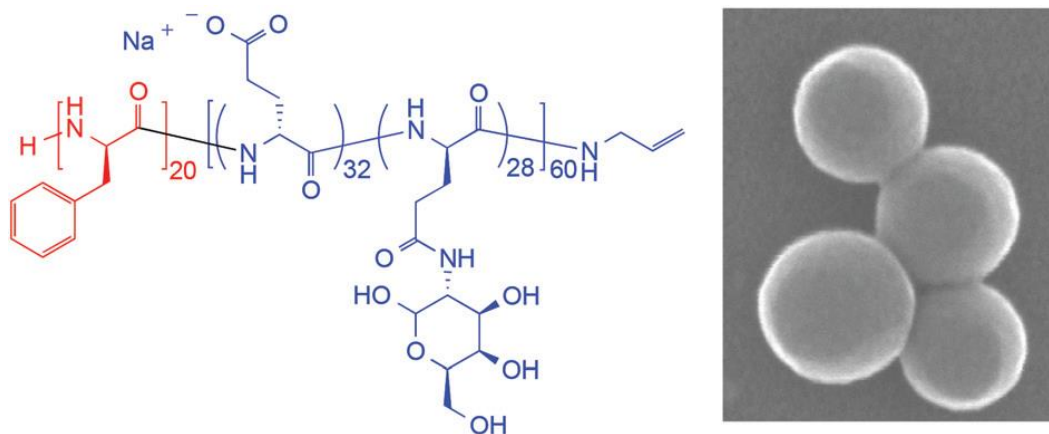


## Chapter 4

### Amphiphilic glycosylated block copolypeptides as macromolecular surfactants in the emulsion polymerization of styrene.



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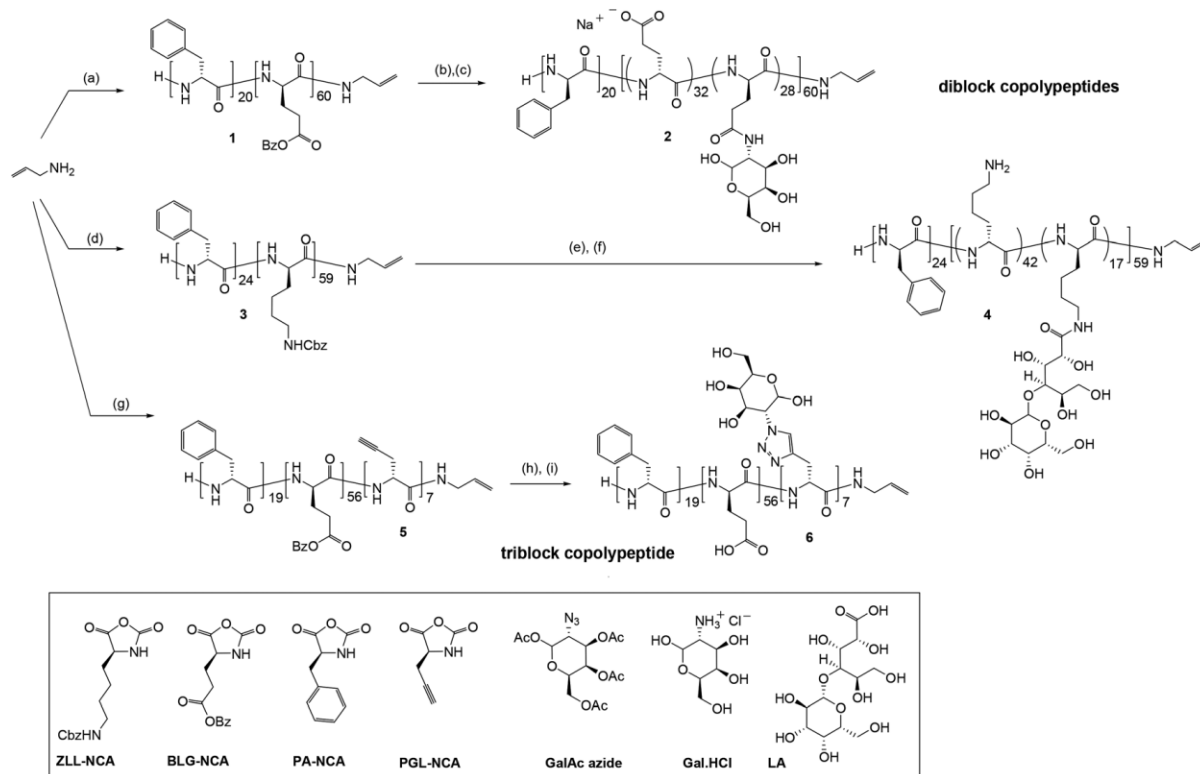
#### 4.1 Abstract

Diblock copolymers consisting of poly(L-phenyl alanine) and poly(benzyl-L-glutamate) or poly(CBZ-L-lysine), respectively, were synthesized via sequential NCA polymerization. After deprotection, subsequent partial glycosylation of the glutamic acid and lysine units with galactosamine hydrochloride or lactobionic acid yielded amphiphilic block copolypeptides. Moreover, a triblock copolymer poly(L-phenyl alanine-*b*-L-benzyl glutamate-*b*-propargylglycine) was obtained and glycosylated by 'click' chemistry. Glycosylated block copolypeptides showed improved water solubility and circular dichroism (CD) confirmed the pH dependence of the helix-coil transition. The block copolypeptides were found to be efficient stabilizers in the emulsion polymerization of styrene offering a facile method for the synthesis of polystyrene nanoparticles in the range of 100-140 nm depending on the block copolymer composition and emulsion concentration. This establishes an example of functional polymer additives fully based on renewable building blocks in nanomaterial synthesis.

## 4.2 Introduction

Over the last decade significant efforts have been made to introduce green approaches in polymer science. Prominent examples include polymers from renewable raw materials and bio-based feedstock.<sup>1-3</sup> Notably most of these renewable polymers are polycondensates and some of these materials have shown academic and industrial merit.<sup>4-7</sup> Another important class of monomers are acrylates and the corresponding polyacrylates are very versatile and adoptable to a range of applications due to their side chain functionality. A class of monomers similar in functionality yet fully bio-based is amino acids. Although it cannot be expected that poly(amino acids) can be a broad substitute for polyacrylics, they might be an alternative in some segments.

Significant advances in the controlled ring-opening polymerisation of amino acid N-carboxyanhydrides (NCA)<sup>8, 9</sup> has fuelled opportunities for the design of well-defined macromolecular structures such as block<sup>9-11</sup>, graft<sup>12</sup>, star (co)polymers<sup>13-16</sup>, polymer brushes<sup>17-19</sup>, etc., which shows parallels to the development in controlled radical polymerisation. While most authors propose such structures in the wider context of biomedical applications, we believe that there is scope in exploring poly(amino acid)s or synthetic polypeptides in non-medical applications. One such area is surface-active amphiphilic block copolymers as surfactants or emulsifiers. A few examples of amphiphilic block copolypeptides have been reported primarily with the aim to study their self-organisation into micelles and vesicles.<sup>20-22</sup> The use of polypeptides as macromolecular emulsifiers is largely unexplored despite the fact that proteins are the stabilizers in natural rubber latex.<sup>23</sup> Reported examples are generally limited to lower molecular weight analogues.<sup>24-28</sup> Only recently Deming has shown that it was possible to stabilize water-in-oil-in-water double emulsions using racemic amphiphilic copolypeptides prepared *via* NCA polymerisation.<sup>29</sup> Here we report glycosylated polypeptide block copolymers as efficient surfactants in emulsion polymerisation. This process was selected as it will provide not only information about the initial stability of an emulsion but also the ability of the amphiphilic block copolypeptides to provide emulsion stability throughout the demanding process of latex formation.



Scheme 4.1 Reagents and conditions: (a) BLG-NCA ( $[M]_0/[I]_0 = 60$ ); PA NCA DMF ( $[M]_0/[I]_0 = 20$ ), HV, 0 °C; (b) HBr (33 wt. % in AcOH), TFA; 0.5 M NaOH (aq); (c) GA.HCl, DMT-MM, DDI (d) ZLL-NCA ( $[M]_0/[I]_0 = 60$ ); PA NCA DMF ( $[M]_0/[I]_0 = 20$ ), HV, 0 °C (e) HBr (33 wt. % in AcOH), TFA; (f) LA, EDC/NHS, pH 4.7, 10 mM MES buffer; DDI (g) PGL NCA ( $[M]_0/[I]_0 = 7$ ); BLG-NCA ( $[M]_0/[I]_0 = 60$ ); PA NCA DMF ( $[M]_0/[I]_0 = 20$ ), HV, 0 °C; (h) GalAc, Cu(PPh<sub>3</sub>)<sub>3</sub>Br, Et<sub>3</sub>N, DMF, 30 °C; (i) HBr (33 wt. % in AcOH), TFA. I = allylamine, HV = high vacuum, DDI = distilled deionized water.

## 4.3 EXPERIMENTAL

### 4.3.1 Materials

All chemicals were purchased from Sigma-Aldrich and used as received unless otherwise noted.  $\gamma$ -Benzyl-L-glutamate and  $\epsilon$ -benzyloxycarbonyl-L-lysine were supplied by Bachem. Anhydrous DMF, chloroform, ethyl acetate, methanol were used directly from the bottle under an inert and dry atmosphere.  $\gamma$ -Benzyl-L-glutamate (BLG),  $\epsilon$ -benzyloxycarbonyl-L-lysine (ZLL), Phenyl alanine (PA) and DL-propargylglycine (PGL) NCA was synthesised following literature procedures.<sup>21, 30, 31</sup> 1- $\beta$ -Azido-2,3,4,6-tetraacetyl-D-galactose (GalAc) was synthesized following a literature procedure.<sup>32</sup>

### 4.3.2 Preparation of copolypeptides PBLG<sub>60</sub>-*b*-PPA<sub>20</sub> (1).

The NCA monomer of BLG (2.5 g, 9.5 mmol) was dissolved in 20 mL anhydrous DMF in a Schlenk tube. The solution was degassed *via* three successive freeze-pump-thaw cycles and kept under vacuum. The reaction flask was immersed in a 0 °C water bath and a solution of 12 µL allylamine in 1 mL anhydrous DMF ( $[M]_0/[I]_0 = 60$ ) was injected through a rubber septum with a syringe. The reaction was left to stir until the BLG NCA had been completely consumed as monitored by ATR-FTIR spectroscopy. The polypeptide was further chain extended *via* the introduction of phenyl alanine NCA (0.60 g, 3.1 mmol,  $[M]_0/[I]_0 = 20$ ) dissolved in 5 mL DMF. After full monomer conversion monitored via ATR FTIR spectroscopy the polymer was precipitated into an excess of diethyl ether, filtered and dried under vacuum. Isolated yield: 2.2 g.

Similar procedures were used to synthesize PZLL<sub>59</sub>-*b*-PPA<sub>24</sub> (3) and PGL<sub>7</sub>-*b*-PBLG<sub>56</sub>-*b*-PPA<sub>19</sub> (5).

### 4.3.3 Block copolypeptide deprotection.

A general procedure was used for the deprotection of both the PBLG and PZLL pendant groups. PBLG<sub>60</sub>-*b*-PPA<sub>20</sub> (2.0 g, 0.13 mmol) was dissolved in trifluoroacetic acid (20 mL). 4 mL of HBr solution (33 wt.% in acetic acid; 3-fold excess with respect to  $\gamma$ -benzyl-L-glutamate repeat units) was added slowly to the reaction at 0 °C. After 2 h, the solution was added to 300 mL diethyl ether and the precipitate washed three times with 50 mL diethyl ether. The subsequent product was dissolved in a 0.5M NaOH solution (aqua) to obtain the polymer in the sodium salt form. The sodium salt polymer was dialyzed against double deionized (DDI) water using Spectra/Por dialysis membranes (MWCO, 3.5 kDa) for 72 h at room temperature. The product PLGA<sub>60</sub>-*b*-PPA<sub>20</sub> was lyophilized and isolated as a white powder. Isolated yield: 1.4 g.

PZLL<sub>59</sub>-*b*-PPA<sub>24</sub> was deprotected in a similar fashion to yield PLL<sub>59</sub>-*b*-PPA<sub>24</sub>.

### 4.3.4 Glycosylation reactions

#### 4.3.4.1 (GA<sub>28</sub>-*r*-PLGA<sub>32</sub>)-*b*-PPA<sub>20</sub> (2)

Glycosylation of the carboxylic acid moieties was achieved according to a modified literature procedure.<sup>33</sup> PLGA<sub>60</sub>-*b*-PPA<sub>20</sub> (1.3 g, 0.12 mmol) and galactosamine hydrochloride (GA.HCl) (0.79 g, 3.7 mmol, 0.5 eq w.r.t. the glutamate repeating units) was dissolved in

deionized water (10 mL) and stirred for 15 min. 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) (1.2 g, 4.4 mmol, 1.2 eq. to Galactosamine HCl) was dissolved in 5 mL of deionized water and added to the reaction mixture. After stirring for 24 h at room temperature 2 mL of a 1M NaOH aqueous solution was added to the reaction mixture and stirred for 3 hours. The mixture was then dialyzed against DDI water using Spectra/Por dialysis membranes (MWCO, 3.5 kDa) for 72 h at room temperature. The polymer was lyophilized and isolated as a white powder. Isolated yield: 1.7 g.

#### 4.3.4.2 (LA<sub>17-r</sub>-PLL<sub>42</sub>)-b-PPA<sub>24</sub> (4)

A general procedure for 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC)/*N*-hydroxysulfosuccinimide (Sulfo-NHS) coupling was employed to attach lactobionic acid to the lysine residues of the amphiphilic copolyptide. Lactobionic acid (LA) (0.7 g, 2 mmol, 0.3 eq w.r.t lysine repeat units), EDC (0.45 g, 2.3 mmol, 1.2 eq w.r.t. the LA) and sulfo-NHS (50 mg, 0.23 mmol, 0.1 eq w.r.t. EDC) were dissolved in 3 mL 10 mM MES buffer (pH 4.7) and stirred for 20 minutes. The latter was added to a solution of PLL<sub>59</sub>-b-PPA<sub>24</sub> (1.2 g, 0.11 mol) in 10 mL of DDI water. The reaction mixture was stirred overnight. The resulting product was purified *via* dialysis against DDI water using Spectra/Por dialysis membranes (MWCO, 3.5 kDa) for 72 h at room temperature. The product was subsequently lyophilized and isolated as a white powder. Isolated yield: 1.5 g.

#### 4.3.4.3 GA<sub>7</sub>-b-PBLG<sub>56</sub>-b-PPA<sub>19</sub> (6)

Glycosylation was achieved via a modified procedure previously utilized in our group.<sup>21</sup> PGL<sub>7</sub>-b-PBLG<sub>56</sub>-b-PPA<sub>19</sub> (1.5 g, 0.66 mmol of alkyne units), 1-β-Azido-2,3,4,6-tetraacetyl-D-galactose 0.3 g, 0.8 mmol, 1.2 eq. to alkyne groups) and triethylamine (40 μL, 0.5 eq.) was dissolved in 20 mL of anhydrous DMF in a Schlenk tube. The mixture was stirred and degassed by bubbling nitrogen for 30 min. (PPh<sub>3</sub>)<sub>3</sub>CuBr (60 mg, 0.1 eq.) was then added and nitrogen was bubbled through the resulting solution for another 30 min. The Schlenk tube was placed in an oil bath at 30 °C for 24 h under nitrogen atmosphere. 100 mg of Amberlite® IR120 hydrogen form ion exchange resin was added and the suspension gently stirred at ambient temperature overnight. After filtration and centrifugation, the polymer solution was precipitated in a 3:2 THF/diethyl ether mixture and washed with THF. Isolated yield: 1.6 g. Subsequent deprotection as described above results in the simultaneous deprotection of the benzyl ester and tetraacetate groups to yield GA<sub>7</sub>-b-PLGA<sub>56</sub>-b-PPA<sub>19</sub>.

#### 4.3.5 Preparation of polystyrene latex via emulsion polymerization.

Styrene was polymerized using a similar method described previously.<sup>34</sup> Batch emulsion polymerizations were all carried out in a three-neck reactor equipped with a reflux condenser, nitrogen inlet and mechanical stirrer. A typical reaction proceeded as follows: GA<sub>28</sub>-*r*-PLGA<sub>32</sub>-*b*-PPA<sub>20</sub> (0.24 g) was added to the reactor under an inert atmosphere and dissolved in 38 mL of distilled water under stirring at 70 °C. The styrene monomer (4.8 g) was deoxygenated separately for 20 min by bubbling nitrogen through it and injected into the reactor. A deoxygenated initiator solution (50 mg of potassium persulfate in 2 mL of water) was injected to start the polymerization. The nitrogen flow was maintained throughout the reaction. Samples were withdrawn at regular times to determine the conversion. A small amount of hydroquinone was added to these aliquots to quench the radical polymerization where the monomer conversion was then determined gravimetrically.

#### 4.3.6 Methods.

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 400 (400 MHz) in DMSO-d<sub>6</sub> and CDCl<sub>3</sub> as solvents. All chemical shifts are reported in parts per million (ppm) with tetramethylsilane (TMS) as an internal reference. Attenuated Total Reflection (ATR) FTIR measurements were performed on a Perkin-Elmer Spectrum 100 instrument. Spectra were obtained from 4 scans with a resolution of 2 cm<sup>-1</sup> in the spectral region of 650 – 4000 cm<sup>-1</sup>. A background measurement was taken before the sample was loaded onto the ATR for measurement. Samples could be characterized in the liquid state without prior sample preparation. Size Exclusion Chromatography (SEC) was performed on an Agilent 1200 system in conjunction with two PSS GRAM analytical (8 x 300 and 8 x 100, 10 μ) columns, a Wyatt Dawn Heleos 8 multi angle light scattering (MALS) detector and Wyatt Optilab rEX differential refractive index (DRI) detector with a 658 nm light source. The eluent was DMF containing 0.1 M LiBr at a flow rate of 1 mL/min. The column temperature was set to 40 °C with the MALS detector at 35 °C and the DRI detector at 40 °C. Molar masses and dispersities were calculated from the MALS signal by the Astra software (Wyatt) using the refractive index increment ( $dn/dc$ ) as calculated relative to the ratio of the homopolymer. The  $dn/dc$  values used for the polymers were experimentally determined or as found in literature.<sup>9, 35</sup> All samples for SEC analysis were filtered through a 0.45 μm PTFE filter (13 mm, PP housing, Whatman) prior to injection. Dynamic light scattering (DLS) experiments were performed at 25°C on a Malvern NanoZS (Malvern Instruments, Malvern

UK) which uses a detection angle of 173°, and a 3 mW He-Ne laser operating at a wavelength of 633 nm. Field emission scanning electron microscopy (FESEM) images were obtained on a Hitachi S5500 scanning electron microscope. CD spectra were recorded on a Jasco J-715 spectropolarimeter at ambient temperature. A quartz cell with 0.1 cm path length was used. Spectra were recorded between 250 and 190 nm. Each spectrum represents the average of five measurements. A baseline was taken from the pure solvent and subtracted from the spectra. The spectra were smoothed using a Savitzky–Golay smoothing filter. For the pH-dependent measurements, a stock solution of the copolypeptide (c.a. 0.3 mg/mL) in 0.01 M NaCl solution was titrated with aqueous HCl or aqueous NaOH. Mean residue ellipticities were calculated using the equation  $[\Theta]_{\text{MRW}} = (\Theta_{\text{MRW}})/(10 \times c \times l)$  with experimental ellipticity  $\Theta$  in mdeg, mean residue weight  $M_{\text{MRW}}$  in g/mol, mass concentration  $c$  in mg/mL and path length  $l$  in cm.<sup>36</sup> Helicities  $f_{\alpha}$  were calculated from the mean residue ellipticities at  $\lambda = 222$  nm using the following equation:  $f_{222} = (-[\Theta_{222}]_{\text{MRW}} + 3000)/39000$  (1).

## 4.4 Results and discussion

### 4.4.1 Synthesis of glycosylated block copolypeptides

With the increasing interest in functional polymer nanoparticles, various strategies are available for preparing polymer colloids via block copolymer-stabilized emulsion polymerisation.<sup>37-39</sup> Previously we demonstrated that hybrid amphiphilic block copolymers comprising a hydrophilic glycosylated polypeptide block and a hydrophobic polystyrene block could efficiently stabilise styrene latex formation.<sup>34</sup> Using a polystyrene block, high compatibility with the styrene latex was achieved. For the purpose of utilizing polypeptide block copolymers as macromolecular stabilisers in this process, it was necessary to carefully select the hydrophobic polypeptide block. In order to obtain uniform polystyrene latex nanoparticles it is critically important that the hydrophobic block remains embedded in the latex particle throughout the polymerisation process.<sup>40</sup> Phenyl-L-alanine (PA) was chosen as the amino acid for this block due to the structural resemblance of the phenyl substituent with styrene.

Initially, two different diblock copolymers were synthesised by sequential NCA polymerisation from poly(benzyl-L-glutamate) (PBLG) and poly(Z-L-lysine) (PZLL), respectively (Scheme 4.1), i.e. PBLG<sub>60</sub>-*b*-PPA<sub>20</sub> (**1**) and PZLL<sub>59</sub>-*b*-PPA<sub>24</sub> (**3**) in DMF at 0 °C under vacuum using allylamine as an initiator.<sup>11</sup> Due to the insolubility of PPA in DMF it

was necessary to first prepare the PBLG or PZLL block to prevent precipitation. The monomer conversion was followed by ATR-FTIR and the PA-NCA was added after complete disappearance of the initial NCAs anhydride peaks ( $1850$  and  $1790\text{ cm}^{-1}$ ). An increase in molecular weight as well as narrow, monomodal distributions are evident from the SEC traces (Figure 4.1 and 4.2; Table 4.1) highlighting the successful synthesis of the block copolymers. Notably, the synthesis of these amphiphilic block copolypeptides is more straightforward than the previously reported synthesis of amphiphilic hybrid block copolymers<sup>34</sup>.

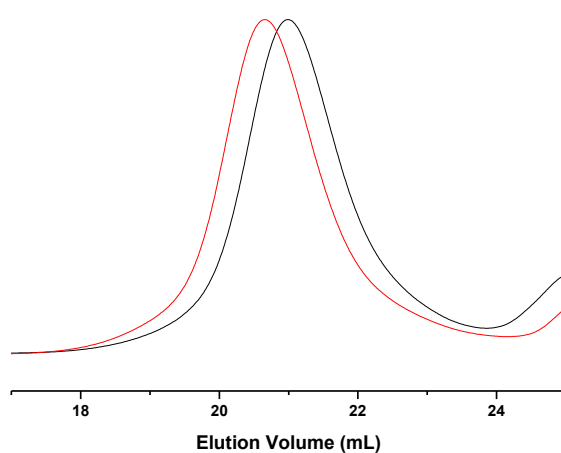


Figure 4. 1 SEC traces of PBLG<sub>60</sub> (red) and PBLG<sub>60</sub>-*b*-PPA<sub>20</sub> (**2**, black)

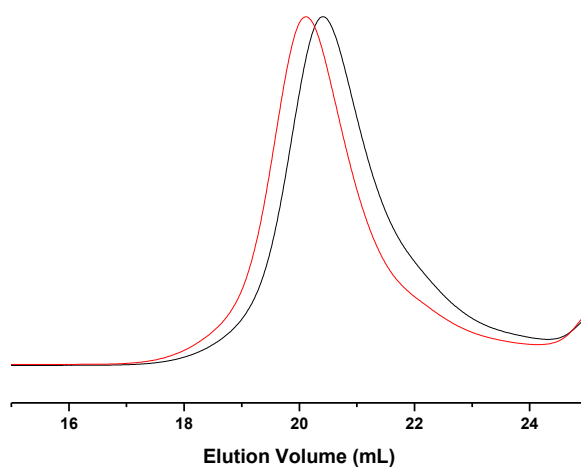


Figure 4. 2 SEC traces of PZLL<sub>59</sub> (black) and PZLL<sub>59</sub>-*b*-PPA<sub>24</sub> (**1**, red).

To enhance amphiphilicity, the deprotected poly(L-glutamic acid) (PGA) and poly(L-lysine) (PLL) blocks were glycosylated using previously reported strategies.<sup>33, 34</sup> The lysine residues of PLL<sub>59</sub>-*b*-PPA<sub>24</sub> were coupled with lactobionic acid (LA) using traditional EDC/NHS coupling chemistry (Scheme 4.1) targeting 30% glycosylation (LA<sub>17</sub>-*r*-PLL<sub>42</sub>-*b*-PPA<sub>24</sub>, **4**). The carboxylic acid residues of PLGA<sub>60</sub>-*b*-PPA<sub>20</sub> were converted to the sodium salt form and galactosamine hydrochloride attached using an aqueous amide coupling approach in the presence of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) (Scheme 1) targeting 50% glycosylation (GA<sub>28</sub>-*r*-PLGA<sub>32</sub>-*b*-PPA<sub>20</sub>, **2**). <sup>1</sup>H-NMR spectra confirm the successful deprotection as well as the glycosylation for both polymers (Figures 4.3 and 4.4).

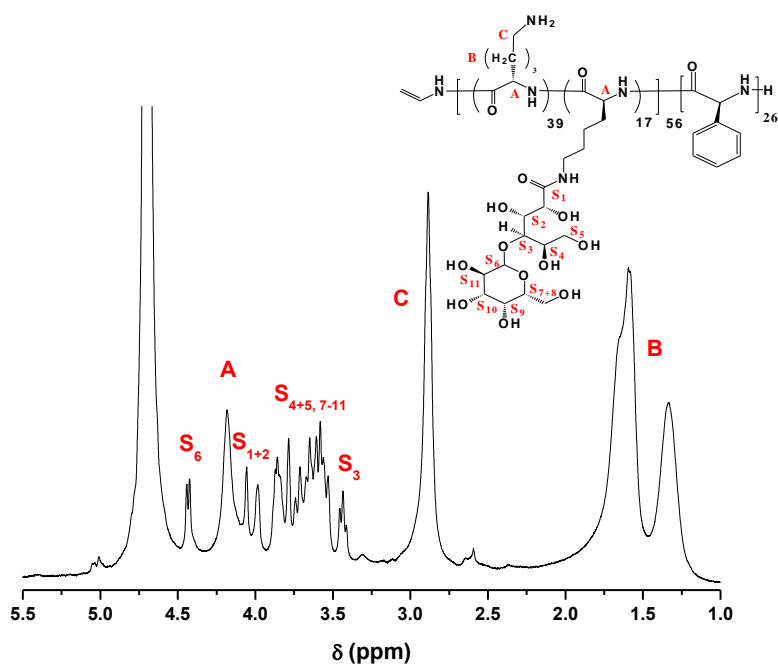


Figure 4. 3 <sup>1</sup>H-NMR spectra (D<sub>2</sub>O, 400 MHz) of LA<sub>17</sub>-*r*-PLL<sub>42</sub>-*b*-PPA<sub>24</sub> (**4**).

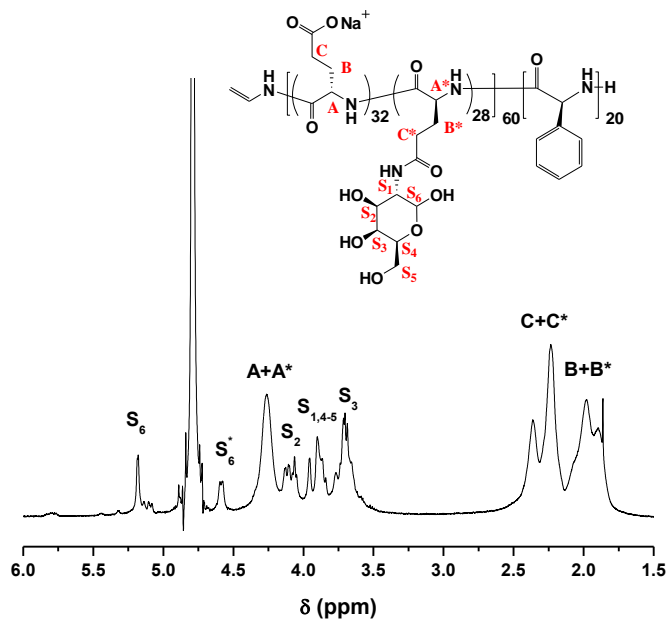


Figure 4. 4  $^1\text{H-NMR}$  spectra (D<sub>2</sub>O, 400 MHz) of GA<sub>28</sub>-*r*-PLGA<sub>32</sub>-*b*-PPA<sub>20</sub> (**2**).

To further illustrate the versatility of the system a triblock copolymer PGL<sub>7</sub>-*b*-PBLG<sub>56</sub>-*b*-PPA<sub>19</sub> (Scheme 1, **5**) was synthesised by sequential NCA polymerisation. Glycosylation was achieved by click chemistry of GA-azide with the alkyne groups of the propargylglycine block to yield GA<sub>7</sub>-*b*-PLGA<sub>56</sub>-*b*-PPA<sub>19</sub> (**6**).<sup>41</sup> While the total composition of this triblock copolyptide is similar to **2**, the amphiphilic block is arranged in a block rather than a random fashion. SEC and  $^1\text{H-NMR}$  analysis confirm the well-defined block structure and efficient glycosylation (Figures 4.5 and 4.6).

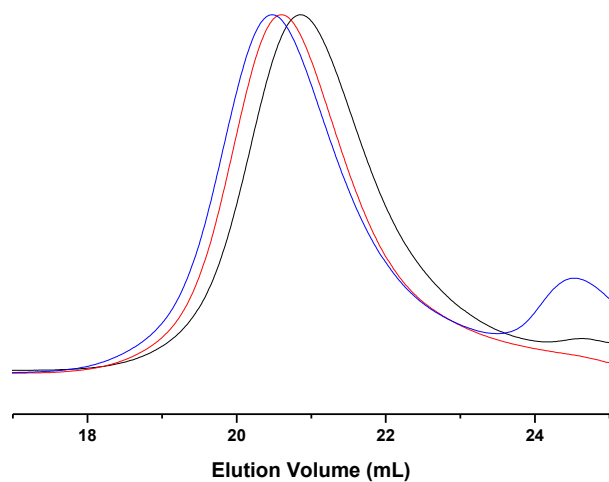


Figure 4. 5 SEC traces of PGL<sub>7</sub>-*b*-PBLG<sub>56</sub> (black), PArg<sub>7</sub>-*b*-PBLG<sub>56</sub>-*b*-PPA<sub>19</sub> (**5**, red) and GalAc<sub>7</sub>-*b*-PBLG<sub>60</sub>-*b*-PPA<sub>19</sub> (blue).

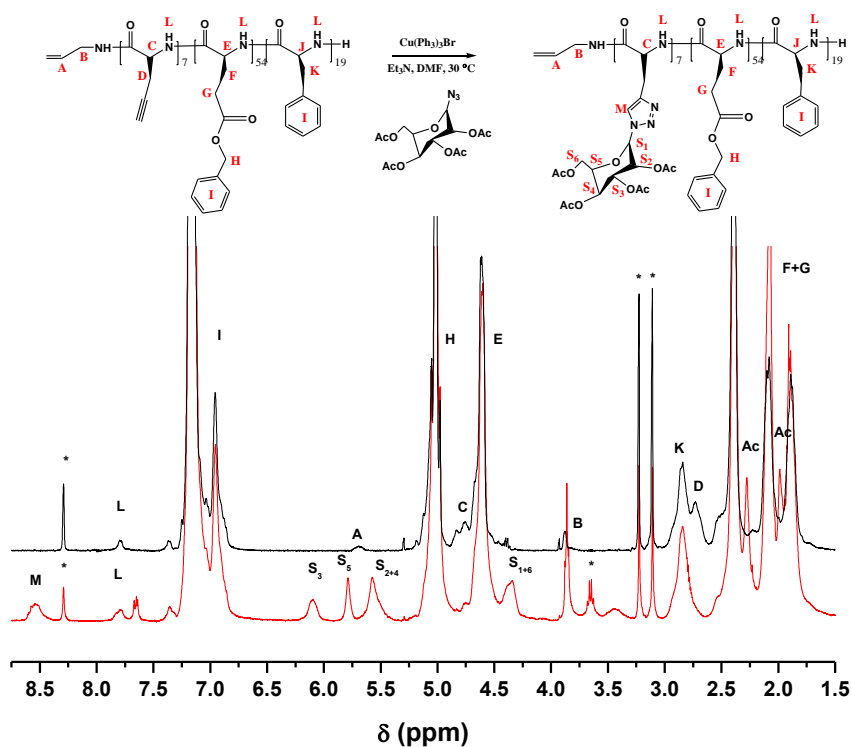


Figure 4. 6 <sup>1</sup>H-NMR spectra (TFA-d, 400 MHz) of PGL<sub>7</sub>-*b*-PBLG<sub>56</sub>-*b*-PPA<sub>19</sub> (**3**, black) and GalAc<sub>7</sub>-*b*-PBLG<sub>56</sub>-*b*-PPA<sub>19</sub> (**5**, red). The \* signals are due to DMF.

Table 4. 1 Polypeptide block copolypeptides

Polymer <sup>(a)</sup>	$[\Pi]_0/[M_1]_0/[M_2]_0$	$M_w$ <sup>(b)</sup> (g/mol)	$\bar{D}$
PBLG <sub>60</sub> - <i>b</i> -PPA <sub>20</sub>	1:60:20	16600	1.07
PZLL <sub>59</sub> - <i>b</i> -PPA <sub>24</sub>	1:60:20	20400	1.07
PGL <sub>7</sub> - <i>b</i> -PBLG <sub>56</sub> - <i>b</i> -PPA <sub>19</sub>	1:7:60:20	17500	1.09

<sup>a</sup>Degree of polymerization was determined by <sup>1</sup>H-NMR spectroscopy (TFA-d) by calculating the ratio of the methine proton of the initiator allylamine (5.6 ppm) to the methylene protons of the polypeptide protecting group for PBLG/PZLL (4.9 – 5.2 ppm) and methylene group of PPA (2.7 – 3.0 ppm). <sup>b</sup>Values obtained from multi angle light scattering (MALS) detection.

#### 4.4.2 Solution properties of glycopolypeptides

Ionic surfactants such as the block copolypeptides presented here are sensitive to solution pH. pH may directly impact the solubility and, unlike conventional surfactants, the secondary structure of the glycopolypeptides. The latter was investigated in aqueous solution by circular dichroism (CD) before and after glycosylation. CD measurements of the glycosylated and native block copolypeptides were compared at different pH values to establish the onset of secondary conformations. Figure 4.7 and A1 (see Appendix A) shows the CD spectra of PLGA<sub>60</sub>-*b*-PPA<sub>20</sub> and (GA<sub>28</sub>-*r*-PLGA<sub>32</sub>)-*b*-PPA<sub>20</sub> respectively. When lowering the pH a transition from a random coil, as evident from a negative Cotton effect with a minimum at ca.  $\lambda = 198$  nm, to an  $\alpha$ -helix at pH 6.32 is apparent from the two minima at  $\lambda = 208$  nm and 222 nm.<sup>42</sup> The transition seems to be solely between random-coil and  $\alpha$ -helices as indicated by an isodichroistic point occurring at  $\lambda = 204$  nm.<sup>43</sup> The native copolypeptide shows a maximum helicity of 35 % near pH 4 after which it precipitates from the solution, as the carboxylic moieties are being protonated.

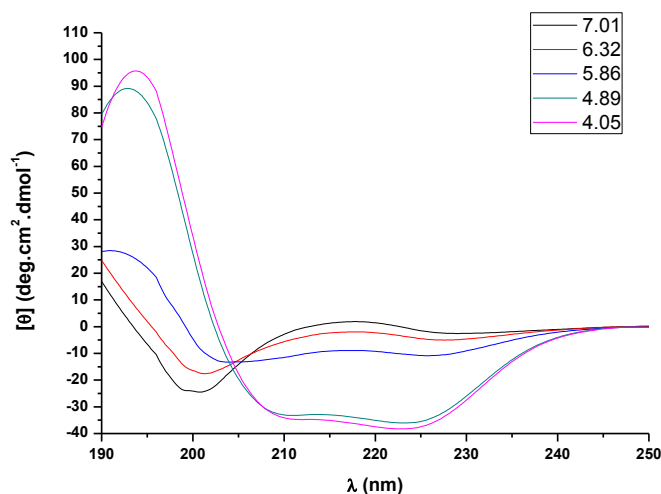


Figure 4. 7 CD spectra of aqueous solutions of PLGA<sub>60</sub>-*b*-PPA<sub>20</sub> as a function of pH

The CD spectra of the glycosylated (GA<sub>28</sub>-*r*-PLGA<sub>32</sub>)-*b*-PPA<sub>20</sub> replicate this trend except for some residual helical conformation still being present at pH 7.10. Glycosylation improves the solubility of the block copolyptide down to pH 3 with a maximum helicity of 44 %. CD measurements of the triblock copolyptides PGL<sub>7</sub>-*b*-PLGA<sub>56</sub>-*b*-PPA<sub>19</sub> and GA<sub>7</sub>-*b*-PLGA<sub>56</sub>-*b*-PPA<sub>19</sub> as shown in Figures A2 and A3 display very similar trends before and after glycosylation. Both materials have the sole transition between a random coil and  $\alpha$ -helical conformation as indicated by the isodichroistic point at  $\lambda = 204$  nm. The onset of the transition is in the region of pH 6 irrespective of glycosylation. Furthermore, improved solubility upon glycosylation at lower pH was noticed.

In the case of PLL block copolyptides, it is known that the PLL block adopts a random coil conformation at low pH while assuming an  $\alpha$ -helical conformation at higher pH as the amino groups get deprotonated.<sup>44</sup> CD spectra of PLL<sub>59</sub>-*b*-PPA<sub>24</sub> and (LA<sub>17</sub>-*r*-PLL<sub>42</sub>)-*b*-PPA<sub>24</sub> (Figure A4 and A5) show exactly this where a transition from random-coil to the  $\alpha$ -helical conformation can be seen as indicated by the two minima at  $\lambda = 208$  nm and 222 nm. Figure 4.8 indicates the calculated helicities for the respective native and glycosylated block copolyptides.

The helicities were only calculated where no precipitation was evident. The increased solubility over the pH range as a whole is evident for the glycosylated copolypeptides. The inherent electrolytic nature of the two respective amphiphiles means that solubility issues occur at the high and low pH limits, something seemingly well counteracted with the attachment of the carbohydrate moieties.

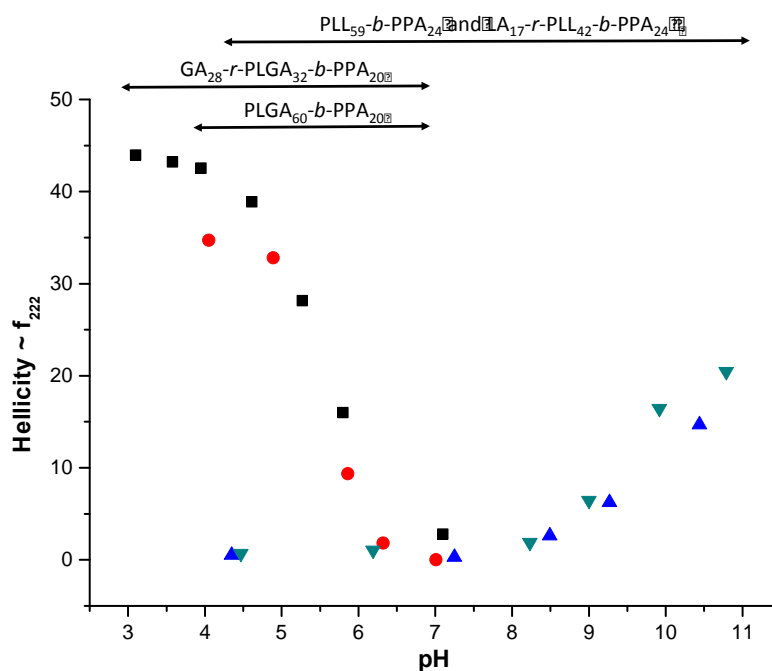


Figure 4. 8 Helicities determined for the block copolypeptides PLGA<sub>60</sub>-b-PPA<sub>20</sub> (●), GA<sub>28</sub>-r-PLGA<sub>32</sub>-b-PPA<sub>20</sub> (■), PLL<sub>59</sub>-b-PPA<sub>24</sub> (▲) and LA<sub>17</sub>-r-PLL<sub>42</sub>-b-PPA<sub>24</sub> (▼) as a function of pH. The arrows indicate the solubility range of the block copolypeptides.

A similar trend was observed for the PGL<sub>7</sub>-b-PLGA<sub>56</sub>-b-PPA<sub>19</sub> and GA<sub>7</sub>-b-PLGA<sub>56</sub>-b-PPA<sub>19</sub>, where the introduction of only 7 repeat units has a clear influence of the solubility below pH 4 (Figure 4.9).

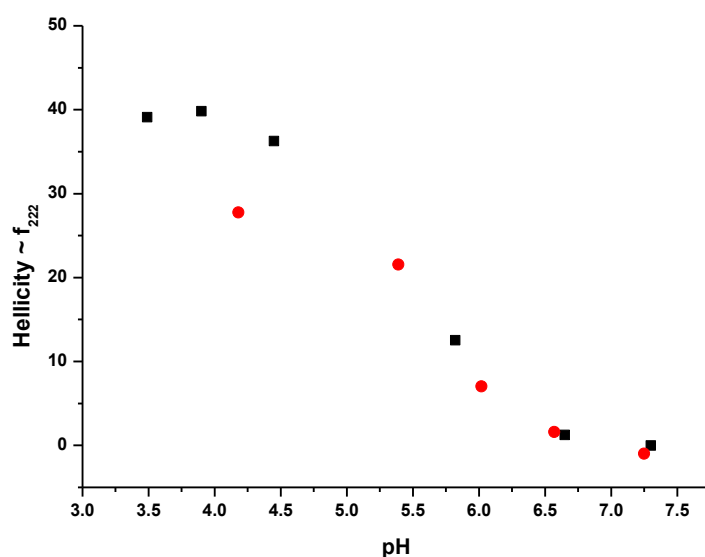


Figure 4.9 Estimated helicities determined for the native copolyptide, PArg<sub>7</sub>-*b*-PBLG<sub>56</sub>-*b*-PPA<sub>19</sub> (●) and its glycosylated counterpart, Gal<sub>7</sub>-*b*-PLGA<sub>56</sub>-*b*-PPA<sub>19</sub> (■) as a function of pH.

#### 4.4.3 Emulsion Polymerisation

To the best of our knowledge, the stabilization of synthetic latexes by an amphiphilic polypeptide has not been shown yet. For this purpose, the three copolypeptides, GA<sub>28</sub>-*r*-PLGA<sub>32</sub>-*b*-PPA<sub>20</sub>, GA<sub>28</sub>-*r*-PLGA<sub>32</sub>-*b*-PPA<sub>20</sub> and LA<sub>17</sub>-*r*-PLL<sub>42</sub>-*b*-PPA<sub>24</sub> were utilized as fully amino acid and carbohydrate based macromolecular surfactants in the aqueous emulsion polymerization of styrene. All reactions were performed at 70 °C and initiated *via* the water-soluble initiator potassium persulfate (KPS) at a concentration of 1 wt% with regard to monomer (1 pphm). Monomer contents of 12 and 15 wt%, and block copolymer contents of 2 and 5 wt% with regard to monomer were used. Under the applied conditions the hydrophilic blocks were in a random coil conformation. It was found that all block copolypeptides could readily stabilize the formed polystyrene particles and only in the case where a low concentration of LA<sub>17</sub>-*r*-PLL<sub>42</sub>-*b*-PPA<sub>24</sub> was used (2 pphm) no stable latex was obtained. This implies that the hydrophilic PLGA blocks result in better stabilization, a result we have observed before.<sup>34</sup>

Conversion-time curves for the emulsion polymerization of styrene stabilized by GA<sub>28</sub>-*r*-PLGA<sub>32</sub>-*b*-PPA<sub>20</sub> are shown in Figure 4.10. The results (especially at higher stabilizer concentrations) show very short nucleation periods (if any), which suggests that preformed

block copolymer micelles act as seeds for the polymerisation. Furthermore, the results in this figure show an increase in the polymerization rate as the copolypeptide concentration is increased. This result is expected as increasing block copolypeptide concentrations lead to higher particle numbers ( $N_p$ ), which in turn lead to a higher rates of polymerization,  $R_p$  ( $R_p \propto N_p$ ).<sup>45</sup> This argument is supported by the dynamic light scattering (DLS) results shown in Table 4.2, where it is clearly seen that increasing the copolypeptide concentration leads to a decrease in particle diameter.

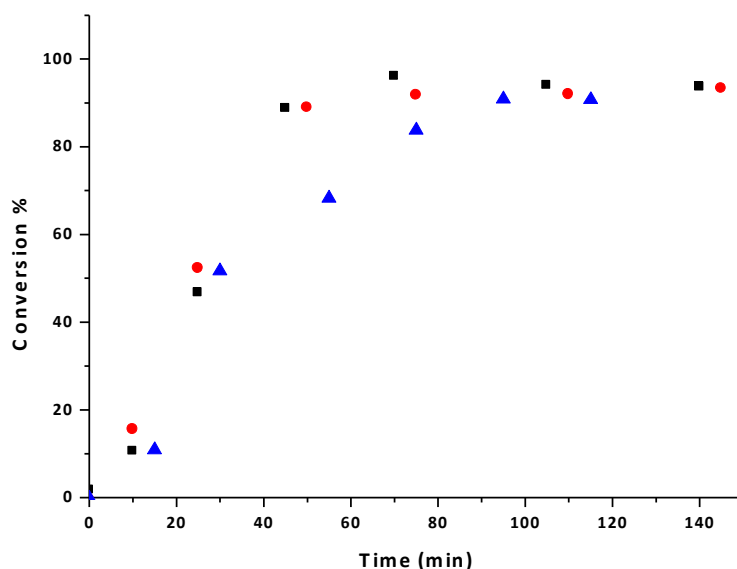


Figure 4. 10 Evolution of monomer conversion with time for the emulsion polymerization of styrene using KPS as initiator (1 wt%) with  $GA_{28-r}PLGA_{32-b}PPA_{20}$  as stabilizer. Monomer content of 12 wt% and surfactant content of 5 wt% (■), monomer content of 15 wt% and surfactant content of 5 wt% (●), monomer content of 15 wt% and surfactant content of 2 wt% (▲).

The triblock,  $GA_7-b-PLGA_{56-b}PPA_{19}$ , acted as an effective stabilizer in the emulsion polymerization of styrene. Figure 4.11 shows the conversion-time curves at varying monomer and copolypeptide wt% content and it is evident that no real differentiation can be made regarding the polymerization rates of any of the three curves. Once again DLS showed no significant difference in particle size at increasing monomer content and constant copolypeptides/monomer ratio. A sharp increase in size to 120 nm is however evident after decreasing the copolypeptides content to 2 pphm, as fewer and thus larger particles are prepared (Table 2).

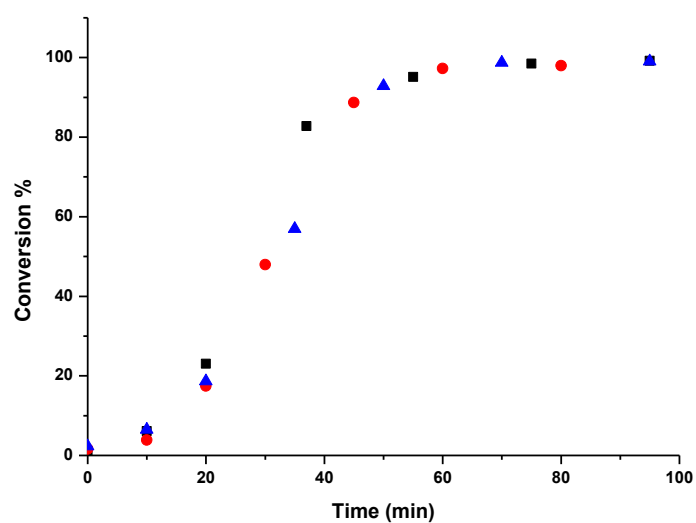


Figure 4. 11 Evolution of monomer conversion with time for the emulsion polymerization of styrene using KPS as initiator (1 wt%), a monomer content of 12 wt% and surfactant content of 5 wt% (■), a monomer content of 15 wt% and surfactant content of 5 wt% (●), a monomer content of 15 wt% and surfactant content of 2 wt% (▲) with  $GA_7$ - $b$ - $PLGA_{56}$ - $b$ - $PPA_{19}$  as stabilizer.

$LA_{17}$ - $r$ - $PLL_{42}$ - $b$ - $PPA_{24}$  was not as effective in stabilizing the polystyrene nanoparticles as its glutamic acid counterparts (Figure 4.12). At low copolyptide/monomer content (i.e. 2 pphm) it was not possible to prepare a stable latex. At copolyptides contents of 5 pphm it was however possible to form stable dispersions where the reaction kinetics are very similar to that of  $GA_{28}$ - $r$ - $PLGA_{32}$ - $b$ - $PPA_{20}$ . Furthermore, DLS determined the particle sizes to be similar to that stabilized by  $GA_{28}$ - $r$ - $PLGA_{32}$ - $b$ - $PPA_{20}$ , concluding that we have comparable particles which in ionic form will be of opposite charge.

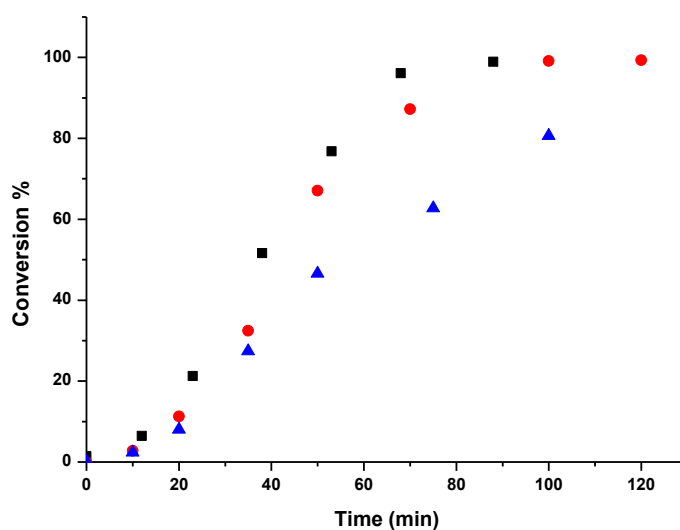


Figure 4. 12 Evolution of monomer conversion with time for the emulsion polymerization of styrene using KPS as initiator (1 wt%), a monomer content of 12 wt% and surfactant content of 5 wt% (■), a monomer content of 15 wt% and surfactant content of 5 wt% (●), a monomer content of 15 wt% and surfactant content of 2 wt% (▲) with LA<sub>17</sub>-*r*-PLL<sub>42</sub>-*b*-PPA<sub>24</sub> as stabilizer.

All particles were further investigated by FE-SEM. The images illustrate the uniformity of the particles in agreement with the low PDIs obtained from DLS (Figure 4.13). Particles sizes (80-100 nm) agree well with sizes obtained by DLS considering the non-hydrated state of the particles surfactant layer in SEM.

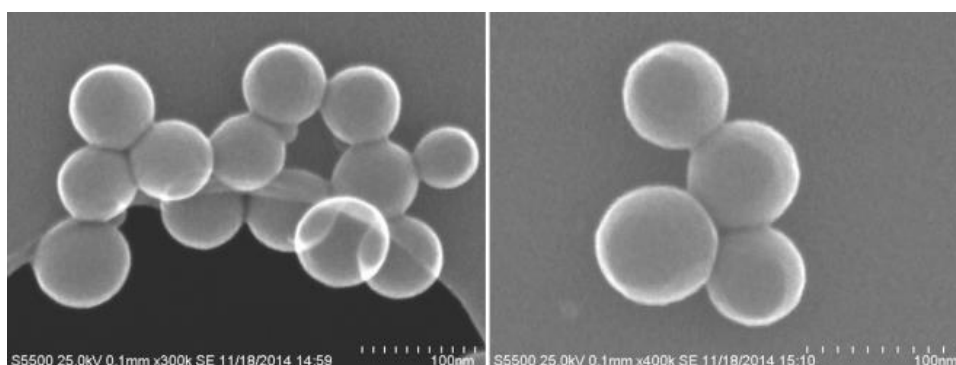


Figure 4. 13 FE-SEM images of polystyrene latex stabilized by GA<sub>28</sub>-*r*-PLGA<sub>32</sub>-*b*-PPA<sub>20</sub> (3) with a monomer content of 12 wt% and surfactant content of 5 wt% (Table 4.2).

Table 4. 2 Results from the emulsion polymerization of styrene using glycosylated block copolymers as surfactants.

Polymer	monomer content <sup>(a)</sup> (Wt%)	[polymer] <sup>(b)</sup> (pphm)	D <sub>z</sub> <sup>(c)</sup> (nm)	PDI <sup>(d)</sup>
GA <sub>28-r</sub> -PLGA <sub>32-b</sub> -PPA <sub>20</sub>	12	5	114	0.08
GA <sub>28-r</sub> -PLGA <sub>32-b</sub> -PPA <sub>20</sub>	15	5	118	0.09
GA <sub>28-r</sub> -PLGA <sub>32-b</sub> -PPA <sub>20</sub>	15	2	140	0.03
GA <sub>7-b</sub> -PLGA <sub>56-b</sub> -PPA <sub>19</sub>	12	5	98	0.03
GA <sub>7-b</sub> -PLGA <sub>56-b</sub> -PPA <sub>19</sub>	15	5	106	0.05
GA <sub>7-b</sub> -PLGA <sub>56-b</sub> -PPA <sub>19</sub>	15	2	120	0.05
LA <sub>17-r</sub> -PLL <sub>42-b</sub> -PPA <sub>24</sub>	12	5	112	0.02
LA <sub>17-r</sub> -PLL <sub>42-b</sub> -PPA <sub>24</sub>	15	5	115	0.02
LA <sub>17-r</sub> -PLL <sub>42-b</sub> -PPA <sub>24</sub>	15	2	n.a.	n.a.

(a) solid content = mass polymer/mass dispersion. (b) 1 pphm = 1 g block copolymer/100 g monomer. (c) Z-average particle diameter from Dynamic Light Scattering (DLS). (d) Polydispersity Index of particle size distribution from cumulants DLS analysis.

## 4.5 Conclusions

Block copolymers consisting of poly(L-phenyl alanine) and partially glycosylated poly(L-glutamic acid) and poly(L-lysine), respectively, were synthesized via sequential NCA polymerization and subsequent polymer glycosylation. The resulting amphiphilic block copolypeptides were found to be efficient stabilizers in the emulsion polymerization of styrene offering a facile method for the synthesis of polystyrene nanoparticles. This signifies an example of functional polymer additives fully based on renewable building blocks in materials applications.

## 4.6 References

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