A library of thermoresponsive PEG-based methacrylate homopolymers: How do the molar mass and number of ethylene glycol groups affect the cloud point?

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ABSTRACT

In this study, a novel library of thermoresponsive homopolymers based on poly (ethylene glycol) (EG) (m)ethyl ether methacrylate monomers is presented. 27 EG based homopolymers were synthesized and three parameters, the molar mass (MM), the number of the ethylene glycol groups in the monomer, and the chemistry of the functional side group were varied to investigate how these affect their thermoresponsive behaviour. The targeted MMs of these polymers are varied from 2560 g mol^{-1}, 5000 g mol^{-1}, 8200 g mol^{-1} to 12000 g mol^{-1}. Seven PEG-based monomers were investigated: ethylene glycol methyl ether methacrylate (MEGMA), ethylene glycol ethyl ether methacrylate (EEGMA), di(ethylene glycol) methyl ether methacrylate (DEGMA), tri(ethylene glycol) methyl ether methacrylate (TEGMA), tri(ethylene glycol) ethyl ether methacrylate (TEGEMA), penta(ethylene glycol) methyl ether methacrylate (PEGMA), nona(ethylene glycol) methyl ether methacrylate (NEGMA). Homopolymers of 2-(dimethylamino) ethyl methacrylate (DMAEMA) were also synthesized for comparison. The cloud points of these homopolymers were tested in different solvents and it was observed that it decreases as the number of EG group was decreased or the MM increased. Interestingly, the end functional group (methoxy or ethoxy) of the side group has an effect as well and is even more dominant than the number of EG groups.

KEYWORDS: thermoresponsive polymers, poly(ethylene glycol), ethylene glycol methacrylate; group transfer polymerization, lower critical solution temperature, LCST, homopolymer, temperature-responsive, MEGMA, EEGMA, DEGMA, PEGMA TEGMA, TEGEMA, NEGMA, OEGMA

INTRODUCTION

In recent years, smart materials have gain significant scientific interest. Smart materials are materials able to respond to external stimulus, such as pH, light, temperature and pressure. Thermoresponsive gels (TRGs) are one kind of smart materials which undergo a sol-gel transition when the temperature changes.[1-5] This thermoresponsive property and their formation of a reversible gels makes TRGs promising materials for many biomedical applications, such as tissue engineering,[6-8] protein purification,[9] 3-D bioprinting,[10, 11] drug delivery,[8, 12] gene therapy,[13] and other industrial applications, for example, sensors,[14, 15] and catalysts.[16]

Thermoresponsive polymers are divided into two groups, the lower critical solution temperature (LCST) polymers and the upper critical solution temperature (UCST) polymers, based on how their solubility changes as the temperature changes.[1, 4] The LCST polymers form homogeneous solutions at low temperatures and at high temperature phase separation occurs (the solution appears cloudy and the polymer might precipitate out of solution). On the other hand, the UCST polymers are not soluble at lower temperature and they are in a transparent homogenous solution at higher temperatures. Our interest lies in LCST polymers because we aim at finding suitable polymers to be used as injectable gels or/and drug delivery.[1, 8] These applications involve mixing the drug or cells with the polymer solution at room temperature *in vitro*; then upon injection, the polymer forms hydrogel *in vivo* due to the higher temperature of human body. LCST polymers with slightly lower than 37°C are ideal for these applications, as these ensure that *in vivo* gelation will take place.[6, 8]

Poly (N-isopropyl acrylamide) (PNIPAAm) is one of most commonly studied LCST polymers with LCST at around 32°C.[3, 4, 17, 18] Many PNIPAAm based polymers form gels around body temperature and thus have been researched extensively for biomedical applications such as controlled cell transplantation, drug delivery system.[19, 20] However, some properties such as the cytotoxicity of the unreacted NIPAAm monomer and NIPAAm oligomers and the possible absorption of proteins have been found to limit the application of PNIPAAm polymers.[21-23]

Poly(ethylene glycol) (m)ethyl ether methacrylate based monomers can offer a great alternative to PNiPAAm based polymers because polyethylene glycol (PEG) is a nontoxic, FDA approved polymer that is resistance to the absorption of proteins.[5, 24, 25] Thus, PEG based (meth)acrylate polymers have gained considerable interest the last 20 years.[15, 24-39] It is known that the number of EG groups will affect the LCST.[5, 24, 26, 36] However, in order to find the PEG based methacrylate alternative to PNIPAAm, a systematic study is needed. This is because it is well-documented that the LCST is affected by the molar mass (MM), thus only well-defined polymers of similar MM and narrow MM distribution should be compared.[18, 40-45] Furthermore, the presence of even small end group of the polymer can affect their thermoresponsive behavior especially if this group is charged, thus all polymers compared should be prepared with the same polymerization method and if possible have a small, non-ionic group.[18, 46-49]

Thus, in this study a library of 27 PEG based methacrylate polymer was synthesized and characterized to investigate the effect of not only of the number of EGs but also the end group on the EG side group while keeping the end functional groups of the polymers the same. To the best of our knowledge, this is the first time that such an extensive library of PEG based homopolymers has been studied. Specifically, seven monomers from PEG based methacrylate family were investigated as shown in Fig.1: ethylene glycol methyl ether methacrylate (MEGMA), ethylene glycol ethyl ether methacrylate (EEGMA), di(ethylene glycol) methyl ether methacrylate (DEGMA), tri(ethylene glycol) methyl ether methacrylate (TEGMA), tri(ethylene glycol) ethyl ether methacrylate (TEGEMA), penta(ethylene glycol) methyl ether methacrylate (PEGMA), and nona(ethylene glycol) methyl ether methacrylate (NEGMA). Homopolymers based on 2-(dimethylamino)ethyl methacrylate (DMAEMA) were also fabricated for comparison. The MMs of the homopolymers was carefully controlled from 2560 g mol^{-1}, 5000 g mol^{-1}, 8200 g mol^{-1} to 12000 g mol^{-1}. After polymerization, the MMs were confirmed by GPC. The cloud points and hydrodynamic diameters of these homopolymers were investigated to reveal how the thermoresponsive properties were influenced. Group transfer polymerization (GTP) [50, 51] was chosen to fabricate the polymers because is a living polymerization method, ideal

for the fabrication of methacrylate in large scales and because the functional group that remains at the end of the polymer backbone is one methyl methacrylate group, a non-ionic group.

EXPERIMENTAL

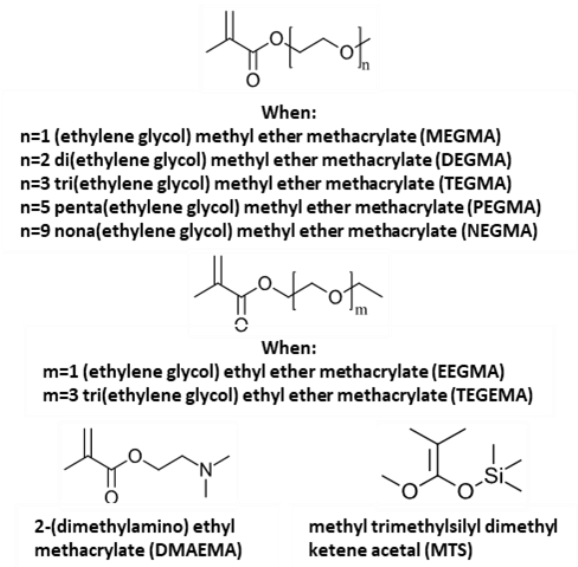

FIGURE 1 Chemical structures and abbreviations of all the monomers investigated.

Materials

The monomers: DMAEMA (MM=157.22 g mol^{-1}, 98%), MEGMA (MM=144.17 g mol^{-1}, 99%), DEGMA (MM = 188.22 g mol^{-1}, 95%), TEGMA (MM=232.27 g mol^{-1}, 93%), PEGMA (MM = 300 g mol^{-1}), NEGMA (MM = 500 g mol^{-1}) were purchased from Aldrich, UK. EEGMA (MM=158.20 g mol^{-1}, 98%) and TEGEMA (MM= 246.30 g mol^{-1}, 95%) were purchased from Tokyo chemical industry, UK. The initiator, methyl trimethylsilyl dimethyl ketene acetal (MTS, 95%), and the polymerization solvent, tetrahydrofuran (THF, HPLC grade, ≥99.9%) and the free radical inhibitor 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH), were purchased from Aldrich, UK. Other chemicals: calcium hydride (CaH_2, ≥90%), aluminum oxide activated basic (Al_2O_3·KOH), deuterated chloroform (chloroform-d, 99.8 atom % D) were purchased from Aldrich, UK. The solvent in chromatography, (THF, GPC grad) and the visual test solvents, phosphate buffered saline (PBS, solution) were purchased from Fischer Scientific. The precipitation solvent n-hexane and ethanol were purchased from VWR chemicals.

Group Transfer Polymerization (GTP). All polymers reported in this study were fabricated using GTP. MTS was used as the initiator while tetrabutylammonium bibenzoate (TBABB) was used as a catalyst. TBABB was in house synthesized, following by Dicker et al.[52]

All the monomers were purified by passing twice through the column with basic aluminum oxide to remove any protic impurities and kept under argon under their distillation prior to polymerization. However, because of their high MMs PEGMA and NEGMA were not be distilled. Thus, the purification and polymerization steps were slightly different, according to Vamvakaki et al.[38, 53-55] Before purification, a 50% vol THF solution of the monomer was prepared. The mixture was then passed through the basic aluminum oxide column twice without adding DPPH to it. After purification, the monomer solution was stirred with calcium hydroxide as other monomers. During polymerization, the monomer solution was filtered before the addition to remove the calcium hydride.

All polymers were synthesized in similar way. Specifically, in a round bottom glass flask that contained the TBABB catalyst and was kept under argon the solvent (THF) and monomer were added. Then the MTS initiator was added at the end. For example, for the first polymer of the MEGMA series 25 mL of THF, 0.5 mL (0.0025mol, 0.43 g) of MTS and 5.9 mL (0.040 mol, 5.8 g) of MEGMA were used. For the rest of the polymers in the same series the amount of MTS added was kept the same (0.5mL) and what varied was the THF and the amount of MEGMA. The concentration of reagents in solution was kept constant at 25wt% for all polymer syntheses as well as the amount of catalyst TBABB (~10mg).

The polymerization was exothermic in all cases and the exotherm was monitored for each reaction that lasted less than 15 minutes. Then

samples for gel permeation (GPC) and nuclear magnetic resonance (NMR) spectroscopy characterization were obtained. Finally, all polymers were precipitated in hexane and then dried in a vacuum oven over a week at room temperature.

Gel Permeation Chromatography (GPC). The gel permeation chromatography (GPC) was used to confirm the MM and the molar mass distribution (MMD) of the polymers synthesized in this project. The GPC machine used in this project was an Agilent, Security GPC system, with a Polymer Standard Service (PSS) SDV analytical linear M column (SDA083005LIM). It was calibrated by poly(methyl methacrylate) (PMMA) standards with molar masses of 2000, 4000, 8000, 20000, 50000, and 100000 $gmol^{-1}$.

The samples were prepared by mixing 10mg of polymer and 1ml of GPC solvent. The solvent was either pure THF or THF with 5% vol of trimethylamine. The samples were filtered before passing through the device to protect the column from blockage.

Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR) After precipitation, the polymer was determined by a 400 MHz Advance Bruker NMR spectrometer instrument to confirm the synthesis. For each sample, around 0.01g of polymer was dissolved in 650µml of d-chloroform.

Cloud point measurements. All the polymers were tested in 2ml of 1 w/w % solutions. The solvent used were deionized water (DI water), PBS, ethanol, 80% water and 20% ethanol. The cloud points of the polymers were at first tested visually (between 20°C to 95°C) and then by measuring the transmittance using a Cary 3500 Compact Peltier UV-Vis System (Agilent, UK).

Dynamic Light Scattering (DLS). The dynamic light scattering was used to determine the sizes of random coil or the micelles formed by the polymers in water solution. The 1wt% water solutions of all the polymers were filtered and test under 25°C. Since the cloud points of TEGEMA polymers are around room temperature, the DLS of those polymers are conducted under 15°C. The instrument used in this project was Zetasizer Nano ZSP (Malvern, UK).

Titration. Since the DMAEMA polymers are also pH-sensitive, the titration was conducted to determine the effective pK_as of these polymers. Firstly, around 5ml of 1wt% solution was made. Then these solutions were titrated between pH 2 and pH 12 using a standard NaOH 0.75 M solution under continuous stirring. The pH was measured using a Fisherbrand Hydrus 400 pH meter. The pK_as were calculated as the pH at 50% ionization.

RESULTS AND DISCUSSION

In total 27 homopolymers with 7 different PEG methacrylate monomers were fabricated and 4 DMAEMA homopolymers were also synthesised for comparison. The target MMs of these polymers were aimed at 2560 g mol^{-1}, 5000 g mol^{-1}, 8000 g mol^{-1} and 12000 g mol^{-1}. The PEG monomers studied were EEGMA, MEGMA, DEGMA, TEGMA, TEGEMA, PEGMA, and NEGMA. Only one polymer was not successfully synthesized, specifically $TEGMA_{51}$ as it will be discussed below and thus is not reported in Table 1.

Molar Mass and Molar Mass Distribution

The MMs and the MMD (dispersity indices, *Đ*) given by GPC of all the homopolymers were presented in Table 1.

As shown in Table 1, the MMDs of these homopolymers were all lower than 1.21 similar to previous GTP polymerizations using PEG based methacrylates, indicating a successful polymerization.[36, 42, 45, 53, 54, 56-58] The MMDs of PEGMA and NEGMA polymers were slightly higher than others which agreed with previous studies. The first reason was the PEGMA and NEGMA are macromonomers with an average MM. The second reason was due to the high viscosity, instead of distillation, these monomers

were filtered before polymerization, thus the remaining impurities in the monomer lead to wider MMDs. For most of the homopolymers, the resulted M_ns were slightly higher (around 15%) than the theoretical MMs. This was due to the deactivation caused by impurities in the flasks and/or the moisture introduced into the flask during the addition of the monomers, solvent and initiator.

Table 1 Molar masses (theoretical and as experimentally determined by GPC) and molar mass distribution of the PEG methacrylate based homopolymers

Sample No.	Chemical structure[a]	Theoretical MM[b] g mol^{-1}	GPC results[c]	
			M_n g mol^{-1} (±250)	M_w/M_n (±0.01)
1	$DEGMA_{13}$	2560	3300	1.12
2	$DEGMA_{27}$	5000	5600	1.14
3	$DEGMA_{44}$	8300	11200	1.13
4	$DEGMA_{63}$	12000	13900	1.12
5	$TEGMA_{10}$	2560	2700	1.17
6	$TEGMA_{21}$	5000	5500	1.17
7	$TEGMA_{35}$	8300	16400	1.08
8	$NEGMA_{5}$	2560	4000	1.21
9	$NEGMA_{10}$	5000	6500	1.14
10	$NEGMA_{16}$	8300	9900	1.09
11	$NEGMA_{24}$	12000	14000	1.14
12	$PEGMA_{8}$	2560	3900	1.20
13	$PEGMA_{16}$	5000	5800	1.16
14	$PEGMA_{27}$	8300	9300	1.18
15	$PEGMA_{40}$	12000	14100	1.21
16	$MEGMA_{16}$	2560	2500	1.14
17	$MEGMA_{34}$	5000	5600	1.11
18	$MEGMA_{56}$	8300	11000	1.06
19	$MEGMA_{83}$	12000	15800	1.08
20	$EEGMA_{15}$	2560	2600	1.11
21	$EEGMA_{31}$	5000	5000	1.12
22	$EEGMA_{51}$	8300	9500	1.08
23	$EEGMA_{75}$	12000	14400	1.12
24	$TEGEMA_{10}$	2560	3000	1.15
25	$TEGEMA_{20}$	5000	6200	1.12
26	$TEGEMA_{33}$	8300	12700	1.11
27	$TEGEMA_{48}$	12000	15800	1.11
28	$DMAEMA_{16}$	2560	3400	1.12
29	$DMAEMA_{31}$	5000	7100	1.13
30	$DMAEMA_{52}$	8300	9400	1.10
31	$DMAEMA_{63}$	10000	13300	1.08

[a] The abbreviations for the eight monomers: (ethylene glycol) methyl ether methacrylate (MEGMA), (ethylene glycol) ethyl ether methacrylate (EEGMA), di(ethylene glycol) methyl ether methacrylate (DEGMA), tri(ethylene glycol) methyl ether methacrylate (TEGMA), tri(ethylene glycol) ethyl ether methacrylate (TEGEMA), penta(ethylene glycol) methyl ether methacrylate (PEGMA), nona(ethylene glycol) methyl ether methacrylate (NEGMA) and 2-(dimethylamino) ethyl methacrylate (DMAEMA).

[b] Theoretical MM = $MM_{monomer}$*DP+100 gmol^{-1}, where the $MM_{monomer}$ was the molar mass of the monomer; the DP was the degree of polymerization of the corresponding homopolymer; the 100 gmol^{-1} was the molar mass of the fragment of the MTS (the initiator) remaining on the polymer backbone.

[C] As determined by GPC using poly(methyl methacrylate) PMMA standards.

It should be noted that the synthesis of the TEGMA polymers was not straight forward. Even though the TEGMA was distilled due to its high MM higher temperature was used and took longer to distill than the other monomers. We believe that as often reported for EG based methacrylate monomers a small percentage of the hydroxy terminated equivalent was present that interfered with the polymerization and thus the higher MM polymer was not obtained after 3 trials. The monomer is no longer commercially available thus the polymerization cannot be repeated. The difficulty of obtaining polymers with TEGMA is also demonstrated by the fact that when we aimed for polymer 7, with a degree of polymerization equal to 35 a polymer with double the MM was obtained.

The GPC chromatograms of MEGMA homopolymers (for samples that were obtained directly from the polymerization flask) are shown in Fig.2. As shown in the figure, there is no shoulder or monomer peak on the curve indicating the 100% conversion of the monomer. The GPC chromatograms of the rest of the homopolymers can be found in Fig. S1.

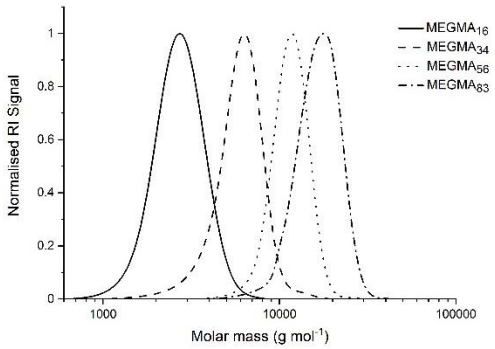

FIGURE 2 GPC chromatograms of MEGMA homopolymers. The theoretical MMs increased from 2560 to 12000 $gmol^{-1}$. The traces of $MEGMA_{16}$, $MEGMA_{34}$, $MEGMA_{56}$, and $MEGMA_{83}$ are shown in black solid, black dashed, black dotted and black dashed dotted lines, respectively.

The ^1H NMR spectrum of $MEGMA_{16}$ in d-chloroform is shown in Fig.3. The characteristic peaks at around 3.5ppm and 4ppm and were labelled as peak d and peak c in the spectrum. These peaks correspond to the protons in the methylene group ($-OCH_2-CH_2-$) of MEGMA homopolymers. The peak e was due to the protons in the methoxy ($-OCH_3$) group. The peak a and b at around 1ppm correspond to the protons on the polymer backbone. Furthermore, one NMR spectra for one homopolymer from each of the 8 monomers is included in the supplementary information. Note that the degree of polymerization is not able to be determined by NMR when using GTP as only an MMA group is left on the polymer chain as it was previously mentioned and the 3 protons of the $-CH_3$ group overlap with the monomer groups.

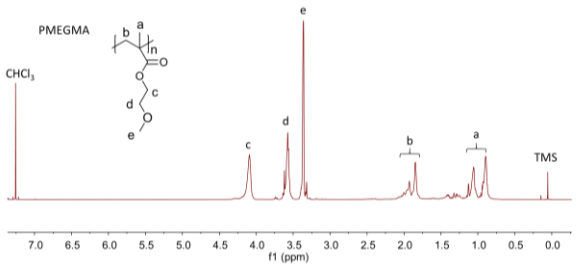

FIGURE 3 ^1H NMR spectrum of $MEGMA_{16}$ in d-chloroform.

Aqueous Solution Properties

Cloud Point

The cloud points of all the homopolymers obtained by visual tests in different solvent are listed in Table 2.

The cloud point was not tested for MEGMA and EEGMA homopolymers because they were not soluble in water, ethanol and $80\%H_2O+20\%C_2H_6O$. This is due to the ethylene glycol groups in these polymers are not sufficient to achieve solubility. For NEGMA homopolymers, no cloud point was observed up to 95°C. This is attributed to the fact that there are nine ethylene glycol groups in the monomer, the homopolymer is too hydrophilic, thus interrupting the "hydrophobic effect".

Table 2 Cloud points of 1%w/w solutions in deionized water, PBS and 80%H_2O+20%C_2H_6O and hydrodynamic diameters in 1%w/w solutions in water of the homopolymers.

Sample No.	Chemical structure	Cloud Point (±1℃)			Hydrodynamic Diameter (nm)	
		Water	PBS	80%H_2O+20%C_2H_6O	$D^d_{theoretical}$	$D_{experimental}$ (±0.5)
1	$DEGMA_{13}$	30	28	60	1.6	3.6
2	$DEGMA_{27}$	29	26	59	2.0	4.2
3	$DEGMA_{44}$	27	25	47	2.9	5.6
4	$DEGMA_{63}$	27	24	46	3.2	6.1
5	$TEGMA_{10}$	63	56	-[a]	1.3	2.7
6	$TEGMA_{21}$	58	44	83	1.8	4.2
7	$TEGMA_{35}$	49	44	83	3.1	6.5
8	$NEGMA_5$	-[a]	-[a]	-[a]	2.2	3.6
9	$NEGMA_{10}$	-[a]	-[a]	-[a]	2.4	4.2
10	$NEGMA_{16}$	-[a]	-[a]	-[a]	2.6	4.9
11	$NEGMA_{24}$	-[a]	-[a]	-[a]	2.8	6.5
12	$PEGMA_8$	76	71	-[a]	2.0	3.3
13	$PEGMA_{16}$	75	71	-[a]	2.2	4.2
14	$PEGMA_{27}$	74	68	-[a]	2.6	5.6
15	$PEGMA_{40}$	71	68	-[a]	2.9	6.5
16	$MEGMA_{16}$	-[b]	-[b]	-[b]	1.6	N/A
17	$MEGMA_{34}$	-[b]	-[b]	-[b]	2.3	N/A
18	$MEGMA_{56}$	-[b]	-[b]	-[b]	3.3	N/A
19	$MEGMA_{83}$	-[b]	-[b]	-[b]	3.9	N/A
20	$EEGMA_{15}$	-[b]	-[b]	-[b]	1.5	N/A
21	$EEGMA_{31}$	-[b]	-[b]	-[b]	2.1	N/A
22	$EEGMA_{51}$	-[b]	-[b]	-[b]	2.9	N/A
23	$EEGMA_{75}$	-[b]	-[b]	-[b]	3.6	N/A
24	$TEGEMA_{10}$	26	22	33	1.3	3.1[c]
25	$TEGEMA_{20}$	24	22	32	1.9	4.2[c]
26	$TEGEMA_{33}$	24	22	29	2.7	6.5[c]
27	$TEGEMA_{48}$	24	22	28	3.0	6.5[c]
28	$DMAEMA_{16}$	46	46	84	1.4	3.6
29	$DMAEMA_{31}$	43	43	72	2.5	4.2
30	$DMAEMA_{52}$	43	36	64	2.9	4.2
31	$DMAEMA_{63}$	41	36	64	3.4	5.6

[a]No cloud point was observed even at 95°C.

[b]These homopolymers were insoluble.

[c]The DLS was conducted under 15°C because the cloud point of TEGEMA homopolymers was around room temperature.

[d] The theoretical hydrodynamic diameter was calculated by assuming that all the soluble polymer chains formed random coils in aqueous solution ($<dg^2>^{1/2} = 2 *(2 * 2.20 * DP_{total}/3)^{1/2} * 0.154$ nm); where DP_{total} is the total degree of polymerization as resulted from GPC result. For PEGMA and NEGMA polymers, the ethylene glycol side chain was long and should be considered when calculating the DP_{total}.

Comparing the homopolymers with similar molar mass and different numbers of PEG groups on the side chain, the cloud points in DI water of the homopolymers were in this sequence: PEGMA > TEGMA > DEGMA > TEGEMA, as shown in Fig.4. This agreed with the

hydrophilicity of the monomers and was expected as summarized in the literature.[5, 24] When comparing DEGMA, TEGMA and PEGMA, the more the ethylene glycol groups in the monomer, the more hydrophilic the polymers are, therefore the higher the cloud point.

Concerning the terminal group on the side chain, i.e. methyl versus ethyl, it is observed that there is a strong effect on the thermoresponsive properties. More specifically, the cloud point of $TEGEMA_{10}$ is 38°C lower than $TEGMA_{10}$. This is because the hydrophobicity of ethyl ether group is much higher than the methyl ether group, therefore the TEGEMA homopolymers are more hydrophobic than TEGMA homopolymers. Furthermore, it is worth noting that, the cloud point of $TEGEMA_{10}$ is 5°C lower than $DEGMA_{13}$, even though it contains more ethylene glycol groups, which demonstrates that the terminal group on the side chain overcomes the effect of the number of EG groups on the side chain.

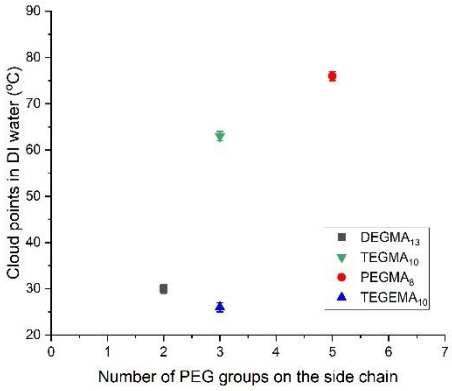

FIGURE 4 Effect of the number of PEG groups on the cloud points of the homopolymers with the same theoretical MM (2560 $gmol^{-1}$). The cloud points were tested in 1%w/w DI water solutions. The cloud points of the MEGMA homopolymer with the same target MM is not presented as the polymer is insoluble in water.

The cloud points of all the homopolymers were plotted against the experimental MMs as shown in Fig.5. The cloud points of these homopolymers decrease as the MMs increase, as expected, and observed before. [18, 40-45] The higher the MM, the higher the diameter of the polymers in solution as confirmed by the DLS in Table 2 thus the easier the aggregation and precipitation as the temperature increases. Therefore, it can be concluded that generally the higher the MM, the lower the cloud points. This effect seems to be more profound for more hydrophilic polymers as longer EG groups are presented and more thermoresponsive group on the polymer.

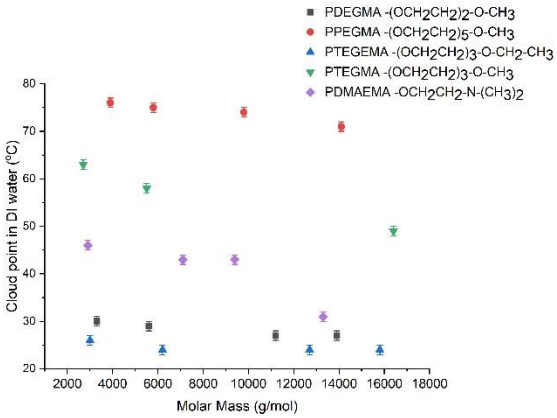

FIGURE 5 Effect of MM on the cloud points of the homopolymers. The cloud points were tested in 1%w/w DI water solutions.

When comparing the DMAEMA, MEGMA and EEGMA homopolymers it was found that both MEGMA and EEGMA polymers were not soluble while DMAEMA homopolymers exhibited cloud points between 35°C to 45°C. The DMAEMA homopolymers cloud point decreased with increasing the MM as expected.

The cloud points of the homopolymers were also tested in other solvents such as PBS, ethanol, and a mixture of 80wt% water and 20wt% ethanol. There was no cloud point observed in the ethanol due to the good solubility of these homopolymers in the ethanol. However, for DEGMA, TEGMA, TEGEMA and DMAEMA homopolymers, cloud points were observed in the mixture of 80wt% water and 20wt% ethanol. The result of DEGMA homopolymers was plotted in Fig.6 below. The same trend was observed for the other homopolymers and the diagram are given in the supplementary information. It was found that the homopolymer showed higher cloud point in DI water than in PBS (around 2-3°C higher), which was consisted with previous

studies.[27, 32] The cloud point increased as much as 30°C in the mixture of 80wt% water and 20wt% ethanol when compared with DI water.

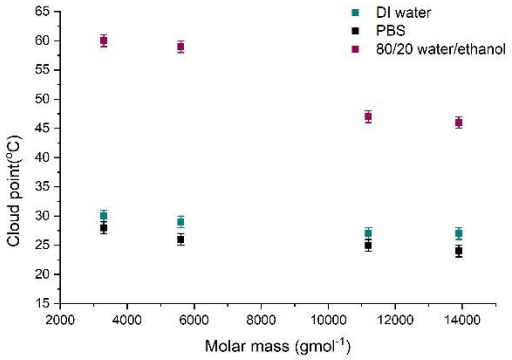

FIGURE. 6 Effect of different solvent on the cloud points of the DEGMA homopolymers.

Hydrodynamic Diameter

Table 2 includes the calculated theoretical hydrodynamic diameters and the experimental hydrodynamic diameters obtained from DLS of these homopolymers. The diameters at the maximum intensity on the DLS histogram were taken as the experimental hydrodynamic diameters. Since the TEGEMA polymers showed cloud points of 23 to 25°C, the solutions were cloudy when firstly tested under room temperature. The results were around 2000-5500nm which indicates that the polymer chains aggregated in the solution under room temperature. To avoid aggregation, the TEGEMA homopolymers were then tested under 15°C and the result was consistent with other homopolymers.

For the soluble homopolymers, the theoretical hydrodynamic diameters were calculated based on Equation.1 by assuming that all the polymer chains formed random coils in aqueous solution. The DP_{exp} was calculated by dividing the experimental MM of the polymer by the MM of the monomer. For DEGMA, TEGMA, TEGEMA and DMAEMA homopolymers, the DP_{total} was considered equal to the DP_{exp}.

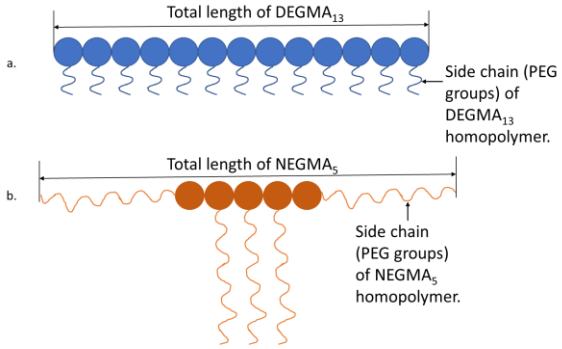

FIGURE 7 Schematic illustrations of the polymer chains of: a) $DEGMA_{13}$ and b) $NEGMA_5$ homopolymers.

However, for PEGMA and NEGMA, as shown in Fig.7, because the length of the PEG side chain is comparable with the backbone length, the length of side chains on each end of the polymer chain should be included in the total length of the polymer chain. The length of EG was considered as 1.5 times of the length of the methacrylate, thus the converted DP of the side chain was 1.5 times of the number of the EG group in the side chain. Therefore, the DP_{total} of NEGMA and PEGMA homopolymer was calculated differently using Equation.2. In Equation.2, n is the number of the EG group in the monomer.

$$\langle dg^2 \rangle^{1/2} = 2 \times \left(2 \times 2.20 \times \frac{DP_{total}}{3}\right)^{1/2} \times 0.154nm \quad (1)$$

$$DP_{total} = DP_{exp} + 2 \times n \times 1.5 \quad (2)$$

The experimental hydrodynamic diameters were close to the theoretical hydrodynamic diameters for all the polymers which indicates that the polymers existed in the solution as a random coil configuration. The hydrodynamic diameters increased as the MM increased. The influence of hydrophilicity of the homopolymers on the hydrodynamic diameters was minor. However, the experimental results were slightly higher than the theoretical ones. This was due to the assumptions made when calculating the theoretical hydrodynamic diameters. When calculating the theoretical hydrodynamic diameters, the polymer chains were assumed coiled and overlapped that this will not be the

case when bulky side group like EG groups are presented. Furthermore, the experimentally determined diameters are hydrodynamic diameters i.e the solvent shell surrounding the polymer is taken into account, thus it is expected that they are larger than the theoretical calculation.

Effective pK_as

The effective pK_as of the DMAEMA homopolymers were determined and were in the range of 7.2 to 7.3 which agreed with previously reported pK_a values of DMAEMA polymers.[36, 42, 44, 53, 54, 56-58]

CONCLUSIONS

In conclusion, a library of 27 homopolymers based on PEG methacrylate monomers and 4 homopolymers based on DMAEMA were successfully synthesized via GTP. The MMs were varied from 2560 g mol^{-1}, 5000 g mol^{-1}, 8200 g mol^{-1} to 12000 g mol^{-1} and the number of PEG groups in the side chain were varied from 1, 2, 3, 5, 9. The thermoresponsive properties of these monomers were investigated and compared. It was observed that the end group of the EG side group has a more dominant effect on the cloud point than the number of the EG or the MM of the polymer. This is an interesting result that can be used to tailor the LCST and gelling temperature of thermoresponsive copolymers.

ACKNOWLEDGEMENTS

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GRAPHICAL ABSTRACT

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A library of thermoresponsive PEG-based methacrylate homopolymers: How do the molar mass and number of ethylene glycol groups affect the cloud point?

Ethylene glycol based methacrylate homopolymers have great potential in many biological applications. Here 27 homopolymers were synthesized and characterized in terms of their thermoresponsive properties. Interestingly the cloud point is not only affected by the number of ethylene glycol group and the molar mass but by also the terminal group on the ethylene glycol functional side group.