Green Enzymatic One-Pot Synthesis of Renewable and Biodegradable Surfactants in Supercritical Carbon Dioxide ($scCO_2$).

Experimental Part

Materials

Glycerol (≥ 99.5% purity) was purchased from Sigma Aldrich (UK) and dried for 24 hours under vacuum (10 mmbar) at room temperature before use and stored over fresh molecular sieves (4Å, particle size 1.6-2.5 mm) at room temperature. Molecular sieves type 3 and 4 Å, 1.6-2.5 mm beads, were purchased from Fisher Scientific (UK) and used as received. Succinic acid (≥ 99.0% purity) was purchased from Sigma Aldrich (UK) and dried for 24 hours under vacuum (10 mmbar) at 50 ºC before use. Lauric acid (≥ 98.0% purity) was purchased from Sigma Aldrich (UK) and used as received. Poly(ethylene glycol) methyl ether with a molecular weight of 350 Da (M-PEG350) was purchased from Sigma Aldrich (UK) and stored over fresh molecular sieves (4Å, particle size 1.6-2.5 mm) at room temperature. Novozyme® 435 (CaLB immobilised on cross-linked acrylic resin beads) was kindly donated by Novozymes™ (Denmark), stored at 4 ºC and dried for 24 hours under vacuum (100 mbar) at room temperature (RT) before use. The commercial surfactants Tween™ 20 (PEG20-sorbitan laurate, ~ 1,200 Da) and NatraGem™ E145 (polyglycerol succinate laurate, ~ 1,300 Da) were supplied by Croda Europe Ltd. (UK). All the solvents were of analytical grade and used as received. Carbon dioxide grade 4.0 (minimum purity 99.99%) was purchased from BOC Special Gases (UK) and used as received.

Methods

Synthesis of poly(glycerol succinate) via melt polycondensation: in the absence of catalyst, and using metal catalyst or enzyme.

The polymerisations of PGLSA were carried out via melt polycondensation with and without enzymatic catalyst. In a typical procedure (1:1 G:SA molar ratio) succinic acid (54.3 mmol, 6.4 g), glycerol (54.3 mmol, 5.0 g), and molecular sieves (size 4Å, 25 wt.% with respect to the total amount of monomers) were added to a 20 mL three-neck round bottom flask without catalyst or with addition of enzyme 25 wt% (calculated from the total weight of polymeric-support and the enzyme) and $Sn(Oct)_2$ 2 wt% of total amount of monomers. The reactions were performed under vacuum and stirred at 300 rpm.

In order to achieve melt conditions and to lower viscosity, all reactions were performed at 120°C. The reactions were stopped by cooling to room temperature. The molecular sieves and the enzyme were removed by filtration after being solubilised in THF and the filtrate was recovered using a rotary evaporator. To ensure complete removal of the solvent, the samples were placed under reduced pressure overnight. The reactions were performed in duplicate to assess reproducibility.

Reagent solubility in supercritical carbon dioxide

To determine visually the effect of $scCO_2$ on the physical state of glycerol and succinic acid, a fixed volume view cell (100 mL) was used. In a typical procedure, 0.5 g of reagent (glycerol or succinic acid) was added to a 1 mL glass vial (previously cut to half of its height) and inserted inside the view cell and fixed in place (see Figure S2). The windows were sealed and clamped to the body and the pressure was raised to 50 bar. The temperature and CO_2 pressure were raised incrementally to the desired conditions or until complete solubilisation.

Enzymatic synthesis of poly(glycerol-succinic acid) using supercritical carbon dioxide

In a typical procedure (1:1 G:SA molar ratio) the succinic acid (16.3 mmol, 1.92 g), glycerol (16.3 mmol, 1.50 g), CaLB (25 wt.% of the total amount of monomers, 0.86 g) and molecular sieves (size 3Å, 25 wt.% with respect to the total amount of monomers, 0.86 g) were added to a stainless steel reaction autoclave (total volume 60 mL). The vessel was sealed and pressurised to 50 bar. Subsequently, the temperature was set to 40 or 60 ºC, and finally the pressure

increased to 275 bar. The reaction was left to run for 24 h and stirred at 150 rpm. To avoid polymer foaming and blockages in the pipework, the reactions were terminated by cooling the vessel in a water/ice bath (~ 0 ºC) before venting. The CO_2 was vented when the pressure was below 20 bar. Conversion of monomers in polymers was quantitative, as observed from NMR spectra and the absence of purification steps. However, due to the sticky nature of the final polymers and the necessity to reduce the use of organic solvent the yield of recovery was in some of the cases lower than the expected. The final product was purified from the catalyst beads and extra washing solvent following the same procedure reported for the previous reaction strategies.

Enzymatic synthesis of lauroyl poly(glycerol-succinate) and PEG-poly(glycerol-succinate) using supercritical carbon dioxide

In a typical procedure, succinic acid (5.4 mmol), glycerol (10.9 mmol), lauric acid (1.5 mmol, 28 mol% to the amount of SA) (or M-PEG (2.4 mmol, 15 mol% to the total amount of SA and G)), CaLB (25 wt.%, of the total amount of monomers and calculated taking into account both the total weight of polymeric-support and the enzyme) and molecular sieves (size 3Å, 25 wt.% of the total amount of monomers) were added to a stainless steel reaction autoclave (total volume 60 mL). The vessel was sealed and pressurised up to 50 bar. Afterwards, the temperature was increased to the desired value (40, 50 or 60 ºC, Table 4.3) before the pressure was increased to 275 bar. The reaction was left to run for 24 h while stirring at 150 rpm. To avoid polymer foaming and consequently blockages in the pipework, the reactions were stopped by cooling the vessel in a water/ice bath (~ 0 ºC) before venting. An excess of glycerol was used to ensure the synthesis of glycerol terminated PGLSA, since lauric acid can only react with the hydroxy moieties. On the other hand, an excess of SA was used to ensure the synthesis of SA terminated PGLSA, since PEG can only react with the carboxylic moieties. The reactions were performed in duplicate to assess reproducibility. Yields ranged from 85 to 95%.

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Analytical Characterisations

Nuclear magnetic resonance spectroscopy (NMR)

All spectra were recorded on a Bruker Advance III HD 400MHz or Bruker Advance 400MHz, with chemical shifts, in ppm, referenced relative to a deuterated solvent (normally chloroform-d1 (7.26 ppm), acetone-d6 (2.05 ppm) or methanol-d3 (3.31 ppm)). Sample concentration was 7 mg/mL. Mestrelab MNova software was used for analysis.

Gel permeation chromatography (GPC)

MW and ***Đ*** were measured using an Agilent 1260 infinity multidetector GPC/SEC (size exclusion chromatography) system with a Wyatt Optilab light scattering detector. The GPC was equipped with two columns; an Agilent PLGEL 5 μm Mixed D (7.5 mm X 300 mm) and a PLGEL 5 μm guard column (7.5 mm X 50 mm). THF was used as the eluent at a flow rate of 1 mL/min and 0.0304 mL/g was used as dn/dc. The software used for analysis was Astra® 6 (Wyatt).

Differential scanning calorimetry (DSC)

A TA Instruments Q2000 was utilised, equipped with an auto-sampler, suitable for use from -90 to 300 ºC, calibrated with indium and sapphire standards. Tzero aluminium pan (TA Instruments) were used for all samples and sealed prior to use. In a normal experiment, a polymeric sample (ca. 2 mg) was cooled to -90 ºC (10 ºC/min) and then heated to 200 ºC (10 ºC/min). Afterwards, the sample was cooled again to -90 ºC (10 ºC/min) and then heated up to 200 ºC (10 ºC/min). The first heating cycle was used to remove the thermal history of the sample and the T_m and T_g were recorded from the second heating cycle. The experiments were carried out under a N_2 flow (50 mL/min). All the data were analysed using the Universal Analysis software.

Tensiometry

Surface tension measurements were made using the Wilhelmy plate method and a bubble tensiometer, measuring equilibrium (static) and dynamic surface tensions, respectively. Measurements were made in duplicate and the commercial surfactants Tween™ 20 and NatraGem™ E145 were assessed for comparison.

For the dynamic surface tension measurements all the synthesised poly(glycerol succinate) polyesters along with the corresponding end-capped polymers (with lauric acid and PEG_{350}) were assessed, typically at 1 wt.%, 0.5 wt.% and 0.1 wt.% in water.

For the equilibrium (static) surface tensions, all the synthesised PGLSA polyesters along with the corresponding end-capped polymers were assessed, typically at 1 wt.% in water.

Critical Aggregation Concentration (CAC)

The CAC was determined using the Wilhelmy plate method. The system contains an automated dispensing unit which enables a high number of measurement points across a broad concentration range (10,000 – 0.1 mg/L). Starting at a concentration of 1 wt.% (10,000 mg/L) in water, the polymer solution was diluted stepwise and surface tension measurements taken at

each concentration. Surface tension *versus* concentration (logarithmic scale) was plotted and the CAC value was determined by the intersection between lines (descending and *quasi*-horizontal lines).

Contact angle measurement

To evaluate the contact angle of the surfactants, the static sessile drop method was used on the surface of a parafilm plate (OCA 15EC model). An aqueous droplet of surfactant was gently dropped on the parafilm surface using a microliter syringe. SCA20 (version 1.60) software was used to analyse the shape and to measure the contact angle of the surfactants.

Degree of Branching defined by ^{1}H-NMR

The DB defined by Frey is the number of actual dendritic units divided by the maximum possible number of dendritic units and is universally applicable for small and large polymer structures.[1]

$$DB_{Frey} = \frac{2 \times D}{2 \times D + L} \times 100 \qquad DB_{Frey} = \frac{2 \times B_0}{2 \times B_0 + B_1} \times 100$$

Equation S1 Degree of branching as defined by Frey[2, 3], for polymers from an AB_n topology. D = dendritic units, L = linear units. Dendritic units correspond to the formation of branched polymers. Degree of branching equation defined by Frey[2, 3], for polymers from an $A_2 + B_3$ topology. In a hyperbranched polymer, it is possible to observe different units in the backbone of the polymer: branched units (B) and linear units (L). In PGLSA branching can occur *via* glycerol (B_3 compound). Thus, when a linear unit is obtained (B_1 unit), polymerisation can occur in the positions H_a and H_b, or H_a and H_c of glycerol (Legend 1). Thus, B_1 can also be described as $L_{a,b}$ and $L_{a,c}$, respectively. The chemical shifts used were: Hb (B0) = 5.28 ppm, Hb (B1) = 5.07 ppm, Hb (B1) = 3.83 ppm.

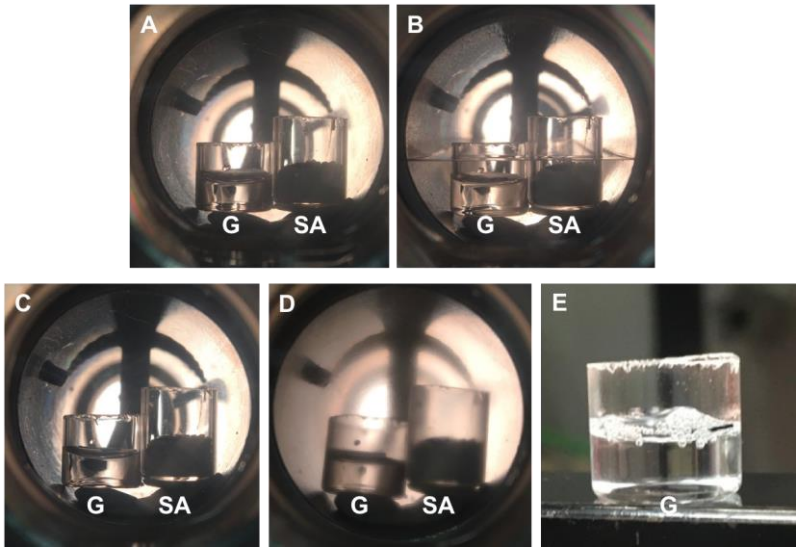
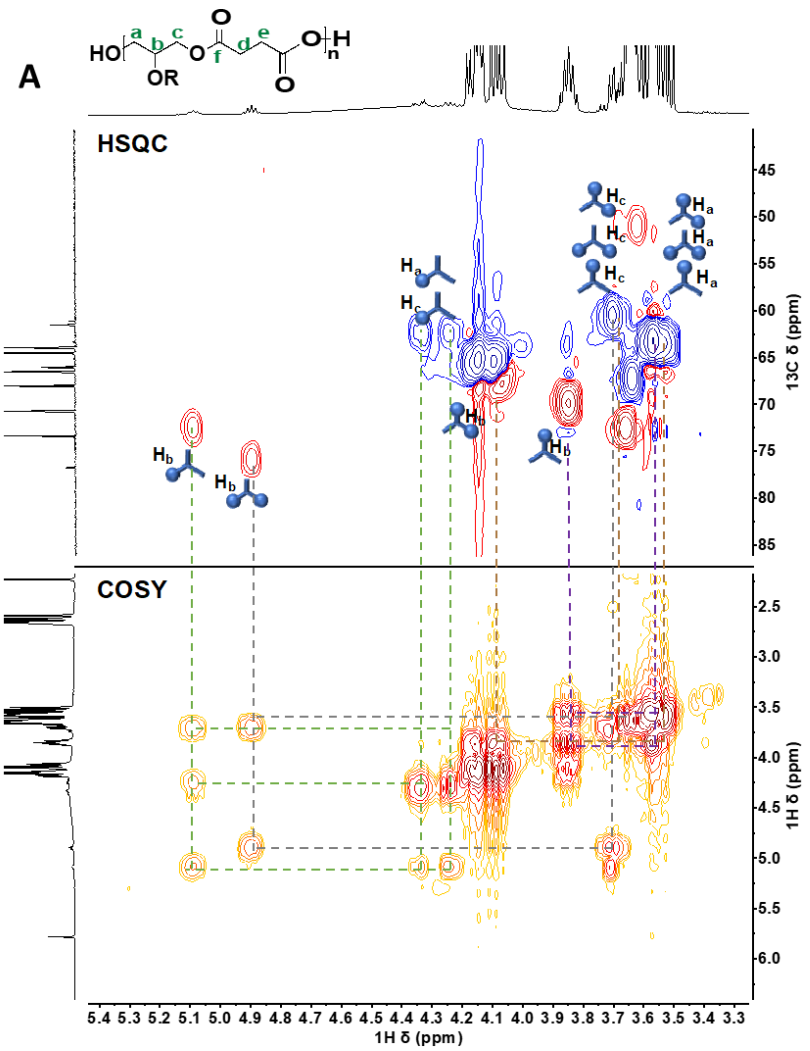


Figure S1 – Visual observation of glycerol (G) and succinic acid (SA) at **A)** at ambient pressure (1 bar) and temperature (25 ºC), **B)** at 65 bar and 27 ºC, **C)** at 275 bar and 40 ºC and **D)** at ambient pressure and temperature upon removal of the CO_2 (after 7 hours under pressure). Picture **E)** shows glycerol, at ambient conditions after 7 hours under supercritical conditions, exposing the evidence of CO_2 being released. It is possible to see that succinic acid and glycerol are not soluble in scCO_2; nonetheless, it is clear that CO_2 is soluble in glycerol due to the presence of "bubbles" after exposure of CO_2.


A
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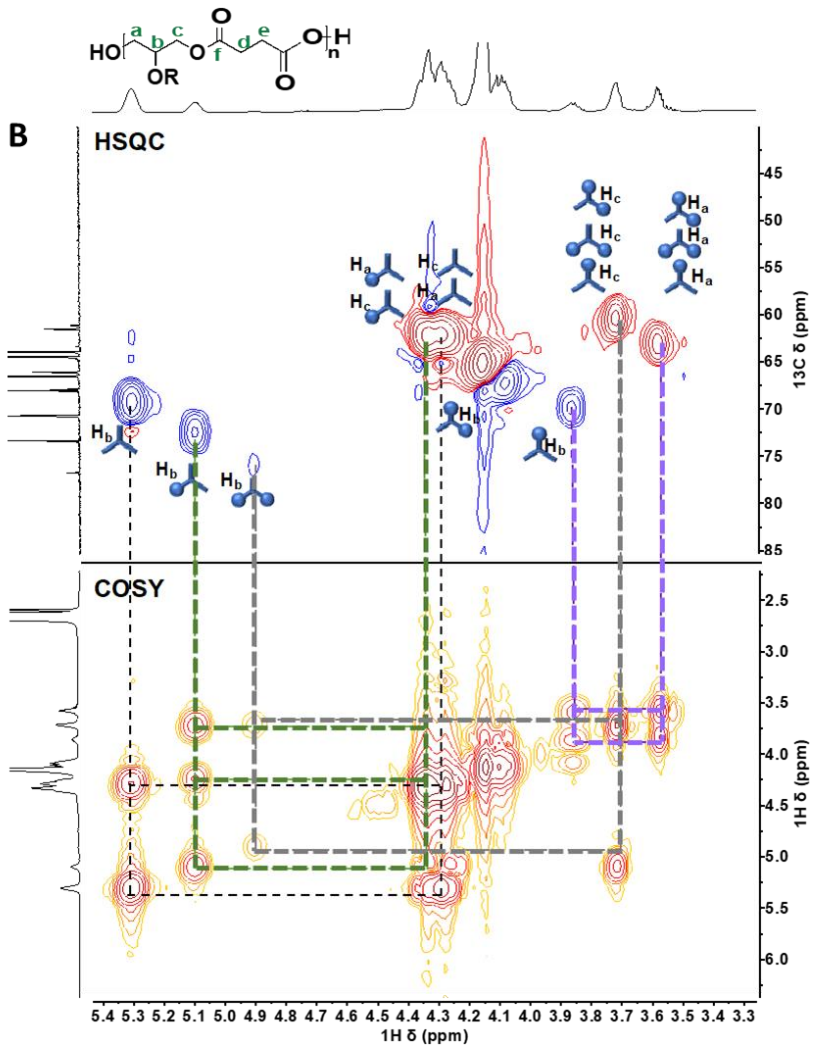
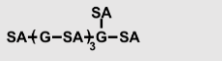
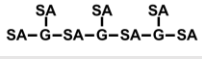
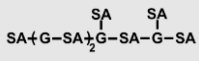
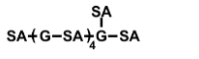
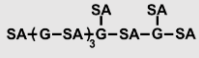
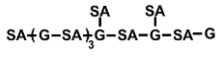


Figure S2 – Sections of the HSQC and COSY NMR spectra showing the assignments of glycerol units in a A) linear polymer synthesised in $scCO_2$ (entry 5, Table 1) and B) branched poly(glycerol succinate) polymer synthesised in the melt (entry 8, Table 2). The combination red-blue for HSQC and yellow-red for COSY are reported simply to distinguish the two types of spectra. For glycerol branching representations refer to **Scheme 2**.

Polymer structure	$(M + Na)^+$	$(M + K)^+$
	937	953
	963	979
	1,037	1,053
	1,111	1,127
	1,211	1,228
	1,285	1,302

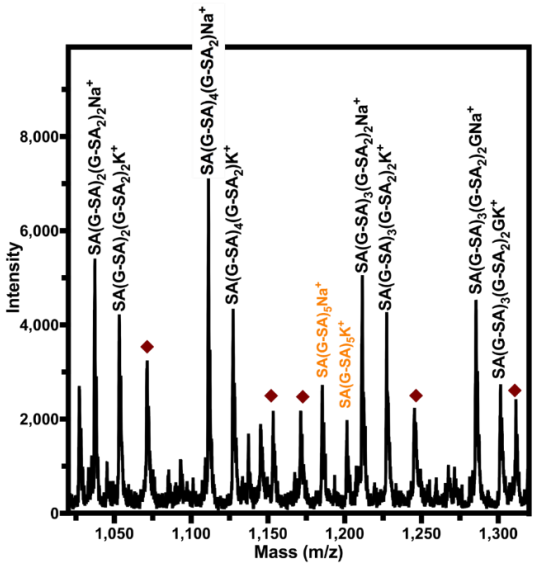


Figure S3 – (TOP) Suggested structures and predicted masses of the sodium and potassium adducts for the branched poly(glycerol succinate) from melt polymerisation at 120°C. Note: G = glycerol; SA = succinic acid. (BOTTOM)Representative section of a MALDI-TOF mass spectrum of K^+ and Na^+ adducts of a branched poly(glycerol succinate). *Note*: ◆ is an unknown peak; the peaks not assigned are assumed to be noise from the baseline spectrum; G = glycerol; SA = succinic acid; orange annotation denotes the linear structure, black annotation denotes the branched structure.

$$M_n^{NMR} = \frac{\frac{I_e\text{-}4}{4} + \frac{I_f\text{-}1}{1} + \frac{I_c}{4}}{3} \times 174 + 56 + 2 \times 200$$

Equation S2 – Average molecular weight of LA-PGLSA calculated through ^{1}H-NMR, using the integration of the peaks H_a (0.88 ppm, terminal methyl group from LA), H_e (I_e, 3.50-3.72 ppm), H_f (I_f, 3.83 and 5.07 ppm) and H_c (I_c, 2.58-2.69 ppm). The values 174, 56 and 200 are for the molar mass of: repeat unit of PGLSA, glycerol unit used as linker (to react with LA) and LA unit, respectively.

$$\text{LA-PGLSA}(\%)^{NMR} = \frac{I_d/4}{I_a/6} \times 100$$

Equation S3 – Percentage of LA attached to the PGSLA backbone.

$$M_n^{NMR} = \frac{\frac{I_e\text{-}31.6}{4} + \frac{I_f}{1} + \frac{I_d\text{-}4}{4}}{3} \times 174 + 82 + 2 \times 350$$

Equation S4 – Average molecular weight of PEG-PGLSA calculated through ^{1}H-NMR, using the integration of the peaks H_a (3.29 ppm, terminal methyl group from M-PEG), H_e (I_e, 3.50-3.72 ppm), H_f (I_f, 3.83 and 5.09 ppm) and H_d (I_d, 2.58-2.69 ppm). The value 31.6 corresponds to the integral of the peak assigned to the –C**H**$_2$– from M-PEG these protons overlap with the protons –CH$_2$– from the glycerol unit (H_e).

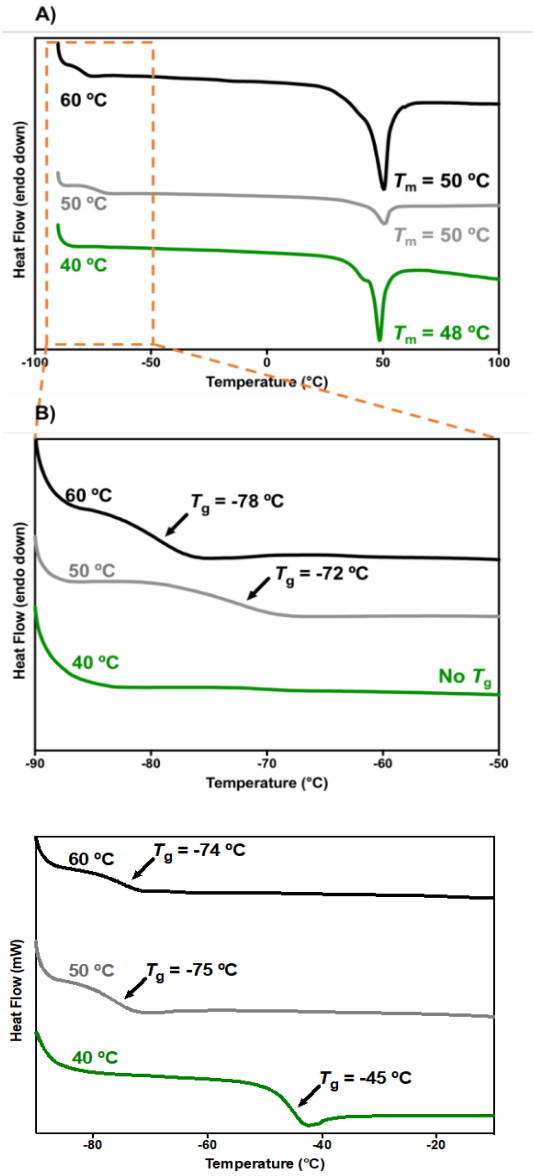


Figure S4. (TOP) DSC traces (2nd heating scans are shown) of the copolymers LA-PGLSA synthesised under enzymatic supercritical conditions at 40, 50 and 60 ºC. A) These traces show the detection of the T_g and T_m of LA-PGLSA; the highlighted region in orange is zoomed in below, in B) to show more clearly the T_g of the LA-PGLSA. (BOTTOM) DSC traces (2nd heating scans are shown) of the copolymers PEG-PGLSA synthesised under enzymatic supercritical conditions at 40, 50 and 60 ºC and shows clearly the detection of the T_g of PEG-PGLSA.

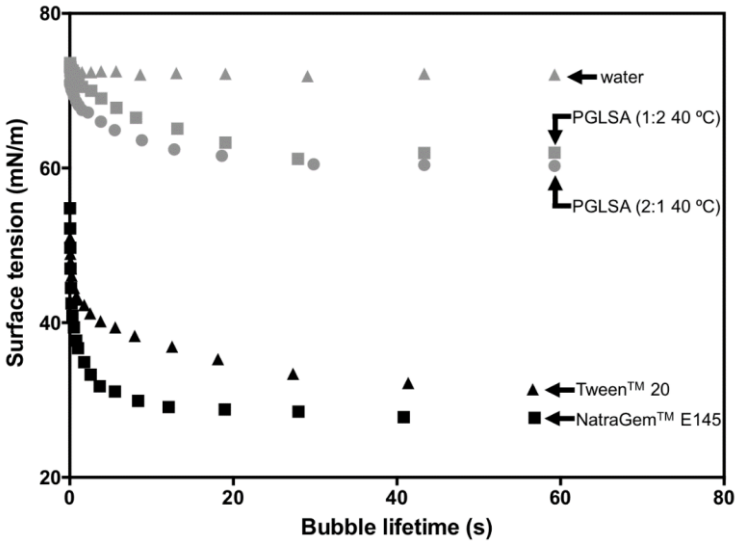


Figure S5– Bubble Tensiometer determination of dynamic surface tension at increasing bubble lifetimes for water, 1 wt.% PGLSA (G:SA 1:2 and 2:1, synthesised at 40 ºC), and commercial surfactants with 1 wt.%, Tween™ 20 and NatraGem™ E145.

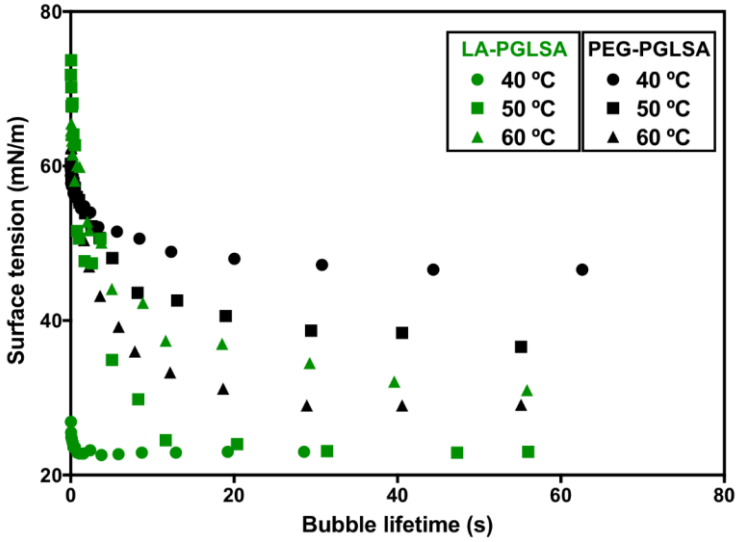


Figure S6 – Bubble Tensiometer determination of dynamic surface tension at increasing bubble lifetimes for LA-PGLSA (1 wt.%) synthesised at different temperatures (40, 50 and 60 ºC, green symbols) and PEG-PGLSA(1 wt.%) synthesised at different temperatures (40, 50 and 60 ºC, black symbols).

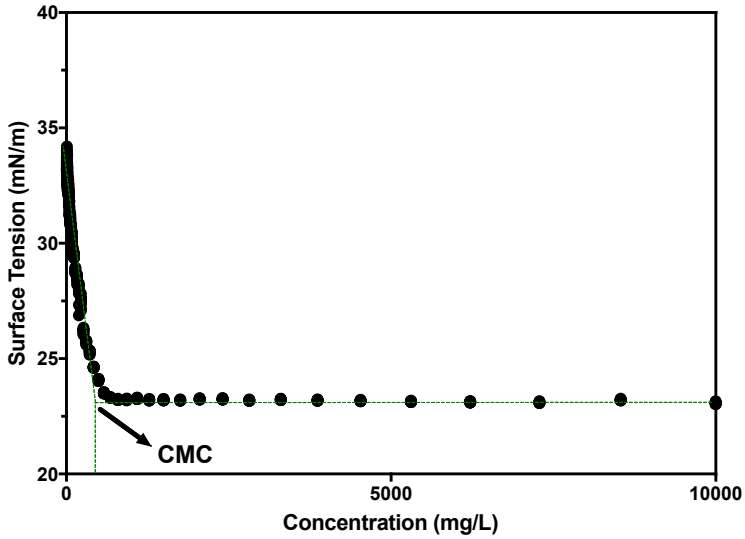


Figure S7 – Surface tension measurements of LA-PEG at 40 ºC at several concentrations. The CMC value is determined by the intersection of the two linear dotted green lines.

References

1. Hawker, C. J.; Lee, R.; Fréchet, J. M. J., One-step synthesis of hyperbranched dendritic polyesters. *J Am Chem Soc* **1991,** *113* (12), 4583-4588.

2. Hölter, D.; Burgath, A.; Frey, H., Degree of branching in hyperbranched polymers. *Acta Polymer* **1997,** *48*, 30-35.

3. Frey, H.; Hölter, D., Degree of branching in hyperbranched polymers. 3. Copolymerization of AB_m-monomers with AB and AB_n-monomers. *Acta Polymer* **1999,** *50*, 67-76.