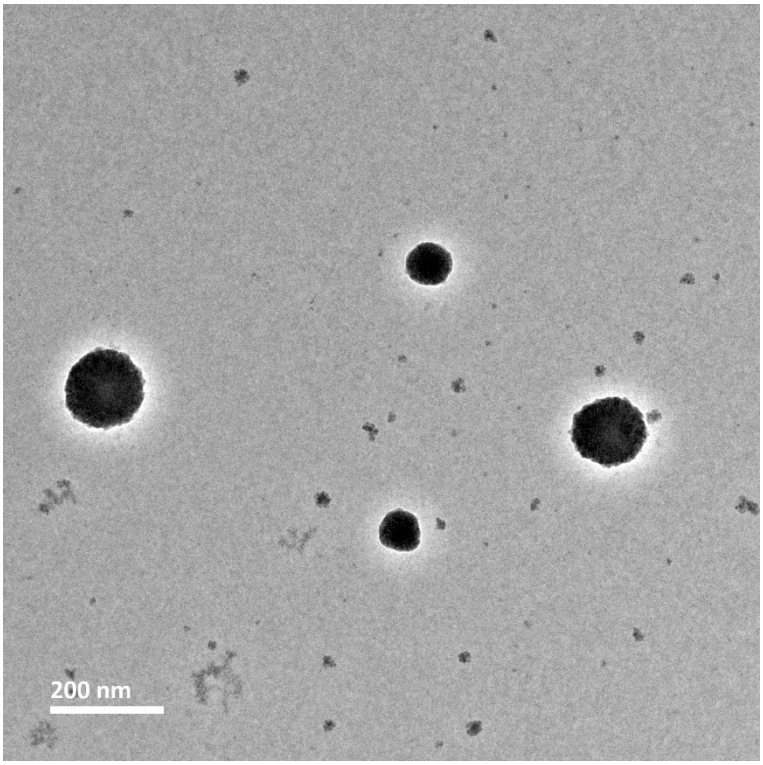
CryoTEM image of PMMA-TBA in water



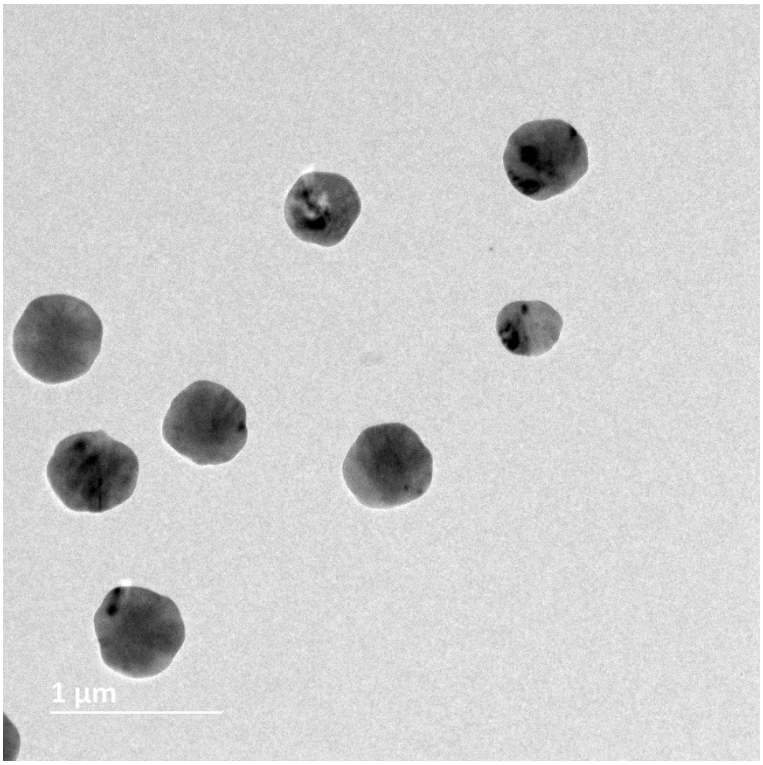
TEM image of PMMA-TBA in THF-water



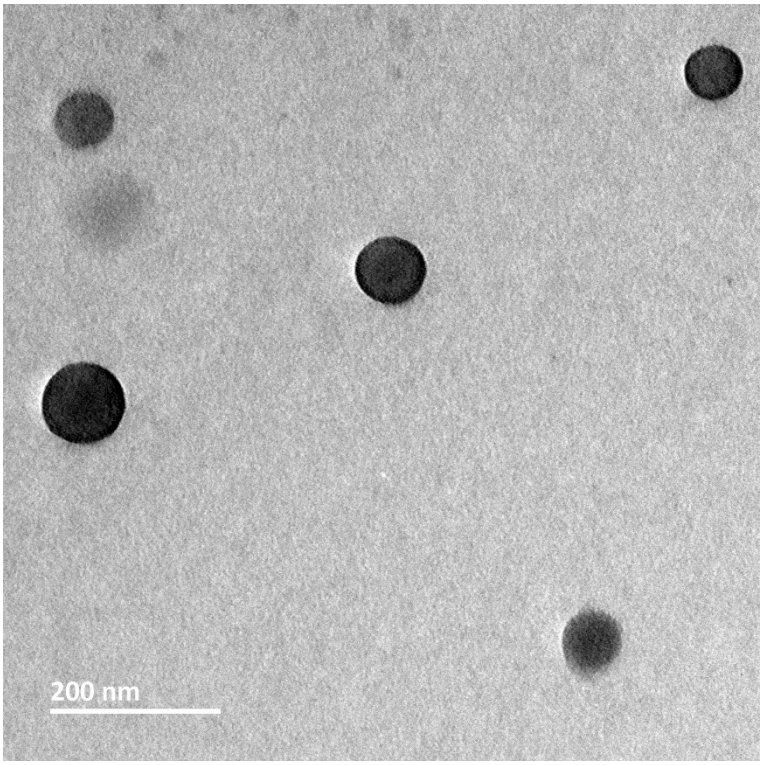
CryoTEM image of PMMA-TBA in THF-water



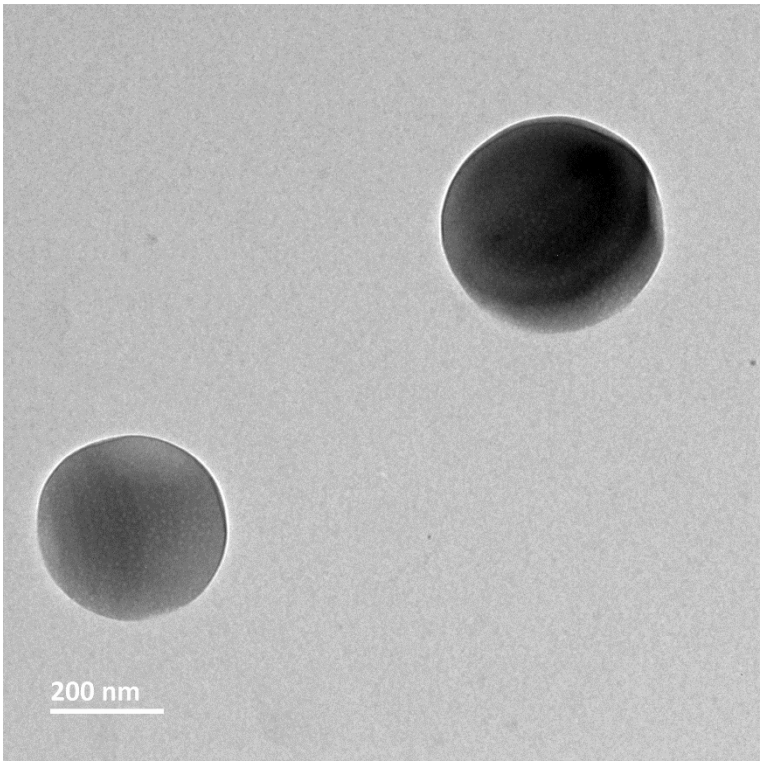
TEM image of PMMA-TBA in ACN-water



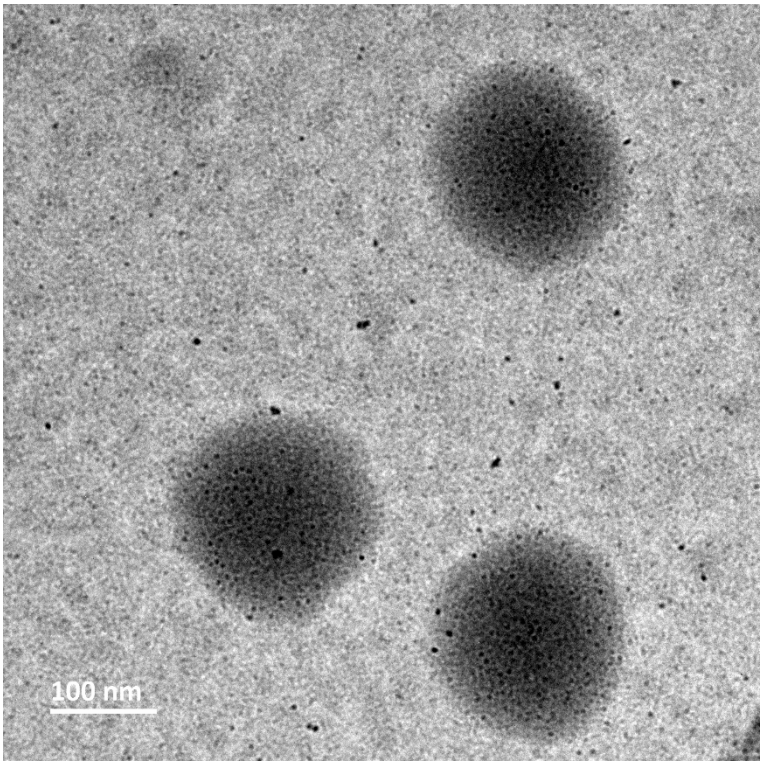
CryoTEM image of PMMA-TBA in ACN-water



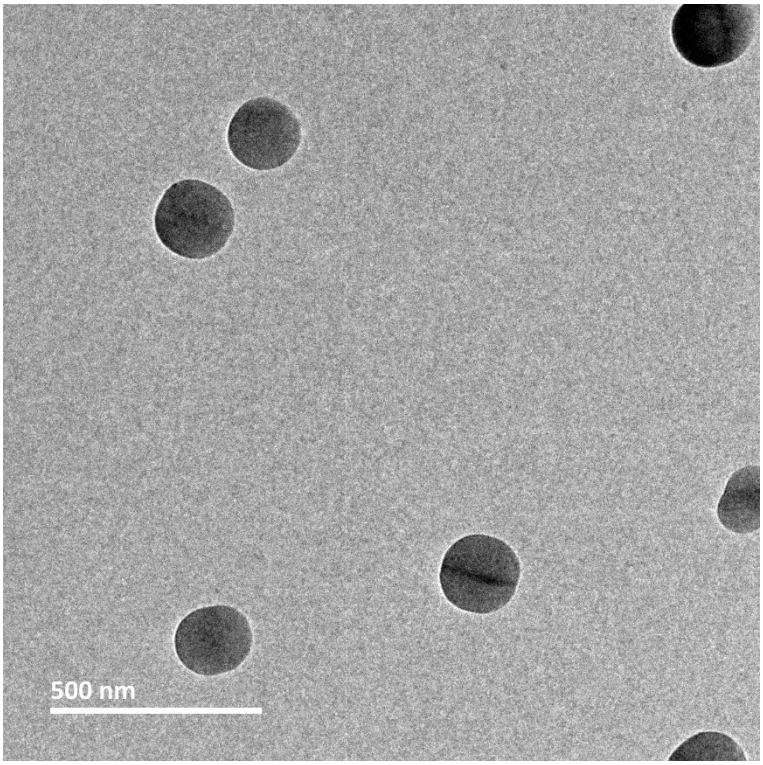
TEM image of PMMA-TBA in DW-water



CryoTEM image of PMMA-TBA in DW-water



TEM image of PMMA-TBA in MeOH-water



CryoTEM image of PMMA-TBA in MeOH-water

Characteristics of different solvents:

Solvent	Nature of solvent	Density (g/ml)	Polarity Index
THF	**Polar aprotic**	**0.888**	**4**
MeOH	**Polar protic**	**0.7913**	**5.1**
ACN	**Polar aprotic**	**0.7822**	**5.8**
1,4 Dioxane	**Polar aprotic**	**1.033**	**4.8**

Order of Polarity: THF<1,4 Dioxane<MeOH<ACN

Order of Density: ACN<MeOH< THF < 1,4 Dioxane

Size and shape of the aggregates of **PMMA-TBA**:

Solvent Mixture	Morphology	Size (TEM)	Size (Cryo-TEM)	Size (SEM)
THF/Water	**Spherical aggregate**	**300 nm**	**350 nm**	**350 nm**
MeOH/Water	**Spherical aggregate**	**250 nm**	**350 nm**	**350 nm**
ACN/Water	**Spherical aggregate**	**100 nm**	**500 nm**	**450 nm**
Dioxane/Water	**Spherical aggregate**	**150 nm**	**250 nm**	**250 nm**