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Degradable Diblock Copolymer Vesicles *via* Radical Ring-Opening Polymerization-Induced Self-Assembly in Aqueous Media

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ABSTRACT: The development of degradable polymeric vesicles holds promise for next-generation controlled delivery systems and responsive materials, particularly for therapy and diagnostics. However, achieving precise control over their synthesis and degradation behavior remains a significant challenge. Here, we report the synthesis of degradable diblock copolymer vesicles *via* radical ring-opening polymerization-induced self-assembly (rROPISA) in aqueous media. This approach employs radical ring-opening copolymerization (rROP) of a thionolactone with a water-miscible vinyl monomer to introduce cleavable thioester groups within the membrane-forming polymer chains. More specifically, a water-soluble poly(*N,N*-dimethylacrylamide) (PDMAC) precursor is chain-extended by statistically copolymerizing dibenzo[*c,e*]oxepane-5-thione (DOT) with 2-methoxyethyl acrylate (MEA) using a reversible addition–fragmentation chain transfer (RAFT) aqueous dispersion polymerization formulation. The disparity in reactivity between DOT and MEA is addressed using a comonomer-starved feed strategy, with MEA acting as a co-solvent for the water-insoluble DOT. This controlled feeding approach ensures a more uniform distribution of DOT repeat units within the hydrophobic core block, significantly improving copolymer composition control, enabling DOT incorporation of up to ~4 mol%, and enhancing overall comonomer conversion. Compared to conventional one-shot batch syntheses, this new method yielded vesicles with superior degradation profiles. Morphological characterization using cryogenic transmission electron microscopy (cryo-TEM), dynamic light scattering (DLS) and small-angle X-ray scattering (SAXS) confirmed the formation of well-defined vesicles. Hydrolytic degradation studies conducted in mildly basic aqueous media led to vesicle disintegration, as indicated by cryo-TEM, DLS and SAXS analyses. Importantly, size exclusion chromatography (SEC) studies revealed the formation of significantly shorter copolymer chains and final molecular weight distributions comparable to that of the non-degradable PDMAC precursor, indicating efficient degradation of the membrane-forming P(MEA-*stat*-DOT) block. This study provides valuable insights regarding the rational design of degradable vinyl-based diblock copolymer vesicles, which augurs well for potential biomedical applications.

INTRODUCTION

Liposomes and block copolymer vesicles (also termed polymersomes) are widely used for drug delivery, gene therapy and theranostics.^{1,2,11–13,3–10} Such nanoparticles can be designed to respond to various physical stimuli, such as light, temperature, pH, shear stress, or magnetic/electric fields.^{14–22} Although synthetic liposomes more closely resemble eukaryotic cell membranes,²³ block copolymer vesicles offer greater structural diversity plus superior mechanical and colloidal stability.^{24–26} Furthermore, vesicle permeability can be readily tuned by varying the molar mass and chemical composition of the membrane-forming block.²³

Despite their extensive development for biomedical applications,^{23,27–33} there are very few examples of (bio)degradable block copolymer vesicles.^{34–37} Such degradability is critical to avoid accumulation within the body, which is likely to lead to long-term toxicity.³⁸ Literature reports of degradable vesicles almost invariably involve either polylactide or poly(ϵ -caprolactone) as the membrane-forming block. These aliphatic polyesters are prepared by ring-opening polymerization (ROP) of cyclic esters in organic solvents.³⁴ Hence such vesicles are typically formulated at relatively low copolymer concentration (~1–5 % w/w) in aqueous solution using post-polymerization processing techniques such as nanoprecipitation,³⁴ the solvent

switch method,³⁵ or thin film rehydration.³⁶ Moreover, such ROP syntheses are often conducted using toxic metal catalysts (e.g., tin octanoate).³⁹ More recently, hydrolytically degradable vesicles have been prepared by post-polymerization processing of block copolymers synthesized by radical ring-opening polymerization (rROP).³⁷ This method enables copolymerization of cyclic monomers such as cyclic ketene acetals (CKAs) with vinyl monomers, thus introducing cleavable ester bonds into the copolymer backbone.^{40,41} However, this approach requires the use of an organic cosolvent and appears to be limited to relatively low copolymer concentrations (1% w/v). Furthermore, CKAs tend to exhibit low reactivity when statistically copolymerized with common vinyl monomers (e.g., acrylates, acrylamides and styrenics) and a significant proportion of non-degradable ring-retained CKAs are produced during polymerization.⁴²

Polymerization-induced self-assembly (PISA) is a powerful technique for the synthesis of well-defined diblock copolymer vesicles^{43,44,53,54,45–52} at relatively high copolymer concentrations (> 20% w/w solids) with tunable membrane thickness⁵⁵ and programmable burst release.⁵⁶ For example, targeting certain membrane-forming blocks can lead to a thermally-induced morphological transition (e.g., vesicle-to-sphere or vesicle-to-worm),⁵⁷

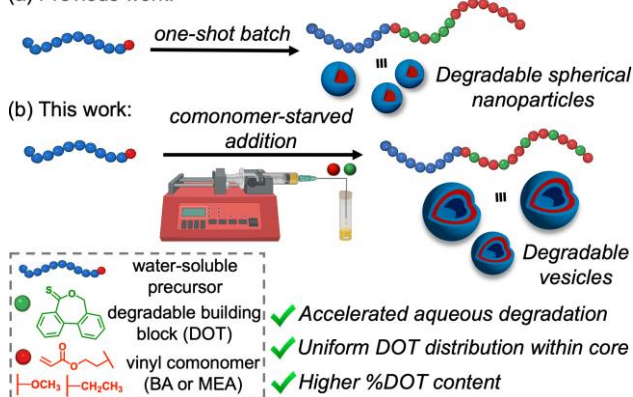
which enables the encapsulation/release of a payload.^{58–60} Recently, PISA has been combined with rROP to produce aqueous dispersions of hydrolytically degradable nanoparticles.^{61–63} However, introducing cyclic comonomers such as CKA leads to incomplete conversion owing to the mismatch in comonomer reactivity.^{40,41} Furthermore, only spherical nanoparticles have been reported to date.⁶⁴ In related work, it has been reported that *N*-carboxyanhydrides (NCA) can be polymerized using an aqueous NCA ROPISA formulation to produce polypeptide-based nanoparticles with various morphologies. However, this approach has not yet been exploited for the design of well-defined unilamellar vesicles.^{51,65–67}

Recently, thionolactones were introduced as an important monomer class that also undergo rROP.^{68–74} Such cyclic monomers have been statistically copolymerized with various vinyl monomers.^{68–71,75,76} The proposed mechanism involves addition of a radical to the C=S double bond of a thionolactone, followed by ring-opening *via* β -scission, reinitiation, and formation of a thioester (–C(=O)S–) group within the copolymer backbone.^{70,77} In principle, selective cleavage of such bonds can be achieved *via* aminolysis, thiolysis, or oxidation.⁶⁹ For example, D’Agosto, Lansalot, Nicolas and co-workers recently used dibenzo[*c,e*]oxepane-5-thione (DOT) to prepare hydrolytically degradable spherical nanoparticles in combination with a reversible addition–fragmentation chain transfer (RAFT) aqueous emulsion polymerization formulation using *n*-butyl acrylate and/or styrene.^{61,63,78} However, there are as yet no literature examples of the PISA synthesis of degradable vesicles directly in aqueous media.^{61,63,78} One well-known limitation of DOT is that it reacts faster than vinyl comonomers during rROP copolymerization. For one-shot batch syntheses, this inevitably results in its rapid initial incorporation. Unfortunately, the resulting non-uniform distribution of DOT repeat units along the copolymer chains adversely affects the observed degradation profile.^{63,78,79}

Herein we report the first example of such degradable vesicles, which are prepared using a formulation that judiciously combines rROP with RAFT aqueous dispersion polymerization.^{80,81} More specifically, 2-methoxyethyl acrylate (MEA) is statistically copolymerized with DOT using poly(*N,N*′-dimethylacrylamide) (PDMAC) as a non-ionic water-soluble precursor block (Scheme 1). Addition of this binary mixture of comonomers under comonomer-starved feed conditions^{82,83} produces: (i) a more uniform distribution of DOT repeat units within the hydrophobic membrane-forming block and (ii) allows a higher thioester content to be achieved with minimal unreacted DOT comonomer. The selection of MEA as the core-forming monomer was important for enabling the successful synthesis of DOT-containing diblock copolymer vesicles. This new approach leads directly to a more uniform degradation profile for the vesicles, which are characterized by cryo-TEM, DLS and SAXS. Finally, trial loading experiments are conducted using Nile Red as a model hydrophobic payload, during which vesicle degradation leads to release of the dye under physiologically relevant concentrations of glutathione and L-cysteine.

Controlled copolymerization of DOT *via* aqueous rROPISA

(a) Previous work:



Scheme 1. Schematic representation of the context for the present study. (a) Representative prior study: a vinyl comonomer (e.g., *n*-butyl acrylate, BA) is copolymerized with DOT in a one-shot batch process using a water-soluble poly(*N*-acryloylmorpholine) precursor to produce degradable spherical nanoparticles *via* RAFT aqueous emulsion copolymerization. (b) This work: a suitable vinyl comonomer (MEA) is used as a cosolvent to dissolve the water-insoluble DOT comonomer: this binary mixture of comonomers is then statistically copolymerized using a water-soluble (poly(*N,N*′-dimethylacrylamide)) precursor, leading to the formation of degradable diblock copolymer vesicles. The judicious use of a comonomer-starved feed strategy allows a higher proportion of DOT to be incorporated while ensuring a more uniform distribution of DOT repeat units within the hydrophobic membrane-forming block. This in turn produces a more uniform degradation profile for the copolymer chains. Abbreviations: rROPISA = radical ring-opening polymerization-induced self-assembly, DOT = dibenzo[*c,e*]oxepane-5-thione, MEA = 2-methoxyethyl acrylate, BA = *n*-butyl acrylate.

RESULTS AND DISCUSSION

MEA was selected as a comonomer for DOT in this study. This choice was guided by three criteria: (i) an acrylic comonomer should ensure optimal statistical copolymerization,⁷⁹ (ii) a liquid monomer was required to act as a cosolvent to ensure molecular dissolution of the water-insoluble DOT, and (iii) MEA has already been used to prepare diblock copolymer vesicles using various aqueous PISA formulations.^{84–87} Moreover, PMEA is a well-known biocompatible synthetic polymer that is FDA-approved for biomedical applications.^{88–91}

Non-degradable Diblock Copolymer Nanoparticles

First, a non-ionic water-soluble poly(*N,N*-dimethylacrylamide) (PDMAC₄₃; $M_n = 5.20$ kDa, $M_w/M_n = 1.15$) precursor was prepared by RAFT solution polymerization using benzyl 2-(dodecylthiocarbonothioylthio)-2-methyl propionamide (Bz-DDMAT). Further details of this synthetic protocol are provided in the Supporting Information (see Experimental Section, plus Figures S1–S3, and Table 1). The RAFT aqueous dispersion polymerization of MEA was then conducted at 70 °C targeting a mean degree of polymerization (DP) of 125, 190, 250, 300, or 400 at 20% w/w solids using 4,4′-azobis(4-cyanovaleric acid) (ACVA) initiator and a [PDMAC₄₃]/[ACVA] molar ratio of 5.0. A gradual increase in turbidity was observed as the MEA polymerization progressed, indicating nanoparticle formation. High MEA conversions (> 95%) were confirmed *via* ¹H NMR spectroscopy studies of reaction mixtures diluted in methanol-*d*₄ (Figure S4, entries 2–6 in Table 1).

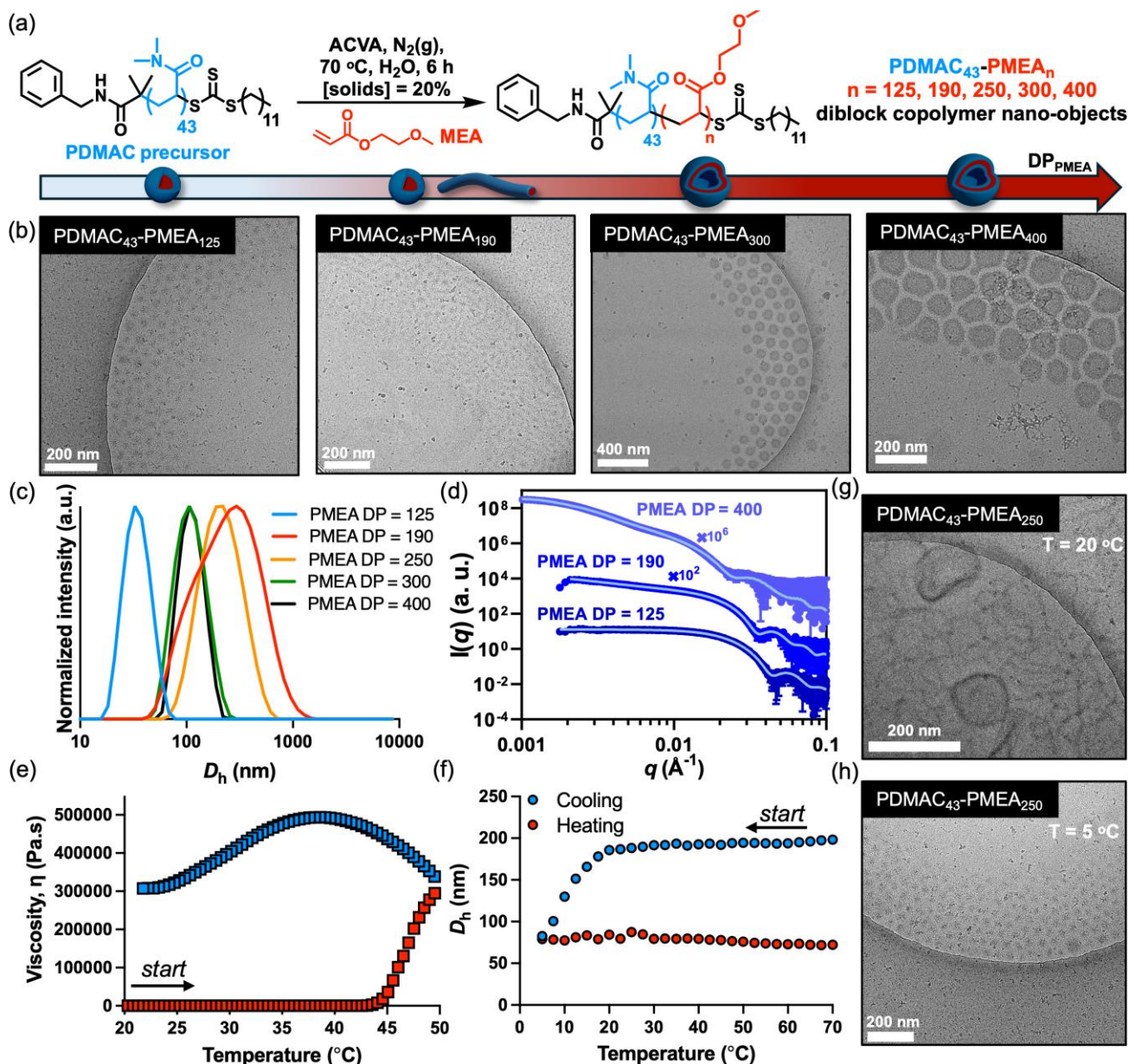


Figure 1. (a) Synthesis of poly(*N,N*'-dimethylacrylamide)-poly(2-methoxyethyl acrylate) (PDMAC₄₃-PMEA₁₂₅₋₄₀₀) nanoparticles by RAFT aqueous dispersion polymerization of MEA targeting 20% w/w solids. (b) Evolution of copolymer morphology from pure spheres (PMEA DP = 125) to a mixture of spheres and worms (PMEA DP = 190), to a mixture of worms and vesicles (PMEA DPs = 300, 400). Representative cryo-TEM images recorded for PDMAC₄₃-PMEA₁₂₅₋₄₀₀ nanoparticles. (c) Intensity-weighted particle size distributions determined by DLS for PDMAC₄₃-PMEA_n ($n = 125-400$) diblock copolymer nanoparticles. (d) SAXS patterns recorded for 1.0% w/w aqueous dispersions of PDMAC₄₃-PMEA₁₂₅ spheres (dark blue data set), a mixed phase of spheres and worms (PDMAC₄₃-PMEA₁₉₀) (blue data set) and pure vesicles PDMAC₄₃-PMEA₄₀₀ (light blue data set). (e) Dispersion viscosity (η) as a function of temperature recorded for a 20% w/w aqueous copolymer dispersion of PDMAC₄₃-PMEA₂₅₀ nanoparticles. (f) Sphere-equivalent hydrodynamic diameter (D_h) as a function of temperature recorded by DLS for a 1.0% w/w aqueous dispersion of PDMAC₄₃-PMEA₂₅₀ nanoparticles during a thermal cycle from 70 °C to 5 °C to 70 °C. Cryo-TEM images recorded for a 1.0% w/w aqueous dispersion of PDMAC₄₃-PMEA₂₅₀ nanoparticles recorded at (g) 20 °C and (h) 5 °C. Abbreviations: ACVA = 4,4'-azobis(4-cyanovaleric acid) initiator.

Size exclusion chromatography (SEC) analysis indicated unimodal molecular weight distributions (Table 1, Figure S5). Copolymer dispersities remained relatively low ($M_w/M_n \leq 1.50$) when targeting a PMEADP of 125, 190, 250 or 300 but a relatively high dispersity ($M_w/M_n = 1.60$) was observed for PMEADP of 400 (Table 1). This is attributed to (i) bimolecular termination and/or (ii) chain transfer to polymer, which is known to be prevalent for acrylic monomers (co)polymerized at 70 °C.⁹² Unfortunately, this relatively high temperature was required to

ensure that the DOT monomer did not precipitate from the reaction mixture when preparing DOT-based vesicles as discussed below.

Dynamic light scattering (DLS) studies suggested the formation of spherical nanoparticles when targeting the lowest PMEADP of 125 (Table 1, Figure 1c). For target PMEADPs of either 190 or 250, relatively broad size distributions were observed, which is consistent with either diblock copolymer worms or nanoparticles comprising more than one morphology.^{45,93} As expected, unimodal size distributions comprising purely vesicles were

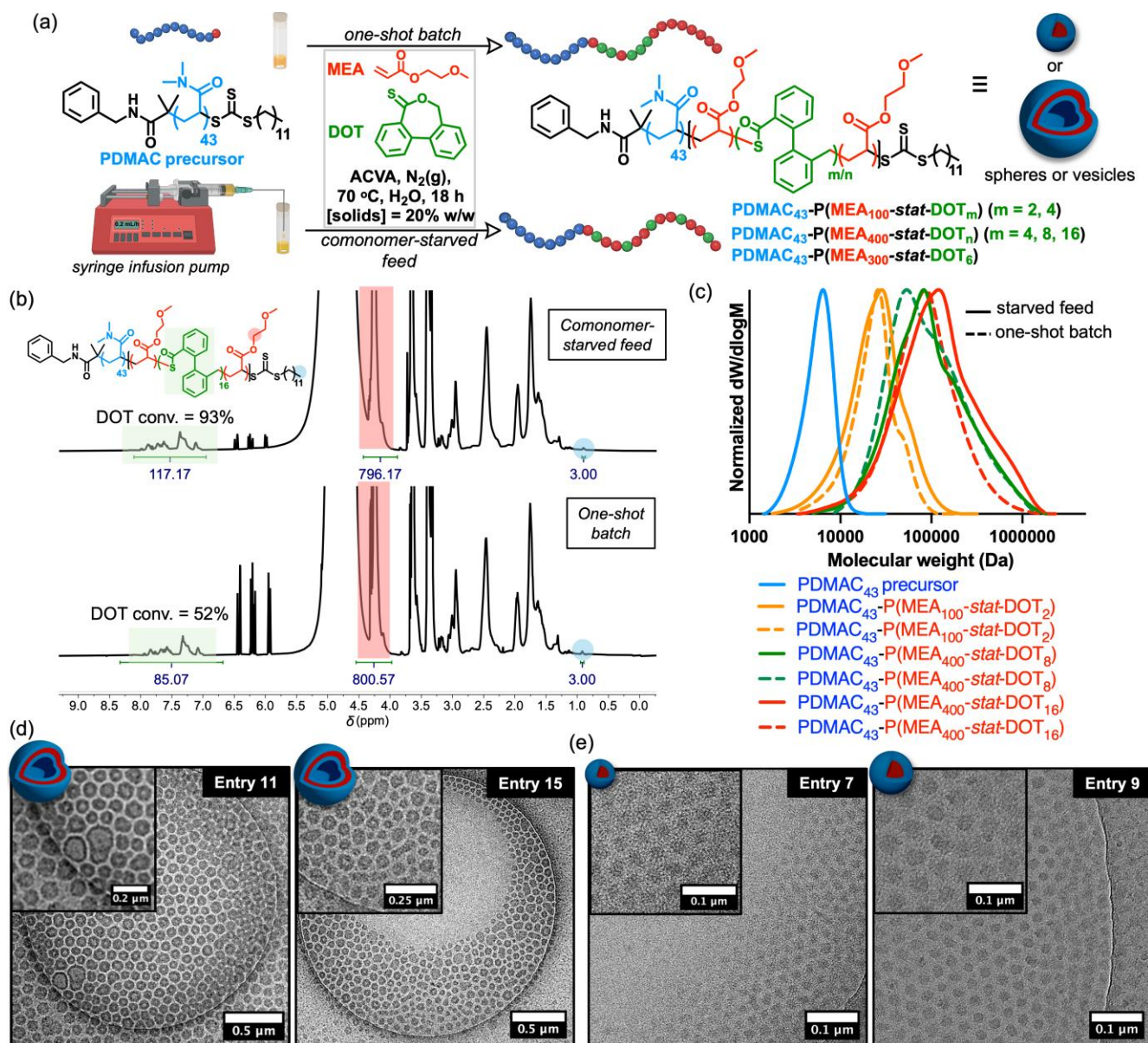


Figure 2. (a) Synthesis of poly(*N,N*-dimethylacrylamide)-poly(2-methoxyethyl acrylate-*stat*-dibenzo[*c,e*]oxepane-5-thione) PDMAC₄₃-P(MEA₁₀₀-*stat*-DOT_{*m*}) spheres (*m* = 2 or 4; entries 7-9 in Table 1), PDMAC₄₃-P(MEA₃₀₀-*stat*-DOT₆) vesicles (entries 10, 11 in Table 1) and PDMAC₄₃-P(MEA₄₀₀-*stat*-DOT_{*n*}) vesicles (*n* = 4, 8 or 16; entries 10-16 in Table 1) by aqueous rROPISA targeting 20% w/w solids. A binary mixture of MEA and DOT was added either by one-shot batch or under comonomer-starved feed conditions using a syringe pump (feed rate = 0.2 mL h⁻¹ over 2 h). (b) ¹H NMR spectra recorded for PDMAC₄₃-P(MEA₄₀₀-*stat*-DOT₁₆) targeting 4 mol% DOT vesicles prepared by either one-shot batch (entry 16 in Table 1) or under comonomer-starved conditions (entry 15 in Table 1). (c) SEC curves (refractive index detector) recorded for the same diblock copolymers prepared by one-shot batch (dashed traces) or under comonomer-starved feed conditions (solid traces), plus the corresponding PDMAC₄₃ precursor. (d) Cryo-TEM images recorded for PDMAC₄₃-P(MEA₃₀₀-*stat*-DOT₆) and PDMAC₄₃-P(MEA₄₀₀-*stat*-DOT₁₆) vesicles (entries 11 and 15, Table 1) and (e) PDMAC₄₃-P(MEA₁₀₀-*stat*-DOT_{*n*}) (*n* = 2 or 4) spheres (entries 7 and 9, Table 1) prepared under comonomer-starved conditions. Insets: magnified images.

obtained when targeting PMEAs of 300 or 400. These tentative morphological assignments were confirmed by cryogenic transmission electron microscopy (cryo-TEM) studies (Figures 1b). More specifically, mainly spheres were observed for a PMEA DP of 125, a PMEA DP of 190 produced a mixture of short worms and spheres, a PMEA DP of 250 produced a mixture of worms and vesicles, and a PMEA DP of 300 or higher afforded pure vesicles. Notably, a pure worm phase was not observed for any of the five target PMEA DPs.⁸⁷ This is understandable given the relatively narrow compositional window

observed for this particular morphology in most PISA formulations.^{57,94} Similar observations were reported by Rieger and co-workers.⁸⁷ However, rheological studies suggested that a pure worm phase may be produced by heating one of the post-polymerization reaction mixtures above ambient temperature (see below). Small-angle X-ray scattering (SAXS) patterns were recorded for 1.0% w/w aqueous dispersions comprising PDMAC₄₃-PMEA₁₂₅, PDMAC₄₃-PMEA₁₉₀ and PDMAC₄₃-PMEA₄₀₀ nanoparticles. This powerful technique can be used to identify the predominant copolymer morphology by inspecting

Table 1. Summary of characterization data for DOT-based diblock copolymers prepared by RAFT (rRO)PISA at 20% w/w solids.

Entry No.	Synthesis Protocol	Target copolymer composition	MEA conv. (%) ^a	No. of MEA units per chain ^a	No. DOT repeat units per chain		M_n kDa ^d	M_w/M_n ^d	DLS D_h (nm) ^e	DLS PDI ^e
					Target ^b	Actual ^c				
1	One-shot batch	PDMAC ₄₃ precursor	–	–	0.0	0.0	5.2	1.15	–	–
2	One-shot batch	PDMAC ₄₃ -PMEA ₁₂₅	>99	125	0.0	0.0	25.8	1.29	33	0.06
3	One-shot batch	PDMAC ₄₃ -PMEA ₁₉₀	>99	190	0.0	0.0	38.5	1.38	214	0.40
4	One-shot batch	PDMAC ₄₃ -PMEA ₂₅₀	96	250	0.0	0.0	39.9	1.47	195	0.21
5	One-shot batch	PDMAC ₄₃ -PMEA ₃₀₀	>99	300	0.0	0.0	50.3	1.30	104	0.10
6	One-shot batch	PDMAC ₄₃ -PMEA ₄₀₀	>99	400	0.0	0.0	69.1	1.60	108	0.09
7	Comonomer-starved feed	PDMAC ₄₃ -P(MEA ₁₀₀ -stat-DOT ₂)	>99	100	2.0	2.0	18.8	1.62	52	0.16
8	One-shot batch	PDMAC ₄₃ -P(MEA ₁₀₀ -stat-DOT ₂)	>99	100	2.0	2.0	19.6	1.38	32	0.05
9	Comonomer-starved feed	PDMAC ₄₃ -P(MEA ₁₀₀ -stat-DOT ₄)	>99	100	4.0	4.1	19.7	1.57	87	0.24
10	One-shot batch	PDMAC ₄₃ -P(MEA ₃₀₀ -stat-DOT ₆)	>99	300	6.0	6.0	66.3	2.91	92.7	0.09
11	Comonomer-starved feed	PDMAC ₄₃ -P(MEA ₃₀₀ -stat-DOT ₆)	>99	300	6.0	6.0	57.2	2.37	113.9	0.04
12	Comonomer-starved feed	PDMAC ₄₃ -P(MEA ₄₀₀ -stat-DOT ₄)	>99	400	4.0	4.0	65.5	2.35	107	0.24
13	Comonomer-starved feed	PDMAC ₄₃ -P(MEA ₄₀₀ -stat-DOT ₈)	98	392	8.0	8.0	66.7	2.60	101	0.04
14	One-shot batch	PDMAC ₄₃ -P(MEA ₄₀₀ -stat-DOT ₈)	>99	400	8.0	8.0	59.9	2.48	182	0.03
15	Comonomer-starved feed	PDMAC ₄₃ -P(MEA ₄₀₀ -stat-DOT ₁₆)	98	392	16.0	14.8	69.3	2.79	131	0.04
16	One-shot batch	PDMAC ₄₃ -P(MEA ₄₀₀ -stat-DOT ₁₆)	95	380	16.0	8.4	59.2	2.04	246	0.10

^a Determined by ¹H NMR spectroscopy. ^b Target DOT content expressed as mean number of DOT repeat units per copolymer chain. ^c Actual DOT content expressed as mean number of DOT repeat units per copolymer chain as estimated by ¹H NMR spectroscopy studies. ^d Apparent number-average molecular weight, M_n , and dispersity (M_w/M_n) calculated by SEC using PMMA standards and DMF eluent. ^e D_h is the z-average hydrodynamic diameter and PD is the polydispersity index, as reported by DLS. [N.B. All copolymers are described in terms of their *target* diblock composition].

the low q gradient in a plot of scattering intensity $I(q)$ vs. scattering vector q .⁹⁵ Spherical particles exhibit a low q gradient of zero, rigid rods (or flexible worms, to a good approximation) possess a gradient of -1 , and vesicles have a gradient of -2 . SAXS patterns were fitted using an appropriate scattering model for each morphology (Figure 1d, Table S1).^{96,97} For the mixed morphology exhibited by PDMAC₄₃-PMEA₁₉₀, a sphere/worm ratio of 5.0 afforded the best data fit. Rheological studies revealed thermoresponsive behavior for a 20% w/w aqueous dispersion of PDMAC₄₃-PMEA₂₅₀ nanoparticles (Figure 1e). A temperature sweep from 20 °C to 50 °C within the linear viscoelastic region (strain amplitude = 1.0%, angular frequency = 10 rad s⁻¹) indicated a maximum in the dispersion viscosity at around 37 °C. According to prior studies by Armes and co-workers, this feature corresponds to the formation of a pure phase comprising linear worms.^{57,98,99} An *irreversible* morphological transition on cooling to 20 °C is evident from the high final viscosity. On the other hand, DLS analysis of a 1.0% w/w aqueous dispersion of the same PDMAC₄₃-PMEA₂₅₀ nanoparticles indicated that an *irreversible* morphological transition from mixed worms/vesicles to pure spheres occurs upon cooling (Figure 1f). Similar observations have been reported for other thermoresponsive diblock copolymer nanoparticles in the literature.^{100,101} Cryo-TEM studies confirmed this change in morphology (Figure 1g, h). Notably, DLS analysis of a 1.0% w/w

aqueous dispersion of PDMAC₄₃-PMEA₄₀₀ vesicles indicated no discernible thermoresponsive behavior (Figure S6). Accordingly, the PDMAC₄₃-PMEA₁₀₀, PDMAC₄₃-PMEA₃₀₀ and PDMAC₄₃-PMEA₄₀₀ formulations were selected as being the most promising when targeting the analogous DOT-based degradable spheres and vesicles.

DOT-based Degradable Diblock Copolymer Nanoparticles.

The aqueous rROPISA synthesis of poly(*N,N*'-dimethylacrylamide)-poly(2-methoxyethyl acrylate-*stat*-dibenzo[*c,e*]oxepane-5-thione) PDMAC₄₃-P(MEA₁₀₀-stat-DOT_{*m*}) spheres (*m* = 2 or 4), PDMAC₄₃-P(MEA₃₀₀-stat-DOT₆) and PDMAC₄₃-P(MEA₄₀₀-stat-DOT_{*n*}) vesicles (*n* = 4, 8 or 16) was conducted using either a traditional one-shot batch protocol⁴⁵ or under comonomer-starved feed conditions (see Experimental Section in the Supporting Information, plus Figures S7-S11). Figure 2 and Table 1 summarize all the copolymer syntheses and the corresponding characterization data. The MEA and DOT comonomers were always added as a binary mixture, which is particularly important given DOT's limited water solubility, which may account for its incomplete conversion in prior one-shot batch syntheses.⁷⁸ Kinetic studies conducted using the one-shot batch protocol confirmed DOT's higher reactivity compared to MEA (Figure S10). Similar observations were previously reported for emulsion rROPISA syntheses.⁶³ The ¹H NMR spectra

shown in Figure 2b highlight the advantages of our new comonomer-starved feed approach: almost complete DOT incorporation (93% conversion) was achieved when targeting PDMAC₄₃-P(MEA₄₀₀-*stat*-DOT₁₆) vesicles (entry number 15, ~4 mol% DOT). In contrast, only 53% DOT conversion (entry number 16, ~2 mol%) was achieved using the one-shot batch protocol under comparable conditions. Moreover, prior studies employing rROPISA techniques reported similarly low levels of DOT incorporation.^{63,78} This is an important difference because it affects the degradation profile of such copolymers (see below). SEC data are summarized in Figure 2c and Table 1. Diblock copolymers prepared using the comonomer-starved feed protocol exhibit higher number molecular weights compared to those observed for the equivalent one-shot batch syntheses. Cryo-TEM images confirmed that the former strategy produced well-defined spheres or vesicles, even when targeting ~4 mol% DOT (PDMAC₄₃-P(MEA₄₀₀-*stat*-DOT₁₆) and PDMAC₄₃-P(MEA₁₀₀-*stat*-DOT₄), Figures 2d and 2e. Notably, targeting such relatively high DOT contents led to much thicker vesicle membranes (Figure 2d, S12). In the case of PDMAC₄₃-P(MEA₁₀₀-*stat*-DOT_m) (*m* = 2 or 4) spheres, targeting a higher DOT content led to the formation of larger spheres (Figure 2e, Table 1). SAXS patterns were also recorded for 1.0% w/w aqueous dispersions of several DOT-based diblock copolymer nanoparticles and satisfactorily fitted using either spherical micelle or vesicle scattering models (Figure S13, Table S1), confirming initial morphology assignments based on cryo-TEM images.

Inspecting the one-shot batch syntheses reported in Table 1 (entry numbers 10, 14 and 16), targeting higher DOT contents leads to narrower molecular weight distributions (i.e., lower dispersities) and larger hydrodynamic diameters (*D_h*). In contrast, the comonomer-starved feed protocol yields relatively well-defined vesicles, irrespective of the DOT content. For example, PDMAC₄₃-P(MEA₄₀₀-*stat*-DOT₁₆) vesicles prepared *via* comonomer-starved feed (entry 15) are significantly smaller and less polydisperse (*D_h* = 131 nm, PDI = 0.04), than those prepared using the one-shot batch method (*D_h* = 246 nm, PDI = 0.10; entry 16). This is presumably because the former synthetic protocol minimizes the mismatch in reactivity between MEA and DOT during their statistical copolymerization. Moreover, the final MEA conversion is consistently high (> 95%) for all copolymerizations regardless of the synthetic strategy, which indicates that the comonomer-starved feed approach does not affect the rate of consumption of this comonomer. Importantly, the comonomer-starved feed strategy enables the incorporation of higher DOT contents without compromising vesicle stability. Thus, it expands the compositional space for the rational design of degradable copolymer vesicles while enabling aqueous PISA syntheses to be conducted even for water-insoluble comonomers such as DOT, which remains a solid at the reaction temperature of 70 °C in the absence of any MEA comonomer.

The hydrolytic degradation of the DOT-containing diblock copolymer vesicles and spheres was studied at 20 °C in the presence of isopropylamine (iPrNH₂) (Figure 3a). Selective hydrolysis of thioester bonds by this primary amine causes chain scission of the hydrophobic block, which in turn leads to a change in copolymer morphology. Figure 3b shows digital photographs recorded for a 20% w/w aqueous dispersion of PDMAC₄₃-P(MEA₄₀₀-*stat*-DOT₁₆) vesicles over time. Initially, a turbid dispersion is observed because vesicles are strong scatterers of visible light.¹⁰² As base-catalyzed degradation progresses, this dispersion gradually becomes transparent (note the presence of the

magnetic flea) as a result of *in situ* vesicle disassembly and the eventual formation of soluble copolymer chains. This change in physical appearance correlates closely with the change in molecular weight reduction and copolymer morphology observed by SEC. SEC analysis reveals a gradual reduction in number-average molecular weight (*M_n*) for a series of PDMAC₄₃-P(MEA₄₀₀-*stat*-DOT_n) diblock copolymers (*n* = 0, 4, 8 or 16) (Figure 3c). For vesicles containing the highest DOT content (*n* = 16), the initial *M_n* of 59.2 kDa is reduced to approximately that of the PDMAC precursor (*M_n* = 5.2 kDa) within 36 h (Figure 3d). Notably, copolymers with higher DOT contents undergo faster degradation. Moreover, copolymers containing fewer DOT repeat units undergo less complete degradation even after 72 h, as evidenced by their higher final *M_n* values (see Figures S14, S15 for additional degradation profiles monitored by SEC and DLS). Diblock copolymer vesicles (PDMAC₄₃-PMEA₄₀₀) containing no DOT repeat units were used as negative control. No significant change in *M_n* over time was observed for such samples, indicating that DOT comonomer incorporation is essential for base-catalyzed degradability.

SEC data recorded for DOT-containing diblock copolymers prepared using the comonomer-starved feed protocol (Figure 3d) indicate uniform degradation profiles. Importantly, the unimodal SEC trace becomes almost indistinguishable from that of the PDMAC precursor within 36 h, indicating near-complete degradation of the hydrophobic P(MEA₄₀₀-*stat*-DOT₁₆) block. In striking contrast, the equivalent copolymer prepared using the one-shot batch method exhibited slower degradation and a multimodal molecular weight distribution after 96 h (Figure 3g, Figure S16a). Moreover, slightly faster degradation kinetics were observed under the same aminolytic conditions for a copolymer prepared using the comonomer-starved feed protocol (entry 13) compared to a copolymer with a similar DOT content prepared by the one-shot batch method (entry 16), see Figure S17. This indicates that the spatial distribution of DOT repeat units along the hydrophobic block strongly influences the subsequent copolymer degradation profile. This is a decisive advantage conferred by the comonomer-starved feed protocol. Cryo-TEM was employed to monitor the corresponding evolution in copolymer morphology for the PDMAC₄₃-P(MEA₄₀₀-*stat*-DOT₁₆) vesicles prepared *via* comonomer-starved feed (Figure 3e). Initially, well-defined vesicles were observed. Within 2 h exposure to iPrNH₂, partial vesicle rupture and membrane-thinning are evident, along with populations of short worms and spheres. After 4 h, only spheres can be observed.¹⁰³ Almost complete copolymer dissociation occurs within 36 h. These observations are consistent with the molecular weight reduction observed in the SEC experiments. DLS studies also confirm vesicle degradation, with a marked reduction in z-average hydrodynamic diameter from ~120 nm for the initial vesicles to less than 20 nm within 36 h (Figure 3f). The corresponding reduction in the scattered light intensity (or derived count rate), confirms vesicle dissolution to produce soluble (co)polymer chains (Figure S16b). Clearly, vesicle degradation profiles can be tuned by systematic degradation of their DOT content. Faster degradation may be advantageous for applications requiring rapid payload release, while slower degradation rates should be more suitable if sustained release is required.¹⁰⁴ The hydrolytic degradation of PDMAC₄₃-P(MEA₄₀₀-*stat*-DOT₁₆) vesicles was also monitored by SAXS (Figure S18a). Like DLS, this scattering technique indicated the formation of significantly smaller particle species over time.

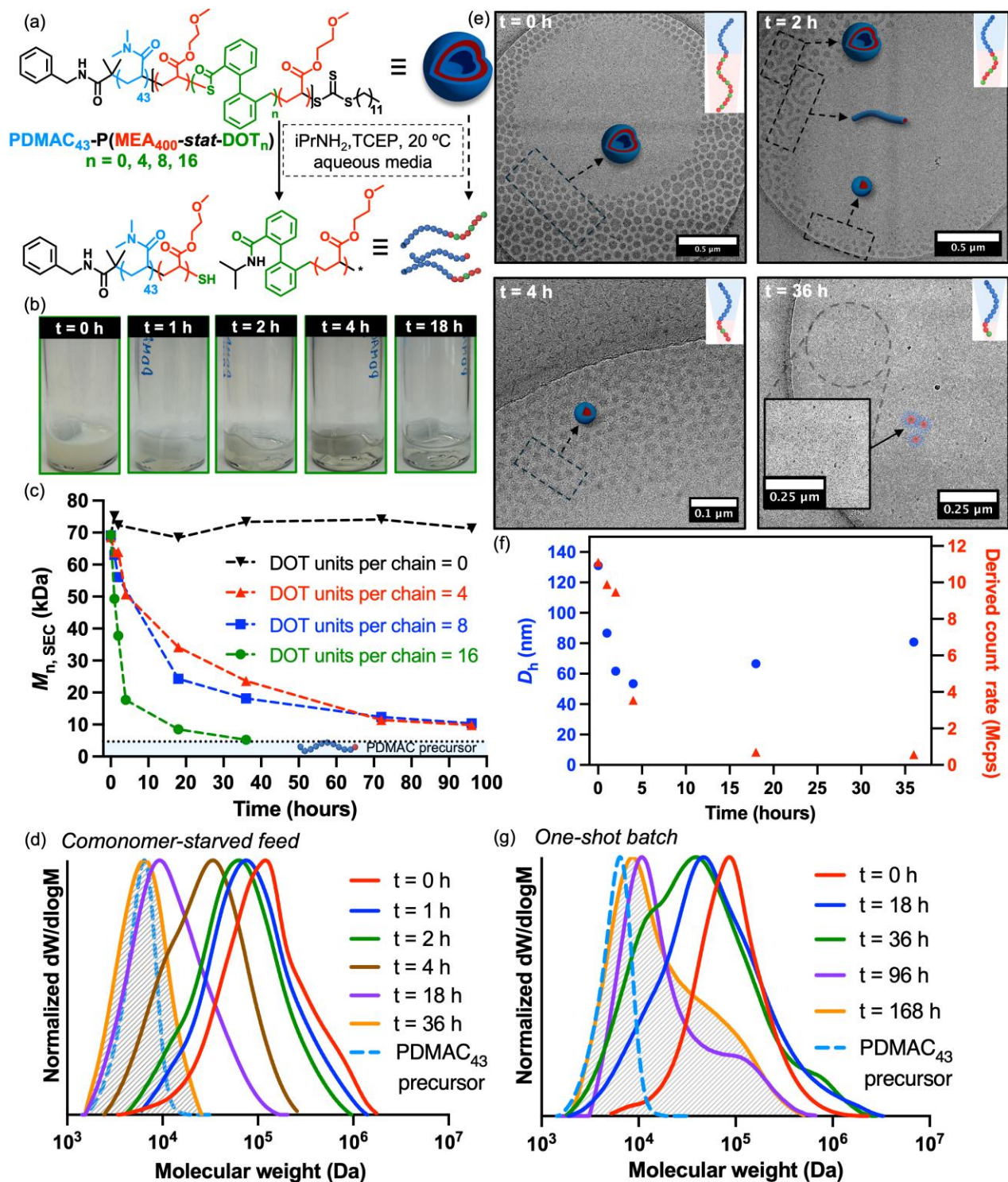


Figure 3. (a) Degradation of PDMAC₄₃-P(MEA₄₀₀-stat-DOT_n) ($n = 0, 4, 8, \text{ or } 16$) vesicles via aminolysis using iPrNH₂. (b) Digital photographs recorded for 20% w/w aqueous dispersions of PDMAC₄₃-P(MEA₄₀₀-stat-DOT₁₆) vesicles prepared via comonomer-starved feed at various time points during their iPrNH₂-catalyzed degradation. (c) Change in apparent number-average molecular weight (M_n) over time determined by SEC analysis of PDMAC₄₃-P(MEA₄₀₀) (black trace), PDMAC₄₃-P(MEA₄₀₀-stat-DOT₄) (red trace), PDMAC₄₃-P(MEA₄₀₀-stat-DOT₈) (blue trace), and PDMAC₄₃-P(MEA₄₀₀-stat-DOT₁₆) (green trace) vesicles prepared under comonomer-starved feed conditions. (d) Normalized SEC curves (refractive index detector) recorded at various time points during the iPrNH₂-catalyzed degradation of a PDMAC₄₃-P(MEA₄₀₀-stat-DOT₁₆) diblock copolymer prepared via comonomer-starved feed plus the corresponding PDMAC₄₃ precursor (dashed light blue trace). (e) Cryo-TEM images recorded after 0, 2, 4 or 36 h during the iPrNH₂-catalyzed degradation of PDMAC₄₃-P(MEA₄₀₀-stat-DOT₁₆) vesicles. (f) Plot of z-average hydrodynamic diameter (D_h) (blue data) and derived count rate (red data) vs. time during the iPrNH₂-catalyzed degradation of PDMAC₄₃-P(MEA₄₀₀-stat-DOT₁₆) vesicles prepared via comonomer-starved feed. (g) Normalized SEC curves (refractive index detector) recorded at various time points during the iPrNH₂-catalyzed degradation of PDMAC₄₃-P(MEA₄₀₀-stat-DOT₁₆) vesicles prepared using the one-shot batch protocol plus the corresponding PDMAC₄₃ precursor (dashed blue trace). Abbreviations: iPrNH₂ = isopropylamine, TCEP = tris(2-carboxyethyl)phosphine.

The degradation of PDMAC₄₃-P(MEA₁₀₀-*stat*-DOT₄) spheres prepared *via* comonomer-starved feed was also investigated using SEC, DLS, and SAXS (Figures S18, S19). SEC analysis indicated a gradual reduction in M_n from 19.7 kDa to 5.5 kDa within 96 h. DLS analysis revealed a reduction in z-average diameter from ~87 nm to ~30 nm within 4 h (Figure S19 c,d). Moreover, higher DLS polydispersities were observed at later time points, suggesting the formation of a population of copolymer chains. Digital photographs recorded for a 20% w/w aqueous dispersion of such PDMAC₄₃-P(MEA₁₀₀-*stat*-DOT₄) spheres indicated a switch from an initial weakly turbid yellow dispersion to a completely transparent solution within 18 h, which is consistent with full degradation. Such decolorization indicates *in situ* removal of the trithiocarbonate end-groups *via* aminolysis.¹⁰⁵ SAXS analysis of these degraded spheres supported these findings, with similar scattering patterns to those for the degraded vesicles being observed within 8 h (Figure S18 c). In summary, the ability to modulate degradation rates by adjusting both the DOT content and synthesis conditions offers a promising platform for designing materials tailored to specific therapeutic needs.

In principle, copolymer degradation should also occur under physiologically relevant concentrations of L-cysteine and glutathione (GSH) (Figure S20).^{106,107} Degradation of hydrophobic DOT-based copolymers has been reported to be effective (albeit rather slow) in the presence of L-cysteine.⁶⁸ In the present study, a 2.0% w/w aqueous dispersion of PDMAC₄₃-P(MEA₄₀₀-*stat*-DOT₈) vesicles was incubated with 10 mM L-cysteine and 10 mM glutathione in PBS at 37 °C. SEC analysis indicated a monotonic reduction in M_n from 66.7 kDa to 16.9 kDa within 96 h, with relatively narrow unimodal distributions. However, DLS data indicated only a rather modest size reduction (from D_h = 103 nm to 96 nm within 36 h with no further change being observed up to 96 h. This is consistent with the observation of persistent turbidity over time, which suggests incomplete degradation on this time scale.

To examine the potential utility of DOT-containing vesicles for the delivery and release of biologically relevant cargos, Nile Red dye was used as a model hydrophobic payload (Figure 4).¹⁰⁸ More specifically, PDMAC₄₃-P(MEA₄₀₀-*stat*-DOT₈) vesicles were lyophilized, resuspended in an aqueous solution containing 0.01% w/w Nile Red, and stirred overnight to load this dye within the vesicle membrane (see Supporting Information for further details). DLS studies indicated a modest increase in vesicle diameter, which is consistent with successful dye uptake (Figure S21). These vesicles were then subjected to the conditions described above, with degradation being monitored over time *via* visible absorption spectroscopy and fluorescence microscopy. Initially, the dye-loaded vesicles exhibited strong absorption at 515 nm, which is characteristic of Nile Red.¹⁰⁹ A significant reduction in this initial absorbance was observed over 96 h owing to the release of Nile Red (Figure 4b), resulting in macroscopic precipitation of this hydrophobic dye. Fluorescence microscopy studies provided complementary information (Figure 4c). Initially, the fluorescence intensity was relatively uniform, with a few of the larger vesicles being visible. After 96 h, a darker background was observed along with highly fluorescent aggregates, which is consistent with precipitation of the hydrophobic Nile Red dye after its release. As a negative control, Nile Red-loaded PDMAC₄₃-PMEA₄₀₀ vesicles were subjected to the same conditions and exhibited no dye release over 96 h, as judged by fluorescence microscopy (Figure S22).

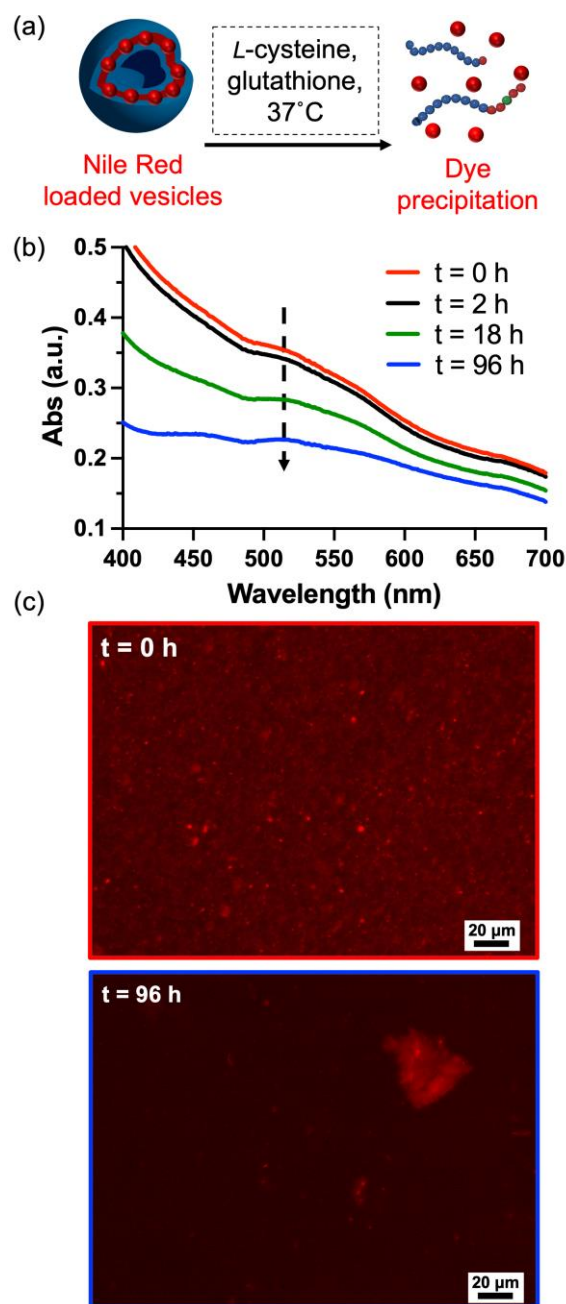


Figure 4. (a) Schematic representation of Nile Red dye (red spheres) loaded within the membrane of PDMAC₄₃-P(MEA₄₀₀-*stat*-DOT₈) vesicles. Subsequent degradation of such dye-loaded vesicles in the presence of 10 mM L-cysteine and 10 mM glutathione in aqueous media leads to macroscopic precipitation of the water-insoluble dye. (b) Evolution of visible absorption spectra of Nile Red loaded within 1.0% w/w aqueous dispersions of PDMAC₄₃-P(MEA₄₀₀-*stat*-DOT₈) vesicles during their degradation over time. (c) Fluorescence micrographs (λ_{EX} = 550 nm, λ_{EM} = 605 nm) recorded for 1.0% w/w aqueous dispersions of Nile Red-loaded PDMAC₄₃-P(MEA₄₀₀-*stat*-DOT₈) vesicles at two time points during their hydrolytic degradation.

CONCLUSIONS

We report the design, synthesis, and characterization of degradable PDMAC-P(MEA-*stat*-DOT) diblock copolymer vesicles. Using a comonomer-starved feed led to a more uniform distribution and higher proportion of degradable DOT repeat

units (up to ~4 mol%) within the water-insoluble block compared to the equivalent traditional one-shot batch syntheses. Importantly, the former strategy also produced a more complete degradation profile: the molecular weight distribution of the degraded copolymer chains was comparable to that observed for the non-degradable PDMAC precursor. Selective cleavage of thioester bonds led to vesicle disassembly over time, with accelerated degradation being observed for copolymer chains containing higher DOT levels. Such tunability was exemplified in model release studies using Nile Red as a model hydrophobic payload, with visible absorption spectroscopy and fluorescence microscopy indicating efficient dye loading and subsequent degradation-triggered release, respectively. Macroscopic precipitation of Nile Red occurs after vesicle disassembly, which suggests that such DOT-functionalized vesicles may be suitable for stimulus-responsive delivery applications.

ASSOCIATED CONTENT

Supporting Information

Supporting Information Available. Characterization techniques, SAXS theory and scattering models, synthetic protocols, additional NMR spectra and SEC curves, tabulated SAXS data, DLS particle size distributions, conversion vs. time curves, additional cryo-TEM images, additional SAXS patterns, additional copolymer degradation data, and fluorescence microscopy images.

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Notes

The authors declare no competing financial interest.

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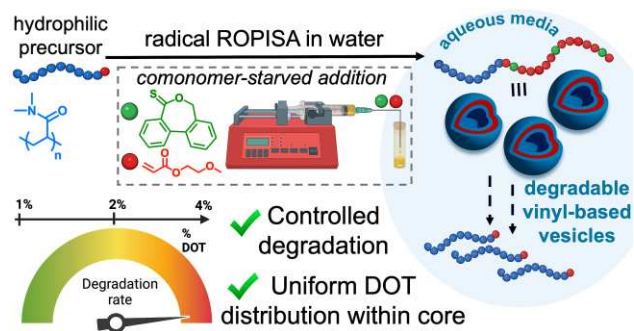


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