Simultaneous enzymatic ring opening polymerisation and RAFT-mediated polymerisation in supercritical CO_2†

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This report presents the first simultaneous, metal-free synthesis of block copolymers through combination of enzymatic ring-opening polymerisation of ε-caprolactone with RAFT-mediated controlled radical polymerisation of styrene.

The combination of various living techniques for the controlled polymerisation of monomers exhibiting different propagation pathways has attracted particular interest over the previous decade. This has been driven by the increasing demand for block copolymers that can self-assemble into micellar structures in water or other solvents. These polymers have particular importance for the pharmaceutical industry as potential drug carriers or controlled release vehicles.[1] While such copolymers have been successfully synthesised in the past, the methodology generally involves multiple reaction and purification steps to achieve the desired species.

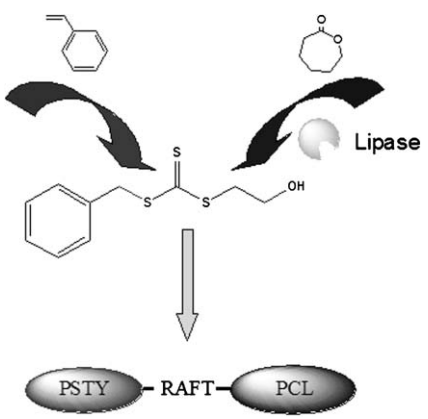
Different approaches have recently been adopted to form polyester based block copolymers. The ring-opening polymerisation (ROP) of lactides or lactones has traditionally been accomplished using metal catalysts, followed by controlled free radical polymerisation of a vinyl monomer. Stenzel and coworkers synthesised a bifunctional RAFT agent, which initially acted as an initiator for the ROP of DL-lactide and then allowed RAFT-controlled free radical polymerisation of *N*-isopropyl acrylamide.[2] However, this method involved purification and isolation of the RAFT terminated polylactide before the free radical step.

More recently, others have shown that enzymatic ROP of ε-caprolactone can be combined with atom transfer radical polymerisation (ATRP)[3,4] or nitroxide mediated polymerisation (NMP)[5] to form copolymers. However, the polymerisation requires two consecutive steps in order to achieve block copolymer. Recently, we have shown that simultaneous enzymatic ROP and ATRP can occur in supercritical CO_2 ($scCO_2$) with good control over the resultant polymer product.[6] This was attributed to the plasticising effect of $scCO_2$ on the polymer. Additionally, the use of $scCO_2$ removes the requirement for toxic organic solvents and results in a clean, solvent-free product. Unfortunately, reactions which utilise ATRP catalysts require purification of the product following the polymerisation to remove the copper catalyst. Another issue surrounding the use of ATRP as a controlling agent is the inhibiting effect that the catalyst can have on the enzyme activity.[7] This can be overcome by careful selection of the ATRP catalyst, however it diminishes the versatility of the ATRP/enzyme coupling.

In order to overcome some of these problems, we have designed a metal-free synthetic strategy for block copolymers. This, combined with the use of $scCO_2$ as the reaction medium, removes the necessity for purification following polymerisation. Additionally, we have shown that RAFT mediated free-radical polymerisation can occur simultaneously with enzymatic ROP leading to a high degree of control over the constituents of the copolymer.

Our approach for the simultaneous preparation of block copolymers is presented below (Scheme 1) and uses an hydroxy-terminated trithiocarbonate.[2] This species is similar to other RAFT agents which have been shown to have low cytotoxicity and are suitable for biomedical applications.[8]

In a typical experiment (supplementary information), a high-pressure reaction vessel was loaded with the monomers (ε-caprolactone and styrene), the RAFT agent ((2-benzylsulfanyl thiocarbonylsulfanyl)ethanol), the enzyme (*Candida Antarctica* lipase B immobilised on an acrylic resin – Novozym-435) and the free radical initiator (azobisisobutyronitrile (AIBN)). The reactions were performed at 27.6 MPa (4000 psi) and 65 °C for 24 hours and in all cases, the RAFT : AIBN molar ratio was kept at 2 : 1. We have previously shown that for successful polymerisation utilising ATRP and enzymatic ROP it is necessary to maintain a homogeneous mixture in the $scCO_2$.[6] This was facilitated by the



Scheme 1 Schematic showing the simultaneous enzymatic catalysed ROP of ε-caprolactone with RAFT-mediated free radical polymerisation of styrene to form block copolymers with a RAFT linker group in $scCO_2$.

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† Electronic supplementary information (ESI) available: Polymerisation procedure, NMR and oxalyl chloride treatment, MALDI-TOF results and GPEC procedure. See DOI: 10.1039/b611626d

Table 1 Block copolymer formation by simultaneous one-pot polymerisation of MMA and ε-caprolactone

Entry	Feed ratio[a] PCL : PSTY	mol% PCL[b]	Conversion[b] %		Block Copolymer[c]		Hydrolysed[c]		Crystallinity[d] %
			PCL	PSTY	M_w	PDI	M_w	PDI	
1[e]	—	100	96	—	36000	1.6	—	—	50
2	3 : 1	90	98	50	17000	2.1	2400	1.12	42
3[f]	3 : 1	92	99	47	15500	1.7	2300	1.09	40
4	1 : 1	65	99	37	11200	1.7	2900	1.14	24
5	1 : 3	35	99	34	4500	1.5	3200	1.25	—
6[g]	1 : 1	60	93	71	8500	1.8	3100	1.15	27

[a] Molar feed ratio. [b] Calculated using ^{1}H NMR. [c] Molecular weight calculated by GPC against polystyrene standards with RI detector. [d] Degree of crystallinity calculated by DSC using enthalpy of melting. [e] Polymerisation of ε-caprolactone using RAFT agent as initiator in absence of styrene (block copolymer molecular weight is the molecular weight of the RAFT-terminated PCL). [f] Repeat of Entry 2. [g] Three times the RAFT and initiator concentration of Entry 4.

excellent plasticising ability of $scCO_2$ in conjunction with the cosolvent effect of ε-caprolactone.[7] In this case, where a RAFT agent was used, we maintained very good control over the polydispersity of the free-radical polymerisation product. This suggests that ε-caprolactone acts as a good cosolvent for polystyrene (PSTY).

The synthesis of block copolymers with reproducible block lengths is an important consideration in copolymer synthesis. For the case presented here, where the RAFT agent also acts as initiator for the enzymatic ROP of ε-caprolactone, the ratio of block lengths can be controlled in a number of ways. The polycaprolactone (PCL) block length can be increased by lowering the number of OH initiators present – *i.e.* decreasing the RAFT concentration. The other method for controlling the block lengths is simply by varying monomer concentrations. Some typical examples are presented where copolymers were prepared by varying the monomer concentration in the feed (Entries 2–5, Table 1). As a result of decreasing the ε-caprolactone feed, smaller PCL block lengths were observed in the copolymer. Indeed, while the polydispersity of the copolymer is relatively high (due to the less well controlled enzymatic ROP of ε-caprolactone), the free radical polymerisation is well controlled with polydispersities typically less than 1.25 being observed for the PSTY block. It is worth noting that the conversion of PSTY is affected by the concentration of ε-caprolactone; lower ratios of caprolactone to styrene result in less styrene conversion (*cf.* Table 1, Entries 2, 4 and 5). Moreover, the highest polydispersity of the PSTY block is observed when the amount of ε-caprolactone in the feed is lowest. This is typical of copolymerisation reactions in $scCO_2$ and shows a loss of control as the cosolvent effect of ε-caprolactone is diminished.[7] At high ε-caprolactone loadings the homogeneity of the polymerisation mixture is maintained, facilitating good control.

In order to determine the effect of RAFT concentration on the copolymer, a reaction was undertaken with three times the concentration of RAFT agent used in the other reactions (Table 1, Entry 6). As expected, the molecular weight of the PCL block decreases due to the much higher concentration of OH-initiating groups (*cf.* Entry 4). Interestingly, the conversion of STY increases, presumably due to the higher concentration of initiator and hence propagating chains. This example clearly shows the extent to which variation in the reaction parameters can be utilised to control the copolymer properties.

The enzymatic ROP of ε-caprolactone in the absence of styrene (Table 1, Entry 1) shows that the RAFT agent is compatible with the enzyme and acts as a good initiator, such that high molecular weight PCL can be obtained with high conversion. The relatively low conversion of PSTY in the copolymers (Table 1, Entries 2–4) is typical of precipitation polymerisation, whereby the heterogeneity of the system inhibits effective transport of monomer to the propagating radical. However, we have shown that good control over the ratio of the block lengths is obtainable in $scCO_2$. Moreover, the good reproducibility of this technique is highlighted by the comparative results obtained in successive experiments (Table 1, Entries 2–3).

One disadvantage of our technique is that potentially both PCL and PSTY homopolymers could be formed; water may initiate PCL and RAFT-capped PSTY might not initiate PCL growth. Thus, the presence of block copolymer was proven using a number of different techniques. Gradient polymer elution chromatography (GPEC) has recently been developed as a tool for distinguishing between homopolymer blends and copolymer.[9] The elution time of PSTY and PCL homopolymers in GPEC depends on their solubility in the gradient solvent and also on their affinity for the stationary phase in the column. Thus, when methanol (a poor solvent for PSTY and PCL) is used as the mobile phase with an increasing gradient of tetrahydrofuran (see supporting information for details), PCL will elute before PSTY due to the greater solubility of PCL in the methanol–tetrahydrofuran mixture (Fig. 1). It follows then, that the copolymer elutes in the period between the two homopolymers. This is clearly seen for the GPEC trace of the copolymer with 92 mol% PCL (Table 1, Entry 4) which falls at longer retention time than PCL, but shorter than PSTY (Fig. 1).

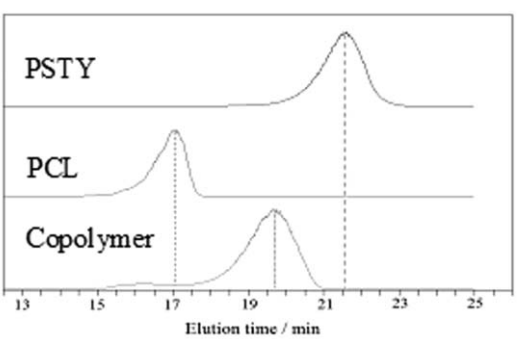

Fig. 1 GPEC traces of PSTY and PCL homopolymers and of the PCL-b-PSTY copolymer. The copolymer elutes at a time between the two homopolymers (gradient details in supporting information).

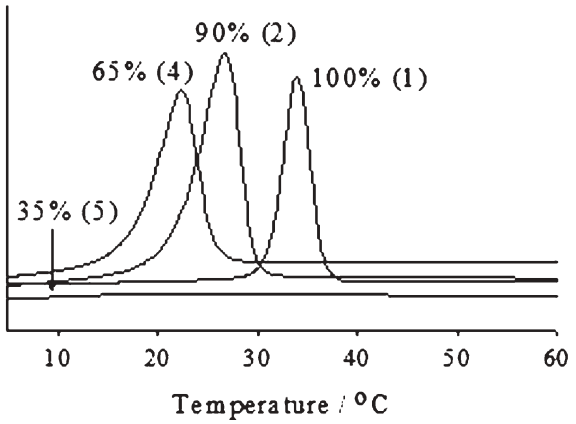

Fig. 2 DSC traces showing the decrease in crystallisation temperature for PCL-b-PSTY with increasing block lengths of PCL in the copolymer (mole% PCL indicated on figure for each curve with corresponding Entry from Table 1 in brackets). Note that no crystallisation peak is evident when PCL contributes 35 mole% or less to the copolymer.

The peak is broadened by the large distribution of PCL block lengths that are present. Additionally, a small peak is evident at similar retention time to homo-PCL. This is due to a small amount of linear and cyclic PCL that is present in the product. Thus, GPEC data clearly demonstrate that block copolymer is formed.

Another common test for the presence of copolymer is the hydrolytic degradation of the polyester from the copolymer.[7] A comparison of the GPC traces (Table 1, Entries 2–6) before and after hydrolysis shows that in all cases, the product after hydrolysis (PSTY block) had a lower molecular weight than the original block copolymer (supporting information). This simple test shows that block copolymer was predominantly formed rather than just a blended mixture of homopolymers. NMR was used to demonstrate the complete removal of PCL from the copolymer following hydrolysis (not shown).

MALDI-TOF data also show that copolymer was indeed formed (supplementary information). Additionally, there was evidence for the formation of low levels of homopolymers of PSTY and PCL. In the case of PCL, both linear and cyclic homopolymer was observed. This is typical of enzymatic ROP of ε-caprolactone and such a product has been observed previously.[4,7]

The effect on the crystallinity of the PCL upon changing the ratio of the block lengths was also investigated. Crystallisation exotherms were measured by differential scanning calorimetry (DSC) for various copolymers (Fig. 2).

The degree of crystallinity of the PCL block was calculated for each copolymer and found to decrease with increasing proportions of PSTY (Table 1). This can be explained by the inability of the PCL chains to effectively pack into crystallites – *i.e.* the disruptive effect of the PSTY block in the copolymer. Indeed, when the PSTY block length becomes much larger than the PCL block length (for example in Table 1, Entry 5), the ability of the PCL to align and form crystallites is totally inhibited and a fully amorphous copolymer is formed. By contrast, when RAFT capped PCL and PSTY (50 : 50 wt%) were physically blended prior to analysis, the degree of crystallinity of pure PCL was not affected, presumably due to phase separation of the two immiscible polymers. The exotherms are measured on the second cooling scan to eliminate the possiblity of kinetically induced blending of homopolymers from the $scCO_2$ processing.

An important factor for consideration in the enzymatic ROP of ε-caprolactone is the potential for initiation by adventitious water in the system. This increasingly becomes an issue when bifunctional initiators are used for copolymer formation without intermediate purification steps. Water initiated PCL in the RAFT mediated reaction was quantified using ^{1}H NMR and end-group functionalisation as previously described[7] (supplementary information). Surprisingly, the amount of water initiated PCL was extremely low, typically less than 1%. This is considerably less than that achieved in analogous reactions involving a bifunctional initiator for ATRP (typically $\sim$ 10%).[7] This suggests that either the system used here was much drier than in previous experiments, or that the bifunctional RAFT initiator has much better access to the active site of the enzyme than those initiators used previously. This may be due to beneficial interactions between the trithiocarbonate moiety of the RAFT agent and the enzyme, such that RAFT hydroxy groups are always present to act as initiator. In any case, this high incidence of initiation greatly decreases the amount of water initiated PCL and hence decreases the propensity for homo-PCL formation.

In this communication, we have presented the first simultaneous, metal-free synthesis of copolymers utilising enzymes and RAFT-mediated polymerisation in $scCO_2$. The low cytotoxicity of the RAFT agent, coupled to the inherent bio-friendly nature of the enzyme, makes this an important technique for the synthesis of polymers for biomedical applications. The combination of these techniques with $scCO_2$ as a solvent makes this approach extremely desirable from a green synthesis point of view.

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