Green Enzymatic One-Pot Synthesis of Renewable and Biodegradable Surfactants in Supercritical Carbon Dioxide (scCO2).

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Abstract

We seek to expand the opportunities to exploit glycerol, a largely untapped renewable feedstock, by exploiting enzymatic catalysis in supercritical carbon dioxide (scCO2). This work highlights a promising and clean approach to bio-renewable amphiphilic polyester-based biodegradable surfactants. We have developed a low temperature (40, 50 and 60 °C), low energy melt processing route to biodegradable, renewable poly(glycerol succinate) (PGLSA) polymers that importantly have a low degree of branching (3% <DB< 11%). Our approach shows significant advantages over traditional melt polycondensation at 110-120°C, where the standard catalyst-free approach led only to highly branched (DB >85%) or insoluble crosslinked materials. We have exploited these linear PGLSA materials to create a library of '*green*' surfactants by end-capping with lauric acid or poly(ethylene glycol). Our approach avoids pre-modification of the monomers and fewer synthetic steps are required. Finally, we evaluate the performance of these new surfactants, focussing upon surface tension, critical aggregation concentration (CAC) and water contact angle.

Introduction

Our growing global population will lead to increased demand for energy and resources.[1, 2] In recent decades, the study of alternative and renewable feedstocks for the synthesis of polymeric materials has received significant attention [3-7] with applications ranging from medical and tissue engineering, food packaging, coatings, cosmetics, surfactants and more.[8] Surfactants are widely used as emulsifiers, detergents and foaming agents across our society, with applications

across home-, personal-, health- and crop-care. Since their introduction in the early 20th century, the production of surfactants from petrochemical sources has continuously increased[8] reaching 18.5 million tons per year, and is forecast to grow at a compound growth rate (CAGR) of 5% from 2018-2023.[9]

There is now an awareness that we need more environmentally-friendly and economically viable surfactants[10] preferably derived from renewable resources[11,12-15] Glycerol is an underexploited by-product of biodiesel production, in particular from the hydrolysis of biomass derived triglycerides (such as palm oil, sunflower oil or rapeseed oil) which results in valuable methyl esters.[16] Between 2007 and 2016, biodiesel production increased by 83% in the European Union.[17] Thus, glycerol is widely available and its price is inversely proportional to the increase in biodiesel production.[18] As a comonomer, we have focussed upon succinic acid (SA) which has been widely employed as a starting material for different applications in the surfactant, food and pharmaceutical industries.[19] Presently, SA is produced from petrochemical feedstocks, but there has been a trend towards production of bio-based SA from biomass (*e.g.* sucrose and glycerol).[20-22] The polycondensation of glycerol and succinic acid to form novel biodegradable materials has been studied previously, but has typically involved energy intensive processes (heat /vacuum), toxic solvents and/or catalysts.[21, 23-26] In addition, if not well-controlled, the polycondensation of glycerol and diacids (succinic acid, azelaic acid and glutaric acid) gives only low conversions and a plethora poorly controlled cross-linked or branched materials.[27-29] Biodegradable and bio-renewable polyesters such as poly(lactic acid) (PLA), poly(glycerol-succinate) (PGLSA) and poly(lactic-*co*-glycolic acid) (PLGA) have been combined with PEG, lauric acid (LA) and palmitic methyl ester to prepare surfactants. However, high temperatures, toxic catalysts and solvents are always required.[25, 27, 30-34]

In this paper we show for the first time the production of a library of biodegradable surfactants derived from glycerol. To do this, we exploited scCO$_2$ to facilitate mild reaction conditions and the allow the use of a lipase CaLB (Novozyme-435) as a chemo- and regio-selective catalyst to yield the linear and low molecular weight polymers desired to construct a range of surfactants. In order to tune the amphiphilic balance of the PLGSA backbone, PEG and lauric acid were then employed as hydrophile and hydrophobe respectively. PEG can be produced from biobased feedstock such as bagasse[35] and lauric acid is a naturally occurring fatty acid. Thus, the entire surfactant molecule can be considered biorenewable and fully biodegradable.

Experimental Section and Materials are reported in the Supporting Information document.

Results and Discussion

The key focus of this work is the design and optimisation of an enzymatic synthesis of PGLSA exploiting $scCO_2$ (Scheme 1) to develop a facile route to linear and low molecular weight polyesters. Avoidance of branching is important because as branching increases, the number of pendant hydroxyl groups on the polymer chain is decreased, and this compromises water solubility.[36, 37] Control experiments were also performed in $scCO_2$ without CaLB and under the more traditional melt polymerisation conditions at 120°C with and without CaLB.

glycerol (G)   succinic acid (SA)   PGLSA
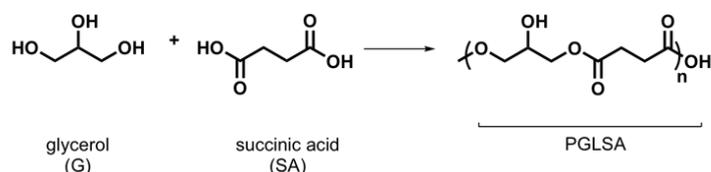
Scheme 1. Schematic representation of the synthesis of PGLSA from glycerol and succinic acid.

Enzymatic polycondensation of poly(glycerol succinate) under supercritical conditions

Previous studies have determined a very low solubility of succinic acid in $scCO_2$[38] and others have investigated the phase equilibrium of the CO_2/glycerol system.[39] In our experiments, the strong interaction and solubility of $scCO_2$ in glycerol is clearly shown by the appearance of bubbles in the liquid glycerol upon depressurisation (Figure S1, E). This interaction offers the opportunity that $scCO_2$ could act as a processing aid in polymerisation, lowering viscosity and improving mass transfer of monomers to the catalyst or enzyme.[36,37] A series of polymerisations of succinic acid and glycerol were trialled using CaLB (25 wt.% wrt monomer

including polymer support) to gauge the effect of temperature (40, 50 and 60 ºC) and molar ratio of the monomers (Table 1).

Table 1 – Synthesis of poly(glycerol succinate) *via* enzyme in scCO$_2$

	Entry	Ratio (Gly:SA)	Yield*	M_n^{GPC} (Da)	Đ	DB[a]	T_g[b]
40 ºC	1	1:1	87%	3,400	3.00	5%	-74 ºC
	2	1:2	67%	1,900	1.48	10%	-44 ºC
	3	2:1	59%	1,100	2.85	11%	-74 ºC
50 ºC	1[a]	1:1	32%	2,600	2.73	13%	-48 ºC
	2[a]	1:2	49%	1,200	1.27	8%	-40 ºC
	3[a]	2:1	85%	2,700	1.84	5%	-56 ºC
60 ºC	4	1:1	84%	1,700	3.41	11%	-55 ºC
	5	1:2	88%	3,500	1.19	7%	-50 ºC
	6	2:1	78%	1,300	3.84	9%	-77 ºC

[a] Degree of Branching determined by Frey's equation S1: $DB_{Frey} = \frac{2 \times D}{2 \times D + L} \times 100$ by integrating the resonances corresponding to the H$_b$ protons from the B$_0$ and B$_1$ structures, using ^{1}H-NMR and focussing on proton H$_b$ (see Figure 1). [b] Measured by DSC.
* yield of recovery: actual amount of material physically recovered from the reactor after reaction.

The molar ratio of the monomers was found to only slightly affect the size and topology of the polymers produced using CaLB in scCO$_2$ with M_n ranging from 1100 to 3500 Da and a very low DB (calculated from ^{1}H-NMR) in the range 5-11 %. The peaks in the ^{1}H-NMR in the regions at 3.5–3.7 ppm, 3.80-3.87 ppm, 4.0–4.2 ppm and 4.8–5.4 ppm are attributed to the protons H$_a$, H$_b$ and H$_c$ of the linear, terminal and branched glycerol units (Scheme 2) and all of our assignments are in accordance with the previous literature [40, 41]. ^{1}H-NMR of the PGLSA product (Figure 1) shows a low intensity for the tri-substituted glycerol unit proton H$_b$ (ca. 5.28 ppm) indicating a low degree of branching (DB) which was determined according to Frey's equation (Supplementary Information) by integrating the resonances corresponding to the H$_b$ protons from the B$_0$ and B$_1$ structures using ^{1}H-NMR (see Scheme 2 and Figure 1). To

understand in more detail the nature of the glycerol ^{1}H pattern the ^{1}H-NMR experiment was combined with 2D-HSQC and COSY NMR techniques (see Figure S2A)
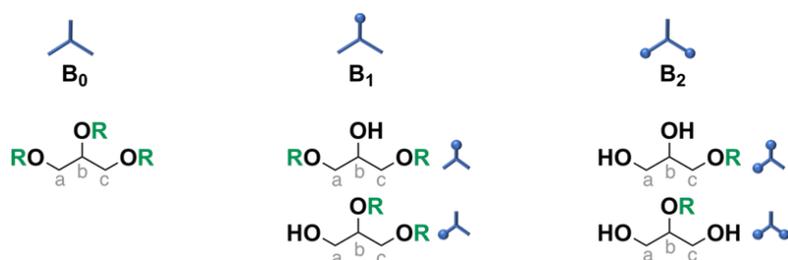
Scheme 2. Visual representation of possible modes of polymerisation showing, linear, branched and terminal units. Structural information on these topologies can be derived from the ^{1}H NMR data relating to H_a, H_b and H_c. The schematic representations of branched, linear and terminal polymer units are as follow: ⅄ = linear glycerol unit; in this case, the polymerisation occurred in positions a and c, ⅄ = linear glycerol unit; in this case, the polymerisation occurred in positions b and c, ⅄ = terminal glycerol in the polymer backbone, leaving the hydroxy groups a and c free, ⅄ = terminal glycerol in the polymer backbone, leaving the hydroxy groups a and b free, ⅄ = trisubstituted glycerol unit in the branched polymer backbone.
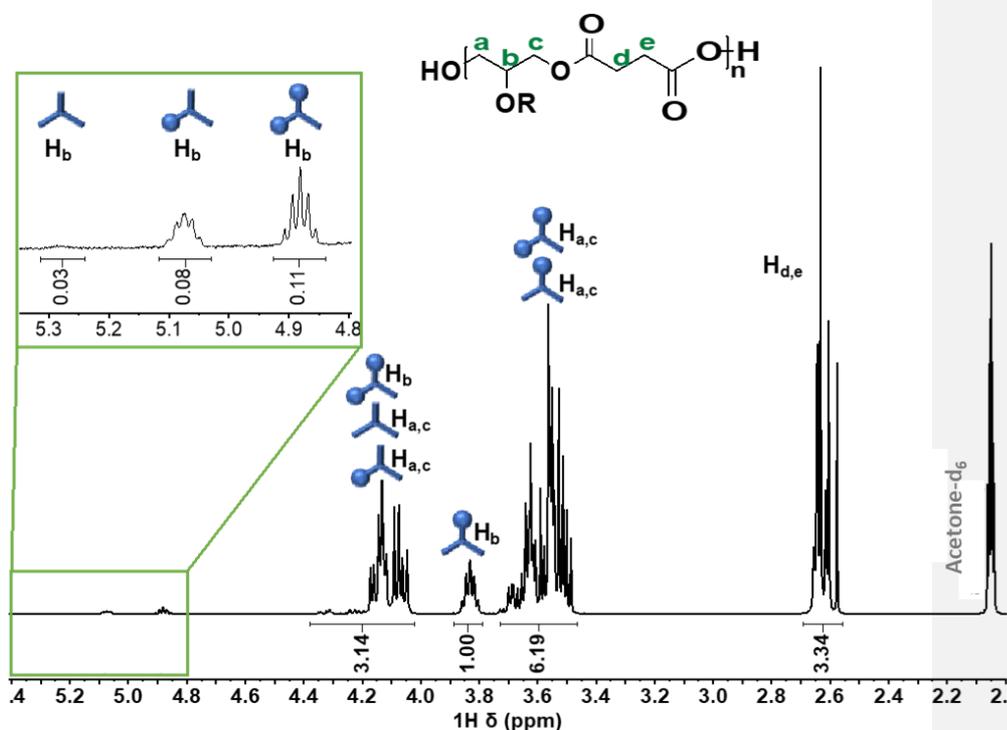
Figure 1 – ^{1}H-NMR spectrum of PGLSA produced by enzymatic catalysis under scCO$_2$ at 60 °C (275 bar) from a G:SA molar ratio of 1:2 (Table 1 entry 5). For glycerol branching representations, refer to Scheme 2. Only a very small presence of tri-substituted glycerol units is observed (proton H$_b$ at ca. 5.3 ppm highlighted in the inset), compared to that obtained for PGLSA synthesised without catalyst (Figure 3)

Using CaLB under supercritical conditions, it was observed that the degree of branching (DB) was not strongly influenced by the molar ratio of alcohol:diacid and no gelation onset was observed at 40 or 60 °C. At 40 °C and with a molar ratio of G:SA 1:1 (Table 1, entry 1), a waxy yellow polymer with low molecular weight (3,400 Da), low DB and broad dispersity ($Đ = 3$) was obtained. Increasing the diacid content or glycerol content (Table 1, entries 2,3) showed an increase in branching, but a decrease in molecular weight and yield.

At 60°C the molecular weight and DB remain generally low and we can be confident that under supercritical conditions the enzyme functions well giving good yields of low molecular weight materials. The materials produced at 50 ºC (chosen as an intermediate temperature) showed the same amorphous behaviour with low DB and low molecular weight at all the monomer ratios explored. Again, there is negligible effect of molar ratio upon branching, highlighting the regioselectivity of the enzyme under these reaction conditions. In all cases the PGLSA showed T_gs from -77 to -40 ºC which was in the expected range for low molecular weight linear polyesters. The Tg obtained is clearly influenced by both molecular weight and DB. Importantly, all the scCO_2 synthesised PGLSA polymers showed molecular weights below 4,000 Da and were soluble in water, thus demonstrating promise for application as renewably sourced biodegradable surfactants. In particular, the bio- and chemical- degradability of polyesters (linear or branched) bearing glycerol and a diacids are well known and broadly reported in literature.[42,43,44,45, 46] As an extra control, reactions were performed in scCO_2 at the same temperature without the enzyme, and no conversion of monomers into polymer could be obtained at 40 – 60 °C.

MALDI-TOF spectrometry was performed in order to further elucidate the PGLSA architecture. If branched polymers are formed, resulting from the esterification of both primary and secondary hydroxy groups of glycerol, oligomers containing tri-substituted units will show characteristic masses different from those of an oligomer containing only linear di-substituted structures. The MALDI-TOF mass spectrum of PGLSA (Figure 2) synthesised at 60 ºC, 275 bar for 24 h with a G:SA molar ratio of 1:2 (Table 1 entry 5) shows peaks that can be assigned to the sodium and potassium adducts for a linear PGLSA; the same pattern was observed for all the polymers synthesised under melt and scCO_2 condition in presence of CaLB. The peaks match well with the predicted masses, confirming a linear structure of PGLSA. In addition, it was still possible to observe some branched structures, which were expected as a consequence of the degree of branching determined from 1H-NMR (DB = 8%).

Polymer structure	$(M + Na)^+$	$(M + K)^+$
SA$\pm$G$-$SA$\pm_5$	1,022	1,038
$\pm$G$-$SA$\pm_6$	1,085	1,101
SA$\pm$G$-$SA$\pm_6$	1,185	1,201
$\pm$G$-$SA$\pm_7$	1,259	1,275
SA$\pm$G$-$SA$\pm_7$	1,359	1,375
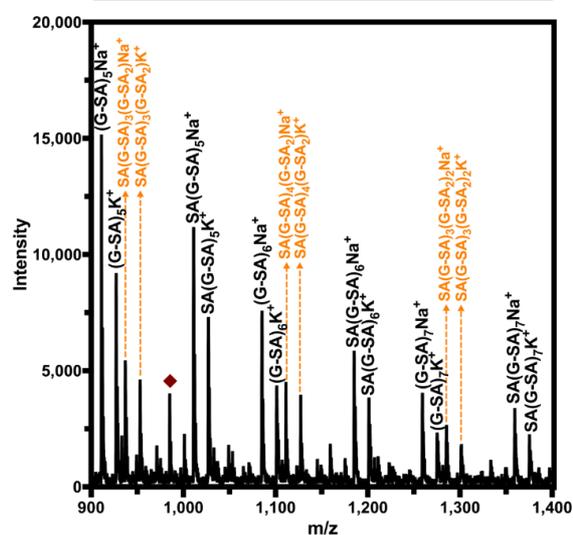
Figure 2– (TOP) Suggested structures and predicted masses of the sodium and potassium adducts for the linear poly(glycerol succinate). Note: G = glycerol; SA = succinic acid. (BOTTOM) MALDI-TOF mass spectrum section of K$^+$ and Na$^+$ adducts of PGLSA synthesised under enzymatic supercritical conditions (). *Note*: ◆ an unknown peak; the peaks not assigned are assumed to be noise from the baseline spectrum; G = glycerol; SA = succinic acid; orange annotation denotes branched structures of PGLSA; black denotes linear structures of PGLSA.

Melt polycondensation of poly(glycerol succinate)

PGLSA was also synthesised without catalyst *via* the more traditional melt polycondensation at 120°C (Table 2, entries 7, 8 and 9). The yield of polymer obtained was generally much lower than that obtained in scCO$_2$. When performed with a 1:1 molar ratio in the absence of catalyst at 120°C, a polymer with a molecular weight up to 3,900 Da ($Đ > 2$) was obtained with DB 18% and this was found to be only partially soluble in water. The increase in branching can clearly be seen (Figure 3). Increasing the SA content to a G:SA molar ratio of 1:1.5 and 1:2, increases DB dramatically. The molecular weight of the polymer also increased, and these materials were found to be insoluble in water, in accordance with previous studies.[47]

Table 2. Synthesis of poly(glycerol succinate) *via* melt polymerisation at 120°C without and with CaLB.

	Entry	Time	G:SA molar ratio	Yield	M_n^{GPC} (Da)	$Đ$	DB[a]	T_g^{b}
No catalyst	7	24 h	1:1	60%	3,900	> 2	18%	-61 ℃
	8	24 h	1:1.5	60%	8,200	> 2	67%	-18 ℃
	9	24 h	1:2	96%	6,200	> 2	65%	-20 ℃
CaLB	10	24 h	1:1	61%	3,700	> 2	13%	-55 ℃
	11	24 h	1:2	54%	14,900	> 2	11%	-50 ℃

[a] determined by Frey's equation S1: $DB_{Frey} = \frac{2 \times D}{2 \times D + L} \times 100$ by integrating the resonances corresponding to the H$_b$ protons from B$_0$ and B$_1$ structures, using ^{1}H-NMR. The chemical shifts used related to H$_b$ the proton are reported in Figure 1. [b] Measured by DSC.

* yield of recovery: actual amount of material physically recovered from the reactor after reaction.
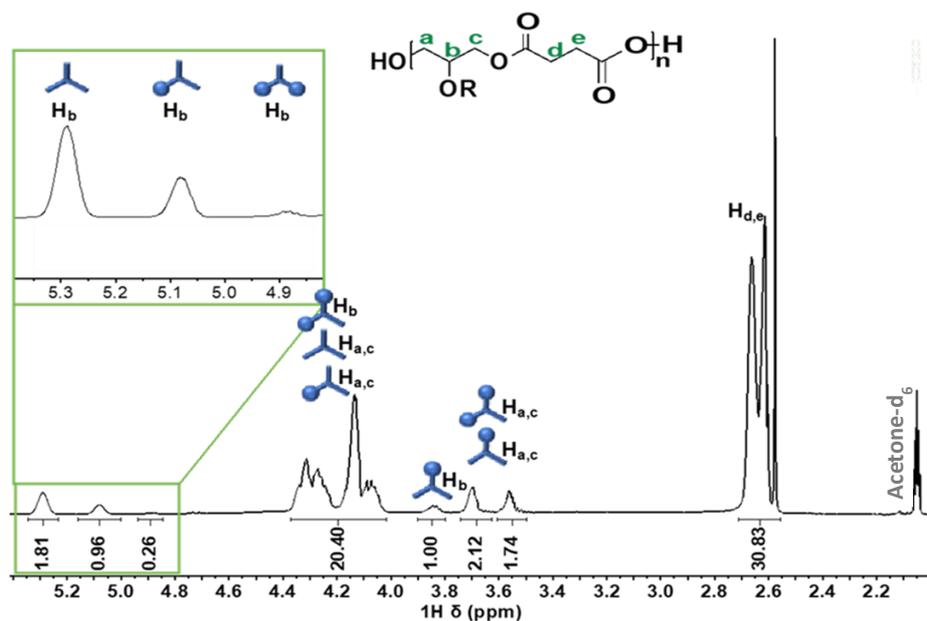
Figure 3. ^{1}H-NMR of PGLSA from melt polymerisation without catalyst (entry 8 Table 2) clearly showing high levels of branching as indicated by the trisubstituted glycerol peak at ca. 5.3 ppm associated with H_b (inset) and the corresponding increase of terminal groups when compared to Figure 1.

The NMR data reveal clearly the increase in branching when moving to melt polymerisation as compared to the polymers produced in scCO$_2$. To understand this in more detail, the branching patterns of the polymeric backbones were analysed by combining ^{1}H-NMR, 2D-HSQC and COSY NMR spectroscopies (see Figure S2 B) to allow the degree of branching to be calculated and compared.

We also probed use of the enzyme under conventional melt conditions, reasoning that the inherent chemo- and regio- selectivity might yield the desired linear polymers (entries 10 and 11 in Table 2). Use of CaLB does indeed significantly lower branching, dropping the DB to 13 and 11%. The molar ratio of the monomers was found to only slightly affect the size and topology of the polymers in the presence of CaLB in scCO$_2$ as well as in melt conditions. On the other hand, when no catalyst was present gelation was seen to occur earlier and at a lower ratio of Gly:SA (Table 2), clearly indicating a tendency towards branched polymer. When CaLB was used as catalyst (in both supercritical and melt conditions) similar DB values were

observed (<15%) but only under supercritical conditions were the yields pushed consistently to above 60 %.

For PGLSA synthesised under melt polycondensation without catalyst (Table 2, entries 7-9), it is clear that the increased molecular weight and higher DB that are obtained lead to higher T_g. Indeed, it is well known that T_g increases with molecular weight[48] and that branching also influences chain interactions[49,50,38]. The T_g's of the PGLSA chains synthesised *via* enzymatic melt polycondensation did not show a dependence upon molecular weight (Table 2), but such T_g values in the range of -50 ºC are strongly indicative of other linear polyesters such as poly(butylene itaconate) and poly(1,5-pentylene adipate)).[38, 51]

The use of CaLB in the melt certainly leads to more linear polyesters containing the desired pendant hydroxy groups. However, the melt condensation process at 120°C is not ideal since the high temperatures lead to degradation of the enzyme which cannot be recycled and tend towards high molecular weight and low yield. Higher molecular weights in general are problematic for developing surfactants because such materials show only low water solubility and cannot be utilised effectively. The use of $scCO_2$ clearly facilitates the synthesis of linear low molecular weight poly(glycerol succinate) at lower temperatures and with reasonable yield. Moreover, it has been shown previously that such supported enzymes can be recycled and reused several times in $scCO_2$. [36,52]

MALDI_TOF analysis for PGLSA synthesised in melt conditions without any catalyst (Figure S3) showed different repeat unit patterns and much more branching compared to the PLGSA polymers obtained in $scCO_2$ with CaLB (Figure 2). This initial screening highlights the crucial combination of enzyme and low temperature in our use of $scCO_2$ leading to linear, low molecular weight (water soluble) poly(glycerol succinate) under mild reaction conditions and with high yield. In the next section we go on to exploit these syntheses to create renewable and biodegradable surfactant from PGLSAs with the addition of lauryl or PEG moieties (Scheme 3).

We also looked carefully at performing the polymerisations in conventional solvent. Clearly the solvent must not be miscible with water to allow ofr removal of water during the

Enzymatic synthesis of lauroyl- and pegylated- poly(glycerol succinate) under supercritical conditions

To create a functional surfactant, it is necessary to find good routes to addition of a range of end groups. Thus, the enzymatic synthesis of lauroyl PGLSA (LA-PGLSA) and pegylated PGLSA (PEG-PGLSA) in $scCO_2$ were performed at 40, 50 and 60 °C, adding PEG or LA to GL and SA in a one-pot reaction exploiting the optimised chemistry developed for the PGLSA scaffold. To explore the temperature effect on the synthesis of the end-capped polymers and broaden the physical properties of the final surfactants, both LA and PEG variations were also performed at 50 °C. The initial syntheses (Table ~~Table~~ 3) were conducted at 275 bar for 24 h, with an excess of glycerol, to ensure glycerol terminated PGLSA (Scheme 2) and promote termination by lauric acid units. For the synthesis of poly(ethylene glycol)-based surfactants, there was an excess of succinic acid, to ensure succinic acid terminated PGLSA, so that the PEG units could add to the carboxylic moieties (Scheme 2).
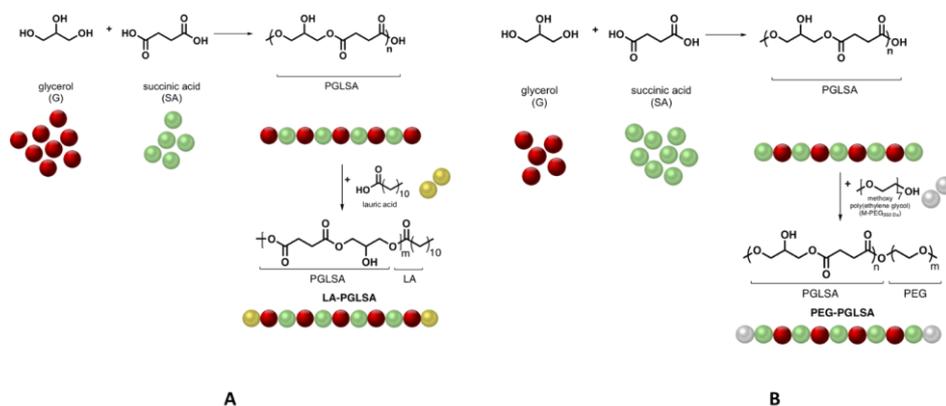
Scheme 3 – Schematic representation of the synthesis of LA-PGLSA, demonstrating use of excess glycerol to ensure the synthesis of glycerol terminated PGLSA (**A**). Schematic representation of the synthesis of PEG-

PGLSA, demonstrating the excess of succinic acid, to ensure the synthesis of succinic acid terminated PGLSA (**B**).

Table 3 – Synthesis of lauroyl and PEG functionalised poly(glycerol succinate) under enzymatic supercritical conditions, at 275 bar.

Entry	T ºC	Ratio (Gly:SA:LA)	Yield	M_n^{GPC} (Da)	Đ	M_n^{NMR} (Da)[a]	DB[b]	T_g	T_m
LA-PGLSA1	40 ºC	2:1:0.15	85%	1,700	1.5	2,200	12%	–	48 ºC
LA-PGLSA2	50 ºC	2:1:0.15	96%	1,400	> 3	2,600	10%	-72 ºC	50 ºC
LA-PGLSA3	60 ºC	2:1:0.15	92%	2,000	1.8	2,800	5%	-78 ºC	50 ºC
PEG-PGLSA4	40 ºC	1:2:0.15	93%	1,200	1.8	2,000	13%	-45 ºC	–
PEG-PGLSA5	50 ºC	1:2:0.15	85%	3,800	>2	2,200	0%	-75 ºC	–
PEG-PGLSA6	60 ºC	1:2:0.15	94%	1,500	>2	2,200	0%	-74 ºC	–

[a] calculated through ¹H-NMR from the ratio between the integrals of the peaks of the polymer backbone and the end-group peak, using; [b] determined by the Frey's equation S1.

* yield of recovery: actual amount of material physically recovered from the reactor after reaction.

For the LA-PGLSA synthesis, the calculation of M_n^{NMR} focussed on the specific NMR signals of the terminal glycerol unit as the linking unit, not those of the repeating unit of the PGLSA backbone. Equation S2 was used to evaluate the successful attachment of LA to the PGLSA backbone. The calculated M_n^{NMR} (Table 3 and Figure 4) were in good agreement with the values obtained through GPC (Table 3). Very positively, all the polymerisations of LA-PGLSA polymers show only low degrees of branching (DB) (Table 3) which is ideal for the potential use as surfactant molecules
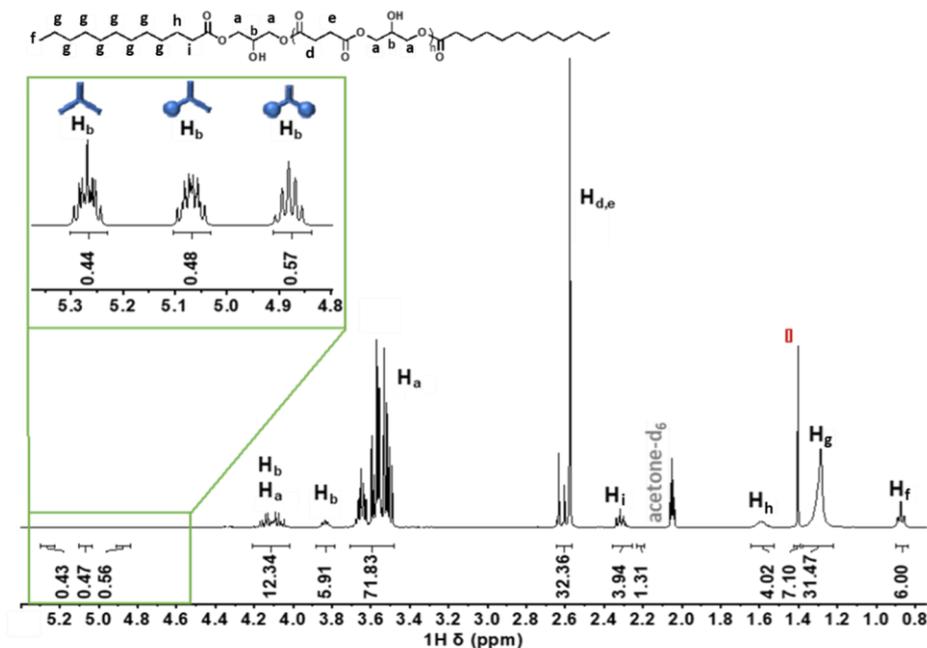
Figure 4 – ¹H-NMR spectrum of LA-PGLSA (entry 1, Table 3). The solvent used was acetone-d_6. Integrals of the peaks of H_f (0.88 ppm, terminal methyl group from LA), and H_a (3.50-3.72 ppm), H_b (3.83 ppm) and $H_{d,e}$ (2.58-2.69 ppm) (backbone of PGLSA) can be used to estimate the average molecular weight of the polymer. The peak at 1.29ppm, H_g, is assigned to the –CH_2– in the LA chain, while peaks at 1.59 and 2.31 ppm, H_h and H_i, are assigned to the –CH_2– close to the carboxy group of LA (–**CH_2–CH_2**–COO–). For glycerol branching representations refer to **Scheme 2**.

The LA-PGLSA synthesised at 40 ℃ (entry LA-PGLSA 1, Table 3; Figure 4) was obtained with 85% yield and a molecular weight of 1,700 Da (M_n^{GPC}) (DB = 12%). These data also show that end-capping *via* enzymatic polycondensation under supercritical conditions is highly efficient with 98.5% of the detected LA moieties attached to the PGLSA backbone. When increasing the temperature to 50 ℃ and 60℃ (Table 3) little effect was noted and hence from a sustainable viewpoint, 40 ℃ was selected as the most energy efficient temperature.

For the PEG-PGLSA system, excess SA ensured the attachment of M-PEG to the terminal units and the calculation of M_n^{NMR} (using Equation S4) focussed on the specific NMR signals of the terminal SA linking unit, not those of the repeating unit of the PGLSA backbone.
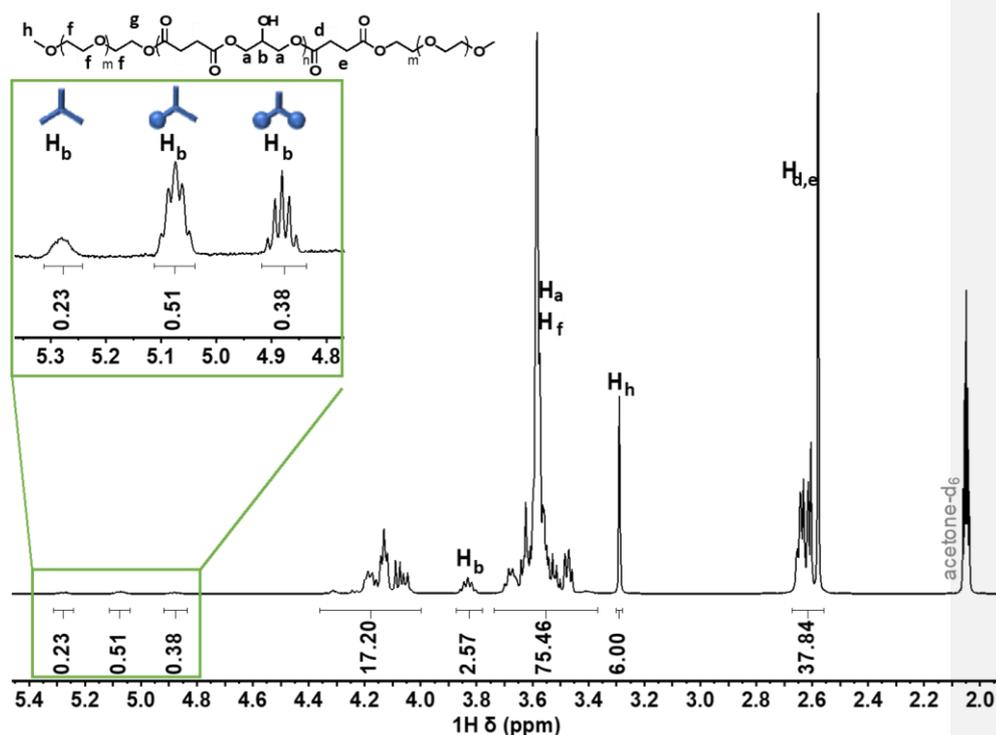
Figure 5 – ^{1}H-NMR spectrum of PEG-PGLSA from entry 4, Table 3. The solvent used was acetone-d_6. Integrals of the peaks of H_h (3.29 ppm, terminal methyl group from M-PEG), and H_a (3.50-3.72 ppm), H_b (3.83 ppm) and $H_{d,e}$ (2.58-2.69 ppm) (backbone of PGLSA) can be used to estimate the average molecular weight of the polymer. The protons H_f, from the M-PEG backbone, overlap with the –CH$_2$– protons from the glycerol unit. For glycerol branching representations refer to **Scheme 2**.

The calculated M_n^{NMR} (Table 3) for the PEG addition also showed good agreement with the values obtained through GPC and indirectly show successful attachment of M-PEG$_{350\ Da}$ to the PGLSA polyester. When varying the temperature, the degree of branching remained low as expected (lower than 15 %) with good yield. Increasing the temperature to 50 ºC (entry PEG-PGLSA 5, Table 3), gave rise to a sudden increase in molecular weight, 3,800 Da (M_n^{GPC}) ($Đ >$ 2, DB = 0%, 85% yield). By contrast, the polymerisation of LA-PGLSA at the same

temperature, 50 ℃ (entry LA-PGLSA 3, Table 3), showed a decreased M_n^{GPC} (1,400 Da) when comparing to 40 ℃ (1,700 Da). Increasing further to a reaction temperature of 60 ℃, (entry PEG-PGLSA 6, Table 3) gave PEG-PGLSA with a molecular weight of 1,500 Da (M_n^{GPC}) ($Đ >$ 2, DB = 0%) and 94% yield was obtained.

It is well known that thermal properties of polymeric structures can be affected not only by the degree of polymerisation, but also by end-groups.[41, 53] Since the LA-PGLSA synthesised polymers all have similar molecular weights and structure, there is no significant difference in their thermal properties and this is consistent with what has been shown previously for similar polyesters[54] e.g. Tween[TM] 20 (also known as polyoxyethylene (20) sorbitan monolaurate), a commercially available non-ionic surfactant[55] with T_g of *ca.* -61 ℃ and a T_m *ca.* -15 ℃. The melting points of LA-PGLSA (*ca.* 48-50 ℃) are close to values obtained by other authors for similar polyesters (linear polyesters based on pentaerythritol, succinic acid and lauric acid)[56] and reflect the interactions of the long alkyl chains of the terminal LA moieties. By contrast, no T_m values were detected for the PEG-PGLSA polymers, reflecting the amorphous nature of the hydrophilic end capping molecule.

The T_g values for the samples synthesised at 40 ℃ (entry PEG-PGLSA 4, Table 3) were higher than those produced at 50 and 60 ℃ (-45 ℃ vs ca. -75 ℃) (entries 5 and 6, Table 3). This appears to show the important influence on the thermal properties of the degree of branching since at 40 ℃, PEG-PGLSA showed a DB of 13%, while at 50 and 60 ℃, no branching was detected. This increase of the degree of branching in the presence of PEG moieties might well increase the entanglements between the polymer chains and lead to higher T_g. No such correlation was observed for the T_g trends for the uncapped PGLSA.

We have demonstrated that the enzymatic synthesis of biorenewable and biodegradable surfactants can be controllable, with low branching values giving linear and water-soluble chains with a range of potential end group functionalities. Moreover, the syntheses with scCO$_2$ are at lower temperatures, and provide a clean and efficient route to new surfactants. These data complement earlier studies that showed the synthesis of PEG-based surfactants under enzymatic scCO$_2$ for azelaic acid and 1,6-hexanediol end-capped with hydrophilic methoxy poly(ethylene glycol) moieties[37] and those based upon sorbitol and lactide.[57] The replacement

of conventional solvents by scCO$_2$ is certainly viable since this introduces opportunities ofr lower temperature processing and the utilisation of enzymatic routes to polymer based surfactants.[58]

Surfactant properties

Amphiphilic, biorenewable and biodegradable polymers can find application in formulations for wetting agents, emulsifiers and detergents, but only if they are able to sufficiently reduce the surface tension of water. The PGLSA polymers (with G:SA ratios of 1:2 and 2:1) that were not end-capped showed only a minimal reduction in the static and dynamic surface tensions and do not show any significant surface-active properties (Figure 6 and S5). By contrast, the end group modified LA and PEG polyesters show great promise as green biodegradable surfactants demonstrating a significant reduction of the dynamic and static surface tension values (Figure 6 and S6) and these compare favourably with benchmark surfactants (Tween[TM] 20 and NatraGem[TM] E145) derived from petrochemical feedstocks.
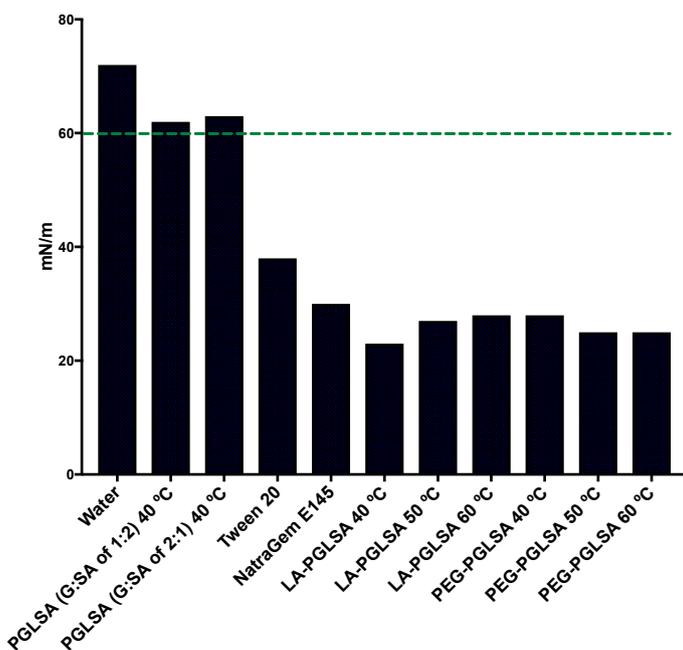
Figure 6 – Static surface tension of water, PGSLA not end-capped (with 1:2 and 2:1 G:SA molar ratio, both at 1 wt.%), commercial surfactants (Tween[TM] 20 – 1,200 Da, NatraGem[TM] E145 – 1,300 Da, both at 1wt.%) and the synthesised end capped surfactants (1 wt.%) at different temperatures. Those the green dashed line are considered to have surface active properties. (LA-PGLSA 40 ºC – 1,700 Da; LA-PGLSA 50 ºC – 1,400 Da; LA-PGLSA 60 ºC – 2,000; PEG-PGLSA40 ºC – 1,200 Da; PEG-PGLSA50 ºC – 3,800 Da; PEG-PGLSA60 ºC – 1,500 Da).

A true understanding of the potential surfactant performance requires assessment of critical aggregation concentrations, the size of the aggregates and an assessment of the water contact angle (Θw) (see supplementary information)

Table 4 – CAC values of synthesised and commercial surfactants.

Entry	Compound	M_n^{GPC}	CAC (μM)	CAC (wt.%)
1	Tween[TM] 20	1,200 Da[a]	73[52]	0.02
2	NatraGem[TM] E145	1,300 Da[a]	312	0.03
3	LA-PGLSA 40ºC	1,700 Da	462	0.05
4	LA-PGLSA 50ºC	1,400 Da	223	0.02
5	LA-PGLSA 60ºC	2,000 Da	838	0.08
6	PEG-PGLSA40ºC	1,200 Da	530	0.05
7	PEG-PGLSA50ºC	3,800 Da	640	0.06
8	PEG-PGLSA60ºC	1,500 Da	>1000	>1

[a] molecular weight determined by the supplier. The CAC measurement in each case was accomplished by automated measurement of the surface tension of the surfactant at a range of concentrations (1-0.007 wt.%), using the static Wilhelmy plate tensiometer. (Figure S7)

In each case we have made comparisons with the commercial samples Tween[TM] 20 and NatraGem[TM] E145; both are known to be efficient surfactants at low concentrations. The CAC values obtained will of course depend upon the chain lengths and degree of branching; both of which determine the size of the polar head of the surfactant in the case of LA-PGLSA. For LA-PGLSA synthesised at 40 ºC (Entry 3 Table 4) we saw a decrease the surface tension to 23 mN/m, with a promising CAC plateau value of 462 mg/L (0.05 wt.%). For LA-PGLSA synthesised at 60 ºC, the surfactant becomes efficient only at much higher concentration (Entry 5, Table 4) and, since the degree of branching at 5% is the lowest, this probably reflects the presence of more hydroxy pendant groups in a larger polar head.

The PEG-PGLSA surfactants gave higher CAC values, likely due to the high hydrophilicity of PEG as an end-group. The PEG-PGLSA at 60ºC showed a surprisingly high CAC value, >1000 mg/L (> 1 wt.%). This might be a consequence the presence of free M-PEG (unattached to the PGLSA) (entry 8, Table 4).

The sizes of the self-assembled structures are obtained from DLS and are in the range of 170-600 nm (entries 3-8 from Table 5). Personal care and cosmetics applications require self-assembled aggregates in a size range between 200-500 nm.[59] Whilst for drug delivery, self-assembled aggregates must typically be smaller than 200 nm;[60] to deliver efficient penetration through blood vessel walls.[61, 62]

Table 5 – Size distribution of surfactant <mark>nanoaggregates</mark> measured by DLS. The measurements were done at surfactant concentrations higher than it CAC value. [a] concentration close to CAC value. [b] value reported from peak 2 because a higher peak was also present in DLS trace, > 5,000 nm, explaining the high Z-average reported.

Entry	Compound	CAC (wt. %)	Concentration (wt. %)	Z-average (d, nm)	PDI
1	Tween™ 20	0.02	0.1	100.5 (±9.2)	0.5 (±0.08)
2	NatraGem™ E145	0.03	0.1	370 (±34.5)	0.4 (±0.04)
3	LA-PGLSA 40°C	0.05	0.1	274 (±25.5)	0.3 (±1.24)
4	LA-PGLSA 50°C	0.02	0.1	543 (±82.2)	0.4 (±0.05)
5	LA-PGLSA 60°C	0.08	0.2	247 (±9.5)	0.2 (±0.03)
6	PEG-PGLSA 40°C	0.05	0.1	631 (±119.7)	0.5 (±0.1)
7	PEG-PGLSA 50°C	0.06	0.1	280 (±18.7)	0.3 (±0.03)
8	PEG-PGLSA 60°C	>1	1[a]	178 (±3.1)	0.3 (±0.01)

The sizes of the self-assembled structures will of course be influenced by the molecular weight of the building blocks, the length of the end-cappers, and the hydrophobicity of the non-polar block.[37] These data did not show any significant size differences between the two types of end-capped PGLSA-based polymers, but promisingly the size distributions were similar to the commercial surfactants NatraGem™ E145 and Tween™ 20 in the range 100-300 nm.

Water contact angle (Θw) is of pivotal importance for different applications, including cleaning, lubrication, coating and printing.[63] Θw values observed for PGLSA polymers not end-capped with LA or PEG were near 100º showing that the bare PGLSA backbones had minimal ability to reduce the interfacial tension between the water solution and the solid surface. On the other hand, the end-capped PGLSA surfactants show promise with contact angles in the same range as Tween[TM] 20 and NatraGem[TM] E145 (Table 6).

Table 6 – Contact angles (left and right) of water.

Entry	Compound	Left angle	Right angle
1	Water	110.0 ± 0.2	109.0 ± 0.3
2	Tween[TM] 20	91.0 ± 2.3	90.0 ± 2.6
3	NatraGem[TM] E145	63.0 ± 2.3	61.0 ± 2.5
4	PGLSA 1:2 40 ºC	103.0 ± 1.1	104.0 ± 2.0
5	PGLSA 2:1 40 ºC	96.0 ± 0.5	96.0 ± 0.6
6	LA-PGLSA 40ºC	75.0 ± 6.2	73.0 ± 7.2
7	LA-PGLSA 50ºC	74.0 ± 5.9	75.0 ± 6.7
8	LA-PGLSA 60ºC	79.0 ± 5.5	78.0 ± 6.0
9	PEG-PGLSA40ºC	88.0 ± 0.9	88.0 ± 0.4
10	PEG-PGLSA50ºC	93.0 ± 0.6	93.0 ± 0.6
11	PEG-PGLSA60ºC	90.0 ± 0.9	90.0 ± 3.8

Commercial surfactants at 0.5 wt.% (Tween[TM] 20 and NatraGem[TM] E145), PGLSA at 0.5 wt.% (with G:SA of 1:2 and 2:1, synthesised at 40 ºC), 0.5 wt.% of LA-PGLSA (synthesised at 40, 50 and 60 ºC) and 0.5 wt.% of PEG-PGLSA(synthesised at 40, 50 and 60 ºC).

These data collectively demonstrate that our PGLSA-based surfactants are effective in reducing the surface tension of water compared with the commercial surfactants and deliver CAC values, aggregate sizes and water contact angles that show promise as biorenewable and biodegradable surfactants.

Conclusions

We have exploited the unique properties of scCO$_2$ to allow melt synthesis of poly(glycerol succinate) PGLSA at very mild temperatures (<60 °C). This allows effective use of an enzyme (CaLB - Novozyme 435) catalyst to deliver linear and low molecular weight PGLSA-based

polymer chains with pendent hydroxy groups. Such low molecular weight and linear materials are of value because they are water soluble and provide the opportunity to develop new types of renewable surfactant. The linearity of the polymers was confirmed by 1H-NMR, COSY and 2D-HSQC NMR, showing that there was minimal esterification on the secondary hydroxyl group of the glycerol monomer. These chains were then exploited by end-capping with lauric acid or poly(ethylene glycol) and the performance of these novel end-capped amphiphilic materials as surfactants has been tested by employing a variety of standard analytical techniques. All of the surfactants showed the ability to decrease the surface tension of water giving competitive surface-active properties when comparing with commercially available surfactants and can be optimised to yield low CAC values, with aggregates in the range 200-600 nm. Contact angle data showed significant reduction in interfacial tension ($75\,^\circ < \theta < 90\,^\circ$) providing the opportunity for application as wetting agents. These preliminary results clearly show that all the synthesised PGLSA-based surfactants that we have developed can form self-assembled aggregates with suitable size for personal care and cosmetic applications.[52]

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