Homo- and co-polymerisation of di(propylene glycol) methyl ether methacrylate – a new monomer

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Experimental

Materials and Methods

OEGMA300 (95%), diPGOH (mixture of four isomers), methacryloyl chloride (97%), triethylamine (Et$_3$N, reagent grade ≥ 99% and HPLC grade), calcium hydrate (CaH$_2$, ≥ 90%), aluminium oxide activated basic, 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH), deuterated chloroform (CDCl$_3$, 99.8%), methyl trimethylsilyl dimethylketene acetal (MTS, 95%), tetrahydrofuran (THF, HPLC grade, ≥ 99.9%), poly)(methyl methacrylate) (PMMA) standard samples (2, 4, 8, 20, 50, 100 kDa), PBS pellets, and *n*-hexane were purchased from Sigma Aldrich.

OEGMA300, is commercially available, was purified according to Vamvakaki et al.[1] Specifically, a 50 v/v% solution in THF was prepared and it was then purified by passing through column of basic aluminum oxide, followed by addition of calcium hydride, which was left to react by stirring for 2h. The OEGMA300 solution was refrigerated prior to use. The purification of the *in-house* synthesised monomer, diPGMA (mixture of isomers), is discussed in the following section. The initiator, MTS, was distilled prior to use, whole the polymerisation solvent, THF, was purified using a solvent purification equipped with an activated alumina column (Pure Solv^{TM} Micro 100 Liter, Sigma Aldrich). All the glassware was dried in an oven at 140 °C prior to use.

Acros Organics, UK, was the provider of benzoic acid, and tetrabutyl ammonium hydroxide (40% in water), compounds used for the synthesis of the GTP catalyst, tetrabutyl ammonium bibenzoate (TBABB), according to Dicker et al.[2]

Monomer Synthesis

The monomer was synthesised via esterification of the corresponding alcohol, diPGOH, and methacryloyl chloride. The reaction was performed by drop-wise addition of methacryloyl chloride (56.6 mL, 0.58 mol), using a dropping funnel, in a 2-neck 2 L round-bottom flask containing diPGOH (75 mL, 0.48 mol), freshly distilled Et_3N (catalyst, 135 mL, 0.97 mol), and freshly purified THF (solvent, 250 mL) at 0 °C. The salt produced ($Et_3NH^+Cl^-$) was removed by vacuum filtration, followed by passage through column of basic alumina and drying in calcium hydride. The monomer was kept under DPPH, and it was distilled twice (between 70-85°C) before use.

Monomer Characterisation

Gas Chromatography - Flame Ionisation Detection (GC-FID)

Both diPGMA and diPGOH were analysed using GC-FID to investigate the number of isomers and relative content. The analysis was performed using a Shimadzu GC-2014 instrument (Shimadzu Scientific instruments, UK), equipped with a Shimadzu AO20i autosampler, and fitted with a polar Stabilwax-DA column (30 m length, 0.32 mm ID and 0.25 μm film thickness), with hydrogen as the carrier gas, supplied by an external hydrogen generator. The sample volume is 1 μL with a 39-split ratio, and the injection and flame temperatures are 250 °C and 300 °C, respectively. The sample was heated from 60 °C to 240 °C at a rate of 5 °C/min, while holding the temperature for 150 s.

Fourier-Transform Infrared (FT-IR) Spectroscopy

diPGMA and diPGOH were analysed via a Nicolet iS10 FT-IR Spectrometer from ThermoScientific (Fisher Scientific UK Ltd, UK).

Nuclear Magnetic Resonance (NMR) Spectroscopy

diPGMA and diPGOH were analysed via ^1H NMR and ^{13}C NMR using a 400-MHz Avance Bruker NMR spectrometer (Bruker, UK Ltd., UK). The samples were prepared as solutions in deuterated chloroform.

Polymer Synthesis and Recovery

The polymers were synthesised via one-pot GTP. As an example, the synthesis of Polymer 2, OEGMA300$_7$-b-diPGMA$_5$-b-OEGMA300$_7$, is presented. Freshly purified THF (solvent, 28 mL), and freshly distilled MTS (initiator, 0.49 mL, 0.002 mol) were syringed in a sealed 100 mL round-bottom flask, which contained TBABB (catalyst, ~10mg) under an inert argon atmosphere. Subsequently, three additions followed: i) solution of OEGMA300 in THF (9.2mL, 0.02 mol of OEGMA300) via direct filtration using polytetrafluoroethylene (PTFE) hydrophilic (0.45 μm pore size, 25 mm diameter, Fisher Scientific), ii) freshly distilled diPGMA (2.6 mL, 0.01 mol), and iii) same as i). The exotherm was monitored after each addition as follows: i) from room temperature to 33.6 °C, ii) from 28.3 °C to 31.7 °C, and iii) from 27.5 °C to 33.2 °C, respectively. After the formation of each block, two aliquots of 0.1mL each were withdrawn for GPC and NMR analysis. The homopolymer was purified by dialysis in methanol, followed by removal of the solvent using rotary evaporator, while the triblock copolymers were precipitated in n-hexane. In both cases, the polymers were vacuum dried at room temperature for a week.

Polymer Characterisation

Gel Permeation Chromatography (GPC)

The final polymers and any precursors were analysed using an Agilent SECcurity GPC system (Agilent technologies UK Ltd), equipped with: i) a guard column (PL1110-1520, PLgel 5 μm, 50 x 7.5 mm, Agilent technologies UK Ltd), ii) MIXED-D column (PL1110-6504, PLgel 5 μm, 300 x 7.5 mm, Agilent technologies UK Ltd, thermostated at 30°C), ii) an Agilent 1250 refractive index (RI) detector, and iii) a "1260 Iso" isocratic pump. THF was used as the mobile phase, which was pumped at a flow rate of 1 mL min^{-1}. The system was calibrated using linear poly(methyl methacrylate) (PMMA) standard samples of 2, 4, 8, 20, 50 and 100 kg mol^{-1}. The samples were prepared in THF, and they were filtered using PTFE syringe filters (0.45 μm, 13 mm diameter, VWR International).

The experimental MM, resulted by GPC, were compared to the targeted MM by

using the following equation:
$$MM^{theor.}(g\,mol^{-1}) = \left(\sum_i MM_i \times DP_i\right) + 100$$
; where

MM$^{theor.}$, MM$_i$ and DP$_i$ stand for theoretical MM, MM of each monomer, and the corresponding degree of polymerisation, while 100 g mol^{-1} is added to consider the methyl methacrylate group that stays on the polymer chain after the completion of the polymerisation.

Proton Nuclear Magnetic Resonance (^{1}H NMR) Spectroscopy

The NMR analysis of the polymers and their precursors (if any) was performed similarly to the NMR on diPGMA and diPGOH. For the analysis, the peak at 3.35 ppm was used for OEGMA300 (three methoxy protons), and the peak at 4.8 ppm for diPGMA (two methylene protons closest to the ester).

Differential Scanning Calorimetry (DSC) Measurements

DSC measurements were performed on a TA Instruments Discovery 2500 DSC instrument with liquid nitrogen cooling. Samples were prepared in T-Zero pans without pin-hole in masses between 2-10 mg. Samples were run between -120 – 100 °C, and cooled again, then the cycle repeated. Samples were run under a flow of nitrogen gas at a heating rate of 50 °C min^{-1}. Samples were analysed using TA Instruments Trios software. The second heating cycle was used to analyse the samples.

Dynamic Light Scattering (DLS)

1 w/w% solutions of the polymers in DI water were analysed using a Zetasizer Nano ZSP (Malvern Instruments Ltd., UK). During the analysis, the samples were analysed three times at each set condition, and the scattered light was collected at a backscatter angle of 173°. The solutions were subjected to: i) size measurements at 25 °C, prior to which the samples were filtered using Nylon syringe filters (0.45 µm pore size, 25 mm in diameter, Fisher Scientific), and ii) temperature ramp size measurements. The samples were left to equilibrate for 120 s prior to each measurement. The hydrodynamic diameters (d_hs) reported are the mean values which correspond to the maximum of the peak by intensity and by number, and are compared to the theoretical diameters, calculated by assuming formation of spherical micelles and considering the following equation:

$$d\ (nm) = \left(DP_{OEGMA300} + DP_{diPGMA}\right) * 0.254$$; where DP stands for degree of polymerisation, and 0.254 nm is the projected length of a methacrylate unit for a polymer in a fully extended configuration. The CP by DLS was calculated as the temperature of 50% aggregation[3] using the DLS data by intensity.

Ultraviolet-Visible (UV-Vis) Spectroscopy

1 w/w% solutions of the polymers in DI water were analysed using an Agilent Cary UV-Vis Compact Peltier UV-Vis spectrometer, during which, the samples were subjected to temperature ramp measurements with a heating rate of 1 °C min^{-1}. Data collection occurred at 550 nm at temperature intervals of 1 °C. The CP by UV-Vis was calculated as the temperature of 50% transmittance.

Visual Observations

1 w/w% solutions of the polymers in DI water were observed visually for a CP by immersing the vials in a water-bath, and raising the temperature while continuously stirring, by using an IKA RCT stirrer hotplate, equipped with an IKA-ETS-D5 temperature controller. The visual CP was determined as the temperature at which the solution turned to cloudy. Similarly, the solutions in PBS were also observed visually for gelation, defined visually as the point at which the sample does not flow upon tube inversion. The sample is observed at every degree Celsius.

Rheological Measurements

15 w/w% solutions in PBS were subjected to oscillatory temperature ramp measurements using a TA Discovery HR-1 hybrid rheometer (TA Instruments UK), equipped with a 40 mm parallel Peltier steel plate (996921) and a solvent trap. The changes in shear storage modulus, G′, and shear loss modulus, G′′ were recorded as the samples were heated with a rate of 1 °C min^{-1}. The analyses were performed at a strain of 1%, and angular frequency of 1 rad s^{-1}, following previously reported values. 20, 25, and 30 w/w% solutions of Polymer 10, which gelled visually, were also subjected to oscillatory temperature ramp measurements.

Critical Micelle Concertation (CMC) Determination

The CMC of all copolymers was determine using fluorescence spectroscopy measurements using pyrene as the probe. Fluorescence emission spectra were measured using an Agilent Technologies Cary Eclipse Fluorescence spectrometer. The measurements were performed using an emission wavelength of 394 nm. The excitation was recorded between 300 and 360 nm at a scan rate of 600 nm min^{-1}. The Intensities of 333 nm and 338 nm were compared. A stock solution of pyrene in filtered acetone 2.5×10^{-4} M was prepared. Aliquots of the pyrene solution (12 µL) were added to volumetric flasks. The flasks were then dried by filtered compressed air. Solutions of various concentrations, from 1×10^{-7} mmol/L to 3×10^{-1} mmol/L of each polymer were prepared in doubly distilled, filtered (0.45 micron) water under dust-free conditions in these volumetric flasks."

Table S1: FT-IR results for the alcohol precursor, diPGOH, and the *in-house* synthesised monomer, diPGMA: the number of peaks present, the characteristic absorption(s), the functional groups, the type of vibration, and the intensity are listed.

No. Peaks	Characteristic Absorption (cm^{-1})	Functional Group	Type of Vibration	Intensity
Alcohol Precursor - diPG				
1	3433.1	O-H (alcohol)	Stretching	Strong, broad
2	2971.6, 2930.6, 2876.5, 2826.3	C-H (alkane)	Stretching	Strong
3	1452.2	C-H (alkane)	Bending	Medium
4	1373.8	O-H (alcohol)	Bending	Medium
5	1202.5	C-O (alcohol)	Stretching	Strong
6	1092.5	C-O (ether)	Stretching	Strong
7	956.4, 854.3	C-H (alkane)	Bending	Strong
Monomer - diPGMA				
1	2978.0, 2930.4, 2876.4, 2824.2	C-H (alkane)	Stretching	Strong
2	1715.2	C=O (ester)	Stretching	Strong
3	1637.1	C=C (Vinyldene)	Stretching	Strong
4	1451.2	C-H (alkane)	Bending	Medium
5	1293.7	C-O (ester)	Stretching	Strong
6	1165.4	C-O (ester)	Stretching	Strong
7	1103.3	C-O (ether)	Stretching	Strong
8	938.3	C-H (alkane)	Bending	Strong
9	813.7	C=C	Bending	Strong

Table S2: GC-FID results, i.e. number of peaks identified, detection time, and integration of each peak, for diPGOH and diPGMA.

| No. Peaks	diPGOH		diPGMA	
	Time (min)	Area (%)	Time (min)	Area (%)
1	4.21	28.22	11.62	56.92
2	4.30	24.36	11.81	1.06
3	4.53	46.72	12.00	40.56
4	4.66	0.40	12.14	0.76
5	4.70	0.31	12.22	0.70
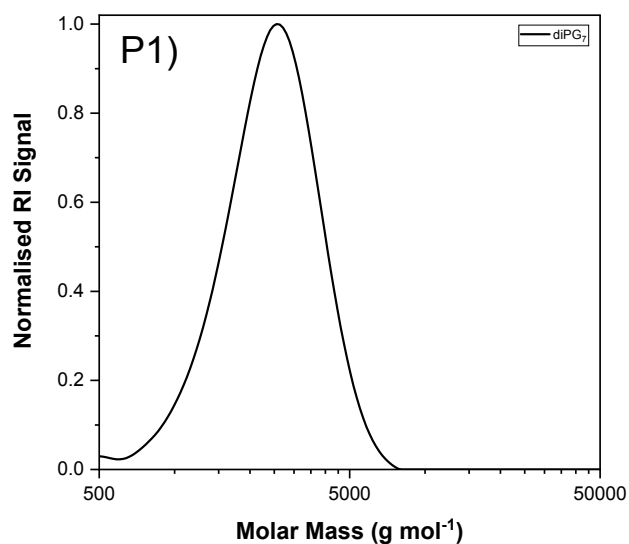
Figure S1: GPC molecular mass distribution of diPGMA homopolymer (P1), in black.
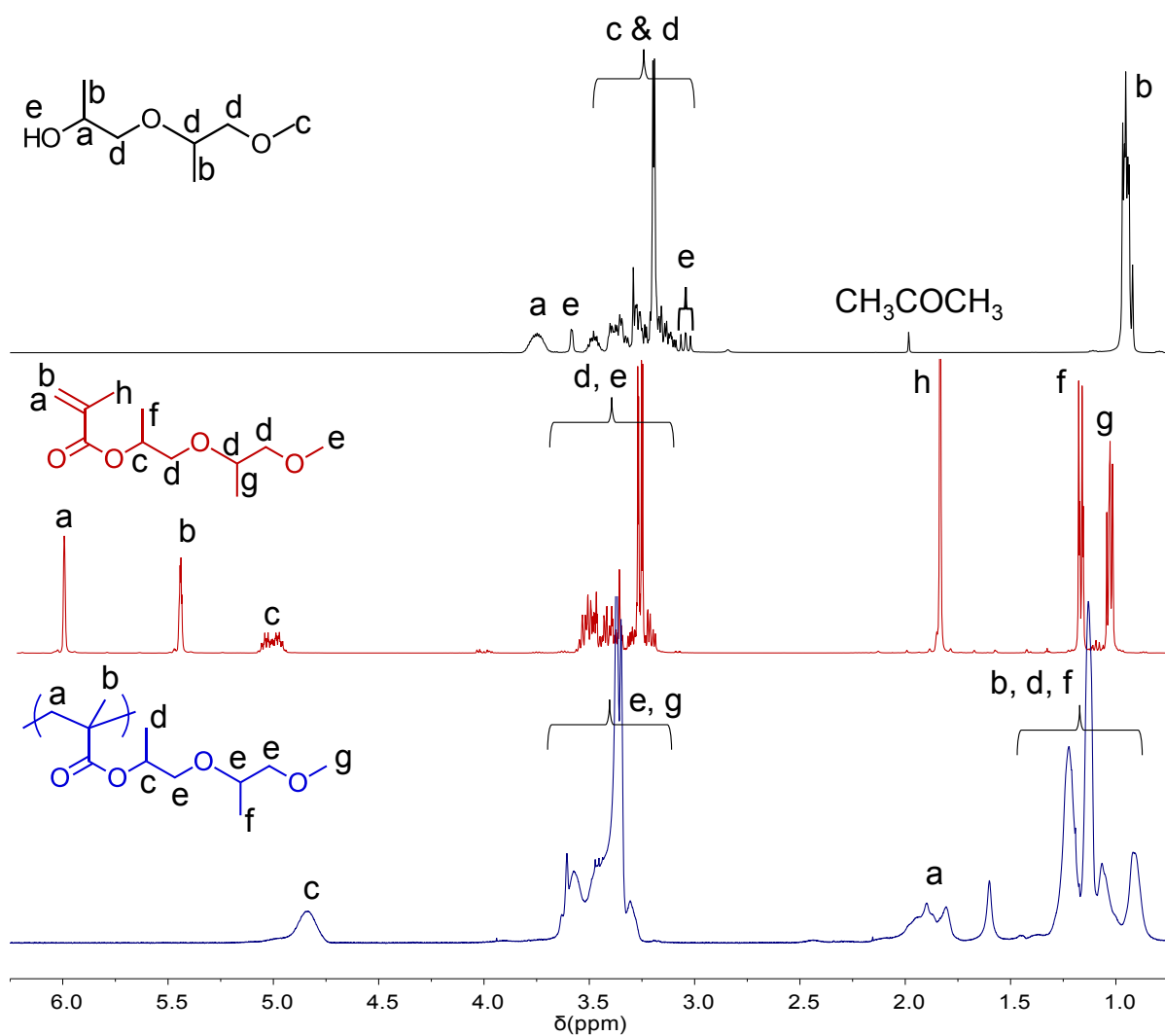
**Figure S2:** ^{1}H NMR spectra of di(propylene glycol) methyl ether (diPGOH) in black (top), di(propylene glycol) methyl ether methacrylate (diPGMA) in red, (middles), and poly[di(propylene glycol) methyl ether methahcrylate] [p(diPGMA)] in blue (bottom). In the analysis, only the most abundant isomer is shown for simplicity.
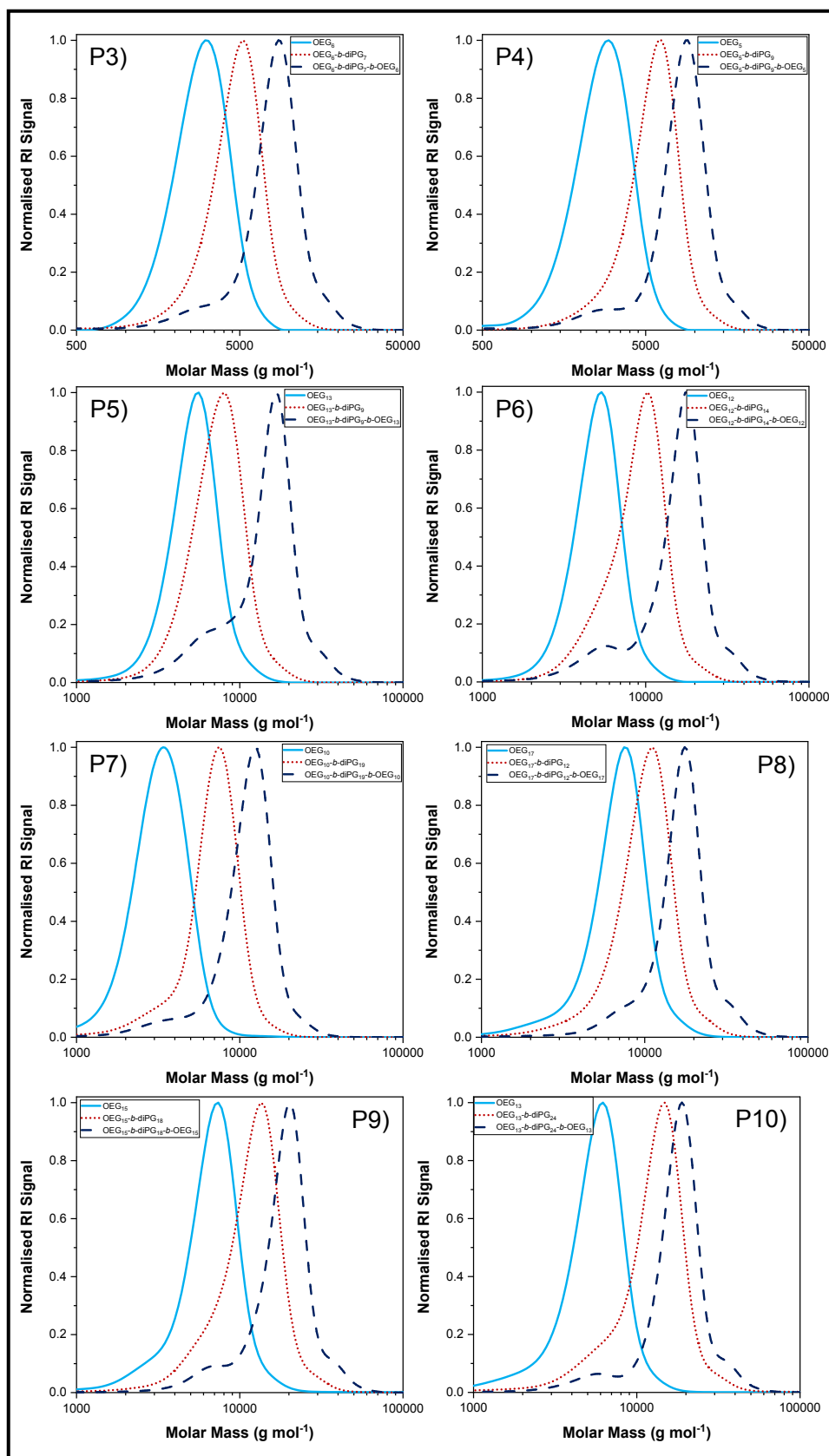
Figure S3: GPC molecular mass distributions of the triblock bipolymers in dark blue dotted line, and their precursors', the homopolymer and diblock copolymer, in light blue solid and in red dashed lines, respectively.
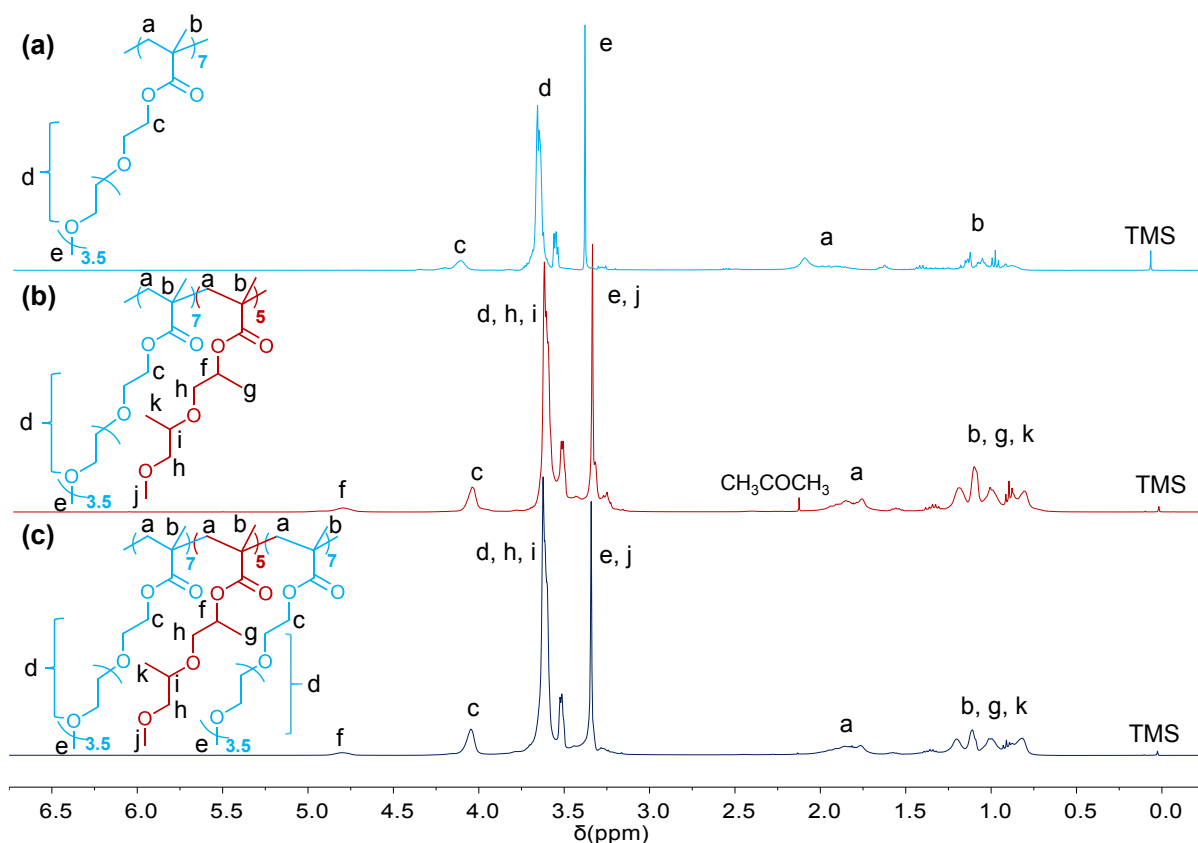
Figure S4: ^{1}H NMR spectra of (a) the homopolymer precursor, OEGMA300$_7$, in light blue (b) the diblock bipolymer precursor OEGMA300$_7$-*b*-diPGMA$_5$, in red and (c) the final triblock bipolymer OEGMA300$_7$-*b*-diPGMA$_5$-*b*-OEGMA300$_7$ (P2), in dark blue. Also, the corresponding chemical structures are shown, with complete assignment of the peaks. <u>*Note: For simplicity, the most abundant isomer of diPGMA is shown above.*</u>
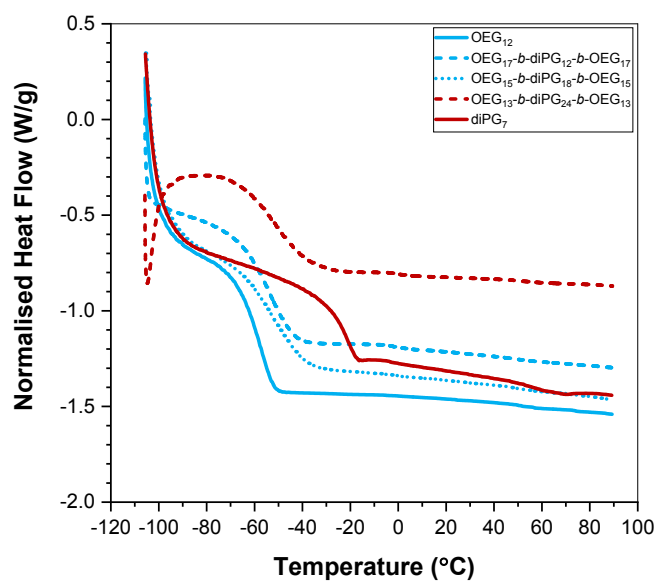
Figure S5: DSC curves of OEGMA300 homopolymer (light blue solid), diPGMA homopolymer (red solid), and the ABA triblock bipolymers with the highest MM studied (13100 g mol^{-1}) and various contents in hydrophobic block (diPGMA increases from 20 w/w%, dashed light blue line, to 30 w/w% dotted light blue line, to 40 w/w% red dashed line, respectively)
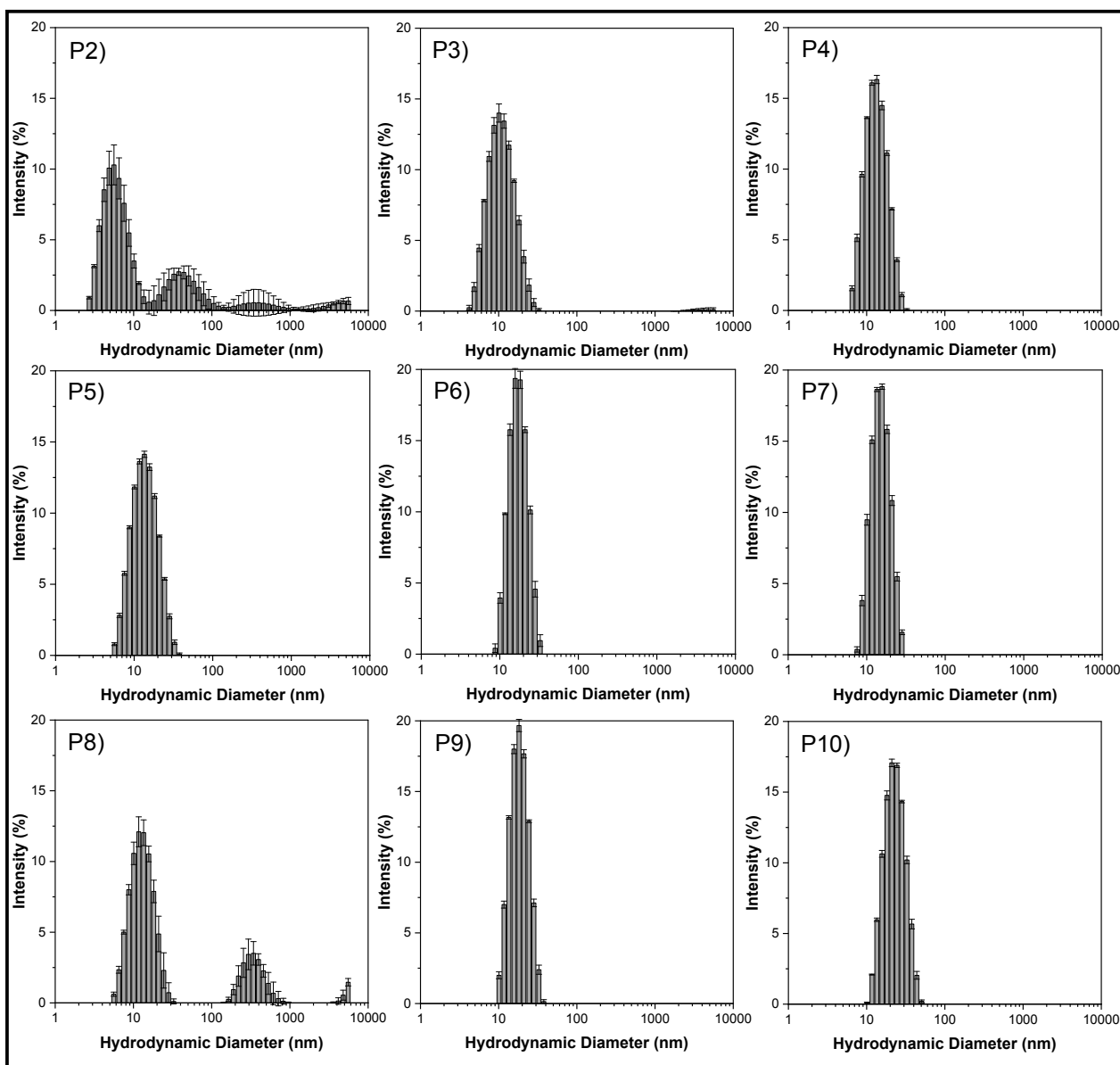
Figure S6: DLS histograms by Intensity of the solutions of the amphiphilic triblock bipolymers at 1 w/w% in deionised water.
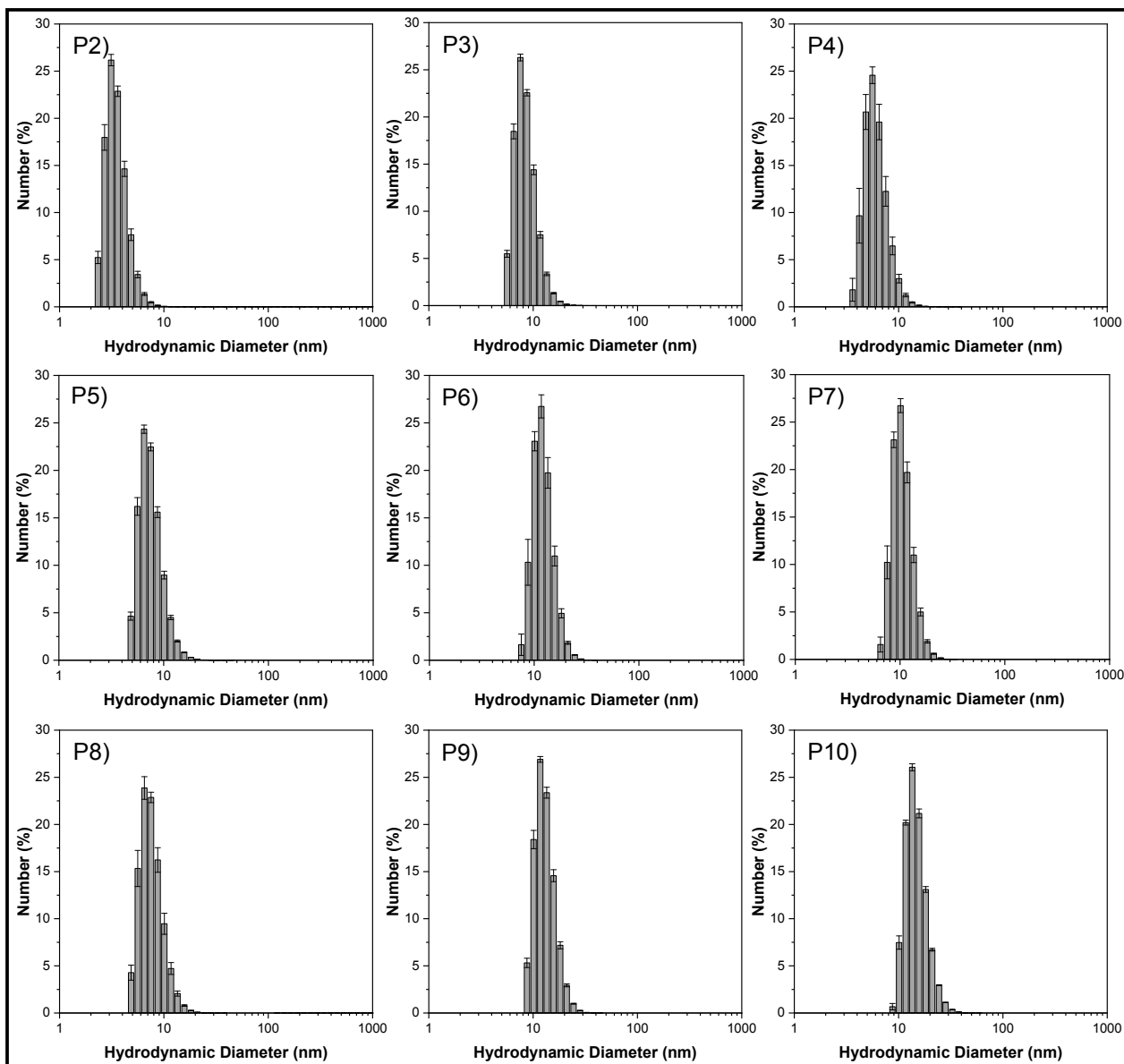
Figure S7: DLS histograms by number of the solutions of the amphiphilic triblock bipolymers at 1 w/w% in deionised water.
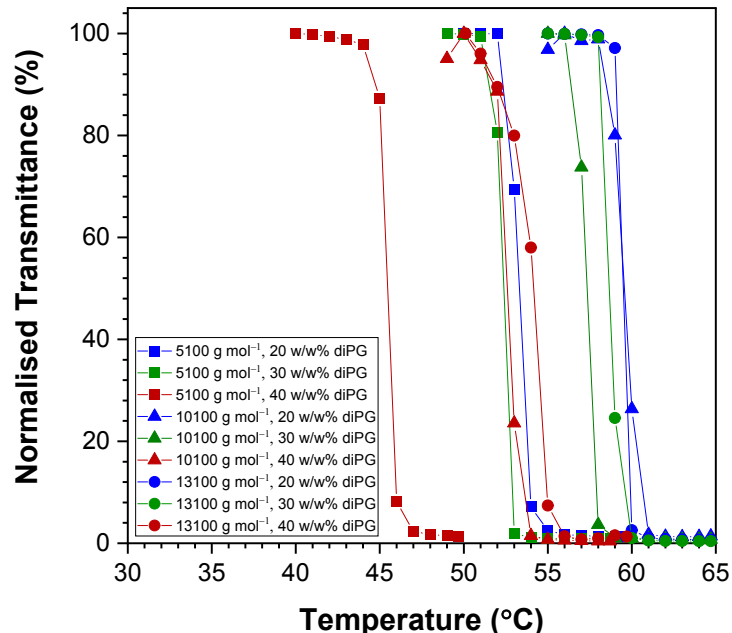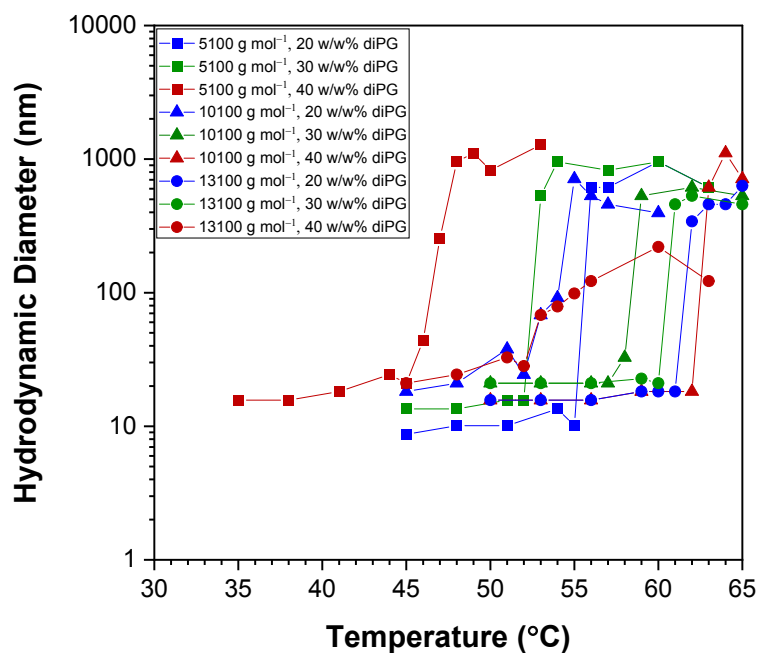
Figure S8: Percentage of normalised transmittance as a function of the temperature, as recorded by UV-Vis on solutions at 1 w/w% in deionised water. The effect of the molar mass is shown in squares, triangles, and circles, for 5100 g mol^{-1}, 10100 g mol^{-1}, and 13100 g mol^{-1}, respectively. The effect of composition is presented, with blue, green, and red corresponding to polymers with content in the hydrophobic diPGMA equal to 20 w/w%, 30 w/w% and 40 w/w%, respectively.

Figure S9: Change in the hydrodynamic diameter (by intensity) as a function of temperature, as recorded in polymer solutions at 1 w/w% in deionised water. The effect of the molar mass is shown in squares, triangles, and circles, for 5100 g mol^{-1}, 10100 g mol^{-1} and 13100 g mol^{-1}, respectively. The effect of composition is presented, with blue, green, and red corresponding to polymers with content in the hydrophobic diPGMA equal to 20 w/w%, 30 w/w% and 40 w/w%, respectively.

Table S3: Hydrodynamic diameters (d_H) and polydispersity indices (PDI), as recorded in 1 w/w% solutions in DI water, at various temperatures.

No.	Temperature (°C)	d_H by Intensity ± 1 (nm)	d_H by Number ± 1 (nm)	PDI
P2	5	**3**	164	3	0.883

	25	**6**	38		3		0.335
	45	**9**	220		6		0.286
	48	**10**	459		6		0.241
	51	**10**	295		7		0.240
	54	**14**	342		9		0.281
	55		10		8		0.221
	56		615		531		0.017
	57		615		615		0.088
	60		955		712		0.032
	63		615		615		1.000
P3	5	4	14	**142**	3		0.912
	25		10		6		0.149
	45		14		9		0.168
	48		14		9		0.143
	51		16		10		0.117
	52		16		12		0.177
	53	12		**531**	12		0.389
	54	9	220	**955**	8	**164**	0.604
	57		825		712		0.271
	60		955		825		0.093
	63		615		615		0.189
P4	5	12	396		2		0.443
	25		14		8		0.098
	35		16		10		0.141
	38		16		9		0.142
	41		18		10		0.126
	44		24		14		0.131
	45		21		12		0.148
	46		44		10		0.180
	47	28	**255**	3580	18		1.000
	48	342	**955**		122	**295**	0.622
	49		1110		712		0.094
	50		825		712		0.021
	53		1280		1110		0.987
P5	5	**12**	2250		3		0.544
	25		14		7		0.113
	50		16		10		0.072
	53		16		10		0.049
	56		16		10		0.063
	59		18		12		0.046
	62		18		14		0.164
	63		615		14		0.300
	64		1110		825		0.027
	65		712		615		1.000
P6	5		16		8		0.196
	25		16		12		0.047
	50		21		14		0.109
	53		21		14		0.103
	56		21		14		0.107

	57		21		16		0.076
	58		33		18		0.319
	59	68	**825**		59		0.587
	62		615		615		1.000
	65		631		459		0.121
P7	5	**14**	1990		7		0.265
	25		16		10		0.070
	45		18		12		0.078
	48		21		14		0.113
	51		38		14		0.143
	52		24		14		0.139
	53		68		33		0.218
	54		91		38		0.300
	55	91	**712**		68		0.630
	56		531		459		0.188
	57		459		459		1.000
	60		396		396		1.000
P8	5	**9**	295		2		0.676
	25	**12**	342		7		0.035
	50		16		10		0.188
	53		16		10		0.180
	56		16		10		0.161
	59		18		10		0.132
	60		18		12		0.138
	61		18		12		0.302
	62		342		10		0.329
	63		459		459		1.000
	64		459		459		1.000
	65		531		531		1.000
P9	5		16		9		0.212
	25		18		12		0.065
	50		21		14		0.087
	53		21		14		0.071
	56		21		14		0.076
	59		23		16		0.060
	60		21		16		0.117
	61	21	51	**459**	10	33	0.765
	62		531		459		0.623
	65		459		459		1.000
P10	5		18		10		0.138
	25		21		14		0.071
	45		21		16		0.076
	48		24		16		0.081
	51		33		16		0.157
	52		28		16		0.131
	53		68		33		0.179
	54		79		38		0.206
	55		99		44		0.231
	56		122		91		0.822

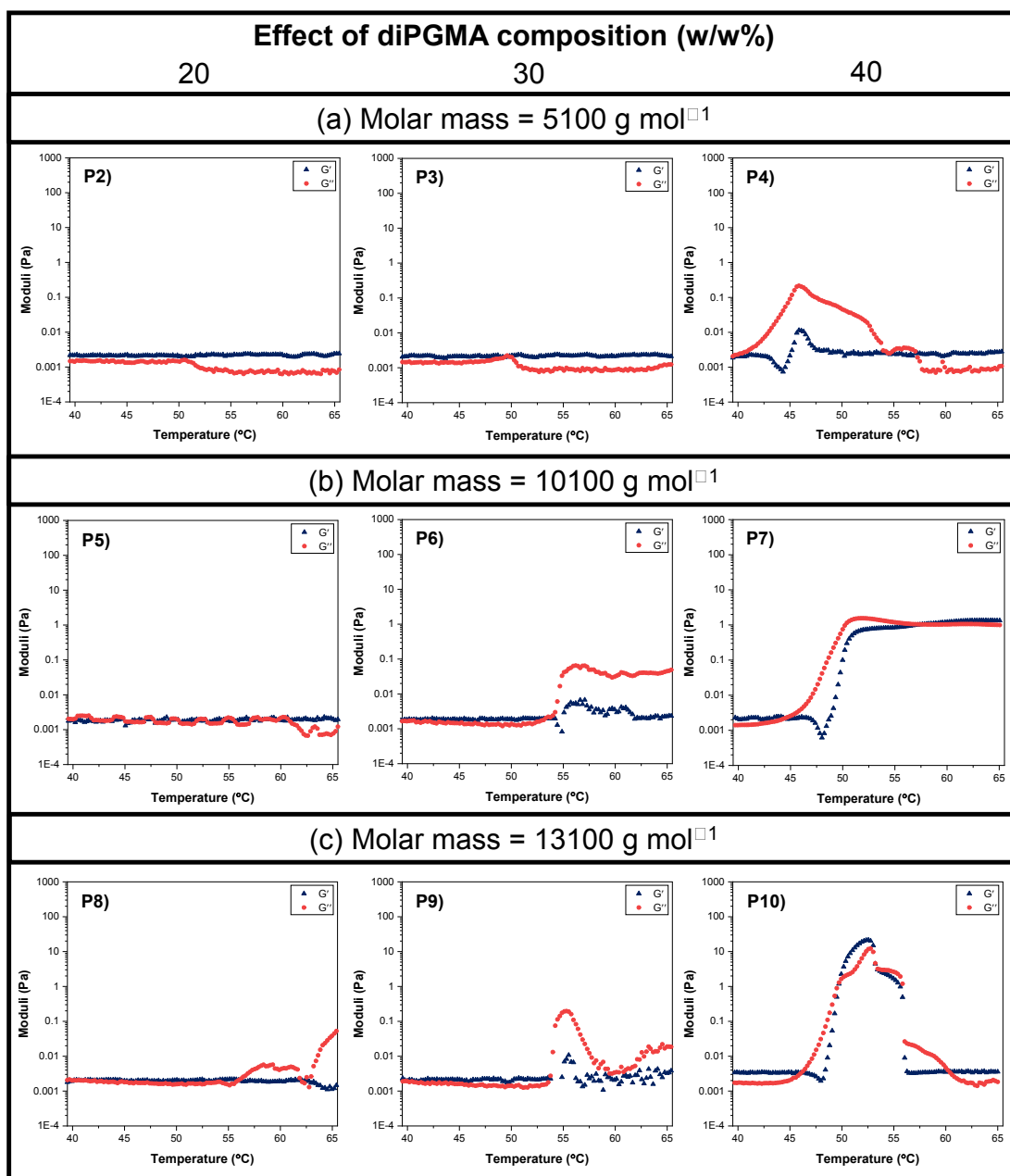|	60	220	190	0.694
	63	122	106	1.000
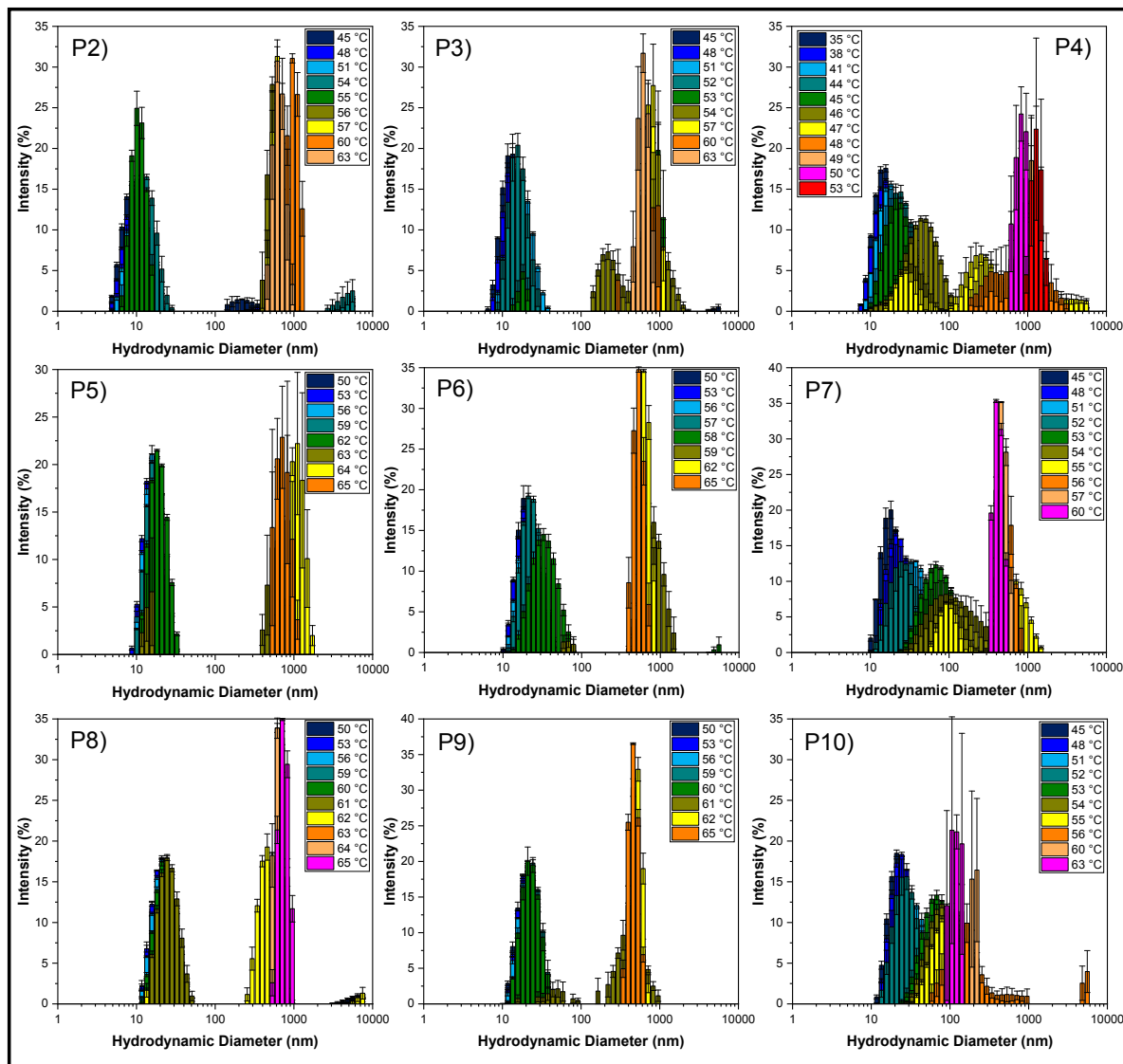
Figure S10: DLS histograms by Intensity of the solutions of the triblock bipolymers at 1 w/w% in deionised water at various temperatures.
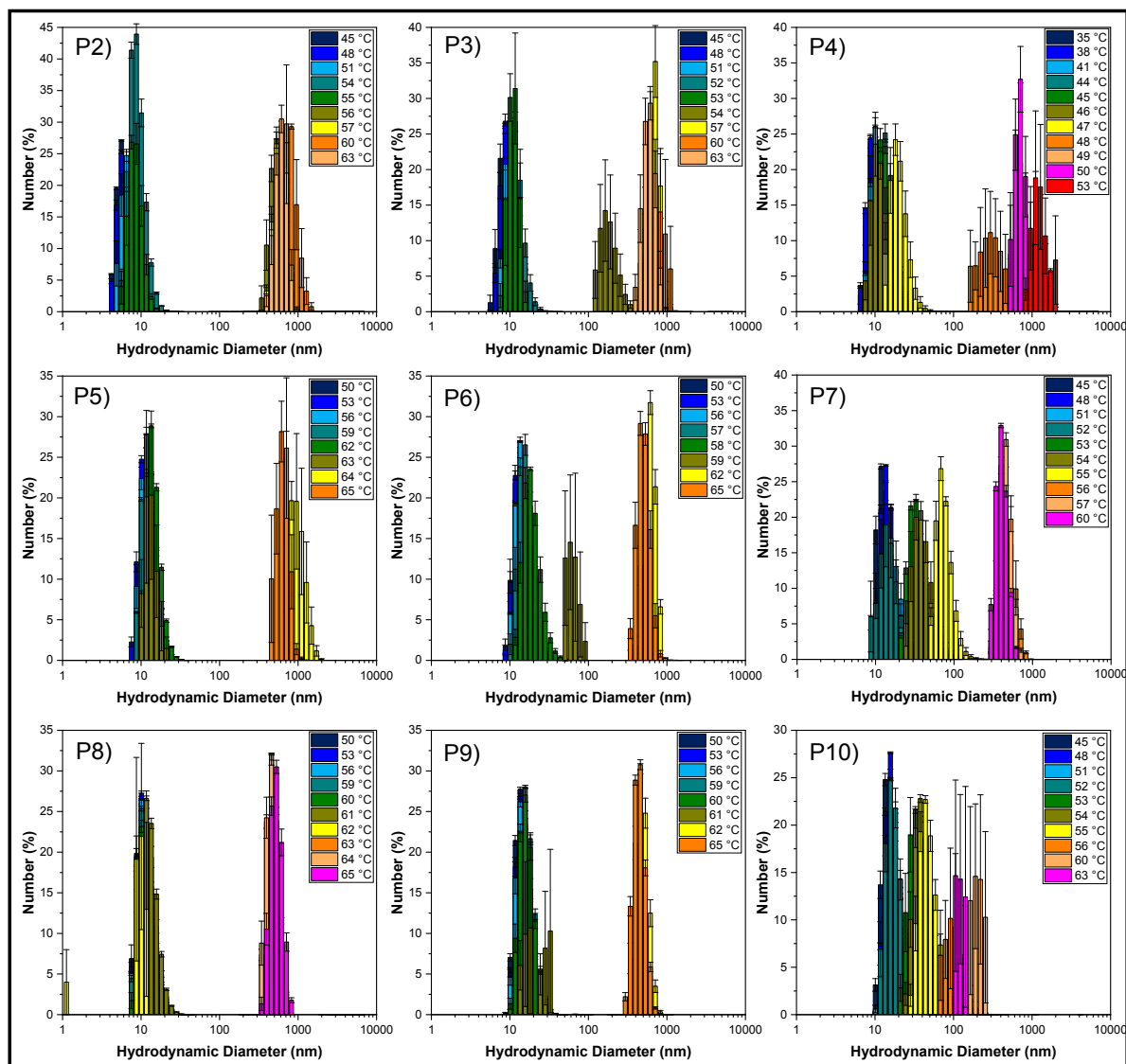
Figure S11: DLS histograms by number of the solutions of the triblock bipolymers at 1 w/w% in deionised water at various temperatures.

Figure S12: Rheological curves of the polymer solutions at 15 w/w% in phosphate buffered saline (PBS), as resulted from temperature ramp measurements. The changes in the shear storage modulus (elastic modulus, G'), and shear loss modulus (viscous modulus, G'') are shown in dark blue triangles, and red circles, respectively. The effect of diPGMA composition is shown from the left to the right, while the effect of molar mass is shown from the top to the bottom.

P10) 13100 g mol^{-1}, 40 w/w% diPGMA
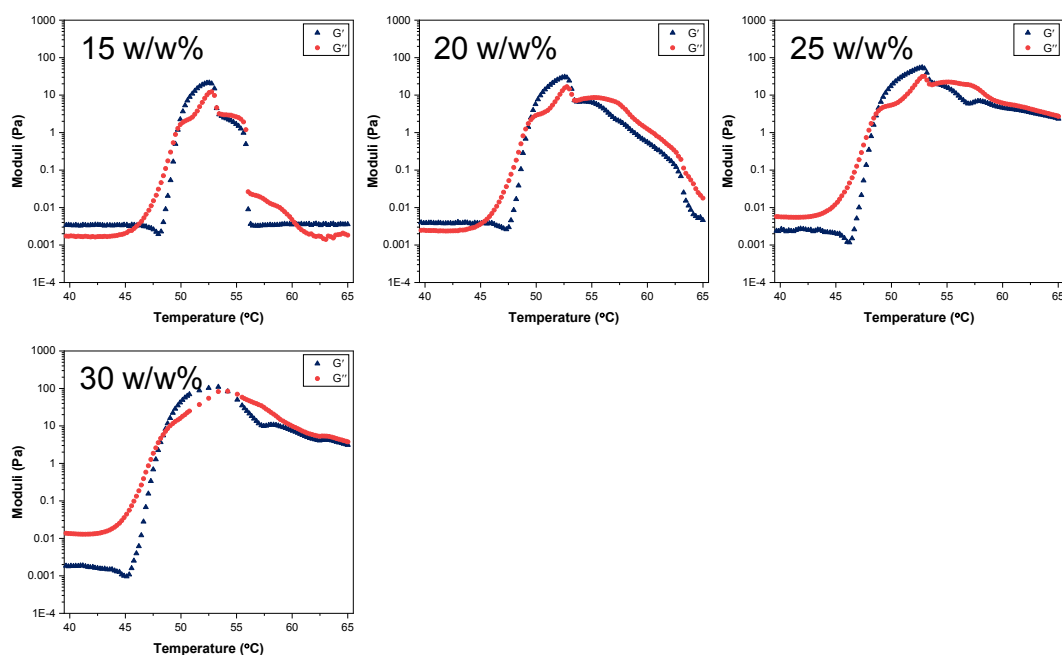
Figure S13: Rheological curves of the thermogelling solutions of Polymer 10 in phosphate buffered saline (PBS), as resulted from temperature ramp measurements, at various concentrations. The changes in the shear storage modulus (elastic modulus, G'), and shear loss modulus (viscous modulus, G'') are shown in dark blue triangles, and red circles, respectively. The corresponding polymer structure is also schematically illustrated, with light blue and red corresponding to the hydrophilic OEGMA300 and the hydrophobic diPGMA units, respectively.

Table S4: Critical micelle concentration (CMC) for all triblock copolymers.

Polymer No	Polymer Chemical Structure	CMC mmol/L
2	OEGMA300$_7$-b-diPGMA$_5$-b-OEGMA300$_7$	0.00123027
3	OEGMA300$_6$-b-diPGMA$_7$-b-OEGMA300$_6$	0.00042954
4	OEGMA300$_5$-b-diPGMA$_9$-b-OEGMA300$_5$	0.00023227
5	OEGMA300$_{13}$-b-diPGMA$_9$-b-OEGMA300$_{13}$	0.00024099
6	OEGMA300$_{12}$-b-diPGMA$_{14}$-b-OEGMA300$_{12}$	0.00008790
7	OEGMA300$_{10}$-b-diPGMA$_{19}$-b-OEGMA300$_{10}$	0.00008453
8	OEGMA300$_{17}$-b-diPGMA$_{12}$-b-OEGMA300$_{17}$	0.00086497
9	OEGMA300$_{15}$-b-diPGMA$_{18}$-b-OEGMA300$_{15}$	0.00007586
10	OEGMA300$_{13}$-b-diPGMA$_{24}$-b-OEGMA300$_{13}$	0.00003981

References

1) M. Vamvakaki, N. C. Billingham and S. P. Armes, Polymer, 1999, 40, 5161-5171.
2) I.B. Dicker, G.M. Cohen, W.B. Farnham, W.R. Hertler, E.D. Laganis, D.Y. Sogah, *Macromolecules,* 1990*, **23**(18)**,** 4034-4041.
3) Q. Zhang, C. Weber, U. S. Schubert and R. Hoogenboom, Mater. Horiz., 2017, 4, 109-116.