Preparation of core-crosslinked linear-dendritic copolymer micelles with enhanced stability and their application for drug solubilisation

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Abstract

In this study we explore the preparation of core-crosslinked micelles of linear-dendritic methoxy-poly(ethylene glycol) (MPEG)-co-poly(ester-sulfide) (PES) polymers to improve the stability of such polymeric micelle systems against premature disintegration and drug release. A series of MPEG-PES copolymers were synthesised via stepwise reactions of acetylation and thiol-ene photoreaction. Surface tension measurement showed that the copolymers with ethenyl surface groups could self-associate in dilute aqueous solutions to form micelles. Crosslinking within the micelle cores in the presence of dithioerythritol (DTT) linker was initiated under UV radiation. The formation of core-crosslinked micelles was confirmed by HPLC in combination with charged aerosol detection (CAD). The copolymers were found to readily hydrolyse under acidic conditions due to the ester-containing dendrons. Drug solubilisation capacities of the micellar solutions were determined using griseofulvin as a poorly water-soluble model drug. The solubility of griseofulvin showed a 10-fold enhancement in 1% w/v micelle solution and increased with the concentration of the copolymers. Drug release studies indicated that a more sustained release of griseofulvin was achieved for the core-crosslinked micelles compared to the non-crosslinked micelles, attributable to greater stability of the crosslinked core structure. The findings of this study present a new pathway towards developing biodegradable polymeric nanocarriers.

1. Introduction

It is known that more than half of potentially useful drug candidates fail to progress to formulation development due to their low solubility in physiological aqueous environment. The poor solubility results in limited gastrointestinal absorption and poor bioavailability. Numerous methods have been investigated in recent decades to improve the water solubility of lipophilic drugs, e.g. control of pH, chemical or physical modification, conjugation with polymeric carriers, and encapsulation in nanoparticles/micelles (Torchilin, 2001; Williams et al., 2013). Polymeric micelles, which can enhance the solubility of drugs by encapsulating drug molecules within the micelle core, have been investigated extensively for pharmaceutical applications (Kwon, 2003; Adams et al., 2003; Attwood et al., 2007). The hydrophobic micelle core provides a suitable domain for the incorporation of a lipophilic drug. The stability and bioavailability of the drug encapsulated is improved due to the unfavoured access to solvents and inert nature of the micelle core. The hydrophilic corona can reduce nonspecific uptake by the reticuloendothelial system and prolong the circulation time of drugs in the body (Gaucher et al., 2010).

Although polymeric micelles are thermodynamically stable, dissociation of the micelles as a result of dilution in biological fluids or under solvent changes is still a concern, leading to premature release of drug. Numerous attempts have been made to improve the stability of polymeric micelle systems. Among the most promising strategies is the introduction of a crosslinking structure by covalently connecting polymer chains in the micelle (O'Reilly et al., 2006; Read and Armes, 2007). The monomers with functional groups are designed and utilised to synthesise a specific block of amphiphilic block copolymer. After micellisation the functional groups undergo crosslinking and thus provide reinforcement to the micellar structure. Various

methods have been developed to facilitate the crosslinking of block copolymers (Nostrum, 2011), e.g. free radical polymerisation (Hu et al., 2009; Wu et al., 2012), addition of bifunctional reagents (Liu et al., 2002; Yue et al., 2012), photochemical reaction (Kim and Youk, 2009; Huang et al., 2016), and disulfide reduction (Li et al, 2015). Zhong and co-workers developed core-crosslinked biodegradable micelles based on poly(ethylene glycol)-poly(2,4,6-trimethoxybenzylidene-pentaerythritol carbonate-co-pyridyl disulfide carbonate) [PEG-P(TMBPEC-co-PDSC)] copolymers (Chen et al., 2015). The hydrophobic core-forming PDSC units contained disulfide bonds that readily crosslinked under the presence of dithioerythritol by the thiol-disulfide exchange reaction. In vitro release studies showed that sustained release was achieved for the crosslinked micelles under physiological conditions with ca. 19.9% of doxorubicin (DOX) released in 24 h. The release of DOX was accelerated in acidic solutions or in the presence of the biological reducing agent glutathione. The synthesis of amphiphilic poly(N-acroyloxysuccinimide)-b-poly(N-isopropylacrylamide)-b-poly(ε-caprolactone) triblock copolymer was reported by Zhang and co-workers using reversible addition fragmentation chain transfer and ring-opening polymerisation (Quan et al., 2011). The hydrophilic poly(N-acroyloxysuccinimide) blocks containing reactive NHS ester groups formed the micelle corona and crosslinked via reaction with cystamine, a bifunctional linker. It was found that media change had no impact on the micelle shape due to the shell-crosslinking. Xiong et al. designed and synthesised the poly (ethylene glycol)-b-poly(acryloyl carbonate)-b-poly(D,L-lactide) (PEG-PAC-PLA) and folate-PEG-PLA block copolymers by sequential ring-opening polymerisation (Xiong et al., 2011). The copolymers formed mixed micelles with a hydrophobic PLA core, a hydrophilic PEG corona and an interfacial PAC layer. The acrylic side groups of the PAC blocks underwent radical polymerisation under UV radiation and thus formed a crosslinked structure

between the micelle core and shell. The crosslinked micelles demonstrated enhanced colloidal stability and smaller size than non-crosslinked micelles. High drug loading efficiencies and sustained release of paclitaxel were obtained in dilute micellar solutions.

Dendrimers are a class of hyperbranched macromolecules with a high degree of uniformity and monodispersity, and multiple surface functional groups (Esfand and Tomalia, 2001; D'Emanuele and Attwood, 2005). Drug molecules can be encapsulated within the dendritic structure or covalently attached to the surface functional groups (D'Emanuele et al., 2013). Linear-dendritic block copolymers, comprising a dendrimer or dendron conjugated to a linear polymer chain, have attracted considerable attention for their applications in drug solubilisation and delivery in the last two decades (Whitton and Gillies, 2015). Gitsov and Fréchet first explored the synthesis of poly(ethylene glycol) (PEG)-dendritic poly(benzyl ether) copolymers (Gitsov and Fréchet, 1993). It was found that the micellisation behavior was dependent on concentration and dendrimer generation. In our earlier study we synthesised triblock linear–dendritic-linear copolymers comprising two poly(oxybutylene)–b–poly(oxyethylene) (BE) copolymers conjugated to a full generation PAMAM dendrimer (Zhou et al., 2009). Significant solubility enhancement of paclitaxel was achieved in dilute micellar solutions of the copolymers (Zhou et al., 2013). Recently, core crosslinking within the linear-dendritic copolymer micelles has been exploited by several groups to prepare stimuli-responsive micelle systems and enhance their stability. Lam and co-workers synthesised linear-dendritic copolymers comprising a hydrophilic PEG and a thiolated poly(L-Lysine) dendron surface functioned with cholic acids (Li et al., 2011). The thiol groups in the dendrons were then oxidised to form disulfide linkage in the micelle core. The core-crosslinked micelles had improved stability in human plasma and in sodium dodecyl sulfate solution. The release study showed that the release of paclitaxel from the

crosslinked micelles was more sustained but accelerated in the presence of glutathione due to the reduction of disulfide bonds. Chen and co-workers have designed and prepared liner-dendritic copolymers consisted of PEG and a PAMAM dendron (Zhang et al., 2014). The surface amino groups of the PAMAM dendron were then partially conjugated with DOX and lipoic acid. After micellisation the lipoic acid moieties in the micelle core were crosslinked via the thiol-disulfide exchange reaction with DTT in borate buffer. The crosslinked micelles were more stable against dilution and high salt concentration. The release of DOX from the crosslinked micelles was slow under neutral conditions but the release rate was increased in acidic solution and in the presence of glutathione due to hydrolysis and disulfide reduction.

Poly(ester-sulfide) dendrimers have been prepared by Hawker and co-workers via a combination of thiol-ene photochemistry and esterification by 4-pentenoic anhydride. Thiol-ene photochemistry has been proved to be efficient for free radical addition of thiol with ethenyl group in the presence of a photo initiator. Sedaghat-Herati and co-workers synthesised linear-dendritic copolymers of methoxy-poly(ethylene glycol) (MPEG) and poly(ester-sulfide) (PES) dendron via acrylation by acryloyl chloride and thiol-ene addition with thioglycerol (Fury et al, 2009). However the thiol-ene reaction was completed via an ionic mechanism (Michael addition). Recent work from the same group employed thiol-ene photochemistry and esterification with 4-pentenoyl chloride to prepare MPEG-PES copolymers (Fury et al., 2013). Up to generation 3.5 PES dendron was constructed to the hydroxyl end of MPEG chain. In the present study we explored the core-crosslinking and stability study of MPEG-PES copolymer micelles and investigated their applications for drug solubilisation and sustained release. This work reported the synthesis and characterisation of linear-dendritic MPEG-PES block copolymer via stepwise reactions of acrylation by acryloyl chloride and thiol-ene photochemistry. The core-crosslinked

133 micelles were prepared by thiol-ene free radical addition in the presence of DTT and Irgacure
134 2959 photoinitiator. The formation and stability of the crosslinked micelles were studied using
135 the CAD-HPLC technique. The solubilisation of griseofulvin in the copolymer micellar solutions
136 was investigated and the drug release profiles were determined by UV assay.
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138 **2. Experimental**
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140 *2.1. Materials*
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142 Poly(ethylene glycol) methyl ether (MPEG) (MW 5000), acryloyl chloride, triethylene
143 amine (TEA), 1-thioglycerol, anhydrous chloroform, 2,2-dimethoxy-2-phenylacetophenone
144 (DMPA), 1,4- dithioerythritol (DTT), 2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone
145 (Irgacure 2959), Sephadex® LH-20 were purchased from Sigma-Aldrich (UK). Spectra/Por®
146 Dialysis Membrane (MWCO 3,500) was from Spectrum Laboratories Inc. (UK) and Slide-A-
147 Lyzer® MINI Dialysis unit (MWCO 2,000) was purchased from Thermo Scientific Inc. NMR
148 grade chloroform-d and deuterium oxide were from Goss Scientific Instruments Ltd.
149 Griseofulvin (97%) was purchased from ACROS Organics UK.
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151 *2.2. Synthesis and characterisation of MPEG-poly(ester-sulfide) copolymers*
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153 The MPEG-poly(ester-sulfide) copolymers were synthesised via acrylation and thiol-ene
154 photochemical reaction. In this paper the MPEG-poly(ester-sulfide) copolymers are noted as
155 MPEG-DEN-G_X (X means dendron generation) for simplicity. Half generation dendrons are

terminated with ethenyl groups while full generation dendrons have hydroxyl surface groups. Thiol-ene photochemical reactions were performed by UV cross-linker, UVItec Ltd (365 nm, 99.99 J cm^{-2}). The copolymers were characterised by ^{1}H and ^{13}C NMR spectroscopy (Bruker Avance 400, Bruker, Coventry, UK).

Synthesis of half generation MPEG-DEN (acrylation)

1 mmol of MPEG (or MPEG-DEN-OH_X) and excess acryloyl chloride (molar ratio of carbonyl chloride : hydroxyl = 1.2:1) was dissolved in 100 ml anhydrous chloroform at 25 °C. Triethylene amine (equal moles to acryloyl chloride) in 5 ml chloroform was added drop-wise in the MPEG solution and stirred at 30 °C for 48 h. The mixture was dried under vacuum, redissolved in 25 ml chloroform, and then suspended in 500 ml diethyl ether. The precipitant was filtered and dried under vacuum. The crude product was dissolved in chloroform and purified by Sephadex LH-20 column (methanol:chloroform 60:40). Product with a yield of over 70 % was recovered after evaporating the solvent.

Synthesis of full generation MPEG-DEN (thiol-ene photoreaction)

MPEG-acrylates (or MPEG-DEN-ene_X) (1mmol) and DMPA (12.2 mg, 0.1 mmol) were dissolved in a mixed solvent of 50 ml chloroform and 5 ml methanol. Excess 1-thioglycerol (molar ratio of thiol : acrylate = 2:1) was added to the solution. The solution was irradiated under UV light for 60 min. The mixture was precipitated into 500 ml diethyl ether and then

filtered and dried under vacuum. The crude product was redissolved in 30 ml chloroform, washed with brine twice and then dried under vacuum.

NMR Characterisation:

MPEG: ^{1}H NMR ($CDCl_3$): 3.38 (s, CH_3O–), 3.45–3.95 (m, –OCH_2CH_2O–, backbone). ^{13}C NMR ($CDCl_3$): 58.72 (CH_3O–), 61.29 (–CH_2*CH_2*OH), 70.48 (–O*CH_2**CH_2*O–), 71.62 (CH_3O*CH_2*–), 72.27 (–*CH_2*CH_2OH).

MPEG-DEN-ene (G0.5, MPEG-acrylate): ^{1}H NMR ($CDCl_3$): 3.38 (s, CH_3O–), 3.45–3.95 (m, –OCH_2CH_2O–), 4.32 (t, –CH_2OCO–), 5.83, 5.86 (dd, –CH=*CH_2* cis), 6.12–6.19 (q, –*CH*=CH_2), 6.41, 6.45 (dd, –CH=*CH_2* trans). ^{13}C NMR ($CDCl_3$): 58.98 (CH_3O–), 63.63 (–CH_2*CH_2*OCO–), 69.05 (–*CH_2*CH_2OCO–), 70.50 (–O*CH_2**CH_2*O–), 71.88 (CH_3O*CH_2*–), 128.25 (–CO–*CH*=CH_2), 130.95 (–CO–CH=*CH_2*), 166.04 (–O*C*O–).

MPEG-DEN-OH_2 (G1): ^{1}H NMR ($CDCl_3$): 2.55–2.75 (m, –$OCOCH_2$–, –S*CH_2*CH(OH)–), 2.85 (t, –CH_2*CH_2*SCH_2–), 3.38 (s, CH_3O–), 3.45–3.95 (m, –OCH_2CH_2O–, –*CH*(OH)–, –*CH_2*OH), 4.25 (t, –CH_2OCO–). ^{13}C NMR ($CDCl_3$): 27.40 (–CO*CH_2*CH_2S–), 34.76 (–$COCH_2$*CH_2*S–), 35.53 (–S*CH_2*CH<), 59.00 (CH_3O–), 63.81 (–CH_2*CH_2*OCO–), 65.27 (–*CH_2*OH), 69.02 (–*CH_2*CH_2OCO–), 70.50 (–O*CH_2**CH_2*O–, –*CH*(OH)–), 71.88 (CH_3O*CH_2*–), 171.84 (–O*C*O–).

MPEG-DEN-ene_2 (G1.5): ^{1}H NMR ($CDCl_3$): 2.60–2.90 (m, –$OCOCH_2$–, –S*CH_2*CH(OH)–, –CH_2*CH_2*SCH_2–), 3.38 (s, CH_3O–), 3.45–3.95 (m, –OCH_2CH_2O–), 4.10–4.50 (m, –CH_2OCO–), 5.10–5.40 (m, >CHOCO–), 5.83–5.90 (m, –CH=*CH_2* cis), 6.05–6.19 (m, –*CH*=CH_2), 6.35–6.50

(m, –CH=*CH*$_2$ trans). ^{13}C NMR ($CDCl_3$): 27.47 (–CO*C*H$_2$CH$_2$S–), 32.23 (–S*C*H$_2$CH<), 34.58 (–COCH$_2$*C*H$_2$S–),58.95 (CH_3O–), 63.64 (>CH*C*H$_2$OCOCH=), 63.79 (–CH$_2$*C*H$_2$OCO–), 68.97 (–*C*H$_2$CH$_2$OCO–), 70.50 (–O*C*H$_2$*C*H$_2$O–), 71.88 (CH_3O*C*H$_2$–), 127.79, 127.91 (–CO–*C*H=CH$_2$), 131.45, 131.66 (–CO–CH=*C*H$_2$), 165.16, 165.47 (–O*C*OCH=CH$_2$), 171.48 (–O*C*OCH$_2$–).

MPEG-DEN-OH$_4$ (G2): ^{1}H NMR ($CDCl_3$): 2.50–2.95 (m, –OCOCH$_2$–, –S*C*H$_2$CH<, –CH$_2$*C*H$_2$SCH$_2$–), 3.38 (s, CH_3O–), 3.40–3.95 (m, –OCH$_2$CH$_2$O–, –*C*H(OH)–, –*C*H$_2$OH), 4.10–4.50 (m, –CH$_2$OCO–), 5.10–5.40 (m, >CHOCO–). ^{13}C NMR ($CDCl_3$): 27.38 (–CO*C*H$_2$CH$_2$S–), 32.13 (–S*C*H$_2$CH(O)CH$_2$–), 34.54 (–COCH$_2$*C*H$_2$S–), 35.42 (–S*C*H$_2$CH(OH)CH$_2$–), 58.96 (CH_3O–), 63.82 (>CH*C*H$_2$OCO–, –CH$_2$*C*H$_2$OCO–), 65.20 (–*C*H$_2$OH), 68.94 (–*C*H$_2$CH$_2$OCO–), 70.50 (–O*C*H$_2$*C*H$_2$O–, –*C*H(OH)–), 71.84 (CH_3O*C*H$_2$–), 171.17, 171.40, 171.62 (–O*C*OCH$_2$–).

MPEG-DEN-ene$_4$ (G2.5): ^{1}H NMR ($CDCl_3$): 2.50–3.00 (m, –OCOCH$_2$–, –S*C*H$_2$CH<, –CH$_2$*C*H$_2$SCH$_2$–), 3.38 (s, CH_3O–), 3.45–3.95 (m, –OCH$_2$CH$_2$O–), 4.10–4.50 (m, –CH$_2$OCO–), 5.10–5.40 (m, >CHOCO–), 5.83–5.90 (m, –CH=*C*H$_2$ cis), 6.05–6.19 (m, –*C*H=CH$_2$), 6.35–6.50 (m, –CH=*C*H$_2$ trans). ^{13}C NMR ($CDCl_3$): 27.40 (–CO*C*H$_2$CH$_2$S–), 32.18 (–S*C*H$_2$CH(O)CH$_2$–), 34.56 (–COCH$_2$*C*H$_2$S–), 58.98 (CH_3O–), 63.65 (>CH*C*H$_2$OCOCH=), 63.81 (>CH*C*H$_2$OCO–, –CH$_2$*C*H$_2$OCO–), 68.98 (–*C*H$_2$CH$_2$OCO–), 70.50 (–O*C*H$_2$*C*H$_2$O–), 71.88 (CH_3O*C*H$_2$–), 127.78, 127.90 (–CO–*C*H=CH$_2$), 131.52, 131.74 (–CO–CH=*C*H$_2$), 165.21, 165.52 (–O*C*OCH=CH$_2$), 170.83, 171.10, 171.53 (–O*C*OCH$_2$–).

Gel permeation chromatography (GPC) was used to characterise the MPEG-DEN copolymers. The GPC system was an Agilent 1260 Infinity with triple detectors and two Agilent

PLgel Mixed-D columns. Dichloromethane was used as eluent at a flow rate of 1ml min^{-1}. The system was calibrated with Agilent EasiVial PS-M and PS standards (M_p 217,900).

2.3. CMC measurement

The critical micelle concentrations (CMC) of the MPEG-poly(ester-sulfide) copolymers at 20 °C were measured by surface tension measurement using the pendant drop method. An FTA1000 video system (First Ten Ångstroms Inc) was used to visualise drops formed on the tip of a 20-gauge stainless-steel needle (aperture 22, brightness and contrast 50%). The tip width of the needle was measured to perform a calibration of the video camera's magnification. Surface tension of aqueous polymer solution ranging in concentration from 0.0001 to 2 % w/v was calculated via drop-shape analysis; measurements were repeated ten times and the results averaged. The standard deviation of the drop-shape analysis was approximately ± 0.5 mN m^{-1} and the measurement error was less than 5%.

2.4. Preparation and stability study of core-crosslinked micelles

1g (0.175 mmol) of MPEG-DEN-G2.5 was dissolved in 25 mL of distilled water. 62 mg (0.4 mmol) of DTT and 4.5 mg (0.02 mmol) of Irgacure 2959 were added in the solution and stirred at RT for 2 h. The solution was irritated under UV (365nm) for 1 h. The completion of crosslinking was assessed by ^{1}H NMR. The solution was dialysised again distilled water (MWCO 3500) overnight and then lyophilized to recover the core-crosslinked micelles.

The crosslinking was investigated by HPLC, using an Agilent 1100 Series HPLC system equipped with a Luna 5 µm, C18 column (250 mm x 4.6 mm) (Phenomenex, Cheshire, UK) at 40 °C. The mobile phase was MeOH:TFA (0.05% w/v) (80:20), with a flow rate of 1.0 ml min^{-1}, and Corona Plus CAD detection (ESA) with gas pressure of 35 psi.

1% w/v solutions of the crosslinked and non-crosslinked copolymers were prepared in phosphate buffer (0.067M, pH 7.4) and hydrochloric acid buffer (0.085M, pH 1.2) and incubated at 37 °C for 48 and 24 h respectively. The solutions were diluted 5 times with 80 % methanol and analysed by the HPLC assay described above.

2.5. Micellar size

Analysis of micelle size distribution of the copolymer solutions before and after crosslinking was conducted using dynamic light scattering (Zetasizer Nano, Malvern Instruments, UK). The polymer solutions (1 % w/v) were prepared in phosphate buffer (0.067M, pH 7.4) and clarified by filtering through a PVDF filter (Millipore, 0.45 µm pore size) into a clean scattering cell.

2.6. Drug solubilisation

The solubilisation method has been reported previously (Crothers et al., 2005). Briefly, saturated drug-loaded solutions were prepared by suspending excess griseofulvin in 5 ml of 1 % w/v copolymer solutions and stirring at 37 °C for three days. The unsolubilised drug was then filtered (Millipore, 0.45 µm). The amount of drug solubilised was determined by UV assay. The

filtrate was diluted 10 times with methanol, and the UV absorbance was determined at optimum wavelength 292 nm (Jenway 7315 spectrophotometer). The absorbance of the polymers at the same dilution was also measured and deducted from the result. Calibration with drug alone provided satisfactory Beer's law plots. All measurements were carried out in triplicate and the results averaged.

2.7. Drug release study

Release of griseofulvin from the micellar solutions was evaluated using a dialysis technique. Aliquots of 100 µl saturated drug-loaded copolymer solutions (1% w/v) were placed into 10 Slide-A-Lyzer® MINI Dialysis units. The dialysis was performed under sink conditions against buffer solutions in a stirring water bath at 37 °C for 24 h. A dialysis unit was taken out at specific time interval. The solution was diluted with methanol and the amount of griseofulvin remained in the unit were measured by the UV assay described above. All measurements were carried out in triplicate and the results averaged. Statistical analysis of the data was carried out using the Student's t-test. Probability values of $p < 0.05$ were considered to be statistically significant.

3. Results and discussion

3.1. Synthesis and Characterisation of MPEG- poly(ester-sulfide) copolymers

As shown in Scheme 1, a series of linear-dendritic copolymers of MPEG and poly(ester-sulfide) dendron were synthesised by stepwise reactions of acrylation and thiol-ene photochemical reaction. Acrylation with acryloyl chloride is efficient to introduce acrylic groups and excess acryloyl chloride was easily removed by evaporation and precipitation. Thiol-ene reaction was classified as a click reaction, which can be performed under mild reaction conditions and has a very high yield. Structures up to generation 2.5 poly(ester-sulfide) dendron was constructed on the hydroxyl end of a linear MPEG by this scheme.

The complete conversion of dendritic surface function at each reaction step was confirmed by ^{1}H and ^{13}C NMR, which indicates the successful formation of the dendritic structure. Representative ^{1}H and ^{13}C NMR spectra of MEPG-DEN copolymers (full generation G2 and half generation G2.5) are shown in Fig. 1. Acrylation of MPEG results in the peaks of two methylene groups next to the hydroxyl end to shift from 72.3 ppm to 69.1ppm and from 61.3 ppm to 63.6 ppm in ^{13}C NMR spectrum, respectively. Three peaks from the acrylic group appear at 128 and 131 ppm for ethenyl group and 166 ppm for carbonyl group. The acrylic group undergoes free radical addition with thiol group in thiol-ene reaction to form hydroxyl-functioned dendrons. Then the two ethenyl peaks downshifted to 27.4 and 34.7 ppm while the carbonyl peak upshifted to 171.8 ppm. Two new peaks located at 35.5 and 65.3 ppm are from the methylene groups of thioglycerol. The CH peak of thioglycerol is overlapped with the big peak of MPEG backbone. Further acrylation of hydroxyl-terminated dendrons results in the two methylenes of thioglycerol to downshift from 35.5 and 65.3 to 32.2 and 63.6 ppm in ^{13}C NMR, respectively. The acrylic groups showed multiple peaks at 128, 131 and 165 ppm due to the slightly different chemical environment when attaching on primary or secondary hydroxyl groups. In ^{1}H NMR, a group of well-separated peaks are shown between 5.8 and 6.5 ppm after acrylation, which are from the

protons of the ethenyl groups. After addition with thiol groups, those peaks are completely removed, indicative of the full conversion of acrylic groups to hydroxyl functional groups.

The MPEG-DEN-G2.5 copolymer was characterised by GPC to determine the molecular weight. As shown in the chromatogram (Fig. 2), the retention time of MPEG-DEN-G2.5 is lower than that of free MPEG, which indicates an increase in the molecular weight. The molecular weight obtained via calibration is 5510 g mol^{-1}, which is smaller than the value calculated from NMR (5702 g mol^{-1}). This discrepancy is thought to be due to the more compact dendritic structure and thus smaller size compared to linear polymers with a similar molecular weight.

3.2. Critical Micelle Concentration

The half generation MPEG-DEN copolymers, comprising a hydrophilic PEG chain and a hydrophobic dendron with ethenyl surface groups, are able to micellise in aqueous solution. The drop-shape analysis method was employed to determine the CMCs of the copolymers. The method is sensitive to measure the surface tension at low concentrations and only requires small quantities of sample (Zhou et al., 2013). However, the measurements were performed at room temperature (approx. 20 °C) due to lack of temperature control accessories.

The CMC of the MPEG-DEN-G2.5 copolymer determined from inflection points in plots of surface tension versus logarithm concentration (Fig. 3) was 0.9 g dm^{-3}. Fury et al. reported the CMC measurement of MPEG-poly(ester-sulfide) copolymer (synthesised using pentenoyl chloride) (Fury et al., 2013). A lower CMC value (7.5 mg dm^{-3}) was found for the G3.5 copolymer with 8 ethenyl groups. This is probably due to the longer alkenyl building units and doubled hydrophobic surface groups. In this paper the micellar properties and solubilisation

characteristics of the copolymers were measured in 1% w/v (10 g dm^{-3}) aqueous solution. It was assumed that micellisation is complete at the concentration and temperature. The surface tension measurement of full generation MPEG-DEN copolymers showed that no CMC was detected at the equivalent concentration range. It indicates that full generation MPEG-DEN copolymers are not able to form micelles due to the hydrophilicity of their hydroxyl surface groups. This is in agreement with the findings reported by Fury et al. (Fury et al, 2013).

3.3. Preparation of core-crosslinked micelles

As shown in Fig. 4, half generation MPEG-DEN copolymers are amphiphiles that can self-associate in dilute aqueous solutions. Hydrophobic poly(ester-sulfide) dendrons form the micelle cores while hydrophilic MPEG chains form the periphery of the micelles. The G2.5 Poly(ester sulphide) dendron has 4 ethenyl surface groups that are suitable for crosslinking via reaction with an appropriate linker. The bithiol linker DTT and photoinitiator Irgacure 2959 were added into a pre-prepared micellar solution of MPEG-DEN-G2.5. DTT and Irgacure 2959 are soluble in a wide range of aqueous and organic solvents. They can be easily dispersed in water and then penetrate into the micelle cores. Thiol-ene photoreaction was initiated by UV radiation and excess DTT was removed by dialysis after crosslinking. The completion of crosslinking was confirmed by ^{1}H NMR. The peaks from the ethenyl groups (5.8–6.5 ppm) disappeared after reaction, which indicates full conversion of the ethenyl groups.

The formation of the core-crosslinked micelles was confirmed by HPLC equipped with a CAD detector. Charged aerosol detection (CAD) has been introduced as a powerful technique in combination with HPLC to analyse compounds without strong UV chromophores (Vehovec and

Obreza, 2010; Almeling et al., 2012). The HPLC eluent is nebulised with a flow of nitrogen to form droplets. The volatile components and mobile phase are then evaporated to obtain analyte particles. The particles are charged by meeting with a secondary stream that has passed a high-voltage platinum wire. The resulting positively-charged particles are collected and measured by an electrometer. CAD detector is mass-dependent and the response is generated regardless of the spectral and physicochemical properties of analytes.

Fig. 5 shows the CAD chromatogram of MPEG, non-crosslinked and crosslinked micelles of MPEG-DEN-G2.5 copolymers. The polymers were analysed by reversed-phase HPLC and separated by the hydrophobic interaction with the stationary phase of the C18 column. MPEG are hydrophilic molecules and eluted fast from the column. MPEG shows a narrow peak due to its low polydispersity and the similar polarity of all the molecules. However the hydrophobic poly(ester-sulfide) dendrons, especially the ethenyl groups, can interact with the stationary phase, which increases the retention time of the linear-dendritic copolymers. The micelles of MPEG-DEN-G2.5 copolymer are unstable upon dilution in mobile phase and could disassociate fully or partially during elution in the column. The molecules of MPEG-DEN-G2.5 copolymer exhibit various polarities as the hydrophobic dendrons are conjugated to MPEG molecules with different chain lengths. Hence the MPEG-DEN-G2.5 copolymer was eluted more slowly and separated into fractions. This explains the multiple broad peaks seen in the chromatogram. Compared to the non-crosslinked micelles, the core-crosslinked MPEG-DEN micelles only show a single peak which is very similar to MPEG but relatively broader. The micelles are stable after crosslinking and remain intact during elution. The crosslinked micelles have a relatively uniform structure and the hydrophobic micelle cores are shielded by the MPEG corona. So they demonstrate similar polarity and thus retention time to MPEG.

3.4. Stability of core-crosslinked micelles

The hydrolysis of the crosslinked and non-crosslinked micelles of MPEG-DEN-G2.5 copolymer was investigated in acidic and neutral solutions. As shown in Fig. 6, both the crosslinked and non-crosslinked micelles have good stability at pH 7.4. No degradation was detected for the crosslinked micelles after 2 days. The peaks of the non-crosslinked micelles were reduced slightly due to the dynamic equilibrium of micellisation and relatively direct exposure of the molecules to the solvent. The MPEG-DEN copolymers contain ester bonds and thus more readily hydrolyse in acidic solutions. The dendritic branches could be fully or partially cleaved from the MPEG chains, which results in an increase of the polarity and a decrease of the retention time. Fig. 7 showed that both micelles demonstrated more apparent changes after incubation at pH 1.2 for 24 h. The peak at 2.1 min is from the chloride ions in the buffer. The non-crosslinked micelles showed clear sign of degradation over the elution range. The peaks of the more hydrophobic fractions were greatly reduced and the copolymers were eluted faster due to the increased hydrophilicity. The crosslinked micelles also showed a similar tendency. The peaks became more narrow and the retention times were slightly decreased. Although the evidence of hydrolysis was found by the CAD technique, the quantitative determination of the degradation of the copolymers is not achievable due to lack of calibration standards. As CAD detection is mass-dependent not concentration-dependent, different compounds could be eluted at the same time and produce very close responsive signals in the detector. However the CAD cannot provide any spectral information to identify the compounds. The results of the stability study indicate that the copolymers are relatively stable under neutral conditions. Crosslinking

405 within the micelle cores could enhance the stability of micelles, limit the access to the
406 surrounding solutions and retard the hydrolysis of the copolymers.
407

408 *3.5. Micellar size*

409

410 The MPEG-DEN copolymers are nonionic polymeric surfactants and hence pH will not
411 have an impact on their micellar properties. The micellar properties of the MPEG-DEN
412 copolymers were measured in aqueous phosphate buffer (0.067 M, pH7.4) to assure the stability
413 of the copolymers during the measurement. The effect of the buffer on the micellar properties is
414 negligible because of its low ionic strength. Dynamic light scattering was used to measure the
415 micellar size of the polymers in dilute aqueous solutions. It was found that the hydrodynamic
416 radius of MPEG molecules in 1% w/v buffer solution at 25 °C is approx. 2.5 nm (Fig. 8). In
417 contrast, the size distribution curve for 1% w/v micellar solution of the MPEG-DEN-G2.5
418 copolymer showed a peak at ca. 12.5 nm (radius) taken as evidence of association to form
419 spherical micelles. The size distribution is within the similar range (diameter 10~100 nm) of the
420 MPEG-G3.5 poly(ester-sulfide) copolymer reported by Fury et al. 2013, which shows a larger
421 peak size at $r \approx 20$ nm due to the larger dendrons with longer alkenyl building units. The core-
422 crosslinked MPEG-DEN-G2.5 micelles show a very similar size distribution to the non-
423 crosslinked micelles under the equivalent conditions, which indicates that the crosslinking within
424 the micelle cores does not affect the micelle size.
425

426 *3.6. Drug solubilisation*

427

The amphiphilic MPEG-DEN copolymers can self-associate to form micelles with a dendritic micelle core and hydrophilic MPEG corona. The hydrophobic cores are the favoured domain for encapsulation of poorly water-soluble drugs. Compared to linear molecules the core-forming dendritic blocks are not able to closely packed due to steric hindrance and thus could lead to a relatively large core size. The MPEG corona is also a possible site for incorporation of guest molecules. Crothers et al. investigated the solubilisation of griseofulvin in solutions of PEG6000 in excess of the solubility in water (Crothers et al., 2005). The solubilisation capacity of 2 mg of griseofulvin per gram of polymer was obtained for a 10 wt.% PEG solution. The solubilisation of griseofulvin showed a linear increase with the concentration of PEG6000 in solutions. In this work the solubilisation of MPEG-DEN copolymers was measured in 1 % w/v solution. The mass fraction of MPEG5000 in the copolymers is less than 0.87. Thus the volume of PEG micelle corona in solution is rather small. So the contribution of MPEG corona to the solubilisation capacities of the copolymers is negligible.

Table 1 shows the solubility of griseofulvin in the buffer solutions of the crosslinked and non-crosslinked MPEG-DEN micelles at 37°C. An approx. 10-fold increase of the solubility was found for both copolymer solutions at 1% w/v. The solubility of griseofulvin in phosphate buffer (0.01 mg ml^{-1}) was deducted for calculation of the solubilisation capacity (S_{cp}, expressed as milligram drug per gram of copolymer). The crosslinked micelles show relatively higher solubilisation capacity than the non-crosslinked ones under equivalent conditions. The micelle size measurement indicates that both micelles have very similar hydrodynamic radii and crosslinking has no impact on the size of micelle cores. It is known that micellisation is a thermodynamic equilibrium and thus the drug molecules encapsulated are possible to diffuse out of the micelle cores. However the escape of drug molecules is considered to be hindered by core-

crosslinking that enhances the stability of the micelles. It should be noted that crosslinking can also affect the diffusion of drug into the micelle. Therefore sufficient suspension time was ensured in order to achieve saturated drug-loaded solutions. It was observed that an approximate doubling of solubility of griseofulvin in the copolymer solutions was obtained by increasing solution concentration from 1 to 2 % w/v. The micellisation was considered to be completed under the measurement conditions due to the low CMC of the copolymers. Hence an increase of concentration was expected to increase the number of micelles. Although the solubility increases the concentration, the solubilisation capacities of the copolymers remain the same as the concentration does not affect the micelle size and thus the number of drug molecules incorporated in each micelle.

3.7. Drug release profiles

The stability study indicates that the MPEG-DEN copolymers have good stability under neutral conditions. Hence the main mechanism of drug release at pH 7.4 is attributed to the diffusion of molecules out of the micelles. Fig. 9 shows the release profiles of griseofulvin from 1% w/v buffer solution (pH 7.4) of crosslinked and non-crosslinked MPEG-DEN-G2.5 copolymers at 37 °C. The sink condition was ensured by refreshing phosphate buffer during dialysis. A rapid release with approximately 36% and 57% drug within the initial 6 h was seen for crosslinked and non-crosslinked copolymer solutions, respectively. The release curves showed that approximately 60% and 85% griseofulvin was released from the copolymer solutions after 24 h of dialysis where a plateau was reached. Drug release from the crosslinked micelles was significantly ($p < 0.05$) more sustained than from the non-crosslinked micelles, attributable to the crosslinking structure within the micelle cores which may hinder the diffusion

of drug molecules. To investigate the effect of hydrolysis of copolymers on the drug release, the release of griseofulvin from 1% w/v crosslinked micellar solution at pH 1.2 was also measured (Fig. 9). A slightly faster release rate was observed under acidic conditions at the initial stage of dialysis and a significant ($p < 0.05$) increase of the amount of drug released (approximately 15%) was found after 24 h. The acceleration of release is due to the degradation of hyperbranched micelle cores or cleavage of the MPEG chains from the micelle corona.

4. Conclusions

Poly(ester-sulfide) dendrons were successfully constructed on the chain ends of MPEG via a combination of acrylation and thiol-ene photochemical reaction. The surface functionality of dendrons was found to have an impact on the physicochemical properties of the linear-dendritic copolymers, especially their micellisation behavior in aqueous solutions. The half generation MPEG-DEN copolymers with ethenyl groups are able to self-associate to form micelles with a hydrophobic hyperbranched core and MPEG corona. Crosslinking within the micelle cores can be initiated by UV radiation via thiol-ene reaction with dithiol linkers, which can reinforce the micelle structure and enhance their stability. The copolymer micelles show good stability under neutral conditions but readily hydrolyse in acidic solutions due to the cleavage of ester bonds in the dendrons. The micelles of the copolymers demonstrated the capability to increase the solubility of poorly water-soluble drugs by incorporation of the drug in the hydrophobic cores. The release profiles from the copolymer solutions indicated that crosslinked micelles showed more sustained release of griseofulvin than the non-crosslinked micelles attributable to the

496 improved stability and crosslinked core structure. This study explores a new strategy on
497 designing biodegradable crosslinked micelles for drug solubilisation and delivery.
498
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503
504 **References**
505
506 Adams, M.L., Lavasanifar, A., Kwon, G.S., 2003. Amphiphilic block copolymers for drug
507 delivery. J. Pharm. Sci. 92, 1343–1355.
508 Almeling, S., Ilko, D., Holzgrabe, U., 2012. Charged aerosol detection in pharmaceutical
509 analysis. J. Pharm. Biomed. Anal. 69, 50–63.
510 Attwood, D., Zhou, Z., Booth, C., 2007. Poly(ethylene oxide) based copolymers: solubilisation
511 capacity and gelation. Expert Opin. Drug Deliv. 4, 533–546.
512 Chen, W., Meng, F., Cheng, R., Deng, C., Feijen, J., Zhong, Z., 2015. Facile construction of
513 dual-bioresponsive biodegradable micelles with superior extracellular stability and
514 activated intracellular drug release. J. Control. Rel. 210, 125–133.
515 Crothers M., Zhou Z., N. Ricardo M.P.S., Yang Z., Taboada P., Chaibundit C., Attwood D.,
516 Booth C., 2005. Solubilisation in aqueous micellar solutions of block copoly(oxyalkylene)s.
517 Int. J. Pharm., 293, 91–100.

518 D'Emanuele, A., Attwood, D., 2005. Dendrimer-drug interactions. Adv. Drug. Deliv. Rev. 57,
519 2147–2162.
520 D'Emanuele, A., Zhou, Z., Attwood, D., Abu-Rmaileh, R. Dendrimers. In: Swarbrick, J. (Ed.),
521 Encyclopaedia of Pharmaceutical Science and Technology. Fourth ed. 2013. CRC press,
522 Taylor and Francis: New York, pp. 799–818.
523 Esfand, R., Tomalia, D.A., 2001. Poly(amidoamine) (PAMAM) dendrimers: from biomimicry to
524 drug delivery and biomedical applications. Drug Disc. Today 6, 427–436.
525 Fury, J. J., Altenhofer, E.F., Sedaghat-Herati, R., 2009. Synthesis of new poly(ethylene glycol)-
526 block-poly(ester sulfide) dendrimers. Tetrahedron Lett. 50, 4205–4207.
527 Fury, J.J., Robison, J., Sedaghat-Herati, R., 2013. Synthesis and self-assembly of new
528 poly(ethylene glycol)-block-poly(ester-sulfide) dendrimers. J. Macromol. Sci., Pure Appl.
529 Chem. 50, 1113–1120.
530 Gauchera, G., Satturwara, P., Jonesa, M., Furtosb, A., Leroux, J.C., 2010. Polymeric micelles for
531 oral drug delivery. Eur. J. Pharm. Biopharm. 76, 147–158.
532 Gitsov, I., Fréchet, J. M. J., 1993. Solution and solid-state properties of hybrid linear-dendritic
533 block copolymers. Macromolecules 26, 6536–6546.
534 Hu, X., Chen, X., Wei, J., Liu, S., Jing, X., 2009. Core crosslinking of biodegradable block
535 copolymer micelles based on poly(ester carbonate). Macromol. Biosci. 9, 456–463.
536 Huang, Y., Sun, R., Luo, Q., Wang, Y., Zhang, K., Deng, X., Zhu W., Li, X., Shen, Z., 2016. In
537 situ fabrication of paclitaxel-loaded core-crosslinked micelles via thiol-ene "click"
538 chemistry for reduction-responsive drug release. J. Polym. Sci. A Polym. Chem. 54, 99–
539 107.

Killops, K.L., Campos, L.M., Hawker, C.J., 2008. Robust, efficient, and orthogonal synthesis of dendrimers via thiol-ene “click” chemistry. J. Am. Chem. Soc. 130, 5062–5064.

Kim, J.S., Youk, J.H., 2009. Preparation of core cross-linked micelles using a photo-cross-linking agent. Polymer 50, 2204–2208.

Kwon, G.S., 2003. Polymeric micelles for delivery of poorly water-soluble compounds. Crit. Rev. Ther. Drug Carrier Syst. 20, 357–403.

Li, Y., Shen, Y., Wang, S., Zhu, D., Du, B., Jiang, J., 2015. Disulfide cross-linked cholic-acid modified PEG–poly(amino acid) block copolymer micelles for controlled drug delivery of doxorubicin. RSC Adv. 5, 30380–30388.

Li, Y., Xiao, K., Luo, J., Xiao, W., Lee, J.S., Gonik, A.M., Kato, J., Dong, T.A., Lam, K.S., 2011. Well-defined, reversible disulfide cross-linked micelles for on-demand paclitaxel delivery. Biomaterials 32, 6633–6645.

Liu, S., Weaver, J.V.M., Save, M., Armes, S.P., 2002. Synthesis of pH-responsive shell cross-linked micelles and their use as nanoreactors for the preparation of gold nanoparticles. Langmuir 18, 8350–8357.

Van Nostrum, C.F., 2011. Covalently cross-linked amphiphilic block copolymer micelles. Soft Matter 7, 3246–3259.

O’Reilly, R.K., Hawker, C.J., Wooley, K.L., 2006. Cross-linked block copolymer micelles: functional nanostructures of great potential and versatility. Chem. Soc. Rev. 35, 1068–1083.

Quan, C., Wei, H., Shi, Y., Li, Z., Cheng, S., Zhang, X., Zhuo, R., 2011. Fabrication of multifunctional shell cross-linked micelles for targeting drug release. Colloid Polym Sci. 289, 667–675.

562 Read, E.S., Armes, S.P., 2007. Recent advances in shell cross-linked micelles. Chem. Commun.
563 3021–3035.
564 Torchilin, V. P., 2001. Structure and design of polymeric surfactant-based drug delivery systems.
565 J. Control. Rel. 73, 137–172.
566 Vehovec, T., Obreza, A., 2010. Review of operating principle and applications of the charged
567 aerosol detector. J. Chromatogr. A 1217, 1549–1556.
568 Williams, H. D., Trevaskis, N. L., Charman, S. A., Shanker, R. M., Charman, W. N., Pouton, C.
569 W., Porter, C. J. H., 2013. Strategies to address low drug solubility in discovery and
570 development. Pharmacol. Rev. 65, 315–499.
571 Whitton, G., Gillies, E.R., 2015. Functional aqueous assemblies of linear-dendron hybrids. J.
572 Polym. Sci. A Polym. Chem. 53, 148–172.
573 Wu, Y., Chen, W., Meng, F., Wang, Z., Cheng, R., Deng, C., Liu, H., Zhong, Z, 2012. Core-
574 crosslinked pH-sensitive degradable micelles: A promising approach to resolve the
575 extracellular stability versus intracellular drug release dilemma. J. Control. Rel. 164, 338–
576 345.
577 Xiong, J., Meng, F., Wang C., Cheng, R., Liu, Z., Zhong, Z., 2011. Folate-conjugated
578 crosslinked biodegradable micelles for receptor-mediated delivery of paclitaxel. J. Mater.
579 Chem. 21, 5786–5794.
580 Yue, J., Wang, R., Liu, S., Wu, S., Xie, Z., Huang, Y., Jing, X., 2012. Reduction-responsive
581 shell-crosslinked micelles prepared from Y-shaped amphiphilic block copolymers as a drug
582 carrier. Soft Matter 8, 7426–7435.

583 Zhang, Y., Xiao, C., Li, M., Ding, J., He, C., Zhuang, X., Chen, X., 2014. Core-cross-linked
584 micellar nanoparticles from a linear-dendritic prodrug for dual-responsive drug delivery.
585 Polym. Chem. 5, 2801–2808.
586 Zhou, Z., D'Emanuele, A., Attwood, D., 2013. Solubility enhancement of paclitaxel using a
587 linear-dendritic block copolymer. Int. J. Pharm. 452, 173–179.
588 Zhou, Z., D'Emanuele, A., Lennon, K., Attwood, D., 2009. Synthesis and micellization of linear–
589 dendritic copolymers and their solubilization ability for poorly water-soluble drugs.
590 Macromolecules 42, 7936–7944.

591

592 **Figure legends**

593

594 Scheme 1. Synthesis of Methoxy-poly(ethylene glycol)-b-poly(ester-sulfide) dendron
595 copolymers.

596

597

598

599 Fig. 1. ^{1}H and ^{13}C NMR spectra of MPEG-DEN-G2.5 (1) and MPEG-DEN-G2 (2). The peaks at
600 52 and 89 ppm in ^{13}C NMR are the noise peaks generated by the instrument.

601

602 Fig. 2. GPC chromatogram of MPEG and MPEG-DEN-G2.5 copolymer.

603

604 Fig. 3. Surface tension versus logarithm concentration (g dm^{-3}) for MPEG-DEN-G2.5 copolymer
605 at 20 °C.

606

607 Fig. 4. Schematic illustration of micellisation of half generation MPEG-DEN copolymers and
608 core-crosslinking of the micelles in the presence of DTT.

609

610 Fig. 5. HPLC chromatogram of MPEG, non-crosslinked and core-crosslinked micelles of
611 MPEG-DEN-G2.5 copolymer detected by CAD.

612

613 Fig. 6. HPLC chromatograms of non-crosslinked (a) and core-crosslinked micelles (b) of MPEG-
614 DEN-G2.5 copolymer at pH 7.4 for 48 h detected by CAD.

615

616 Fig. 7. HPLC chromatograms of non-crosslinked (a) and core-crosslinked micelles (b) of MPEG-
617 DEN-G2.5 copolymer at pH 1.2 for 24 h detected by CAD.

618

619 Fig. 8. Intensity fraction distributions of logarithm hydrodynamic diameter of MPEG (■) and
620 MPEG-DEN-G2.5 copolymer (□) in a 1% (w/v) buffer solution at 37 °C.

621

622 Fig. 9. Drug release profiles from 1% w/v micellar solutions of MPEG-DEN-G2.5 copolymer at
623 37 °C: non-crosslinked micelles at pH 7.4 (●), crosslinked micelles at pH 7.4 (♦) and pH 1.2 (■)
624 (mean ± SD, n=3). * indicates a significant difference ($p < 0.05$) from crosslinked micelles at pH
625 7.4.

626

627 Table 1. Solubility of griseofulvin in copolymer solutions at various concentrations

628

MPEG-DEN-G2.5	Conc. /%w/v	S /mg ml^{-1}	Scp /mg g^{-1} [a]
Non-crosslinked micelles	1.0	0.101	9.1
	2.0	0.210	9.5
Crosslinked micelles	1.0	0.123	11.3
	2.0	0.242	11.1

a. Measurement uncertainty ± 1 mg g^{-1}.

scheme 1

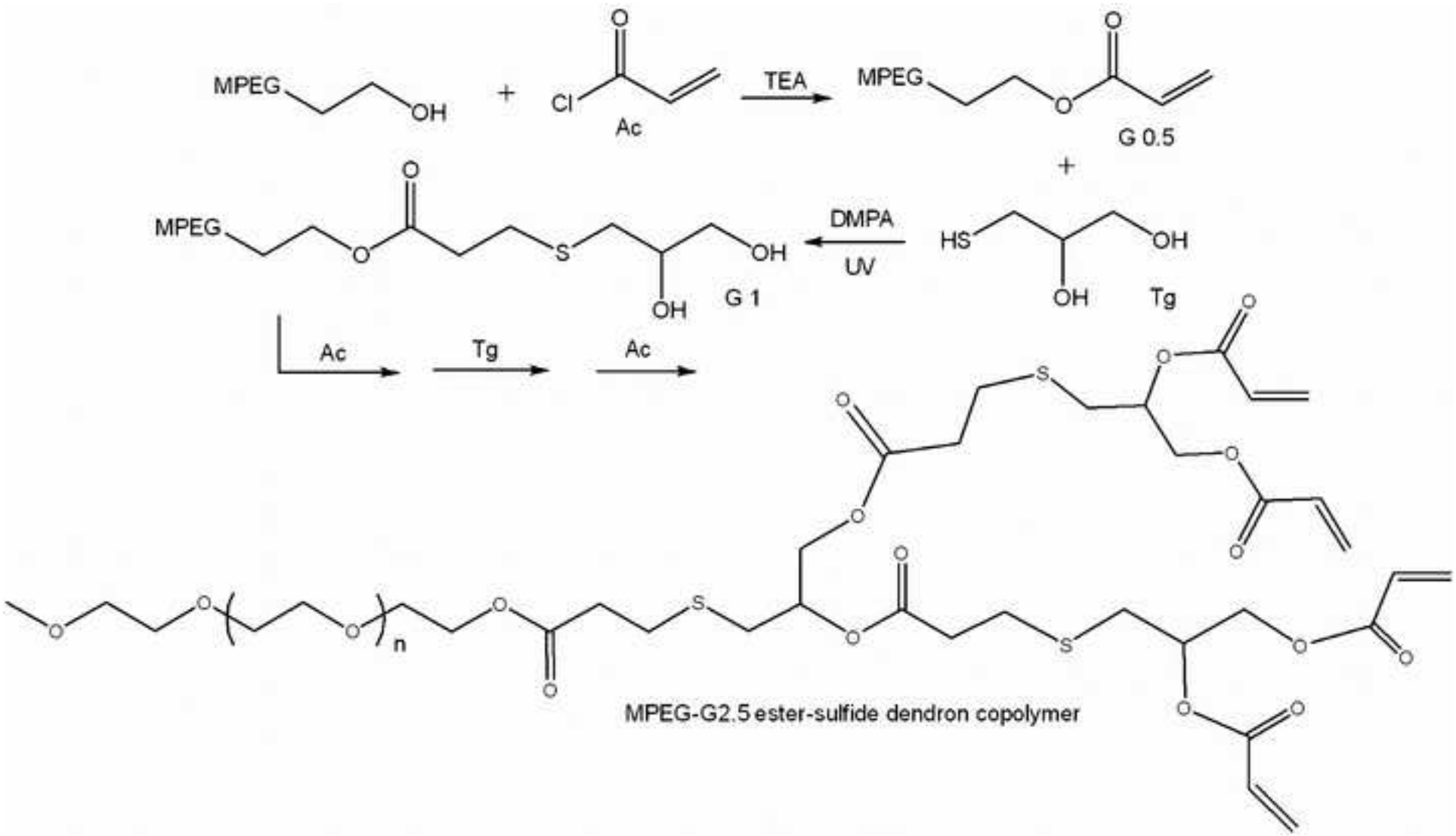
MPEG
OH
Cl
Ac
TEA
MPEG
G 0.5
DMPA
UV
HS
OH
OH
Tg
MPEG
OH
OH
G 1
Ac
Tg
Ac
n
MPEG-G2.5 ester-sulfide dendron copolymer

Figure 1

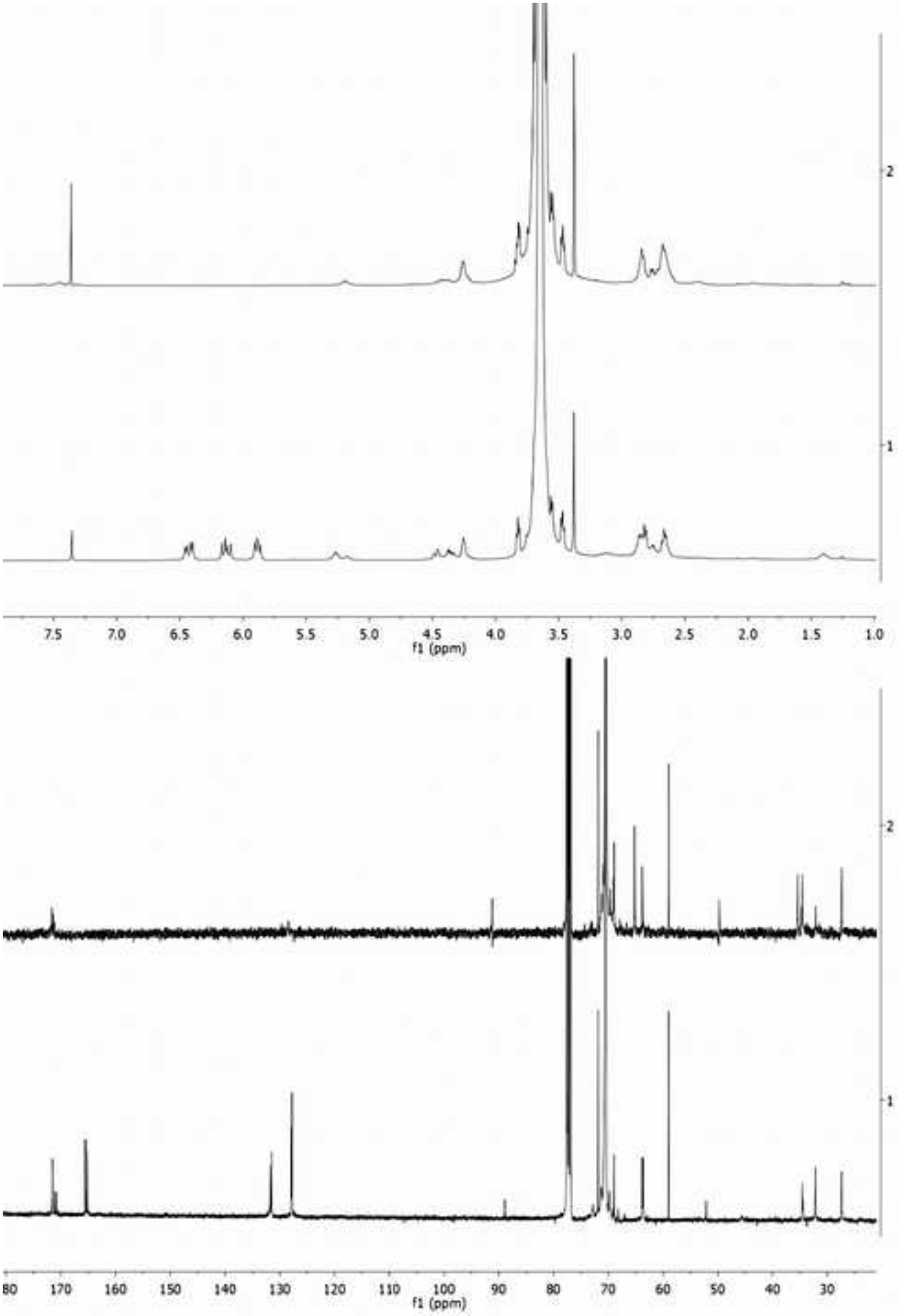
2
1
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)
2
1
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
f1 (ppm)

Figure 2

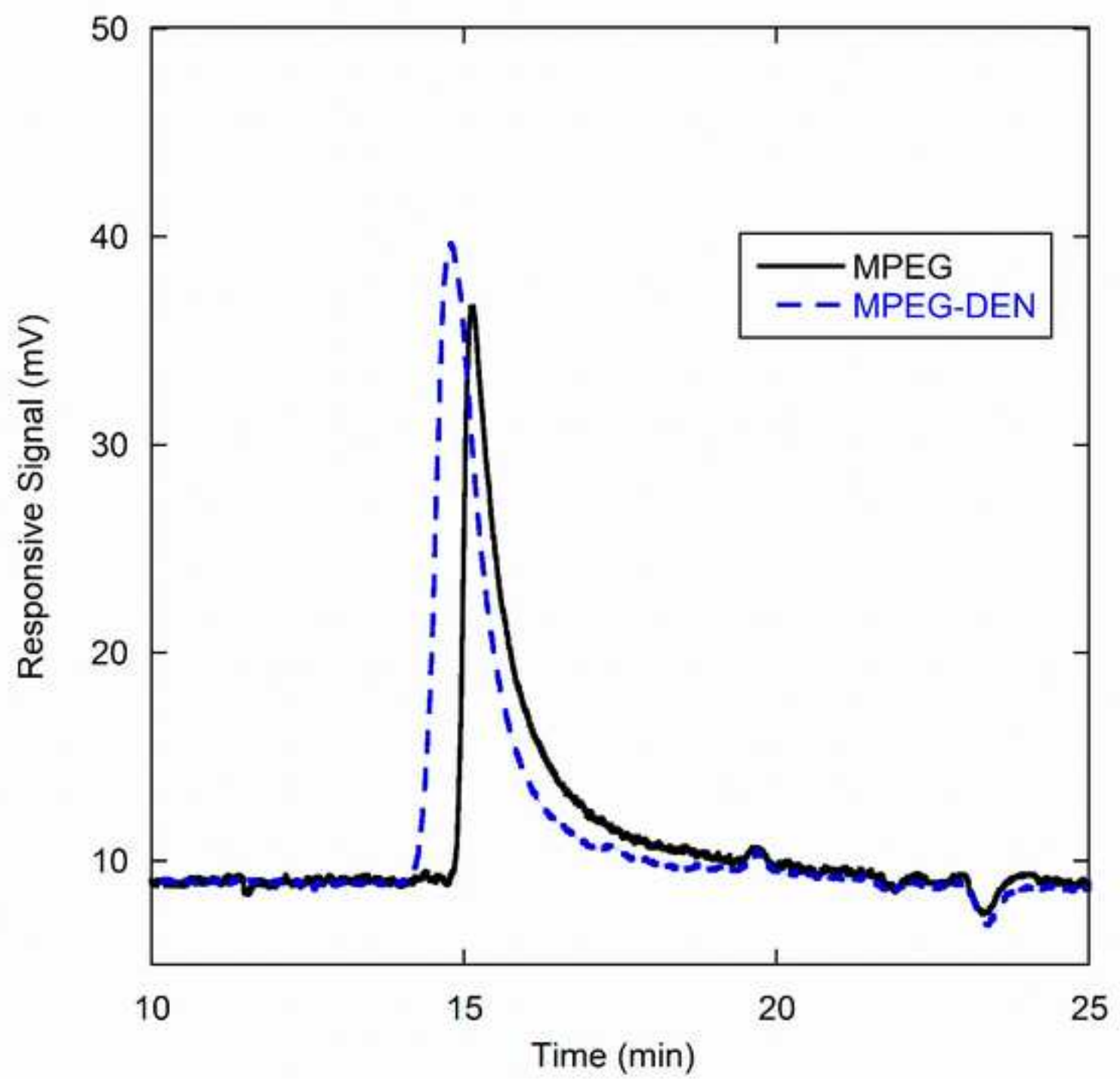
50
40
30
20
10
Responsive Signal (mV)
MPEG
MPEG-DEN
10
15
20
25
Time (min)

Figure 3

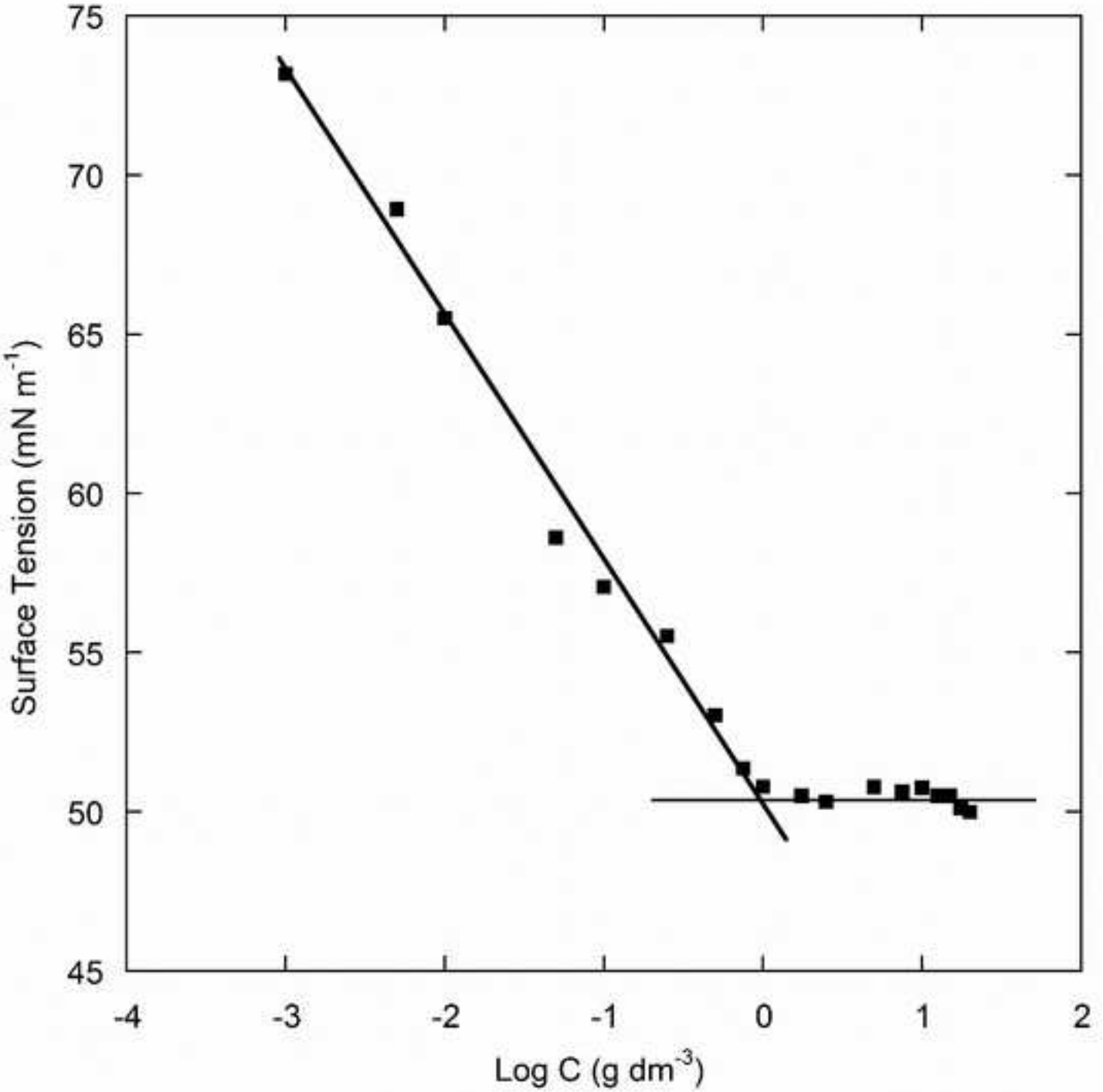

Figure 4

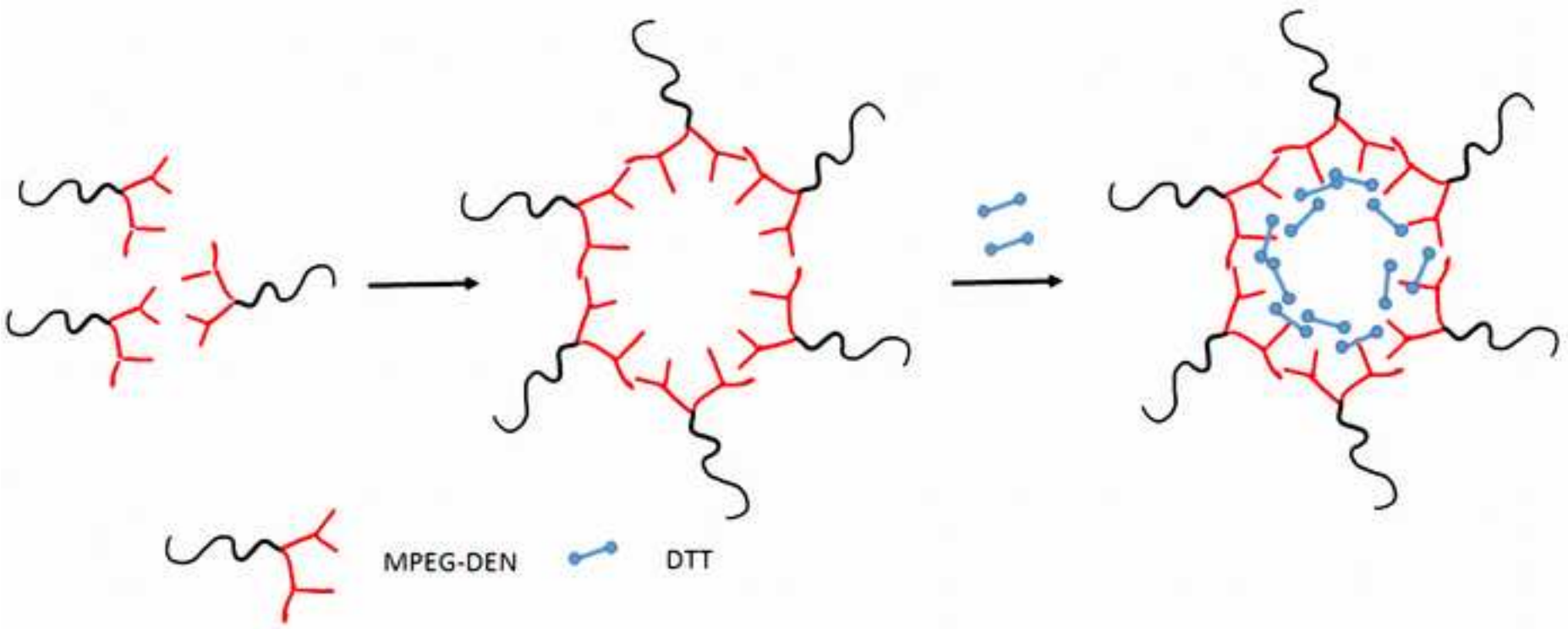

Figure 5

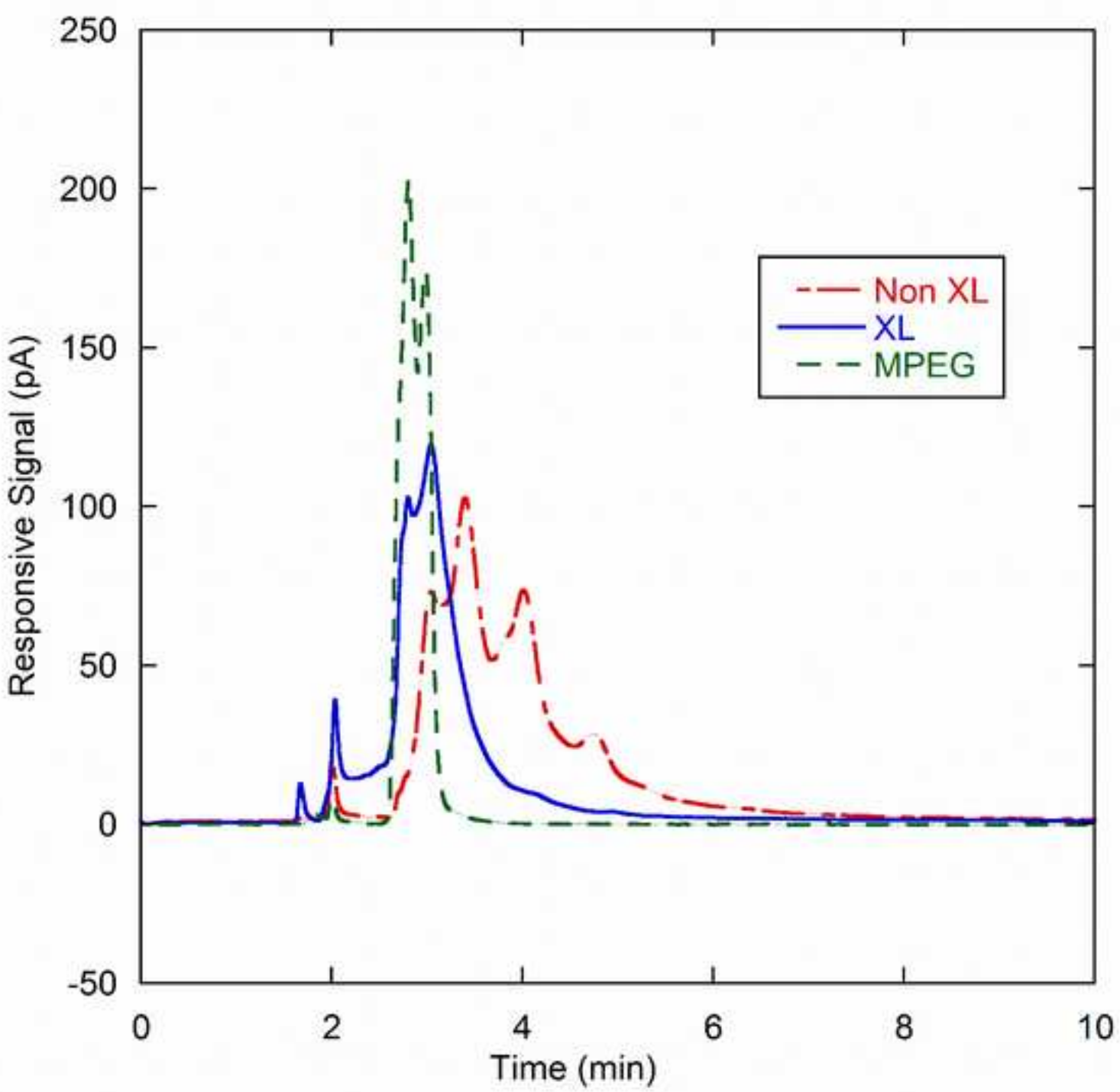
250
200
150
100
50
0
-50
Responsive Signal (pA)
0
2
4
6
8
10
Time (min)
Non XL
XL
MPEG

Figure 6

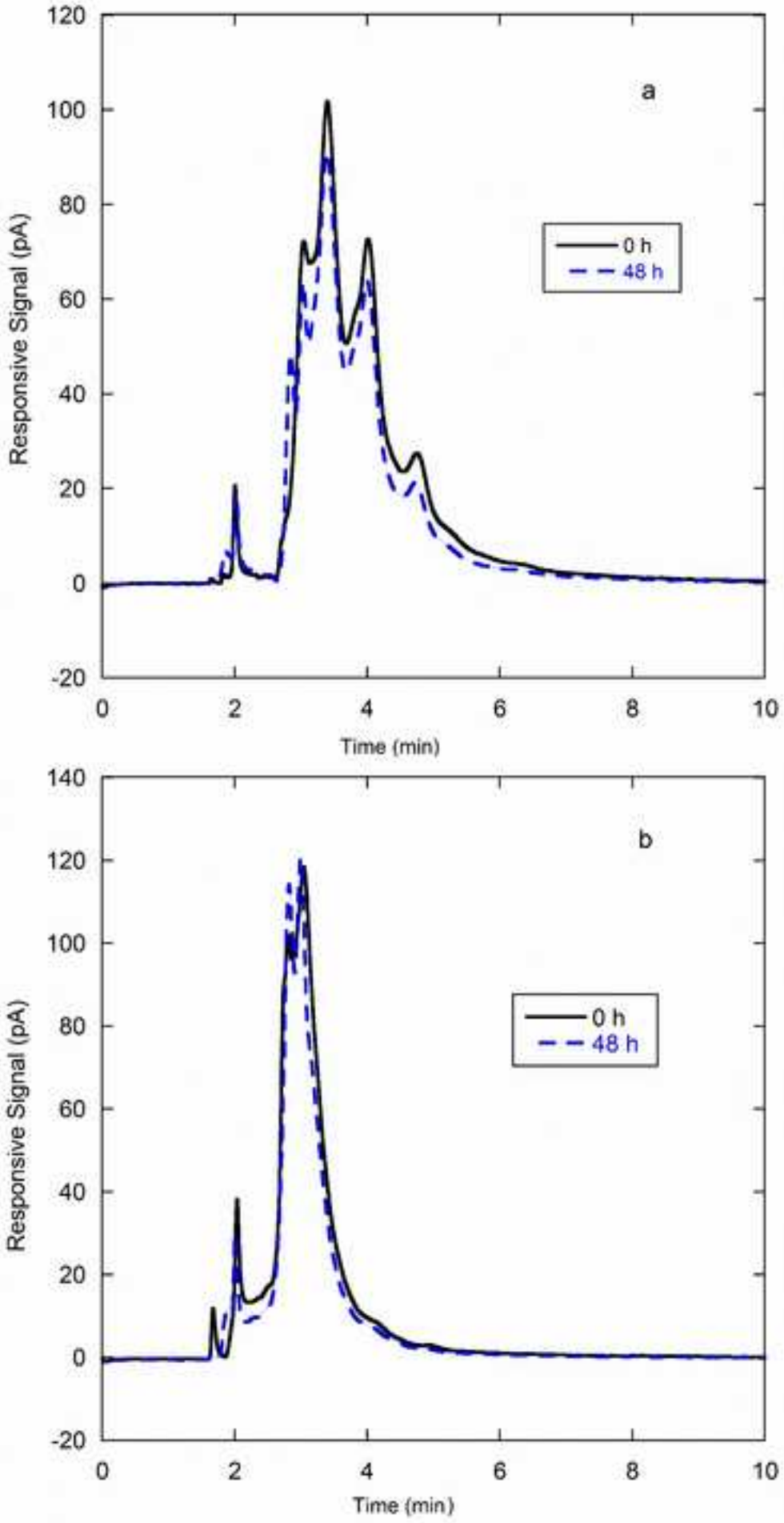
a
0 h
48 h
Responsive Signal (pA)
Time (min)
b
0 h
48 h
Responsive Signal (pA)
Time (min)

Figure 7

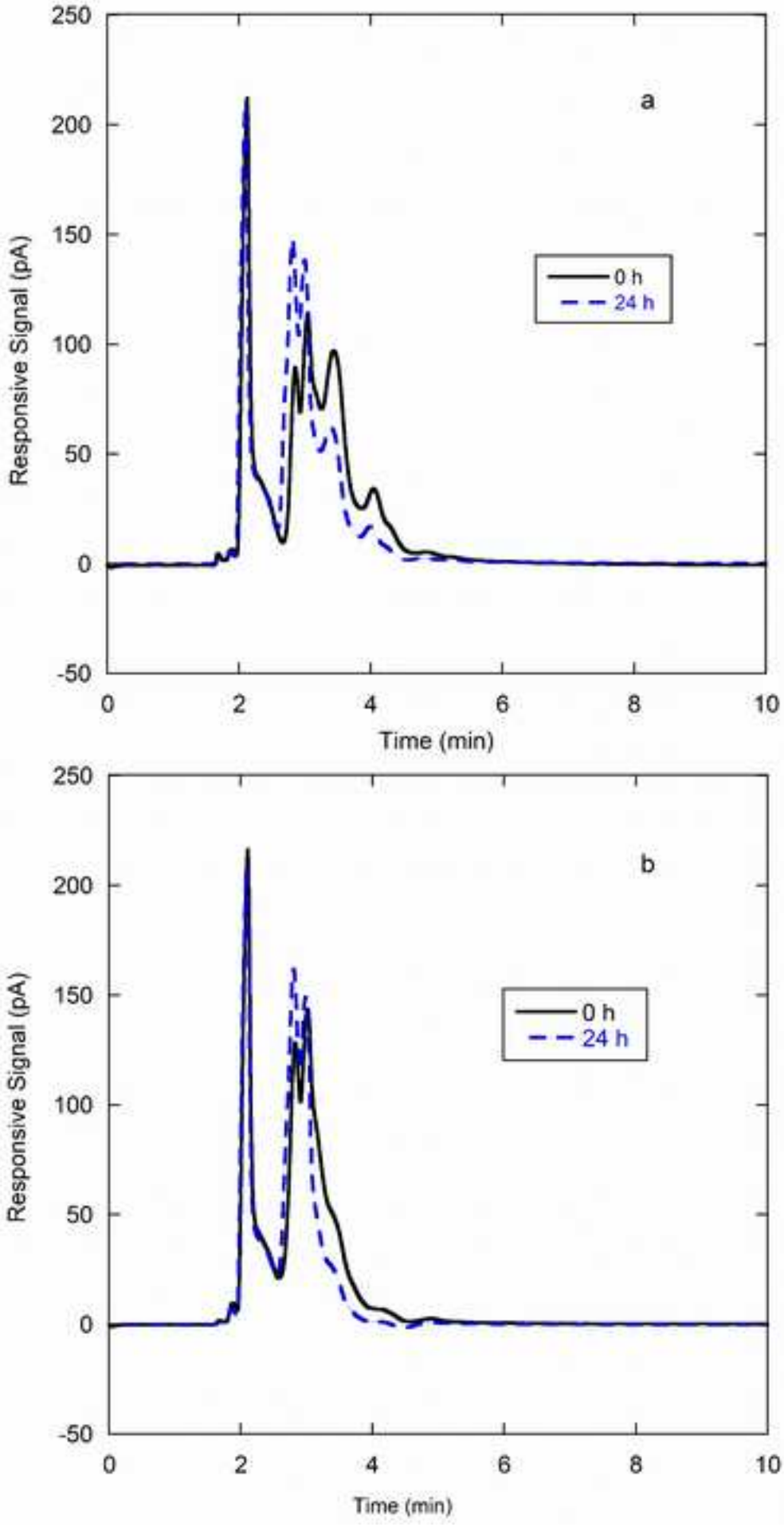
a
0 h
24 h
Responsive Signal (pA)
Time (min)
250
200
150
100
50
0
-50
0
2
4
6
8
10
b
0 h
24 h
Responsive Signal (pA)
Time (min)

Figure 8

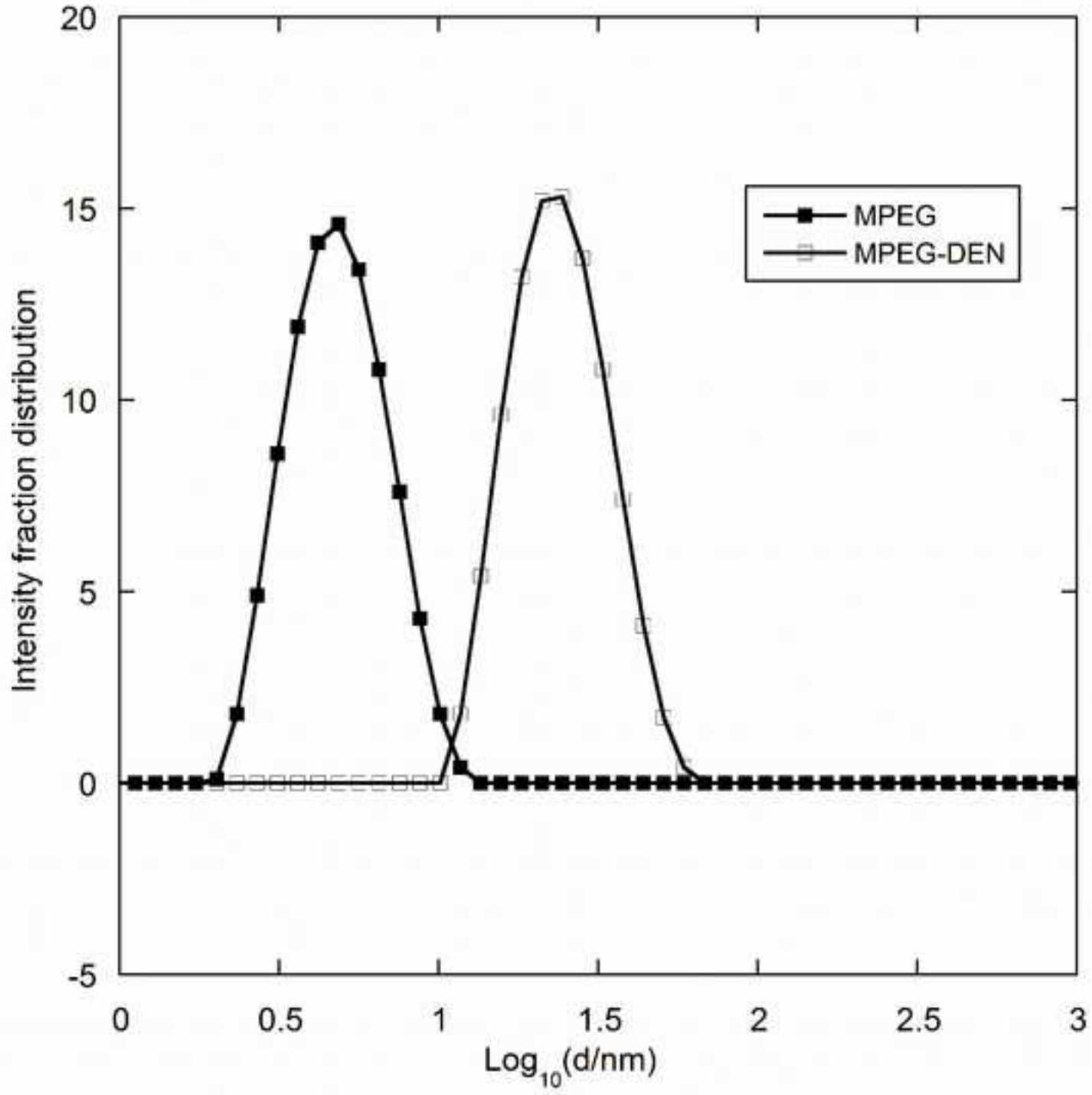
20
15
10
5
0
-5
0
0.5
1
1.5
2
2.5
3
Intensity fraction distribution
Log10(d/nm)
MPEG
MPEG-DEN

Figure 9