

## RESEARCH ARTICLE

# Liquid–liquid phase separation in aqueous solutions of poly(ethylene glycol) methacrylate homopolymers

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**Abstract**

Here, the liquid–liquid phase separation (LLPS) in aqueous solutions containing poly(ethylene glycol) (PEG) methacrylate homopolymers is reported for the first time. In this study, the thermoresponse of concentrated solutions of DEGMA<sub>60</sub> (two ethylene glycol, EG, groups) TEGMA<sub>71</sub> (three EG groups), OEGMA<sub>300<sub>x</sub></sub> (4.5 in average EG groups) of varying molar masses (MM), and OEGMA<sub>500<sub>28</sub></sub> (nine in average EG groups) is discussed. Interestingly, the temperature of LLPS ( $T_{LLPS}$ ) is controlled by the length of the PEG side chain, the MM of the OEGMA<sub>300<sub>x</sub></sub> and the polymer concentration. More specifically, the transition temperature decreases with: (i) Decrease in the length of the PEG side chain, (ii) increase in MM of the OEGMA<sub>300<sub>x</sub></sub>, and increase in concentration. In addition, LLPS is also observed in mixtures of OEGMA<sub>300<sub>x</sub></sub> with Pluronic<sup>®</sup> F127. In conclusion, these systems present a thermally induced LLPS, with the transition temperature being finely tuned to room temperature when DEGMA is used. These systems find potential use in numerous applications, varying from purification to “water-in-water” emulsions.

**KEYWORDS**

aqueous two-phase system, liquid–liquid phase separation, poly(ethylene glycol) methacrylate, thermoresponsive

## 1 | INTRODUCTION

Liquid–liquid phase separation (LLPS) is a process during which solutions of macromolecules phase separate into two liquid phases which differ in the concentration of (some of) their components.<sup>1</sup> LLPS is highly important in biology as it is responsible for the formation of the different compartments inside a cell.<sup>2</sup> Synthetic macromolecules mimic their biological counterparts by presenting LLPS when in aqueous solutions. LLPS is also referred in the literature as aqueous two-phase systems (ATPS), and it finds utility in the purification of biological macromolecules,

such as proteins and nucleic acids,<sup>3–6</sup> in the formation of “water-in-water” emulsions,<sup>5,7</sup> in liquid–liquid extraction using two aqueous phases,<sup>6,8</sup> in porous membrane formation,<sup>9</sup> and in the synthesis of membranellar organelles.<sup>10</sup> LLPS offers the advantage of avoiding the use of oil/organic phase.

LLPS can be categorized depending on the number and chemical nature of the components being present in the blend. The simpler case of LLPS is when a dense phase, that is, a phase in which the concentration of a specific macromolecule is high, and a dilute phase, that is, a phase with low concentration of the same macromolecule, are

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formed. As an example, a polymer solution under the appropriate conditions might phase separate by presenting LLPS, thus a polymer-rich phase (bottom phase), and a polymer-lean (solvent-rich) phase (top phase) are formed. This transition takes place when the water–water and molecule–molecule interactions are energetically favorable over the water–molecule interactions.<sup>1</sup> A well-studied type of LLPS is when polymer/salt mixtures<sup>4,11</sup> and surfactant/salt mixtures<sup>12</sup> are concerned, including ionic liquids, that is, salts in the liquid state,<sup>13</sup> with PEG/salt solutions being the most popular. In this case, a polymer-rich and a salt-rich phase are formed when LLPS takes place. A third category of LLPS is when mixtures of two incompatible polymers,<sup>7</sup> such as poly(ethylene glycol) (PEG) and dextran,<sup>4,7</sup> separate into two phases, which are both polymer rich, with each polymer being dominant in one of the phases.

As previously mentioned, LLPS has been well-reported in aqueous solutions of PEG and salts.<sup>3,14–17</sup> This has been either thermally-induced, that is, by increasing the temperature, or salt-induced, that is, by increasing the salt concentration at room temperature. In one of the studies, concentrated solutions (50 w/w%) of PEG homopolymers with MM around 1000 g mol<sup>-1</sup> and 4000 g mol<sup>-1</sup> mixed with sodium citrate (30 w/w%) and ammonium sulfate (40 w/w%) presented LLPS.<sup>17</sup> It has generally been observed that LLPS is favored as the concentration of the salt increases<sup>16</sup> and as the MM of PEG increases.<sup>14–16</sup> Sodium chloride and propionic acid sodium salt were also added to aqueous PEG solutions of MM varying from 2180 to 719,000 g mol<sup>-1</sup>, and LLPS was thermally induced.<sup>16</sup> However, it should be noted that this thermally-driven LLPS was observed at temperatures close to or higher than 100 °C.<sup>16</sup> Similarly, Ferreira et al. observed that the higher the salt concentration, the lower the polymer concentration needed for LLPS, when studying systems containing PEG with MM 8000 g mol<sup>-1</sup>, sodium sulphate, sodium chloride, and potassium chloride.<sup>18</sup> Wysoczanska and Macedo have come to similar conclusions by testing solutions of PEG with MM varying from 4000 to 8000 g mol<sup>-1</sup>, with potassium citrate and potassium sodium tartrate as additives, with the higher MM PEG favoring LLPS.<sup>14</sup>

LLPS has also been reported in systems other than PEG. Modeling studies on the LLPS of aqueous solutions consisting of *N*-isopropionamide were performed by Mochizuki et al.<sup>19</sup> In this study, spontaneous LLPS was observed using molecular dynamic simulations, with the solution destabilizing as the temperature was approaching the point of thermoresponse. It was concluded that the contribution of the polymer aggregation becomes dominant over the contribution of the separation of the polymer chains from the water molecules, as the polymer concentration decreases. Da Vela et al. have studied the kinetics of

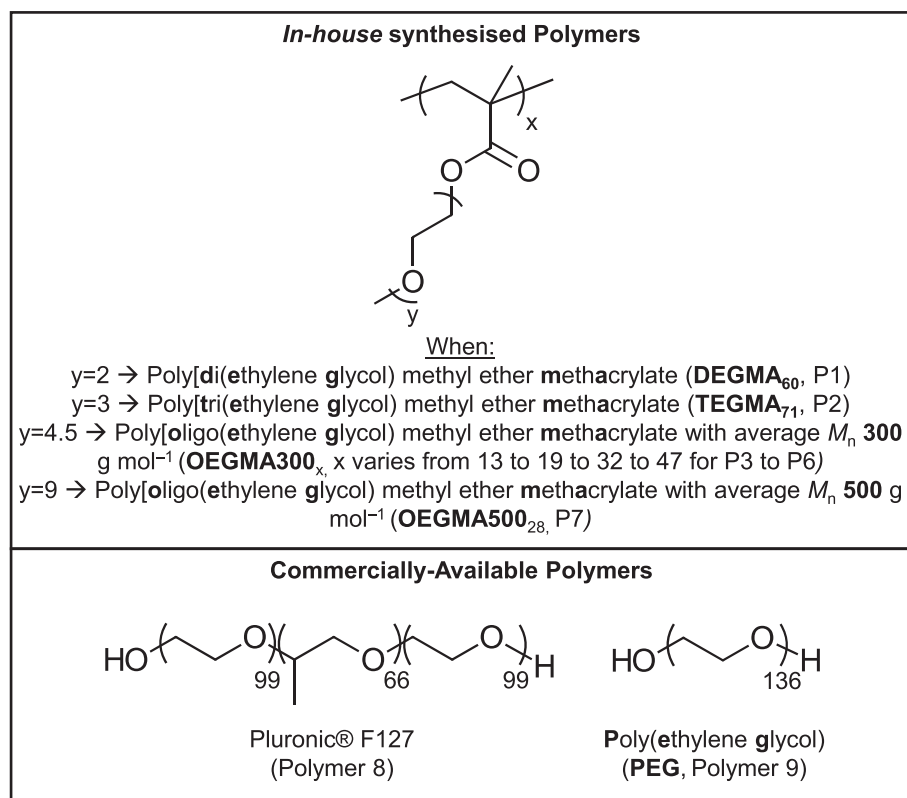
LLPS on protein solutions exhibiting lower critical solution temperature (LCST).<sup>20</sup> This study was performed via a combination of ultra-small-angle x-ray scattering and very-small-angle neutron scattering.<sup>20</sup> Phase separation in mixtures of poly(ethyl glycidyl ether) with an ionic liquid, namely 1-ethyl-3-methylimidazolium bis(trifluoromethane sulfonyl) amide,<sup>21</sup> as well as hyperbranched polyglycerol bearing imidazolium salt<sup>22</sup> were also reported. LLPS induced by changes in the pH and salt concentration has also been reported by Patrickios et al. in aqueous systems containing poly(vinyl alcohol) and methacrylic polyampholytes.<sup>23</sup> In addition, Khutoryanskaya et al. have observed LLPS on random copolymers of 2-hydroxyethyl methacrylate and 2-hydroxyethyl acrylate.<sup>24</sup>

Here, to the best of our knowledge, we report for the first time LLPS in concentrated aqueous solutions of PEG-based methacrylate homopolymers and their mixtures with Pluronic<sup>®</sup> F127, a commercially available thermoresponsive polymer, used as a thermogelling, foaming and de-foaming agent, injectable gel, and 3-D printable material.<sup>25–31</sup> Even though the LCST, often used interchangeably with the term “cloud point (CP),” defined as the temperature at which the solution turns from transparent to cloudy, has been extensively observed in the past, the LLPS in such systems has not been previously reported. Several PEG-based methacrylate homopolymers were tested, including di(ethylene glycol) methyl ether methacrylate (DEGMA), tri(ethylene glycol) methyl ether methacrylate (TEGMA), oligo(ethylene glycol) methyl ether methacrylate with average  $M_n$  300 g mol<sup>-1</sup> (OEGMA300) of various MM, and oligo(ethylene glycol) methyl ether methacrylate with average  $M_n$  500 g mol<sup>-1</sup> (OEGMA500), Figure 1 and Table 1. A PEG homopolymer was tested for comparison. Mixtures of Pluronic<sup>®</sup> F127 with OEGMA300<sub>x</sub> were also tested and presented LLPS. The trends are summarized and discussed below.

## 2 | RESULTS AND DISCUSSION

### 2.1 | Phase transitions in concentrated aqueous solutions

As previously mentioned, seven *in-house* synthesized PEG-based methacrylate polymers were used, specifically, one DEGMA homopolymer (DEGMA<sub>60</sub>, P1), one TEGMA homopolymer (TEGMA<sub>71</sub>, P2), four OEGMA300<sub>x</sub> homopolymers of various MM values (P3 to P6), and one OEGMA500 homopolymer (OEGMA500<sub>28</sub>, P7). In addition, two commercially available PEG-based polymers were used in this study, namely Pluronic<sup>®</sup> F127 (P8), which is a thermogelling polymer, and a PEG homopolymer (P9). These polymers were investigated in concentrated solutions



**FIGURE 1** Chemical structures, names, and abbreviations of the polymers used in this study

No.	Manufacturer	Polymer structure <sup>a</sup>	$M_n$ (g mol <sup>-1</sup> )	$\mathcal{D}$
P1	<i>In-house</i> <sup>i</sup>	DEGMA <sub>60</sub>	11,200	1.13
P2		TEGMA <sub>71</sub>	16,400	1.08
P3		OEGMA300 <sub>13</sub>	3900	1.16
P4		OEGMA300 <sub>19</sub>	5800	1.16
P5		OEGMA300 <sub>32</sub>	9800	1.18
P6		OEGMA300 <sub>47</sub>	14,100	1.21
P7		OEGMA500 <sub>28</sub>	14,000	1.14
P8	Sigma Aldrich	EG <sub>99</sub> - <i>b</i> -PG <sub>66</sub> - <i>b</i> -EG <sub>99</sub>	12,600 <sup>b</sup>	c
P9	SERVA Electrophoresis	EG <sub>136</sub>	6000 <sup>b</sup>	c

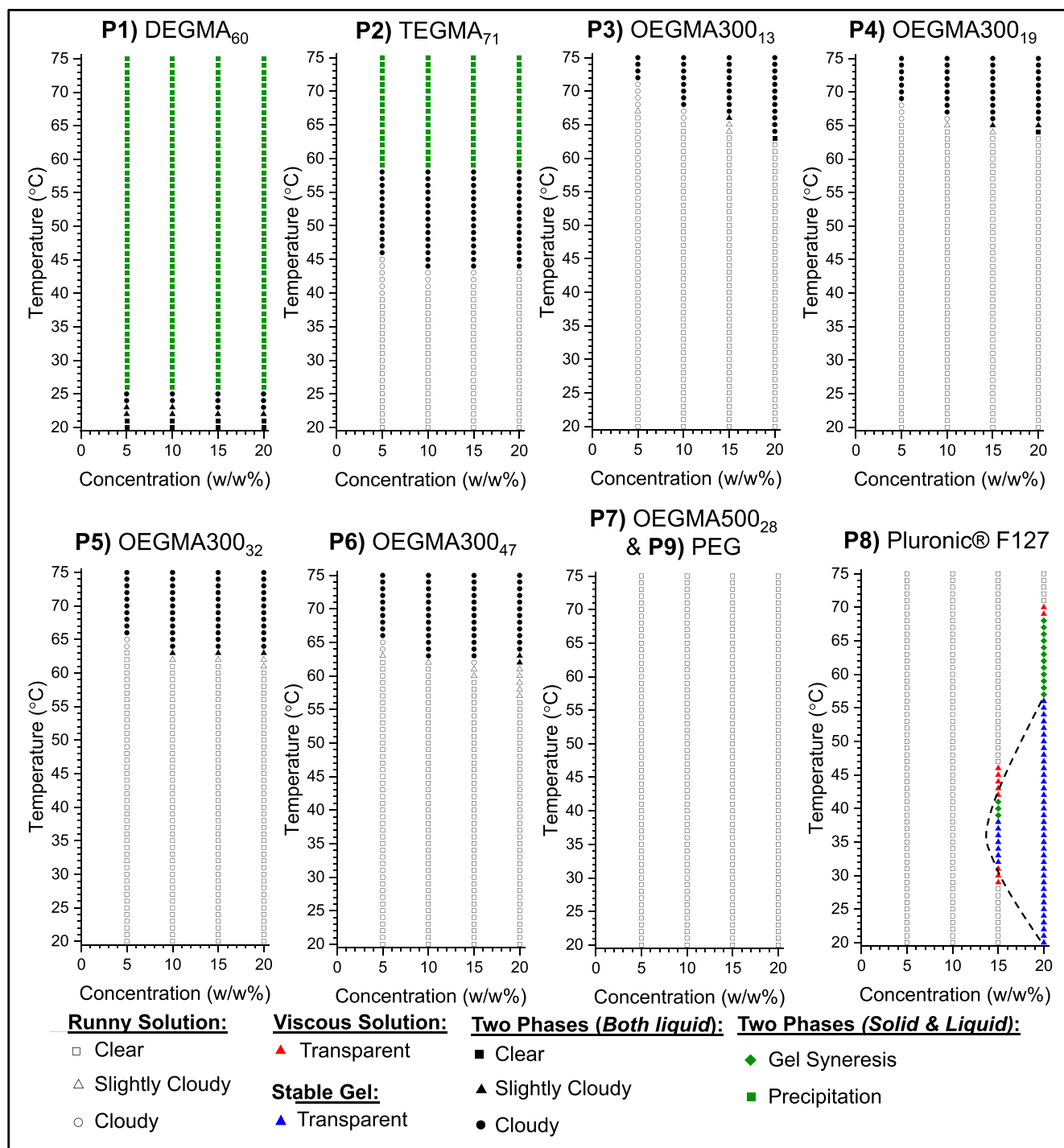
**TABLE 1** Experimental polymer structures, and experimental molar mass (number-average molar mass,  $M_n$ ) and dispersity indices, where available

*Note:* The superscript letter indicates the experimental polymer structures of the *in-house* synthesized polymers denote the experimental degrees of polymerization, as calculated by using the experimental molar mass and composition values, resulted by gel permeation chromatography (GPC) and proton nuclear magnetic resonance (<sup>1</sup>H NMR) spectroscopy, respectively. The GPC was in THF and six well-defined poly(methyl methacrylate) standard samples (2, 4, 8, 20, 50 and 100 kDa) were used for the calibration.

in phosphate buffered saline, Figure 2. They were inspected visually for the state of the sample, for example, liquid and the cloudiness, Figure 3.

The concentrated solutions of the homopolymers did not form a gel, as expected, Figure 2. Thus, the samples were only observed visually for any changes regarding their homogeneity and transparency/cloudiness that is, no tube inversion. Concerning the methacrylate homopolymers with varying length of the PEG side chain, the thermoresponse is observed at higher temperatures as the length of the PEG

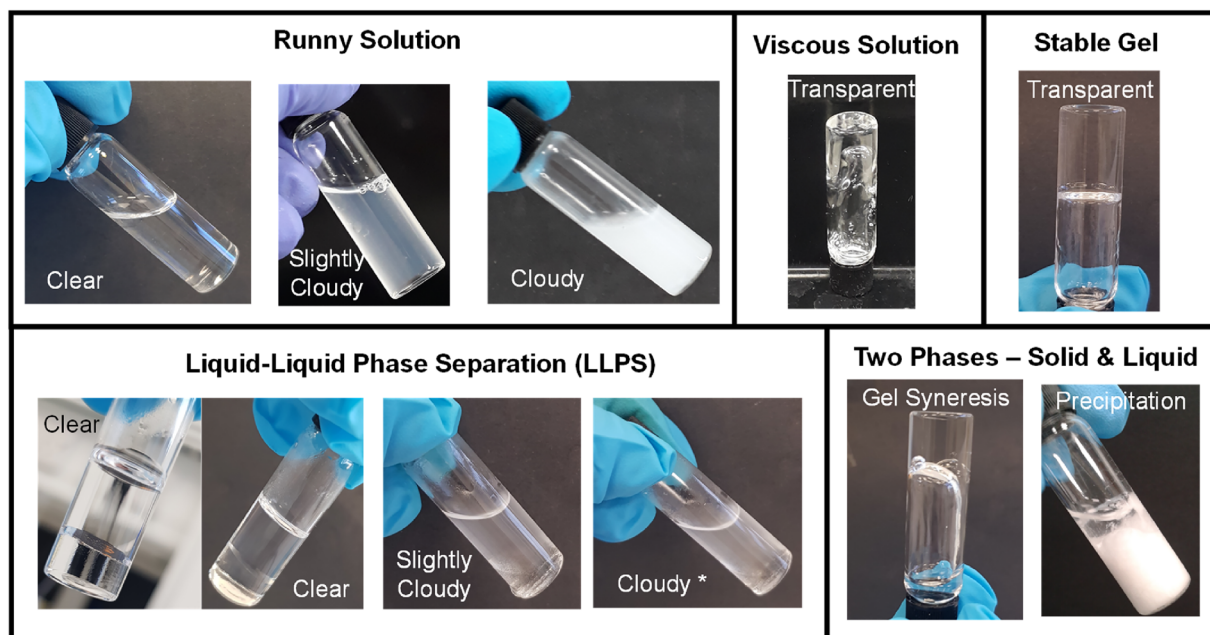
side chain and thus the hydrophilicity increases, with the OEGMA500<sub>28</sub> being too hydrophilic to respond to temperature. In addition, DEGMA<sub>60</sub> and TEGMA<sub>71</sub> precipitate out of solution as the temperature is increased, which is not the case for OEGMA300<sub>x</sub>, due to the increased hydrophilicity of its structure. Interestingly, LLPS was observed for all the ones which presented thermoresponse, which becomes more pronounced as the content in polymer increases. Notably, the DEGMA<sub>60</sub> solutions are in this state that is, LLPS, at 20 °C, however when at 5 °C, only the 20 w/w% solution



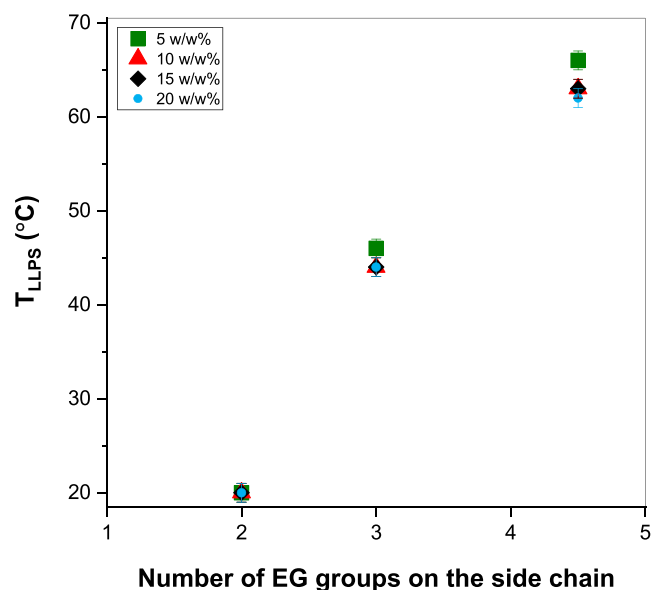
**FIGURE 2** Phase diagrams of DEGMA<sub>60</sub> (P1), TEGMA<sub>71</sub> (P2), OEGMA<sub>300</sub><sub>x</sub> (P3-P6), OEGMA<sub>500</sub><sub>28</sub> (P7), Pluronic® F127 (P8), and PEG (P9) in phosphate buffered saline (PBS). The following transitions are reported: (A) Runny solution state in white (clear: Square, slightly cloudy: Triangle, and cloudy: Circle), (B) Viscous solution state in red (transparent: Triangle and cloudy: Circle), (C) Stable gel in blue (transparent: Triangle and cloudy: Circle), (D) LLPS in black (clear: Square, slightly cloudy: Triangle, and cloudy: Circle), and (E) Phase separation into insoluble solid and supernatant liquid in green (gel syneresis: Rhombus and precipitation: Square). The gelation area of Pluronic® F127 is approximately indicated by black dashed line. Note that (P7) and (P9) reported the same phase diagram at the temperatures and concentrations tested

is in this state, while the less concentrated solutions present only one layer (data not presented in the phase diagram). This process is repeatable under thermal cycling, that is, homogeneous solution, followed by LLPS and precipitation

are observed upon heating, while a transition from precipitation to LLPS to homogeneous solution is observed upon cooling, Figure S1. Interestingly, the temperature at which LLPS ( $T_{LLPS}$ ) is observed strongly depends on the length of



**FIGURE 3** Pictures showing the phase transitions detected during the visual tests. \*The solution was cloudy when in the water-bath and the LLPS could not be detected, that is, both liquid phases were totally cloudy. However, the solution faded quickly as soon as removed from the water-bath, and at this point the two phases could be identified. In the picture the cloudiness has faded thus the solution appears slightly cloudy with LLPS



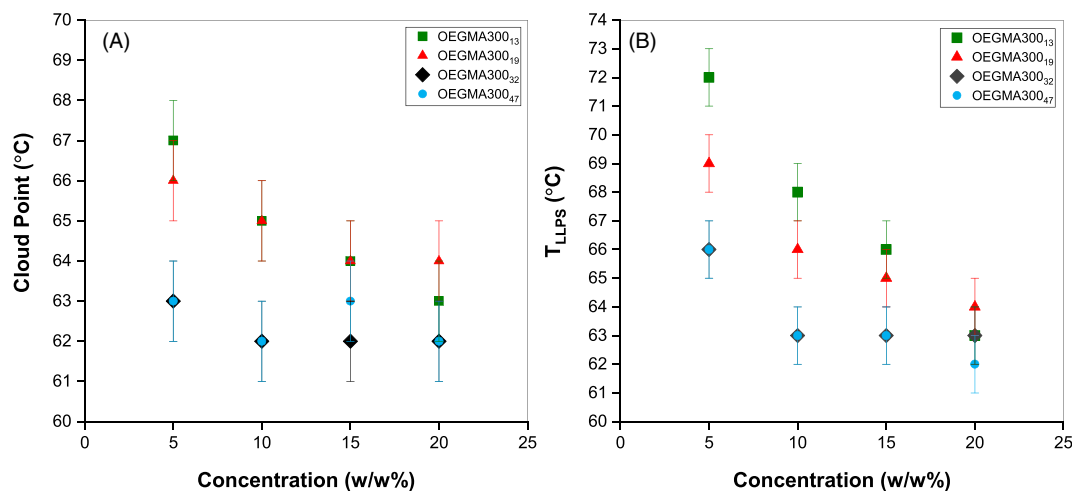
**FIGURE 4** Temperature of liquid–liquid phase separation ( $T_{LLPS}$ ) as a function of the number of the EG groups on the side chain of the methacrylate homopolymers. The results at different concentrations are shown in green squares (5 w/w%), red triangles (10 w/w%), black rhombi (15 w/w%), and light blue circles (20 w/w%)

the PEG side chain, Figure 4. More specifically, while the DEGMA<sub>60</sub> solutions present LLPS at room temperature, the  $T_{LLPS}$  increases to 44–46 °C (concentration dependent) for TEGMA<sub>71</sub> to 62–66 °C (concentration dependent) for

OEGMA300<sub>47</sub>, which is the one with comparable total molar mass. This is attributed to the increased hydrophilicity of the structure as there are more EG groups. On the other hand, in most of the cases, the polymer concentration does not affect significantly the  $T_{LLPS}$ , as it will be discussed in more detail in the following paragraphs.

The effects of the degree of polymerization of OEGMA300<sub>x</sub> and the concentration on the CP are presented in Figure 5A. As can be seen, the concentration does not affect the CP, within the error of the ultraviolet–visible (UV–Vis) experiment, similarly to the polymers of shorter side chains, namely DEGMA<sub>60</sub> and TEGMA<sub>71</sub>. As the experiments were performed at relatively high concentrations, the data points might be close to the local minimum of the well-established in the literature curve of the cloud point versus concentration, that is, close to the LCST point. It should be reminded that the LCST is defined as the minimum point in this curve, even though the terms LCST and CP are often used interchangeably. On the other hand, by increasing the DP, the CP decreases, as it has been previously observed for thermoresponsive homopolymers at low concentrations (normally ~1 w/w%).<sup>32–36</sup> This trend is more pronounced at low concentrations (up to 15 w/w%) as at 20 w/w%, the CPs are equal within the experimental error ( $\pm 1^\circ\text{C}$ ).

Both the concentration and the DP of OEGMA300<sub>x</sub> affect the  $T_{LLPS}$ , Figure 5B, as  $T_{LLPS}$  decreases with increased concentration and DP.



**FIGURE 5** Trends identified on the OEGMA300<sub>x</sub> homopolymer solutions in phosphate buffered saline (PBS): (A) Cloud point (CP) by UV-vis as a function of polymer concentration, and (B) Temperature of LLPS ( $T_{LLPS}$ ) by visual tests as a function of polymer concentration. The effect of the degree of polymerization (DP) on the CP and  $T_{LLPS}$  are shown as follows: OEGMA300<sub>13</sub> in green squares, OEGMA300<sub>19</sub> in red triangles, OEGMA300<sub>32</sub> in black rhombi and OEGMA300<sub>47</sub> in light blue circles

Similar to the effect on the CP (Figure 5A), the differences are less pronounced (i) at high concentration, as at 20 w/w%, the  $T_{LLPS}$  values are equal within the experimental error, and (ii) at DP between 32 and 47, as the two  $T_{LLPS}$  values are equal at any concentration. Therefore, it can be concluded that the CP and  $T_{LLPS}$  decrease as a function of both concentration and DP, until a plateau is reached, above which, further increase does not influence the phase behavior. This may be attributed to the increased hydrophobicity of the structure when longer OEGMA300<sub>x</sub> chains are studied, which moves the local minimum to lower concentration values, thus the  $T_{LLPS}$  values detected are similar. This may also explain the independence of the  $T_{LLPS}$  on the concentration in the case of DEGMA<sub>60</sub> and TEGMA<sub>71</sub>, as the polymers are undoubtedly more hydrophobic than OEGMA300<sub>x</sub>, thus the values balance around the local minimum of the curve.

As a comparison, solutions of a TEGMA homopolymer with lower MM than TEGMA<sub>71</sub>, specifically, TEGMA<sub>24</sub> (not included on the table), were tested for LLPS. The  $T_{LLPS}$  was observed at around 46–48 °C, concentration dependent, which is slightly higher than the  $T_{LLPS}$  of the TEGMA<sub>71</sub> solutions (44–46 °C). This confirms that the  $T_{LLPS}$  decreases with increased DP of the polymer.

LLPS was also observed in concentrated OEGMA300<sub>x</sub> solutions in DI water, Figure S2. It is generally observed that the transitions are presented at higher temperatures in DI water compared to PBS, as expected, due to the ionic strength effect.<sup>37–39</sup>

The temperature-induced LLPS indicates a polymer-rich and a solvent-rich (polymer-lean) phase. Similar observations have been previously made in PEG solutions,<sup>3,14–18,40</sup> as discussed in the introduction, in

which the LLPS was either thermally induced, that is, by heating up the solution or salt-induced that is, by adding salts to induce LLPS at room temperature. Nevertheless, to the best of our knowledge, this is the first time that this behavior is reported in PEG-based methacrylate homopolymer solutions. Interestingly, in the case of PEG-based methacrylate homopolymer solutions, this transition is observed in deionized water, Figure S2 that is, in the absence of any salt additive, while when decreasing the length of the PEG side chain from 4.5 to 2, the LLPS is observed at room temperature.

To investigate the LLPS of OEGMA300<sub>x</sub> solutions, <sup>1</sup>H NMR technique was implemented. More specifically, the 20 w/w% OEGMA300<sub>47</sub> in PBS was heated to the appropriate temperature and LLPS was induced. The two liquid phases were immediately separated, and the samples were subjected to freeze-drying, which revealed a different morphology, with the bottom phase being transparent viscous liquid, while the top phase was a white powder. The morphology agrees with the hypothesis of a polymer-rich bottom phase and solvent-rich top phase, as OEGMA300<sub>47</sub> is a transparent viscous liquid, while the PBS salts are white powder. In addition to this observation, the bottom phase was soluble in deuterated chloroform, while the top phase was not, thus analysis in deuterated water was performed. <sup>1</sup>H NMR analysis (Figures S3, S4) confirmed that both phases contain OEGMA300<sub>x</sub>, even though the intensity of the peaks corresponding to OEGMA300<sub>x</sub> was much lower in the top phase compared to the bottom one. Thus, <sup>1</sup>H NMR, in combination with the solubility and morphology differences support that the bottom phase was polymer-rich, while the top phase was solvent-rich.

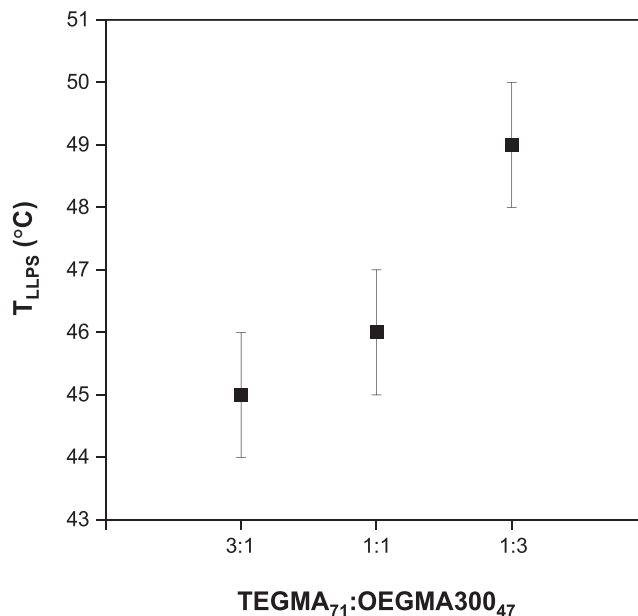
The concentrated solutions of the two commercially available polymers were also tested visually, Figure 2. The phase diagram of Pluronic® F127 presents a gelation area with critical gelation concentration at 15 w/w%, in high agreement with the literature.<sup>30,37,41,42</sup> The gel destabilizes at higher temperature, by returning to the solution phase. On the other hand, PEG<sub>136</sub> solutions in PBS showed no thermoresponse up to 80 °C, similarly to the solutions of Pluronic® F127 below 15 w/w%, which contrasts with the PEG-based methacrylate homopolymers. This difference can be attributed to the methacrylate backbone increasing the hydrophobicity of the structure, thus thermoresponse is detected at temperatures lower than 80 °C. This agrees with the previous studies on aqueous PEG solutions, in which thermoresponse was observed at high temperatures (CP ≈ 100–175 °C, depending on the MM),<sup>16</sup> as PEG is highly hydrophilic due to its high number of ether oxygens that along with the hydroxyl end groups enable significant hydrogen bonding with water.<sup>43,44</sup> The studies reporting LLPS in PEG solutions at lower temperatures, even at room temperature in some cases, concern either higher MM PEG polymers,<sup>3</sup> or highly concentrated polymer (50w/w%)<sup>14,17</sup> and salt solutions (at least 5 w/w%<sup>14</sup> or in the order of M,<sup>15</sup> as opposed to mM, which is in PBS).

## 2.2 | Phase transitions in concentrated aqueous solutions—Mixtures of homopolymers

Mixtures of TEGMA<sub>71</sub> and OEGMA<sub>300</sub><sub>47</sub>, which have comparable  $M_n$ , were investigated for LLPS. The total polymer concentration was kept constant at 20 w/w% in PBS, while the ratio of the TEGMA<sub>71</sub> to OEGMA<sub>300</sub><sub>47</sub> was varied from 1:3, to 1:1 to 3:1. As a reminder, TEGMA<sub>71</sub> solutions present LLPS around 44–46 °C, while the solutions of OEGMA<sub>300</sub><sub>47</sub> present LLPS around 62–66 °C. When mixed, LLPS was promoted when the temperature increased, above the CP, and the  $T_{LLPS}$  was controlled by the ratio of TEGMA<sub>71</sub> to OEGMA<sub>300</sub><sub>47</sub>, Figure 6. The LLPS of the mixture was presented at temperatures close to the LLPS of the TEGMA<sub>71</sub> solutions, with the  $T_{LLPS}$  increasing from 45 °C to 49 °C as the content in the hydrophilic OEGMA<sub>300</sub><sub>47</sub> increases. Therefore, these mixtures present LLPS in which both phases are polymer-rich, with the bottom phase consisting mainly of TEGMA<sub>71</sub>, while the top phase of OEGMA<sub>300</sub><sub>47</sub>.

## 2.3 | Phase transitions in concentrated aqueous solutions—Homopolymers as additives in solutions of Pluronic® F127

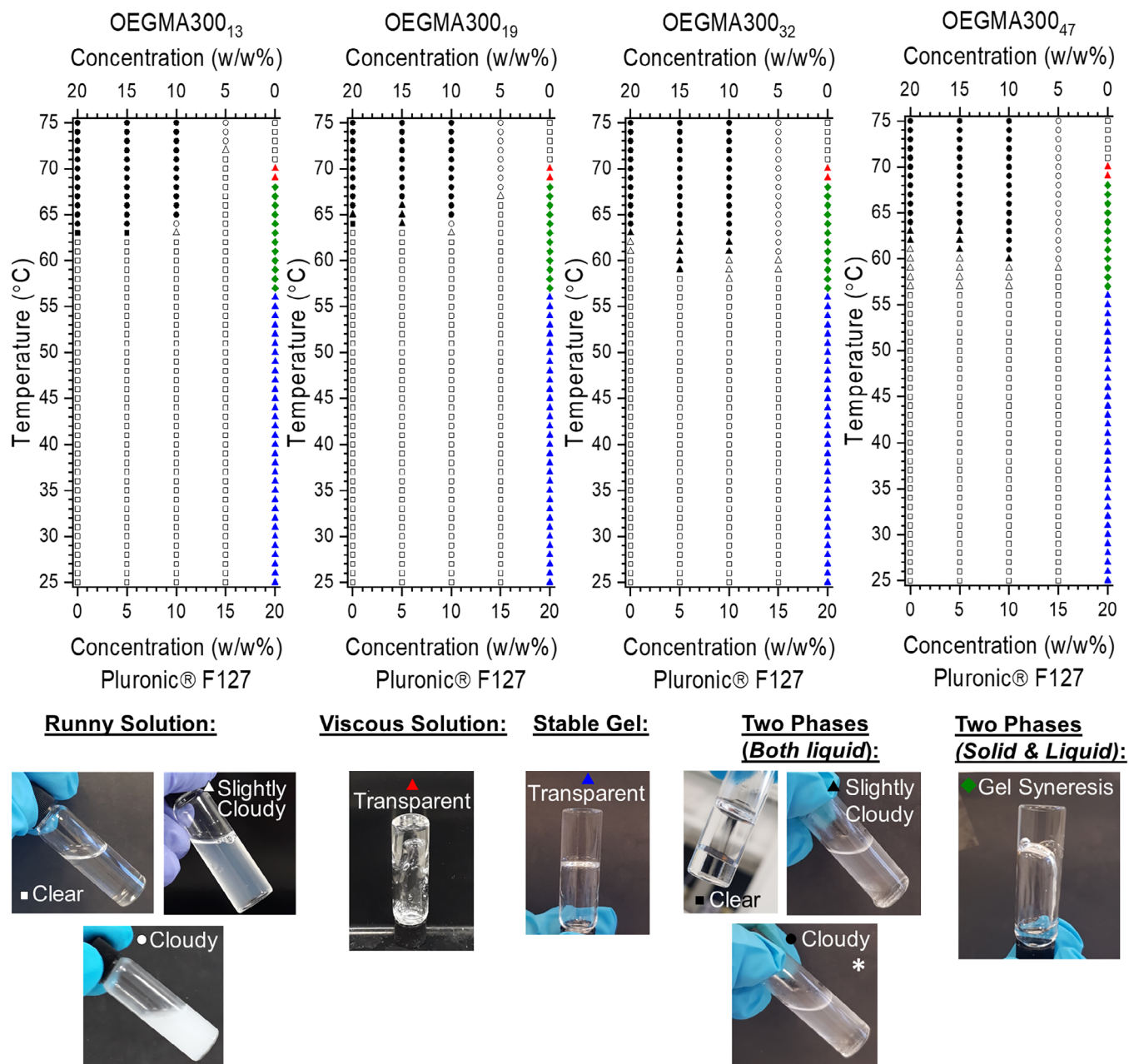
Mixtures of OEGMA<sub>300</sub><sub>x</sub> with Pluronic® F127 were also investigated, with total polymer concentration at 20 w/w



**FIGURE 6** Temperature of liquid–liquid phase separation ( $T_{LLPS}$ ) as a function of the ratio of TEGMA<sub>71</sub> to OEGMA<sub>300</sub><sub>47</sub>, with total polymer concentration equal to 20 w/w% in PBS

% in PBS, while the concentration ratio of Pluronic® F127/OEGMA<sub>300</sub><sub>x</sub> was varied from 1:3, 1:1, and 3:1, Figure 7. The transitions of 20 w/w% solutions of OEGMA<sub>300</sub><sub>x</sub> and Pluronic® F127 are also presented for comparison.

When OEGMA<sub>300</sub><sub>x</sub> was added in aqueous solutions of Pluronic® F127, this abates the gelation of Pluronic® F127 and interestingly, LLPS is observed, similarly to the OEGMA<sub>300</sub><sub>x</sub> in PBS. The higher the concentration in OEGMA<sub>300</sub><sub>x</sub> within a formulation, the more distinct the LLPS was. This might explain the lack of this transition in the 15 w/w% Pluronic® F127 || 5 w/w% OEGMA<sub>300</sub><sub>x</sub>, in which the content in OEGMA<sub>300</sub><sub>x</sub> was not high enough to promote LLPS, or the bottom phase, that is, OEGMA<sub>300</sub><sub>x</sub>-rich phase, was too small to be observed. In addition, variations in CP and  $T_{LLPS}$  with the DP of OEGMA<sub>300</sub><sub>x</sub> were observed, Figure 8. The effect of the concentration ratio is also presented: (i) 5 w/w% OEGMA<sub>300</sub><sub>x</sub> || 15 w/w% Pluronic® F127 in green squares (if any), (ii) 10 w/w% OEGMA<sub>300</sub><sub>x</sub> || 10 w/w% Pluronic® F127 in red triangles, (iii) 15 w/w% OEGMA<sub>300</sub><sub>x</sub> || 5 w/w% Pluronic® F127 in black rhombi, and (iv) 20 w/w% OEGMA<sub>300</sub><sub>x</sub> in light blue circles. A clear trend can be established regarding the 5 w/w% OEGMA<sub>300</sub><sub>x</sub> || 15 w/w% Pluronic® F127, the CP of which decreased from 73 °C to 60 °C as the DP increased from 13 to 47. At higher OEGMA<sub>300</sub><sub>x</sub> concentrations, this difference becomes insignificant, with CP being at ≈ 63 °C. Therefore, it is concluded that at low concentrations of OEGMA<sub>300</sub><sub>x</sub>, increasing the MM decreases the CP, while this is not the case at high concentrations OEGMA<sub>300</sub><sub>x</sub>. Concerning the LLPS, when OEGMA<sub>300</sub><sub>13</sub> and OEGMA<sub>300</sub><sub>19</sub> are



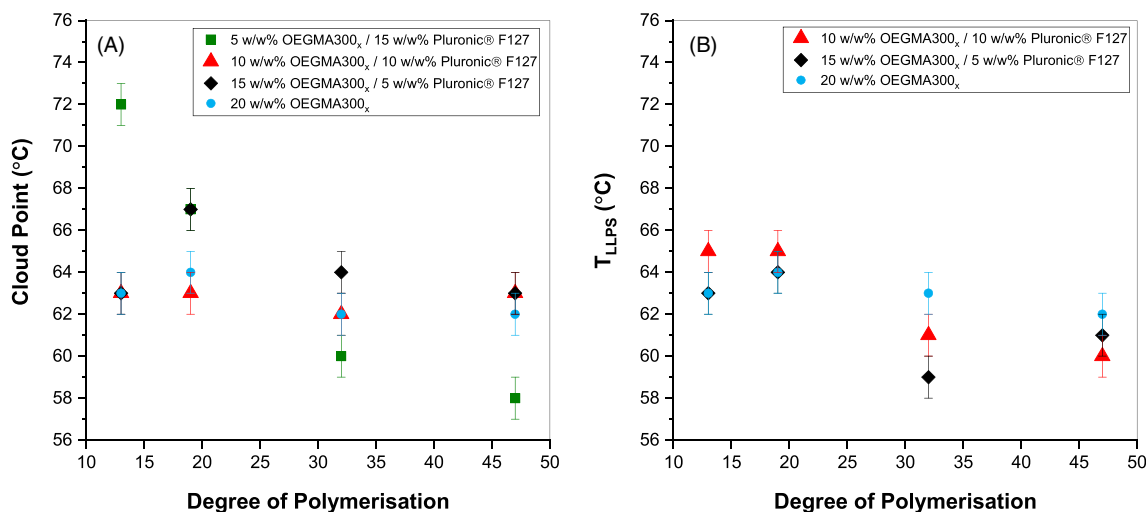
**FIGURE 7** Phase diagrams in phosphate buffered saline (PBS) of mixtures of Pluronic® F127 (P7) with OEGMA300<sub>13</sub> (P3), OEGMA300<sub>19</sub> (P4), OEGMA300<sub>32</sub> (P5), and OEGMA300<sub>47</sub> (P6) from left to right. The mixtures were prepared at total polymer concentration 20 w/w% and a ratio of Pluronic® F127/ OEGMA300<sub>x</sub> equal to 1:3, 1:1, and 3:1. The phase transitions at 20 w/w% concentration of Pluronic® F127, OEGMA300<sub>13</sub>, OEGMA300<sub>19</sub>, OEGMA300<sub>32</sub>, and OEGMA300<sub>47</sub> in PBS without any polymeric additives are also presented for completion. The following transitions are reported: (A) runny solution state in white (clear: Square, slightly cloudy: Triangle, and cloudy: Circle), (B) transparent viscous solution state in red triangles, (C) Transparent stable gel in blue triangles, (D) LLPS in black (clear: Square, slightly cloudy: Triangle, and cloudy: Circle), and (e) Phase separation into insoluble solid and supernatant liquid in green (gel syneresis: Rhombus). Photographs of the different transitions are reported. \*The solution was cloudy when in the water-bath and the LLPS could not be detected, that is, both liquid phases were totally cloudy. However, the solution faded quickly as soon as removed from the water-bath, and at this point the two phases could be identified. In the picture the cloudiness has faded thus the solution appears slightly cloudy with LLPS

added in the mixture, the  $T_{LLPS}$  is detected at  $\approx 64$  °C, while the  $T_{LLPS}$  is slightly lower for the mixtures of OEGMA300<sub>47</sub> with Pluronic® F127.

The LLPS was investigated by <sup>1</sup>H NMR spectroscopy, similarly to the solutions of OEGMA300<sub>x</sub> in PBS. This

was applied in the 5 w/w% Pluronic® F127 || 15 w/w% OEGMA300<sub>47</sub> formulation, and upon freeze-drying the top phase existed as white powder, while the bottom phase was a transparent viscous liquid; the increased viscosity of the bottom phase indicated the presence of





**FIGURE 8** Trends identified on the mixtures of Pluronic® F127 (P8) and OEGMA300 homopolymers (P3-P6) in phosphate buffered saline (PBS) with total polymer concentration at 20 w/w%.: (A) Cloud point (CP) by UV-vis as a function of the degree of polymerization of OEGMA300<sub>x</sub>, and (B) temperature of LLPS ( $T_{LLPS}$ ) by visual tests as a function of the degree of polymerization of OEGMA300<sub>x</sub>. The effect of the concentration ratio of OEGMA300<sub>x</sub>/Pluronic® F127 is shown as follows: 5 w/w% OEGMA300<sub>x</sub>/15 w/w% Pluronic® F127 (ratio 3:1) in green squares, 10 w/w% OEGMA300<sub>x</sub>/10 w/w% Pluronic® F127 (ratio 1:1) in red triangles, and 15 w/w% OEGMA300<sub>x</sub>/5 w/w% Pluronic® F127 (ratio 1:3) in black rhombi. The transitions of the OEGMA300<sub>x</sub> solutions at 20 w/w% in PBS are also shown in light blue circles for completion

OEGMA300<sub>47</sub>. <sup>1</sup>H NMR analysis confirmed that the bottom phase only consisted of OEGMA300<sub>47</sub>, while the top phase consisted of both Pluronic® F127 and OEGMA300<sub>47</sub> at a mass ratio of Pluronic® F127 to OEGMA300<sub>47</sub> equal to 1.6 (molar ratio equal to 1.2) (see Figures S5, S6, S7 for the <sup>1</sup>H NMR spectra of the bottom phase, top phase, and Pluronic® F127, respectively). Therefore, it is qualitatively and quantitatively confirmed that the concentration of Pluronic® F127 in the top phase is higher than OEGMA300<sub>47</sub>, since the latter is mainly forming the bottom phase.

To investigate whether the disruption of the gel was caused by the incompatibility of the PEG and PPG chains of Pluronic® F127 and the PEG side chains of OEGMA300<sub>x</sub>, the phase transitions were investigated in mixtures of a PEG homopolymer, specifically EG<sub>132</sub> (Polymer 9). Similar to the OEGMA<sub>x</sub> investigations, the total polymer concentration was kept constant at 20 w/w% in PBS, while the concentration ratio of Pluronic® F127 to EG<sub>132</sub> was varied from 1:3, 1:1 and 3:1. All formulations containing PEG homopolymer remained transparent runny solutions up to 80 °C, Figure S8 and no thermoresponse was observed. Therefore, it is concluded that the use of PEG as polymeric additive in solutions of Pluronic® F127 prevented gelation for all concentrations and temperatures studied. When compared to the mixtures of OEGMA300<sub>x</sub> with Pluronic® F127, it is concluded that the methacrylate backbone on the OEGMA polymers enhances the incompatibility with Pluronic® F127, thus promoting LLPS, which is not the case for the PEG/Pluronic® F127 mixtures.

PEG homopolymers have been previously studied as polymeric additives in Pluronic® solutions,<sup>41,45–48</sup> with the disruption of gelation by the addition of PEG being consistent with what has been previously reported in the literature.<sup>45,46</sup> More specifically, it has been observed that the addition of PEG with  $MM \geq 2000 \text{ g mol}^{-1}$  increases the polydispersity in micelle size, which is attributed to the formation of micelle clustering. This micelle clustering is formed by the incorporation of sufficiently long PEG chains in the PEG shell of multiple micelles formed by Pluronic® F127, and thus it hinders the formation of well-defined micelles, and increases the gelation temperature. Accordingly, critical gelation concentration (CGC) has also been found to increase on addition of PEG with  $MM \geq 2000 \text{ g mol}^{-1}$  to solutions of Pluronic® F127. More specifically, the CGC of an aqueous solution of Pluronic® F127 increased from 15.2 to 21 w/w% following the addition of 5 w/w% PEG with  $MM = 6000 \text{ g mol}^{-1}$ . This corresponds with the lack of gelation observed in this study, as the 15 w/w% F127/5 w/w% PEG ( $M_n \approx 6000 \text{ g mol}^{-1}$ ) sample contained the highest concentration of F127 and was below the critical gelation concentration value observed by Ricardo et al.<sup>45,46</sup>

### 3 | CONCLUSION

In this study, the thermoresponsive properties of concentrated solutions of PEG-based methacrylate homopolymers have been investigated. More specifically, DEGMA<sub>60</sub>,

TEGMA<sub>71</sub>, OEGMA300<sub>13</sub>, OEGMA300<sub>19</sub>, OEGMA300<sub>32</sub>, OEGMA300<sub>47</sub>, and OEGMA500<sub>28</sub> were investigated. Interestingly, LLPS is reported for the first time in aqueous solutions of PEG-based methacrylate homopolymers and their mixtures with Pluronic® F127. This indicates the formation of a polymer-rich and a solvent-rich phase when the homopolymer solutions are concerned, while when mixtures of polymers present LLPS, then both phases are polymer-rich with a different polymer being dominant in each phase. Interestingly, the temperature at which LLPS occurs ( $T_{LLPS}$ ) is tuned by the length of the PEG side chain, with the transition decreasing from  $\approx 65$  °C to 20 °C as the degree of polymerization decreases from 4.5 (OEGMA300<sub>x</sub>) to 2 (DEGMA<sub>60</sub>). In addition, the temperature at which LLPS is observed in OEGMA300<sub>x</sub> solutions is controlled by the MM of the OEGMA300<sub>x</sub> and polymer concentration. Therefore, thermally induced LLPS in aqueous solutions of methacrylate PEG derivatives is reported, which can find potential use in several applications, such as purification, extraction and “water-in-water” emulsions.

## 4 | EXPERIMENTAL SECTION

### 4.1 | Materials

Sigma Aldrich Ltd., UK, was the provider of Pluronic® F127 ( $M_n \approx 12,600$  g mol<sup>-1</sup>,  $\approx 70\%$  EG), and phosphate buffered saline (PBS) tablets, while phosphate buffer saline (PBS 10 $\times$  solution) was purchased from Fischer Scientific UK Ltd, Loughborough, UK, and PEG homopolymer ( $M_n \approx 6000$  g mol<sup>-1</sup>) was purchased from SERVA Electrophoresis GmbH, Germany. The methacrylate homopolymers were *in-house* synthesized, via GTP, with their detailed synthesis reported elsewhere.<sup>32</sup> The structural properties of the commercially-available polymers are provided by the manufacturer, while the structural properties of the *in-house* synthesized polymers have been determined via gel permeation chromatography (GPC) and proton nuclear magnetic resonance (<sup>1</sup>H NMR) spectroscopy, Table 1.

### 4.2 | Sample Preparation

Concentrated solutions of the polymers in PBS were prepared and investigated (5, 10, 15, and 20 w/w%). Concentrated (20 w/w%) stock solutions of the polymers in PBS were prepared, and they were used to prepare the mixtures at a constant total polymer concentration at 20 w/w%. The mixtures were prepared with concentration ratio of additive 1: additive

2 equal to 1:3, 1:1 and 3:1, for example, 5w/w% additive 1 and 15 w/w% additive 2 for ratio 1:3.

### 4.3 | Visual tests

The visual tests were performed from 20 to 80 °C, with visual observations being recorded every 1 °C. This was performed using an IKA RCT basic stirrer hotplate, an IKA ETS-D5 temperature controller, and a continuously stirred water bath. Several phases were detected and recorded, as follows: (i) runny solutions (clear, slightly cloudy, and cloudy), (ii) transparent viscous solution, (iii) transparent stable gel, (iv) LLPS (clear, slightly cloudy, and cloudy), and (v) phase separation into insoluble solid and supernatant liquid (gel syneresis and precipitation), Figure 3.

### 4.4 | UV-Vis spectroscopy

UV-Vis spectroscopy was implemented to determine the cloud points (CP) of the concentrated solutions of OEGMA300<sub>x</sub> and the mixtures of OEGMA300<sub>x</sub> with Pluronic® F127. For this experiment, an Agilent Cary UV-Vis rate of 1 °C min<sup>-1</sup> while continuously stirring, in order to prevent the LLPS. The data were collected every 1 °C at 550 nm, and the CP was determined as the temperature at which the transmittance was 50%.

### 4.5 | <sup>1</sup>H-NMR spectroscopy

<sup>1</sup>H NMR spectroscopy was implemented to investigate the LLPS. In this experiment, two samples were analyzed: (i) 20 w/w% PEGMA<sub>47</sub> in PBS and (ii) mixture of 5 w/w% Pluronic® F127 and 15 w/w% PEGMA<sub>47</sub> in PBS. The samples were heated to induce LLPS and the two phases formed were separated. This resulted in four samples, namely top and bottom phase of 20 w/w% PEGMA<sub>47</sub>, and top and bottom phase of the mixture. The samples were subjected to freeze dry using a Labogene ScanVac CoolSafe freeze drier. The dried samples were dissolved in either deuterated chloroform (CDCl<sub>3</sub>) or deuterium oxide (D<sub>2</sub>O), and they were analyzed using a JEOL 400 MHz NMR spectrometer. The sample of Pluronic® F127 in CDCl<sub>3</sub> was also analyzed for comparison.

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## DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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