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

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*Keywords:* Linear-dendritic copolymers, Thiol-ene reaction, Micellisation, Core-crosslinking, Charged aerosol detection, Drug solubilisation.

19 **Abstract**

20

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

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39

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## 41 **1. Introduction**

42

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

56 Although polymeric micelles are thermodynamically stable, dissociation of the micelles as  
57 a result of dilution in biological fluids or under solvent changes is still a concern, leading to  
58 premature release of drug. Numerous attempts have been made to improve the stability of  
59 polymeric micelle systems. Among the most promising strategies is the introduction of a  
60 crosslinking structure by covalently connecting polymer chains in the micelle (O'Reilly et al.,  
61 2006; Read and Armes, 2007). The monomers with functional groups are designed and utilised  
62 to synthesise a specific block of amphiphilic block copolymer. After micellisation the functional  
63 groups undergo crosslinking and thus provide reinforcement to the micellar structure. Various

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

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

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

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

119 Poly(ester-sulfide) dendrimers have been prepared by Hawker and co-workers via a  
120 combination of thiol-ene photochemistry and esterification by 4-pentenoic anhydride. Thiol-ene  
121 photochemistry has been proved to be efficient for free radical addition of thiol with ethenyl  
122 group in the presence of a photo initiator. Sedaghat-Herati and co-workers synthesised linear-  
123 dendritic copolymers of methoxy-poly(ethylene glycol) (MPEG) and poly(ester-sulfide) (PES)  
124 dendron via acrylation by acryloyl chloride and thiol-ene addition with thioglycerol (Fury et al,  
125 2009). However the thiol-ene reaction was completed via an ionic mechanism (Michael addition).  
126 Recent work from the same group employed thiol-ene photochemistry and esterification with 4-  
127 pentenoyl chloride to prepare MPEG-PES copolymers (Fury et al., 2013). Up to generation 3.5  
128 PES dendron was constructed to the hydroxyl end of MPEG chain. In the present study we  
129 explored the core-crosslinking and stability study of MPEG-PES copolymer micelles and  
130 investigated their applications for drug solubilisation and sustained release. This work reported  
131 the synthesis and characterisation of linear-dendritic MPEG-PES block copolymer via stepwise  
132 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.

137

## 138 **2. Experimental**

139

### 140 *2.1. Materials*

141

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.

150

### 151 *2.2. Synthesis and characterisation of MPEG-poly(ester-sulfide) copolymers*

152

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<sub>X</sub> (X means dendron generation) for simplicity. Half generation dendrons are



156 terminated with ethenyl groups while full generation dendrons have hydroxyl surface groups.  
157 Thiol-ene photochemical reactions were performed by UV cross-linker, UVItec Ltd (365 nm,  
158 99.99 J cm<sup>-2</sup>). The copolymers were characterised by <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy (Bruker  
159 Avance 400, Bruker, Coventry, UK).

160

161       Synthesis of half generation MPEG-DEN (acrylation)

162

163       1 mmol of MPEG (or MPEG-DEN-OH<sub>x</sub>) and excess acryloyl chloride (molar ratio of  
164 carbonyl chloride : hydroxyl = 1.2:1) was dissolved in 100 ml anhydrous chloroform at 25 °C.  
165 Triethylene amine (equal moles to acryloyl chloride) in 5 ml chloroform was added drop-wise in  
166 the MPEG solution and stirred at 30 °C for 48 h. The mixture was dried under vacuum,  
167 redissolved in 25 ml chloroform, and then suspended in 500 ml diethyl ether. The precipitant was  
168 filtered and dried under vacuum. The crude product was dissolved in chloroform and purified by  
169 Sephadex LH-20 column (methanol:chloroform 60:40). Product with a yield of over 70 % was  
170 recovered after evaporating the solvent.

171

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

173

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

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

180

181 NMR Characterisation:

182 MPEG:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 3.38 (s,  $\text{CH}_3\text{O}$ -), 3.45–3.95 (m,  $-\text{OCH}_2\text{CH}_2\text{O}$ -, backbone).  $^{13}\text{C}$

183 NMR ( $\text{CDCl}_3$ ): 58.72 ( $\text{CH}_3\text{O}$ -), 61.29 ( $-\text{CH}_2\text{CH}_2\text{OH}$ ), 70.48 ( $-\text{OCH}_2\text{CH}_2\text{O}$ -), 71.62

184 ( $\text{CH}_3\text{OCH}_2$ -), 72.27 ( $-\text{CH}_2\text{CH}_2\text{OH}$ ).

185

186 MPEG-DEN-ene (G0.5, MPEG-acrylate):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 3.38 (s,  $\text{CH}_3\text{O}$ -), 3.45–3.95

187 (m,  $-\text{OCH}_2\text{CH}_2\text{O}$ -), 4.32 (t,  $-\text{CH}_2\text{OCO}$ -), 5.83, 5.86 (dd,  $-\text{CH}=\text{CH}_2$  cis), 6.12–6.19 (q,  $-\text{CH}=\text{CH}_2$ ),

188 6.41, 6.45 (dd,  $-\text{CH}=\text{CH}_2$  trans).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 58.98 ( $\text{CH}_3\text{O}$ -), 63.63 ( $-\text{CH}_2\text{CH}_2\text{OCO}$ -),

189 69.05 ( $-\text{CH}_2\text{CH}_2\text{OCO}$ -), 70.50 ( $-\text{OCH}_2\text{CH}_2\text{O}$ -), 71.88 ( $\text{CH}_3\text{OCH}_2$ -), 128.25 ( $-\text{CO}-\text{CH}=\text{CH}_2$ ),

190 130.95 ( $-\text{CO}-\text{CH}=\text{CH}_2$ ), 166.04 ( $-\text{OCO}$ -).

191

192 MPEG-DEN- $\text{OH}_2$  (G1):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 2.55–2.75 (m,  $-\text{OCOCH}_2$ -,  $-\text{SCH}_2\text{CH}(\text{OH})$ -),

193 2.85 (t,  $-\text{CH}_2\text{CH}_2\text{SCH}_2$ -), 3.38 (s,  $\text{CH}_3\text{O}$ -), 3.45–3.95 (m,  $-\text{OCH}_2\text{CH}_2\text{O}$ -,  $-\text{CH}(\text{OH})$ -,  $-\text{CH}_2\text{OH}$ ),

194 4.25 (t,  $-\text{CH}_2\text{OCO}$ -).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 27.40 ( $-\text{COCH}_2\text{CH}_2\text{S}$ -), 34.76 ( $-\text{COCH}_2\text{CH}_2\text{S}$ -),

195 35.53 ( $-\text{SCH}_2\text{CH}$ ), 59.00 ( $\text{CH}_3\text{O}$ -), 63.81 ( $-\text{CH}_2\text{CH}_2\text{OCO}$ -), 65.27 ( $-\text{CH}_2\text{OH}$ ), 69.02 ( $-\text{CH}_2\text{CH}_2\text{OCO}$ -),

196 70.50 ( $-\text{OCH}_2\text{CH}_2\text{O}$ -,  $-\text{CH}(\text{OH})$ -), 71.88 ( $\text{CH}_3\text{OCH}_2$ -), 171.84 ( $-\text{OCO}$ -).

197

198 MPEG-DEN-ene<sub>2</sub> (G1.5):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 2.60–2.90 (m,  $-\text{OCOCH}_2$ -,  $-\text{SCH}_2\text{CH}(\text{OH})$ -),

199  $-\text{CH}_2\text{CH}_2\text{SCH}_2$ -), 3.38 (s,  $\text{CH}_3\text{O}$ -), 3.45–3.95 (m,  $-\text{OCH}_2\text{CH}_2\text{O}$ -), 4.10–4.50 (m,  $-\text{CH}_2\text{OCO}$ -),

200 5.10–5.40 (m,  $>\text{CHOCO}$ -), 5.83–5.90 (m,  $-\text{CH}=\text{CH}_2$  cis), 6.05–6.19 (m,  $-\text{CH}=\text{CH}_2$ ), 6.35–6.50

201 (m,  $-\text{CH}=\text{CH}_2$  trans).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 27.47 ( $-\text{COCH}_2\text{CH}_2\text{S}-$ ), 32.23 ( $-\text{SCH}_2\text{CH}<$ ), 34.58 ( $-\text{COCH}_2\text{CH}_2\text{S}-$ ), 58.95 ( $\text{CH}_3\text{O}-$ ), 63.64 ( $>\text{CHCH}_2\text{OCOCH}=\text{}$ ), 63.79 ( $-\text{CH}_2\text{CH}_2\text{OCO}-$ ), 68.97 ( $-\text{CH}_2\text{CH}_2\text{OCO}-$ ), 70.50 ( $-\text{OCH}_2\text{CH}_2\text{O}-$ ), 71.88 ( $\text{CH}_3\text{OCH}_2-$ ), 127.79, 127.91 ( $-\text{CO}-\text{CH}=\text{CH}_2$ ), 131.45, 131.66 ( $-\text{CO}-\text{CH}=\text{CH}_2$ ), 165.16, 165.47 ( $-\text{OCOCH}=\text{CH}_2$ ), 171.48 ( $-\text{OCOCH}_2-$ ).

205

206 MPEG-DEN-OH<sub>4</sub> (G2):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 2.50–2.95 (m,  $-\text{OCOCH}_2-$ ,  $-\text{SCH}_2\text{CH}<$ ,  $-\text{CH}_2\text{CH}_2\text{SCH}_2-$ ), 3.38 (s,  $\text{CH}_3\text{O}-$ ), 3.40–3.95 (m,  $-\text{OCH}_2\text{CH}_2\text{O}-$ ,  $-\text{CH}(\text{OH})-$ ,  $-\text{CH}_2\text{OH}$ ), 4.10–4.50 (m,  $-\text{CH}_2\text{OCO}-$ ), 5.10–5.40 (m,  $>\text{CHOCO}-$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 27.38 ( $-\text{COCH}_2\text{CH}_2\text{S}-$ ), 32.13 ( $-\text{SCH}_2\text{CH}(\text{O})\text{CH}_2-$ ), 34.54 ( $-\text{COCH}_2\text{CH}_2\text{S}-$ ), 35.42 ( $-\text{SCH}_2\text{CH}(\text{OH})\text{CH}_2-$ ), 58.96 ( $\text{CH}_3\text{O}-$ ), 63.82 ( $>\text{CHCH}_2\text{OCO}-$ ,  $-\text{CH}_2\text{CH}_2\text{OCO}-$ ), 65.20 ( $-\text{CH}_2\text{OH}$ ), 68.94 ( $-\text{CH}_2\text{CH}_2\text{OCO}-$ ), 70.50 ( $-\text{OCH}_2\text{CH}_2\text{O}-$ ,  $-\text{CH}(\text{OH})-$ ), 71.84 ( $\text{CH}_3\text{OCH}_2-$ ), 171.17, 171.40, 171.62 ( $-\text{OCOCH}_2-$ ).

212

213 MPEG-DEN-ene<sub>4</sub> (G2.5):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 2.50–3.00 (m,  $-\text{OCOCH}_2-$ ,  $-\text{SCH}_2\text{CH}<$ ,  $-\text{CH}_2\text{CH}_2\text{SCH}_2-$ ), 3.38 (s,  $\text{CH}_3\text{O}-$ ), 3.45–3.95 (m,  $-\text{OCH}_2\text{CH}_2\text{O}-$ ), 4.10–4.50 (m,  $-\text{CH}_2\text{OCO}-$ ), 5.10–5.40 (m,  $>\text{CHOCO}-$ ), 5.83–5.90 (m,  $-\text{CH}=\text{CH}_2$  cis), 6.05–6.19 (m,  $-\text{CH}=\text{CH}_2$ ), 6.35–6.50 (m,  $-\text{CH}=\text{CH}_2$  trans).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 27.40 ( $-\text{COCH}_2\text{CH}_2\text{S}-$ ), 32.18 ( $-\text{SCH}_2\text{CH}(\text{O})\text{CH}_2-$ ), 34.56 ( $-\text{COCH}_2\text{CH}_2\text{S}-$ ), 58.98 ( $\text{CH}_3\text{O}-$ ), 63.65 ( $>\text{CHCH}_2\text{OCOCH}=\text{}$ ), 63.81 ( $>\text{CHCH}_2\text{OCO}-$ ,  $-\text{CH}_2\text{CH}_2\text{OCO}-$ ), 68.98 ( $-\text{CH}_2\text{CH}_2\text{OCO}-$ ), 70.50 ( $-\text{OCH}_2\text{CH}_2\text{O}-$ ), 71.88 ( $\text{CH}_3\text{OCH}_2-$ ), 127.78, 127.90 ( $-\text{CO}-\text{CH}=\text{CH}_2$ ), 131.52, 131.74 ( $-\text{CO}-\text{CH}=\text{CH}_2$ ), 165.21, 165.52 ( $-\text{OCOCH}=\text{CH}_2$ ), 170.83, 171.10, 171.53 ( $-\text{OCOCH}_2-$ ).

221

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

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

226

### 227 2.3. CMC measurement

228

229 The critical micelle concentrations (CMC) of the MPEG-poly(ester-sulfide) copolymers at  
230  $20\text{ }^\circ\text{C}$  were measured by surface tension measurement using the pendant drop method. An  
231 FTA1000 video system (First Ten Ångstroms Inc) was used to visualise drops formed on the tip  
232 of a 20-gauge stainless-steel needle (aperture 22, brightness and contrast 50%). The tip width of  
233 the needle was measured to perform a calibration of the video camera's magnification. Surface  
234 tension of aqueous polymer solution ranging in concentration from 0.0001 to 2 % w/v was  
235 calculated via drop-shape analysis; measurements were repeated ten times and the results  
236 averaged. The standard deviation of the drop-shape analysis was approximately  $\pm 0.5\text{ mN m}^{-1}$   
237 and the measurement error was less than 5%.

238

### 239 2.4. *Preparation and stability study of core-crosslinked micelles*

240

241 1g (0.175 mmol) of MPEG-DEN-G2.5 was dissolved in 25 mL of distilled water. 62 mg  
242 (0.4 mmol) of DTT and 4.5 mg (0.02 mmol) of Irgacure 2959 were added in the solution and  
243 stirred at RT for 2 h. The solution was irradiated under UV (365nm) for 1 h. The completion of  
244 crosslinking was assessed by  $^1\text{H}$  NMR. The solution was dialysed against distilled water  
245 (MWCO 3500) overnight and then lyophilized to recover the core-crosslinked micelles.

246 The crosslinking was investigated by HPLC, using an Agilent 1100 Series HPLC system  
247 equipped with a Luna 5  $\mu\text{m}$ , C18 column (250 mm x 4.6 mm) (Phenomenex, Cheshire, UK) at  
248 40 °C. The mobile phase was MeOH:TFA (0.05% w/v) (80:20), with a flow rate of 1.0 ml min<sup>-1</sup>,  
249 and Corona Plus CAD detection (ESA) with gas pressure of 35 psi.

250 1% w/v solutions of the crosslinked and non-crosslinked copolymers were prepared in  
251 phosphate buffer (0.067M, pH 7.4) and hydrochloric acid buffer (0.085M, pH 1.2) and incubated  
252 at 37 °C for 48 and 24 h respectively. The solutions were diluted 5 times with 80 % methanol  
253 and analysed by the HPLC assay described above.

254

#### 255 2.5. *Micellar size*

256

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

262

#### 263 2.6. *Drug solubilisation*

264

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

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

274

### 275 2.7. *Drug release study*

276

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

285

## 286 3. **Results and discussion**

287

### 288 3.1. *Synthesis and Characterisation of MPEG- poly(ester-sulfide) copolymers*

289

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

297 The complete conversion of dendritic surface function at each reaction step was confirmed  
298 by  $^1\text{H}$  and  $^{13}\text{C}$  NMR, which indicates the successful formation of the dendritic structure.  
299 Representative  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of MPEG-DEN copolymers (full generation G2 and half  
300 generation G2.5) are shown in Fig. 1. Acrylation of MPEG results in the peaks of two methylene  
301 groups next to the hydroxyl end to shift from 72.3 ppm to 69.1 ppm and from 61.3 ppm to 63.6  
302 ppm in  $^{13}\text{C}$  NMR spectrum, respectively. Three peaks from the acrylic group appear at 128 and  
303 131 ppm for ethenyl group and 166 ppm for carbonyl group. The acrylic group undergoes free  
304 radical addition with thiol group in thiol-ene reaction to form hydroxyl-functioned dendrons.  
305 Then the two ethenyl peaks downshifted to 27.4 and 34.7 ppm while the carbonyl peak upshifted  
306 to 171.8 ppm. Two new peaks located at 35.5 and 65.3 ppm are from the methylene groups of  
307 thioglycerol. The CH peak of thioglycerol is overlapped with the big peak of MPEG backbone.  
308 Further acrylation of hydroxyl-terminated dendrons results in the two methylenes of thioglycerol  
309 to downshift from 35.5 and 65.3 to 32.2 and 63.6 ppm in  $^{13}\text{C}$  NMR, respectively. The acrylic  
310 groups showed multiple peaks at 128, 131 and 165 ppm due to the slightly different chemical  
311 environment when attaching on primary or secondary hydroxyl groups. In  $^1\text{H}$  NMR, a group of  
312 well-separated peaks are shown between 5.8 and 6.5 ppm after acrylation, which are from the

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

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

321

### 322 3.2. *Critical Micelle Concentration*

323

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

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



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

342

### 343 3.3. *Preparation of core-crosslinked micelles*

344

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

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

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

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

382

383 *3.4. Stability of core-crosslinked micelles*

384

385 The hydrolysis of the crosslinked and non-crosslinked micelles of MPEG-DEN-G2.5  
386 copolymer was investigated in acidic and neutral solutions. As shown in Fig. 6, both the  
387 crosslinked and non-crosslinked micelles have good stability at pH 7.4. No degradation was  
388 detected for the crosslinked micelles after 2 days. The peaks of the non-crosslinked micelles  
389 were reduced slightly due to the dynamic equilibrium of micellisation and relatively direct  
390 exposure of the molecules to the solvent. The MPEG-DEN copolymers contain ester bonds and  
391 thus more readily hydrolyse in acidic solutions. The dendritic branches could be fully or partially  
392 cleaved from the MPEG chains, which results in an increase of the polarity and a decrease of the  
393 retention time. Fig. 7 showed that both micelles demonstrated more apparent changes after  
394 incubation at pH 1.2 for 24 h. The peak at 2.1 min is from the chloride ions in the buffer. The  
395 non-crosslinked micelles showed clear sign of degradation over the elution range. The peaks of  
396 the more hydrophobic fractions were greatly reduced and the copolymers were eluted faster due  
397 to the increased hydrophilicity. The crosslinked micelles also showed a similar tendency. The  
398 peaks became more narrow and the retention times were slightly decreased. Although the  
399 evidence of hydrolysis was found by the CAD technique, the quantitative determination of the  
400 degradation of the copolymers is not achievable due to lack of calibration standards. As CAD  
401 detection is mass-dependent not concentration-dependent, different compounds could be eluted  
402 at the same time and produce very close responsive signals in the detector. However the CAD  
403 cannot provide any spectral information to identify the compounds. The results of the stability  
404 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

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

441 Table 1 shows the solubility of griseofulvin in the buffer solutions of the crosslinked and  
442 non-crosslinked MPEG-DEN micelles at 37°C. An approx. 10-fold increase of the solubility was  
443 found for both copolymer solutions at 1% w/v. The solubility of griseofulvin in phosphate buffer  
444 ( $0.01 \text{ mg ml}^{-1}$ ) was deducted for calculation of the solubilisation capacity ( $S_{cp}$ , expressed as  
445 milligram drug per gram of copolymer). The crosslinked micelles show relatively higher  
446 solubilisation capacity than the non-crosslinked ones under equivalent conditions. The micelle  
447 size measurement indicates that both micelles have very similar hydrodynamic radii and  
448 crosslinking has no impact on the size of micelle cores. It is known that micellisation is a  
449 thermodynamic equilibrium and thus the drug molecules encapsulated are possible to diffuse out  
450 of the micelle cores. However the escape of drug molecules is considered to be hindered by core-

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

461

### 462 3.7. *Drug release profiles*

463 The stability study indicates that the MPEG-DEN copolymers have good stability under  
464 neutral conditions. Hence the main mechanism of drug release at pH 7.4 is attributed to the  
465 diffusion of molecules out of the micelles. Fig. 9 shows the release profiles of griseofulvin from  
466 1% w/v buffer solution (pH 7.4) of crosslinked and non-crosslinked MPEG-DEN-G2.5  
467 copolymers at 37 °C. The sink condition was ensured by refreshing phosphate buffer during  
468 dialysis. A rapid release with approximately 36% and 57% drug within the initial 6 h was seen  
469 for crosslinked and non-crosslinked copolymer solutions, respectively. The release curves  
470 showed that approximately 60% and 85% griseofulvin was released from the copolymer  
471 solutions after 24 h of dialysis where a plateau was reached. Drug release from the crosslinked  
472 micelles was significantly ( $p < 0.05$ ) more sustained than from the non-crosslinked micelles,  
473 attributable to the crosslinking structure within the micelle cores which may hinder the diffusion

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

480

#### 481 **4. Conclusions**

482

483 Poly(ester-sulfide) dendrons were successfully constructed on the chain ends of MPEG via  
484 a combination of acrylation and thiol-ene photochemical reaction. The surface functionality of  
485 dendrons was found to have an impact on the physicochemical properties of the linear-dendritic  
486 copolymers, especially their micellisation behavior in aqueous solutions. The half generation  
487 MPEG-DEN copolymers with ethenyl groups are able to self-associate to form micelles with a  
488 hydrophobic hyperbranched core and MPEG corona. Crosslinking within the micelle cores can  
489 be initiated by UV radiation via thiol-ene reaction with dithiol linkers, which can reinforce the  
490 micelle structure and enhance their stability. The copolymer micelles show good stability under  
491 neutral conditions but readily hydrolyse in acidic solutions due to the cleavage of ester bonds in  
492 the dendrons. The micelles of the copolymers demonstrated the capability to increase the  
493 solubility of poorly water-soluble drugs by incorporation of the drug in the hydrophobic cores.  
494 The release profiles from the copolymer solutions indicated that crosslinked micelles showed  
495 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.

- 540 Killops, K.L., Campos, L.M., Hawker, C.J., 2008. Robust, efficient, and orthogonal synthesis of  
541 dendrimers via thiol-ene “click” chemistry. *J. Am. Chem. Soc.* 130, 5062–5064.
- 542 Kim, J.S., Youk, J.H., 2009. Preparation of core cross-linked micelles using a photo-cross-  
543 linking agent. *Polymer* 50, 2204–2208.
- 544 Kwon, G.S., 2003. Polymeric micelles for delivery of poorly water-soluble compounds. *Crit.*  
545 *Rev. Ther. Drug Carrier Syst.* 20, 357–403.
- 546 Li, Y., Shen, Y., Wang, S., Zhu, D., Du, B., Jiang, J., 2015. Disulfide cross-linked cholic-acid  
547 modified PEG–poly(amino acid) block copolymer micelles for controlled drug delivery of  
548 doxorubicin. *RSC Adv.* 5, 30380–30388.
- 549 Li, Y., Xiao, K., Luo, J., Xiao, W., Lee, J.S., Gonik, A.M., Kato, J., Dong, T.A., Lam, K.S., 2011.  
550 Well-defined, reversible disulfide cross-linked micelles for on-demand paclitaxel delivery.  
551 *Biomaterials* 32, 6633–6645.
- 552 Liu, S., Weaver, J.V.M., Save, M., Armes, S.P., 2002. Synthesis of pH-responsive shell cross-  
553 linked micelles and their use as nanoreactors for the preparation of gold nanoparticles.  
554 *Langmuir* 18, 8350–8357.
- 555 Van Nostrum, C.F., 2011. Covalently cross-linked amphiphilic block copolymer micelles. *Soft*  
556 *Matter* 7, 3246–3259.
- 557 O’Reilly, R.K., Hawker, C.J., Wooley, K.L., 2006. Cross-linked block copolymer micelles:  
558 functional nanostructures of great potential and versatility. *Chem. Soc. Rev.* 35, 1068–1083.
- 559 Quan, C., Wei, H., Shi, Y., Li, Z., Cheng, S., Zhang, X., Zhuo, R., 2011. Fabrication of  
560 multifunctional shell cross-linked micelles for targeting drug release. *Colloid Polym Sci.*  
561 289, 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.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of MPEG-DEN-G2.5 (1) and MPEG-DEN-G2 (2). The peaks at  
600 52 and 89 ppm in  $^{13}\text{C}$  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 ( $\text{g dm}^{-3}$ ) 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 <sup>-1</sup>	Scp /mg g <sup>-1</sup> 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

629 a. Measurement uncertainty  $\pm 1 \text{ mg g}^{-1}$ .

630

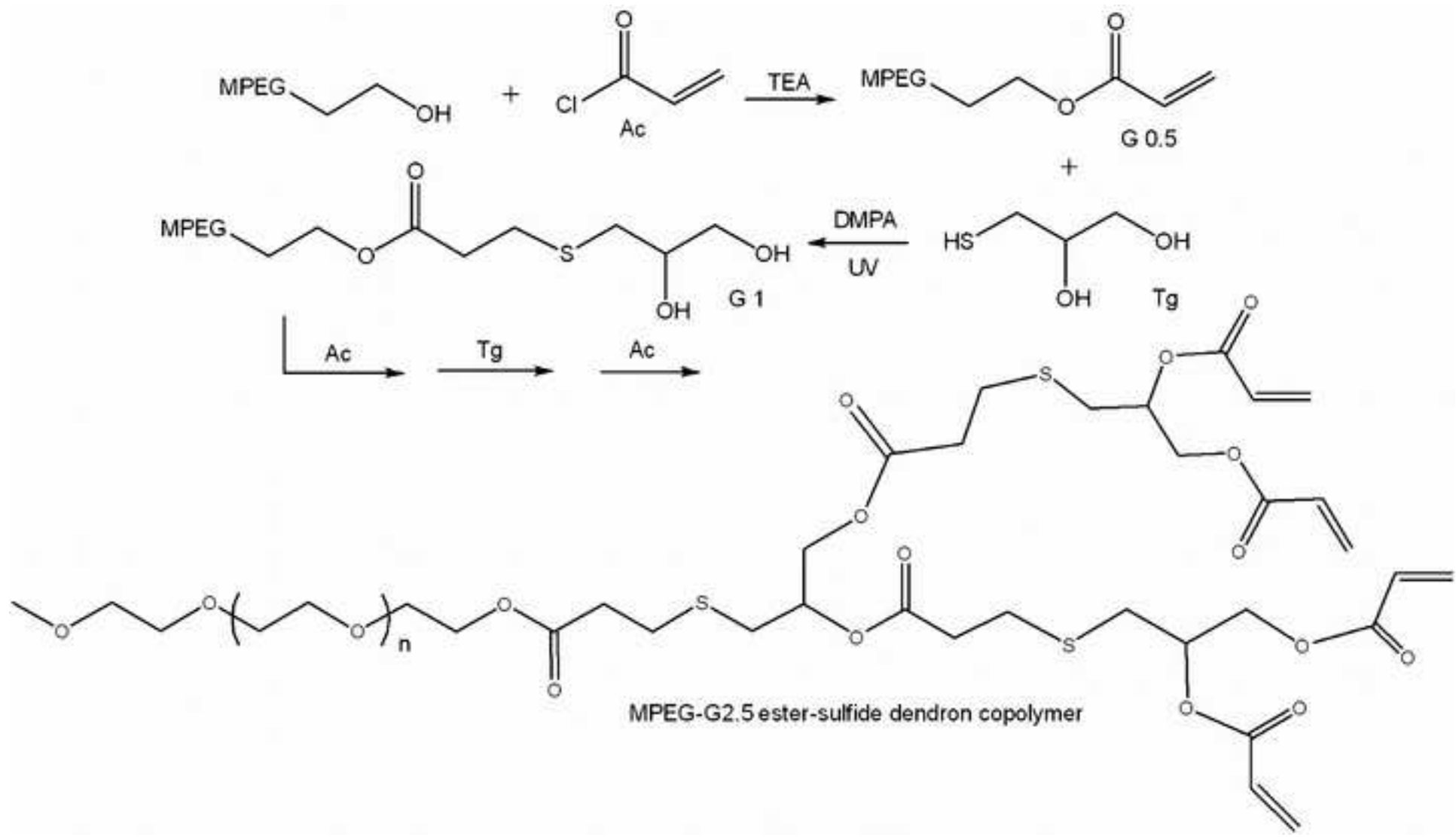


Figure 1

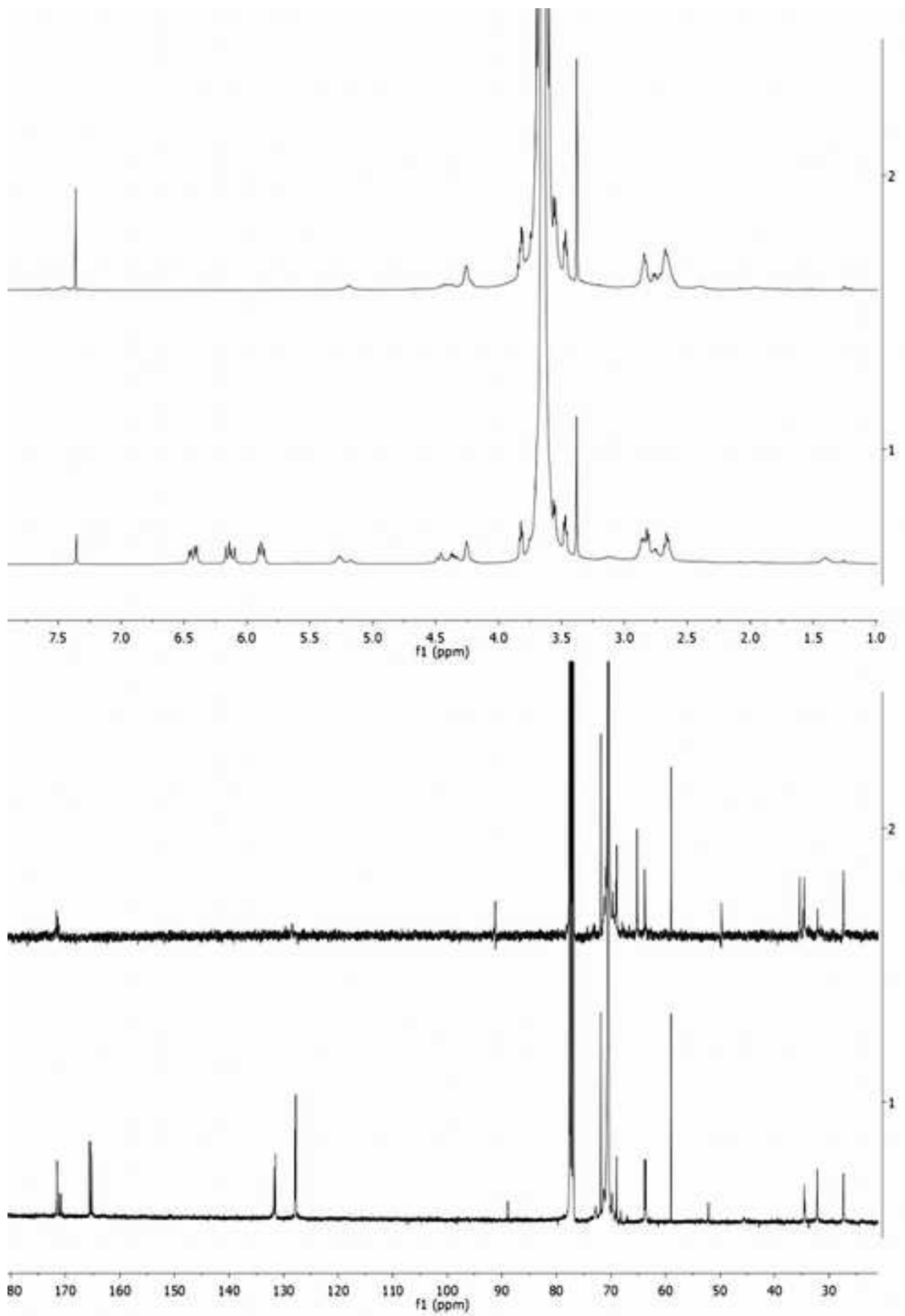




Figure 2

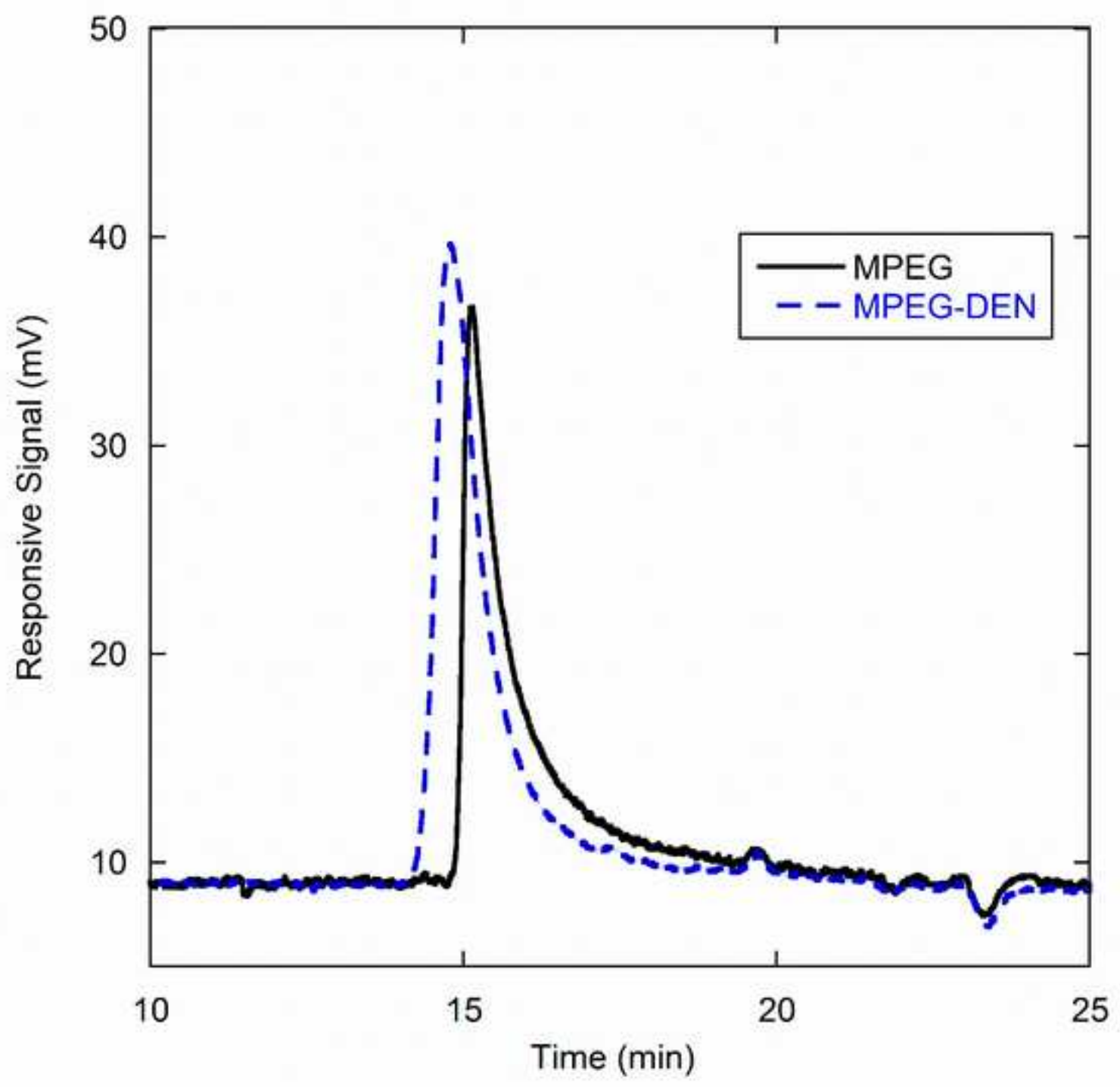


Figure 3

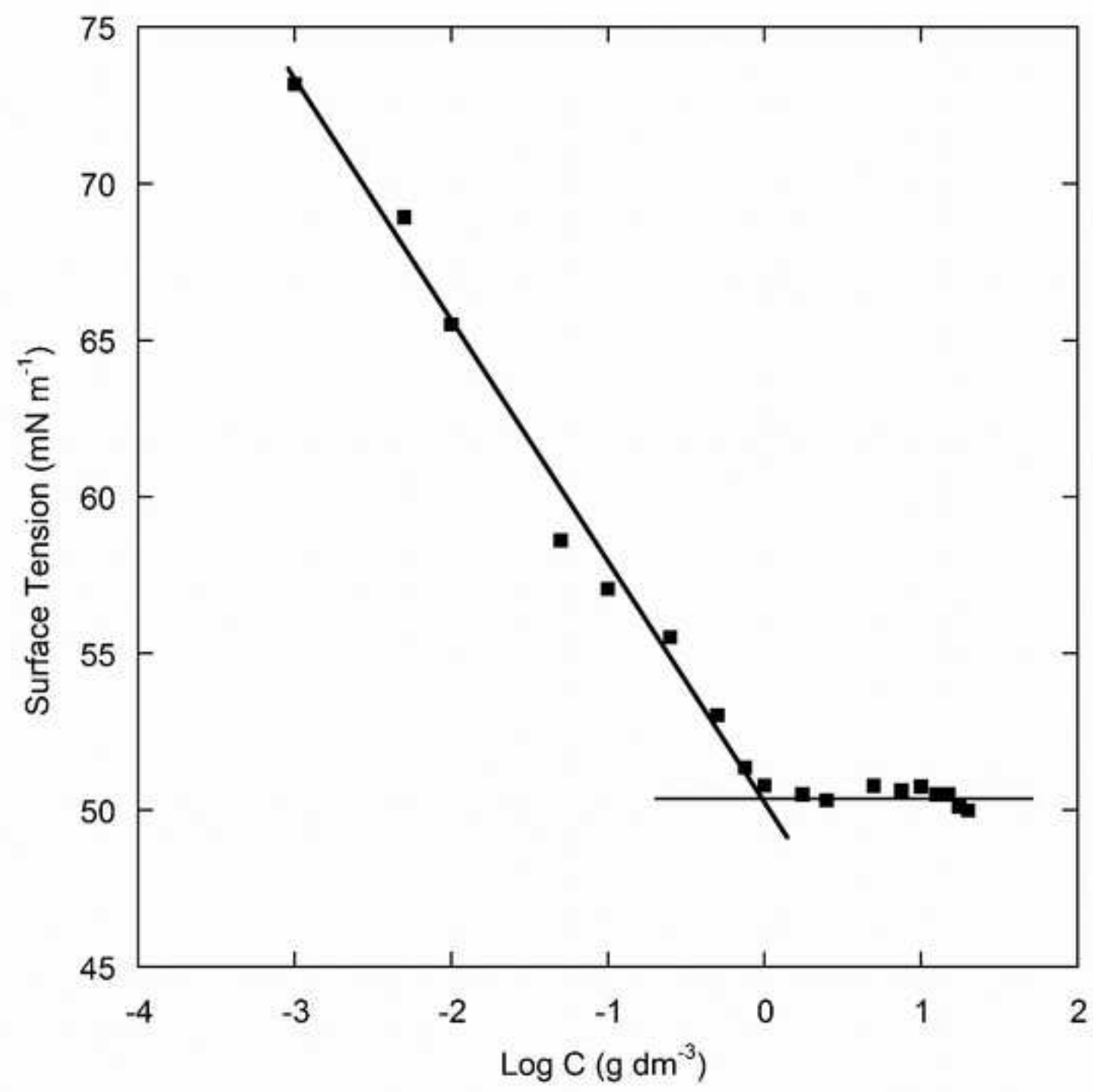


Figure 4

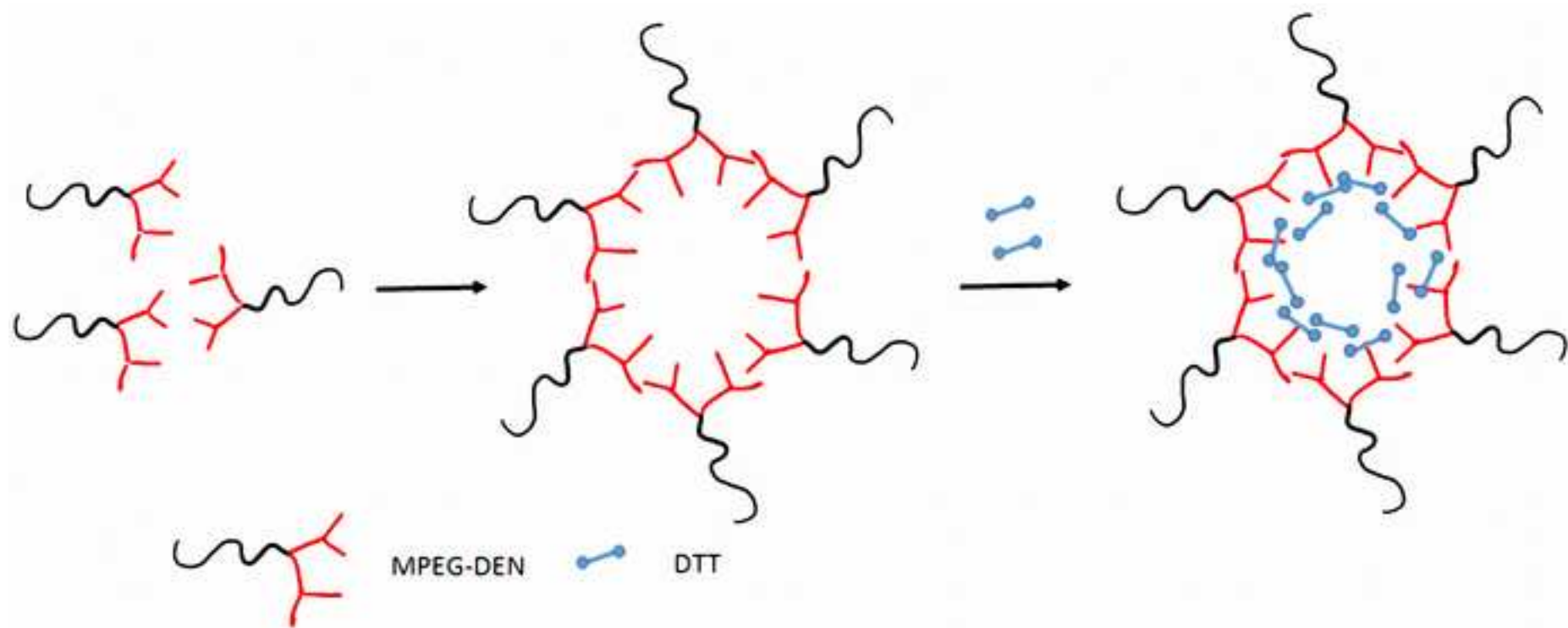


Figure 5

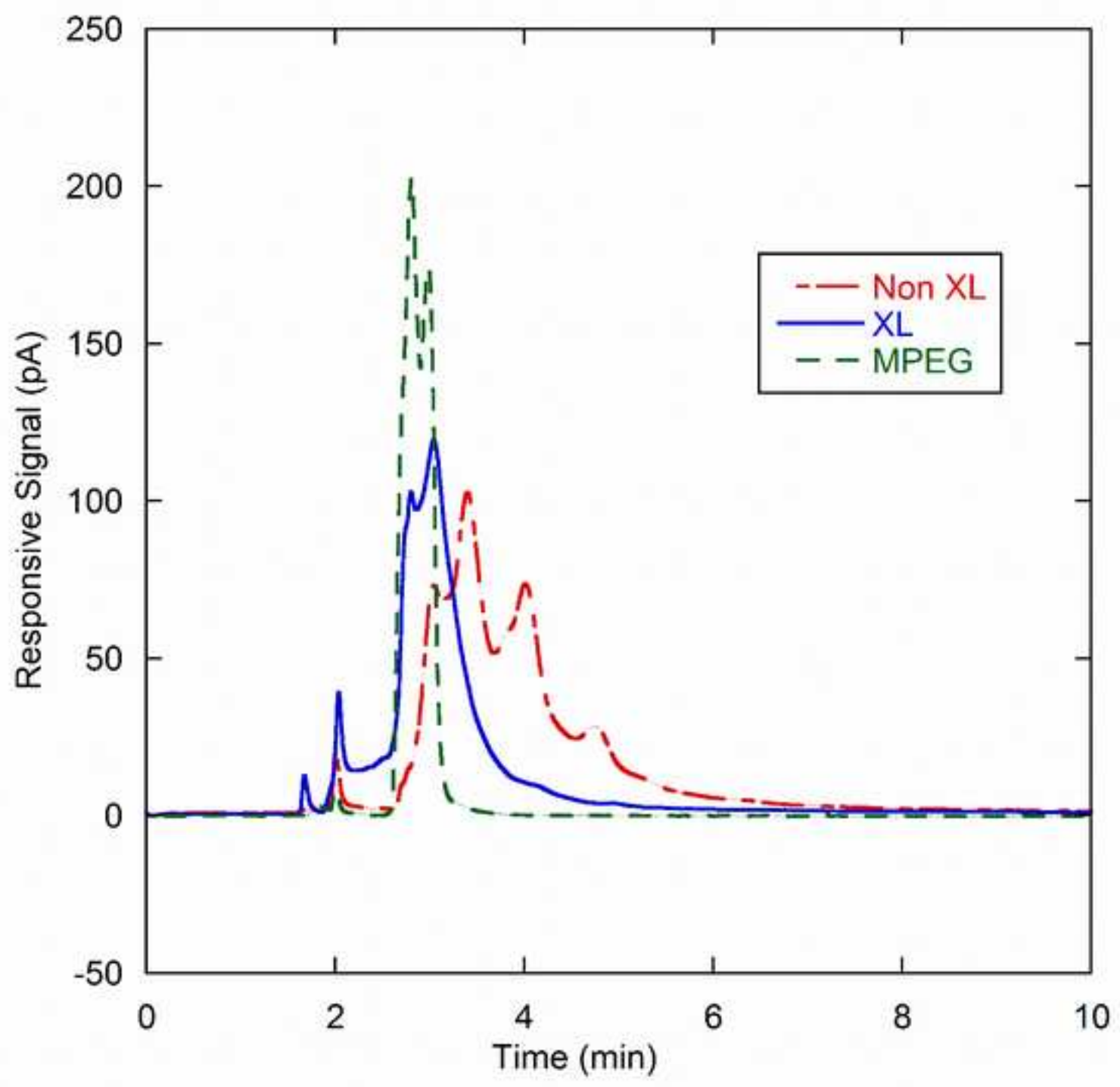


Figure 6

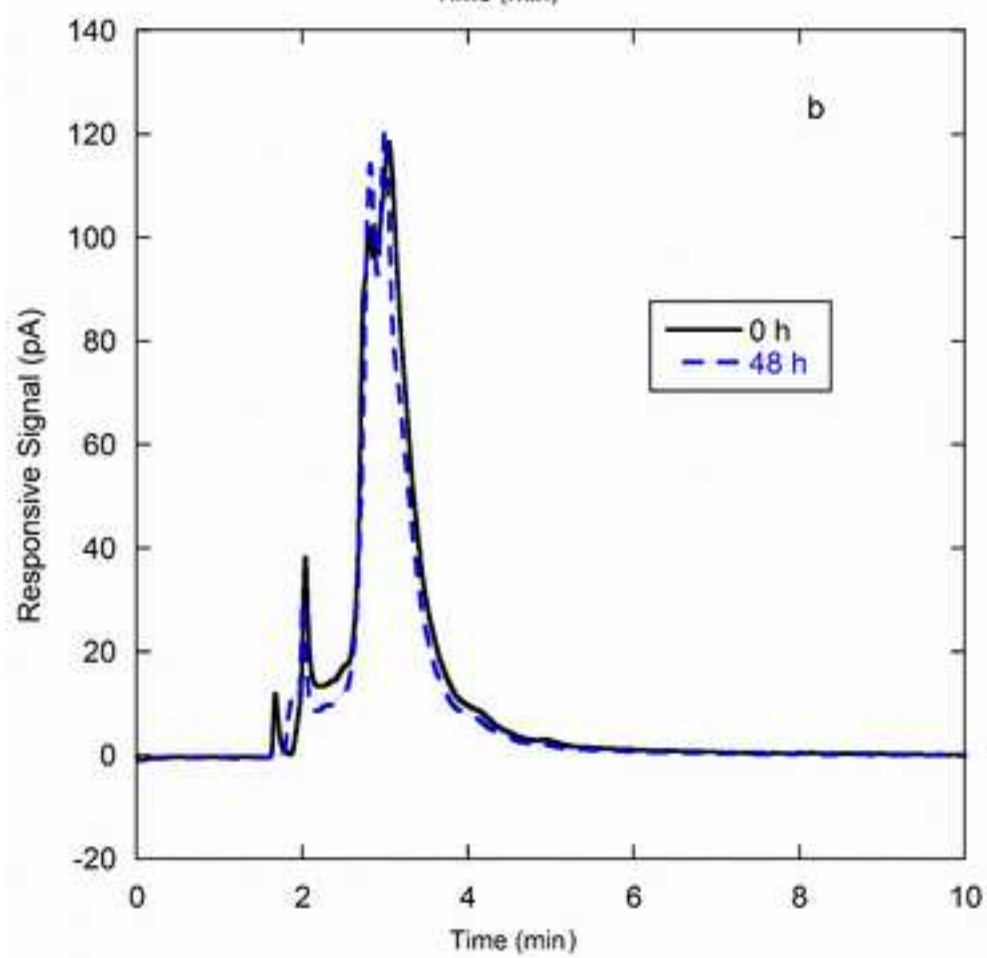
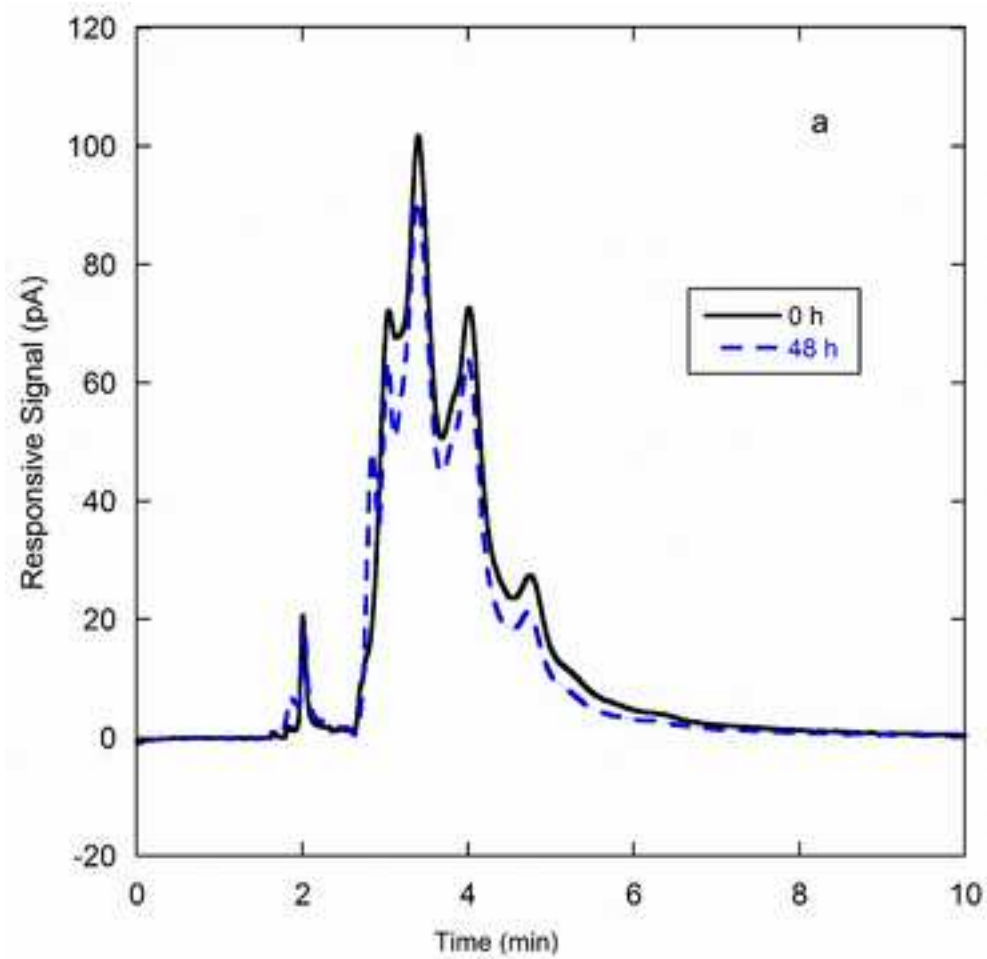


Figure 7

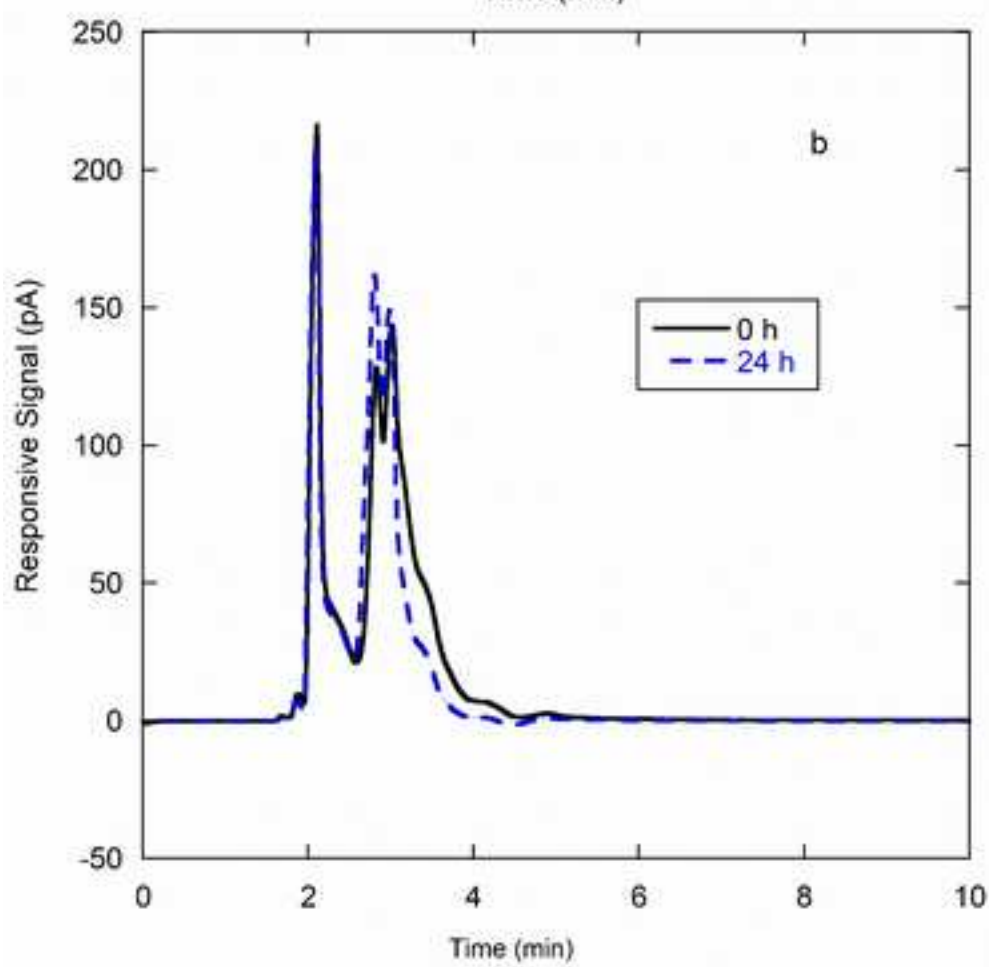
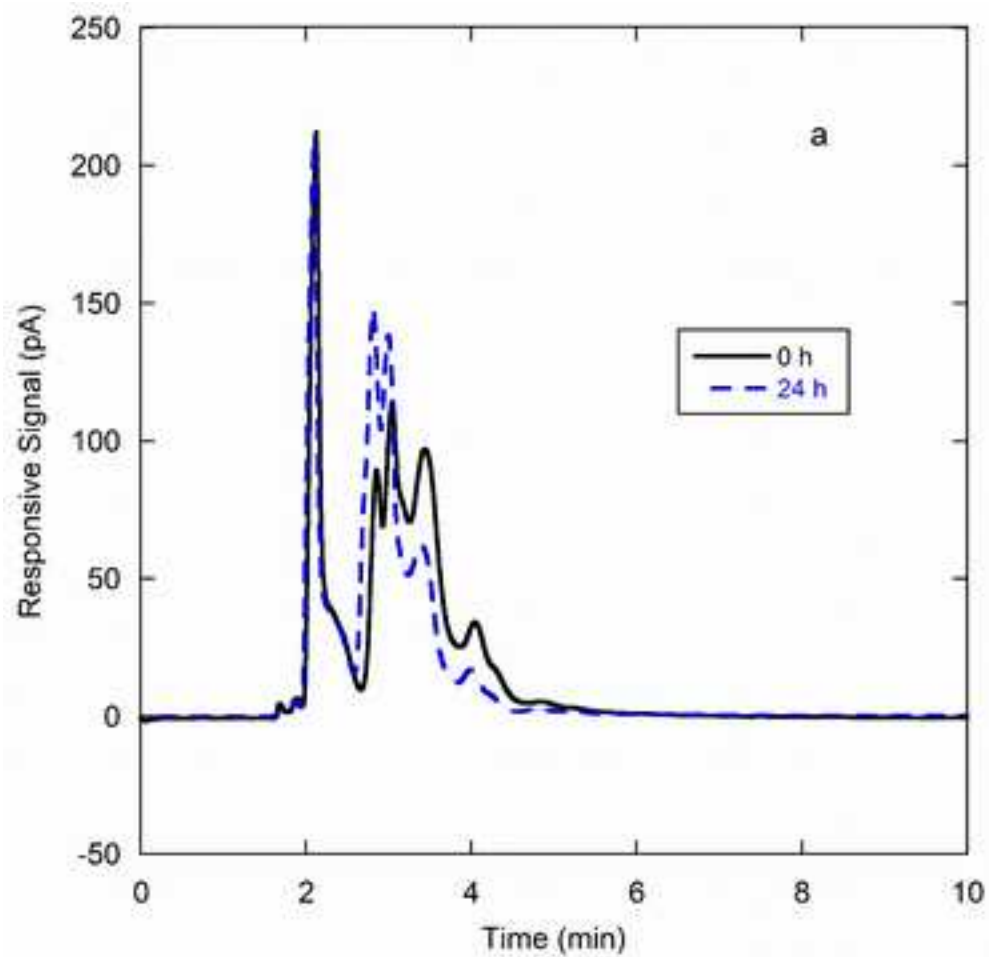


Figure 8

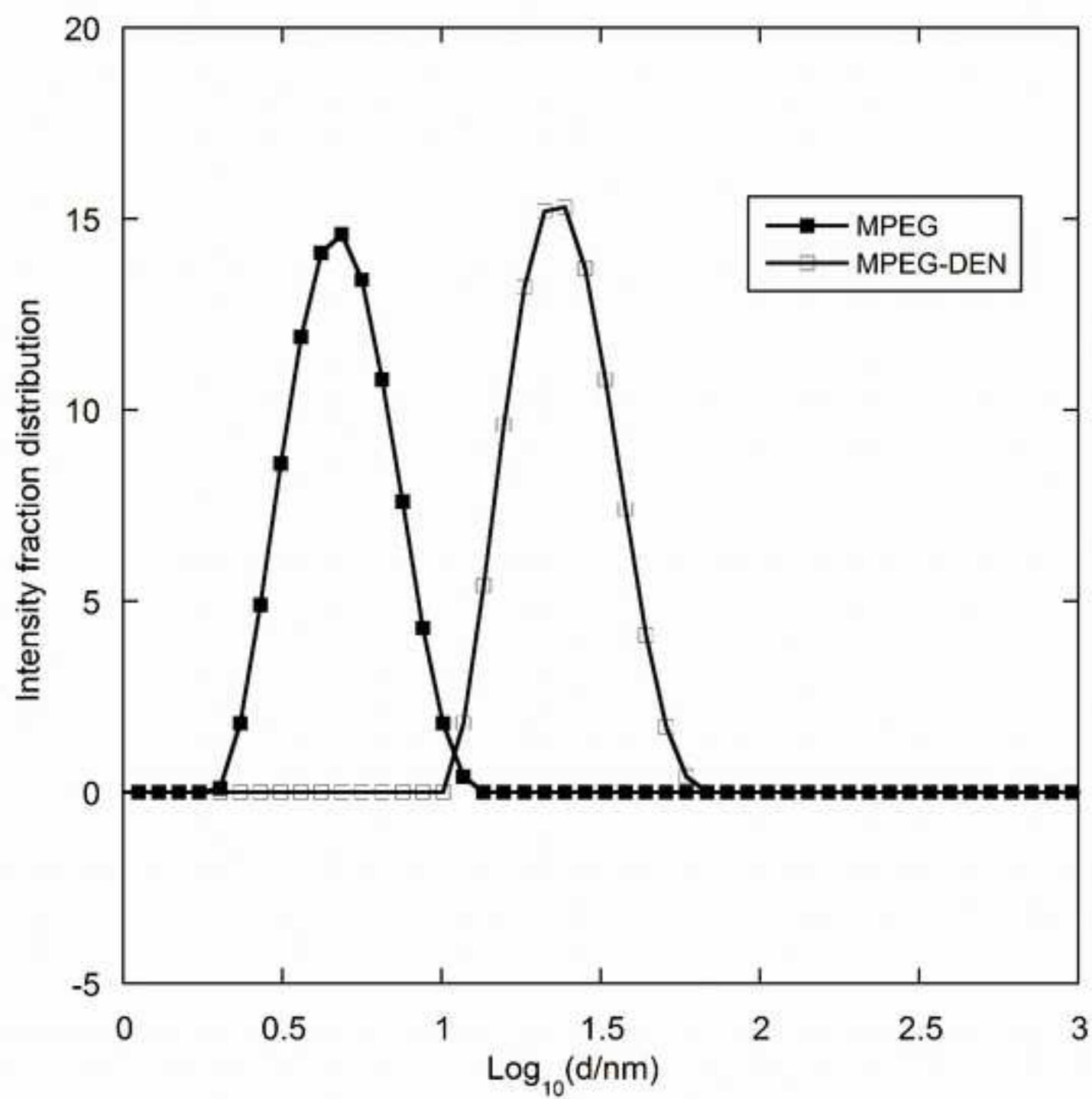


Figure 9

