Supercritical CO_2: an effective medium for the chemo-enzymatic synthesis of block copolymers?

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In this review, we describe the combination of enzymatic polymerisation and controlled free radical polymerisation in supercritical carbon dioxide. This combination facilitates the preparation of a range of block and graft copolymers, some of which cannot easily be obtained by conventional polymer synthesis. Biocatalysis in polymer science provides significant new opportunities and will open up a very broad range of new polymeric materials.

Introduction

Achieving well-defined macromolecular architectures *via* controlled synthesis is a major challenge in polymer science. The search for new materials requires the continuous development of novel polymers and polymerisation techniques. Therefore, new synthetic strategies, catalysts and sustainable processes must be integrated into the chemist's synthetic toolbox to enable the preparation of the next generation of new functional materials. These will be utilised in high technology applications from photonics to resorbable biomaterials. The application of enzymes offers many interesting opportunities in polymer chemistry. However, biocatalysis cannot replace all chemical pathways. Therefore, the challenge is the development of mutually compatible chemo- and biocatalytic methods. The successful combination of such different polymerisation techniques will be a powerful and elegant approach to the synthesis of next generation materials. In our research, we have explored the integration of biocatalytic polymerisation with traditional controlled radical polymerisation techniques. Our ultimate goal is to combine this methodology with supercritical carbon dioxide ($scCO_2$) as the reaction medium. The use of $scCO_2$ removes the requirement for toxic organic solvents and is seen as an acceptable green alternative. Additionally, the low viscosity of $scCO_2$ offers specific processing advantages for many processes.

In the field of organic synthesis, enzymes have been in use for more than seven decades as catalysts for preparation of functional organic compounds.[1] This area really took off in the 1980s[2] and over the previous decade, enzyme mediated polymerisation has emerged as an important method for the preparation of polymers.[3] Enzymes are an attractive alternative to conventional chemical polymerisation catalysts because of their high stereo-, regio- and chemoselectivities. Moreover, their ability to operate under mild conditions and their inherent recyclability and biocompatibility add to their attractiveness. Whilst traditional catalysts often contain toxic and enviromentally-damaging metal species, enzymatic catalysis is seen as an attractive alternative.[4] Enzymatic polymerisations have received more attention in recent years as the structural variation of synthetic targets in polymers has led to the development of highly selective polymerisations. This has been fueled by the increasing demand for the production of controlled and functional polymers for materials science. Enzyme catalysis has provided a new synthetic strategy for

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Silvia Villarroya

Silvia Villarroya obtained her BSc (1997) and PhD (2002) from Universidad Autonoma de Barcelona (Spain), the latter supervised by Prof. Marcial Moreno-Mañas. After one year as a postdoctoral researcher at Life Science DSM (Geleen, The Netherlands) she then moved to Nottingham to join the European Research Network Biomade as a postdoctoral researcher with Howdle. She was then awarded a European Marie Curie Fellowship to focus upon the combination of enzymatic and free radical polymerizations in supercritical carbon dioxide.

Kristofer J. Thurecht

Kristofer J. Thurecht obtained his BSc (Hons) (2001) and PhD (2005) from the University of Queensland (Australia), supervised by Prof. Andrew Whittaker. He was awarded a Postdoctoral Fellowship by the Dutch Polymer Institute with Howdle at the University of Nottingham. His current research is focussed upon the synthesis of polymers in $scCO_2$ using RAFT.

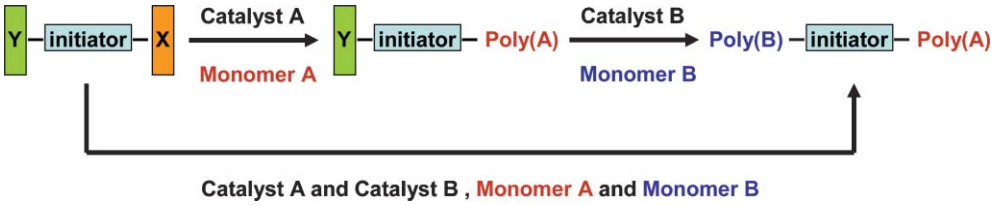


Fig. 1 Schematic representation of the bifunctional initiator system.

polymers. In particular this is so for those that are difficult to produce by conventional chemical catalysis such as chiral polymers and polyesters from macrolactones.[5] Perhaps one of the most investigated class of enzymes for polymer synthesis is lipases. These have been shown to catalyse effectively the ring-opening polymerisation of lactones, lactides and cyclic carbonates to the corresponding biodegradable polyesters[6] and polycarbonates.[7]

Supercritical fluids (SCFs) have been the focus of much research as potential replacements for conventional organic solvents.[8] Of these, supercritical carbon dioxide ($scCO_2$) has received by far the most attention primarily because it is cheap, environmentally-friendly, non-toxic, non-flammable and its critical parameters are easily obtainable (T_C = 31.0 °C, P_C = 73.8 bar). Generally speaking, the properties of supercritical fluids lie between those of a liquid and a gas. For example, their densities are comparable to those of liquids, whilst diffusivities and viscosities approach those of gases. Furthermore, the ability to manipulate these physical properties by slight variations in pressure or temperature is unique to supercritical fluids. Exhaustive research has clearly demonstrated that the activity of enzymes is dependent on solvent properties.[9] What naturally follows is that supercritical fluids are attractive media in which to perform and control enzymatic reactions and enzymatic polymerisations. The key features are the tunability of the solvent – by manipulating temperature and pressure, and the enhancement of mass transfer rates of reactants to the active sites on enzymes – brought about by the gas-like diffusivities and viscosities. $ScCO_2$ is generally accepted as a poor solvent for most polymers.[10] However, $scCO_2$ can plasticize and liquefy polymers such as poly(caprolactone) (PCL) under very mild conditions. The attraction of combining natural catalysts with natural solvents has been the driving force behind a growing body of literature concerning the use of enzymes in supercritical carbon dioxide. A number of reviews of biocatalysis in SCFs have been published documenting the progress of the whole field.[11]

Block copolymers are a fascinating class of polymeric materials made by covalent bonding of two or more chemically different polymer chains.[12] Their synthesis is particularly suited to the combination of two different polymerisation techniques. The use of a bifunctional initiator is an elegant synthetic approach to prepare such block copolymers through the combination of mechanistically incompatible monomers (Fig. 1). Recently, Bernaerts and Du Prez published a extensive review with a complete overview of the state-of-the-art on such heterofunctional initiators for polymerisation.[13] Other reviews describe bi- and polyfunctional initiators for free radical polymerisation.[14] The use of multifunctional initiators provides the opportunity to combine different polymerisation techniques without the need for intermediate transformation and protection steps. We have adopted a bifunctional initiator strategy for the synthesis of block copolymers by combination of enzymatic polymerisation with other free radical controlled polymerisations. In this feature article, we present the state-of-the-art for the synthesis of functional polymeric materials in $scCO_2$. The particular approaches presented in this report range from classical block copolymers to graft copolymers and include exhaustive $scCO_2$ studies on:

Andreas Heise

Andreas Heise obtained his PhD (1997) from the University of Hannover (Germany) under Prof. Henning Menzel. He was awarded a two year postdoctoral fellowship by the Deutsche Forschungsgemeinschaft (DFG) with Dr Robert Miller at the IBM Almaden Research Center and Prof. Curtis Frank at Stanford University. In 1999 he joined DSM (The Netherlands), where he is heading the activities for the application of biotechnology in performance materials and is the coordinator of a European Research Network (Biomade). Andreas also holds a part time position at TU Eindhoven, where he supervises a research group on bioinspired materials and methodologies.

Steven M. Howdle

Steve Howdle is a native of Rotherham, South Yorkshire, and obtained a first degree in Chemistry (Manchester 1986) and a PhD on "Spectroscopy in Liquefied Noble Gases" (Nottingham 1989). Steve's interests focus on the utilisation of $scCO_2$ for polymer synthesis, polymer processing and preparation of novel polymeric materials for tissue engineering and drug delivery. A more detailed description and some movies of supercritical fluids can be viewed at http://www.nottingham.ac.uk/~pczctg/index.hml.

(a) enzymatic ring-opening polymerisation (eROP) of lactones;

(b) living polymerisation of vinyl monomers;

(c) simultaneous eROP and ATRP; and;

(d) simultaneous eROP and RAFT-mediated controlled radical polymerisation

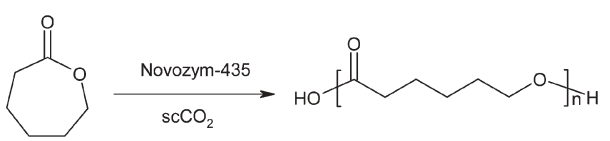
Comparisons will be drawn between our research in $scCO_2$ and similar experiments carried out in conventional solvents to justify whether the use of this alternative solvent can bring about any advantage over the traditional routes.

eROP of lactones in supercritical carbon dioxide

Ring-opening polymerisation of lactones is more commonly performed in organic solvents or in bulk using a Lewis acid catalyst.[15] Recently there has been much interest in the replacement of such catalysts by enzymes. In particular, the synthesis of polyesters by *in vitro* lipase-catalysed ROP of lactones has been well documented.[16] The focus of such studies has been to eliminate the use of potentially toxic Lewis acid type catalysts that are used traditionally. A wide range of enzymes have been screened for the ROP of lactones, but the Lipase B from *Candida Antartica*, supported on macroporous beads (Novozym-435), has proved to be most active. The polymerisation of ε-caprolactone, a seven-membered lactone, is a good model system to test the activity of an enzyme for lactone ROP. Many studies report that ROP of ε-CL using Novozym-435 in bulk gives PCL of relatively low molecular weight and with polydispersity greater than 2 (typically M_n = 3400–8500 g mol^{-1}, PDI = 2.1–2.5).[17] More recently, a considerable improvement was obtained with Novozym-435 catalysis by selecting toluene as the organic medium and using a ratio of ε-CL to toluene of 1 : 2 (w/w). This resulted in PCL in 86% yield with M_n and M_w/M_n of 44800 g mol^{-1} and 1.7, respectively.[18] Other research groups have reported similar results for enzymatic ring opening polymerisation of lactones in traditional solvents.[3]

The key question addressed in our research was whether lipase-catalysed lactone polymerisations could function effectively in $scCO_2$ and whether by using $scCO_2$ any advantage might be gained over working in conventional solvents. Enzymes have been utilized extensively in $scCO_2$ for processes ranging from acidolysis to chiral synthesis of esters and there is an extensive literature describing recent progress.[19]

Recently, we demonstrated that $scCO_2$ is an environmentally benign replacement for organic solvents in the enzyme-catalysed ROP of ε-CL (Scheme 1).[20] The monomer ε-CL is soluble in the supercritical fluid containing the dispersed enzyme beads, and PCL precipitates out during the polymerisation reaction. Despite the insolubility of the product in the medium, it is very effectively plasticised by $scCO_2$, a fact that significantly improves the mass transport of monomer and the progress of polymerisation. Optimisation of the reaction conditions were carried out by changing the different parameters of temperature, pressure, reaction time and enzyme loading. The best results with respect to molecular weight and yield were obtained after 24 h at a relatively low $scCO_2$ density of 0.50 g cm^{-3} (pressure at 1500 psi (103 bar) and temperature of 35 °C). These optimum conditions were achieved by finding a balance between the solubilising power of the solvent and the best temperature for enzyme activity in $scCO_2$. In general, these polymerisations in $scCO_2$ produce similar molecular weights to those carried out in toluene, but in $scCO_2$ the PDI of the PCL was lower and the yields were higher. For instance, when the reaction was carried out at 35 °C and 1500 psi (103 bar) for 24 h with a 10 wt% loading of Novozym-435, PCL homopolymer was obtained in a very high yield (98%) with an average molecular weight of 40 000 and a polydispersity of 1.5.[19] The optimised results presented here are also a significant step beyond those presented earlier by Kobayashi for Novozym-435 catalysed ROP of ε-CL using $scCO_2$ as the solvent, where the highest M_n was 17000 with high polydispersity of 4.0.[21]



Scheme 1 eROP of CL using Novozym-435 in $scCO_2$. *Reaction conditions*: 5 mL CL, 0.5 g Novozym-435 at 35 °C and 1500 psi (103 bar) for 24 h.

Recently, the kinetics of the enzymatic polymerisation with Novozym-435 in $scCO_2$ were investigated using a new, high pressure sampling autoclave.[22] The reaction was found to be approximately first order with respect to monomer up to 80% conversion. The results show that high molecular weight polymer can be obtained (up to 50 kDa) with polydispersities in the range of 2. The relatively poor molecular weight control was attributed to the large degree of enzyme-catalyzed transesterification that forms both cyclic species (intramolecular transesterification) and linear polymer (intermolecular transesterification). This effect has also been observed for eROP of caprolactone in conventional solvents.[23]

Another advantage of the $scCO_2$ approach is that the whole process can be carried out using only $scCO_2$. This means that both the synthesis of the polymer and any subsequent recycling or cleaning steps can be achieved *in situ*. For example, the polymer can be purified at the completion of the reaction by supercritical extraction of residual monomer, low molecular weight oligomers and cyclic products by simply flushing with $scCO_2$. By this strategy, the use of organic solvents to purify the polymer is eliminated. In all of the published studies in conventional solvents or in the bulk, the polymer product must be purified by dissolution in an organic solvent and reprecipitation *via* an antisolvent, to remove residual monomer and low molecular weight oligomers. However, by using the extractive properties of $scCO_2$, the polymer can be purified without using any other solvents. The reaction parameters in $scCO_2$ are quite simple. Typically, the autoclave and reaction mixture are purged with $scCO_2$ at 4000 psi (274.7 bar) and 35 °C for 4 h with a flow rate of approximately 5 mL of CO_2 min^{-1}. The extracted materials were identified as monomer and oligomers by NMR and GPC. The polymer was also analyzed by GPC and NMR before and after extraction to demonstrate that all signals caused by oligomeric reaction product disappeared after the extraction step –highlighting the

success of this method. Such a step would be important in the manufacture of biodegradable polymers for biomedical applications. In addition, the enzyme can be recycled and reused. In conventional solvents, the enzyme is separated from the product PCL by dissolving and filtering to recover the supported enzyme beads. In standard experiments the supported enzyme beads are free inside the autoclave and are trapped within the resultant PCL product, and must be separated by use of dissolution and filtering. Our aim was to recycle the enzyme without use of conventional solvents. To achieve this, the enzyme beads were placed in a small wire mesh filter pot, attached to the shaft of the motor-driven stirrer blade. When the reaction was terminated and before the pressure was released, the pot was spun vigorously for 1 h to remove any polymer trapped inside the pot or left on the surface of the enzyme beads. The spin cleaning was repeated a further three times leaving the enzymes beads clean of any adhered PCL and ready to be used in a subsequent polymerisation. The ease of removal is attributed to the plasticisation (liquefaction) of the PCL which under the spin cycle simply falls into the bottom of the autoclave. The enzyme retains activity despite the prolonged exposure to $scCO_2$ and repeated pressurisation and depressurisation cycles. Reusing the cleaned enzyme in different polymerisation cycles led to reproducible high molecular weight polymer and retention of high yields.[19]

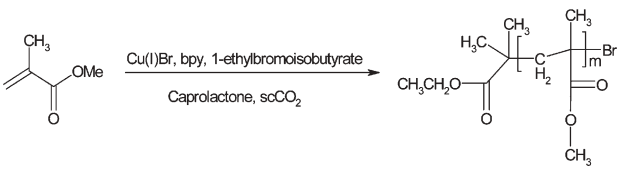
These results clearly demonstrate that a one-pot, semicontinuous batch synthesis of PCL can be carried out using only $scCO_2$ as the processing solvent. This combination of natural enzyme catalyst and clean $scCO_2$ could be described as a truly ''green'' process for the formation of high molecular weight, biodegradable PCL.

Controlled polymerisation of vinyl monomers in supercritical carbon dioxide

The advent of living polymerisation over the previous decades has dramatically improved the control that synthetic chemists have over polymerisation reactions. This has been most apparent for free radical polymerisation where atom transfer radical polymerisation (ATRP),[24] nitroxide-mediated polymerisation (NMP)[25] and reversible-addition fragmentation chain-transfer mediated polymerisation (RAFT)[26] have been utilised.

The application of these techniques to $scCO_2$ polymerisation has been limited. This can be attributed mainly to the inability to perform solution or bulk phase reactions in which these controlling agents are most effective. To date, the most effective polymerisations in $scCO_2$ have been dispersion-type, where the insolubility of polymer leads to heterogeneous systems. Hence, the application of living polymerisation in $scCO_2$ has been limited to the synthesis of CO_2-philic and soluble polymers, such as poly(fluoromethyl(meth)acrylate),[27] or as a grafting-type mechanism for a macro-stabiliser in dispersion polymerisations.[28]

Recently, we have shown that living solution/precipitation polymerisations of a variety of monomers can take place in $scCO_2$ resulting in well-defined polymer with very low polydispersity. We found that the key to success was the use of ε-caprolactone, which acted as a very effective cosolvent in these systems. The use of ε-CL as a liquid solvent for ATRP reactions has previously been reported,[29] and we have extended this application to $scCO_2$. Our use of ε-CL as the cosolvent ensured that the polymerisation medium remained homogeneous, even to relatively high monomer conversion. This has been effectively achieved using ATRP for (meth)acrylate, styrenes and many others, resulting in well-controlled polymerisation and low polydispersities.[30] We have shown that ATRP works effectively for radical polymerisation carried out in $scCO_2$ for the synthesis of poly(methylmethacrylate) PMMA[31] (Scheme 2), poly(perfluorooctylmethacrylate) PFOMA[32] and poly(methyl methacrylate-*co*-2-hydroxyethyl methacrylate) copolymers (P(MMA-*co*-HEMA)).[33] Recently, we also showed that a similar effect is observed for RAFT mediated polymerisation of styrene in $scCO_2$, where good control was observed when ε-caprolactone was used as cosolvent.[34]



Scheme 2 ATRP of MMA in $scCO_2$. *Reaction conditions*: 3 mL MMA, 5 mL CL, 33 μL initiator, 32 mg $Cu^{I}Br$, 70 mg bpy, at 35 °C, 1500 psi (103 bar) for 20 h.

High pressure polymerisations were also carried out in a view cell equipped with sapphire windows. This afforded visual observation of the phase behaviour during the polymerisation. For ATRP, the polymerisation medium was a homogeneous brown solution and no visible precipitation was observed at any time during the polymerisation. An important aspect here is the solubility of the catalyst system [$Cu^{I}Br$/bpy]. This catalyst is not soluble in pure $scCO_2$. However, it readily dissolves with the assistance of ε-CL. In the absence of cosolvent, the monomer conversion is very low because of the precipitation of polymer, and this lack of control leads to high-molecular weight PMMA. When ε-CL is used as cosolvent, the ATRP of MMA was successful in $scCO_2$, achieving over 50% yield. This simple co-solvent approach eliminates the necessity for fluorinated reagents.[35]

The cooperative effect observed for living polymerisation of vinyl monomers using ε-caprolactone as a cosolvent in $scCO_2$ leads on to the next section in which the ε-caprolactone acts as both cosolvent and monomer.

Block copolymers using dual initiators

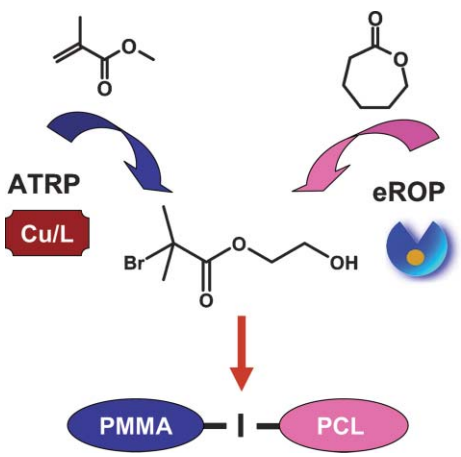
The use of a dual initiator or more generally a heterofunctional initiator shows many advantages compared to the classical methods for the synthesis of block copolymers. A bifunctional initiator with two fundamentally different initiating groups in the same molecule allows the polymerisation of two mechanistically incompatible monomers without the need for intermediate transformation and protection steps. The feasibility of this approach has been successfully demonstrated for the

combination of various chemical polymerisations.[12] A requirement for this strategy is that each initiating group is stable under the different polymerisation conditions. The catalysts must be compatible and must tolerate the other catalysts and monomers present in the system. The majority of copolymer syntheses using bifunctional initiator have required sequential reaction steps by synthesising first one, then the other polymer. Recently it has been shown that a one-step procedure is also possible,[36] but this had not been achieved for an enzyme catalysed process. For the simultaneous one-pot approach, the reaction temperature must remain constant during the whole process and the kinetics must be controlled.

The focus of this review is the use of bifunctional initiators to combine enzymatic polymerisation with other controlled polymerisation techniques such as ATRP, NMP and RAFT. Dual initiators for the combination of enzymatic ROP and controlled radical polymerisation must contain two functionalities: a hydroxyl group—which in the presence of the enzyme can be used as the initiating site for the eROP of cyclic lactones and lactides—and an initiating moiety for controlled radical polymerisation such as a nitroxide (NMP), activated halide (ATRP) or a reversible chain transfer agent (RAFT).

ATRP-eROP

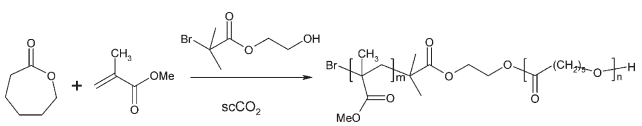
A major contribution to development of such dual processes has been made by Heise and co-workers who reported the combination of eROP of ε-CL and methyl substituted derivatives and ATRP of MMA and styrene.[37] The bifunctional character can be illustrated (Scheme 3) by the presence of an activated bromine group for the free radical polymerisation (ATRP) and a primary hydroxyl group for eROP. In conventional solvents, the successful synthesis of block copolymers in two successive reactions was reported.[34] This procedure required purification of the intermediate macroinitiator. The reaction can be also performed by a one-pot cascade polymerisation approach, where the two polymerisation reactions proceed consecutively without intermediate work up. In this case, the compartmentalization of both techniques is achieved by adding one of the catalysts after a specific time. For this process ε-CL, *tert*-butyl methacrylate (*t*-BMA), Novozym-435 and the bifunctional initiator were heated at 60 °C to initiate the eROP and obtain a PCL block end-capped with a bromine atom. After 2 h, $Cu^{I}Br$/bpy was added in order to activate the ATRP to complete block copolymer formation. While this methodology greatly improved the process, a simultaneous one-pot approach has not been successful in organic solvents. The combination of eROP and ATRP in a one-pot cascade reaction where all the components are present in the flask from the beginning of the reaction seems to be more challenging. The enzymatic polymerisation of ε-CL in the presence of MMA inevitably results in transesterification, and thus in the incorporation of methacrylate groups to a high extent. Moreover, even the methanol released in this transesterification process acts as an active component in the reaction, competing as an initiator for eROP and leading to a significant presence of PCL molecules with methyl ester end-groups.[38]



Scheme 3 Schematic showing the simultaneous enzymatic catalysed ROP of ε-caprolactone with ATRP of methylmethacrylate to form block copolymers with the bifunctional initiator in $scCO_2$.

Wang and co-workers also contributed to this field by using a novel bifunctional initiator 2,2,2-trichloroethanol for the synthesis of AB-type diblock copolymer polycaprolactone-*block*-polystyrene (PCL-*b*-PSt) by combination of enzymatic ROP and ATRP in organic solvent.[39] Trichloromethyl-teminated PCL macromolecules were subsequently employed as macroinitiators in the ATRP of styrene using standard catalysts for ATRP to afford well-defined diblock copolymer. The authors have demonstrated a successful two-step sequential route for the synthesis of the block copolymers. Experiments aiming at cascade copolymerisation by a one-pot approach are currently in progress.

The limitation shown for a simultaneous one-pot approach in organic solvents has been overcome by performing the reaction in $scCO_2$. We have reported the single-step, simultaneous, one-pot chemoenzymatic synthesis of block copolymers combining ATRP and eROP in $scCO_2$.[31] eROP of ε-CL and ATRP of MMA were integrated into a one-step synthetic route to yield poly(ε-CL-*block*-MMA) (PCL-*b*-PMMA) using a bifunctional initiator (Scheme 4). It was demonstrated that the enzymatic polymerisation step and the ATRP proceeded concurrently. An exhaustive study has been carried out, not only to investigate and optimise all different the parameters in the synthesis of block copolymers, but also to understand the interaction between the two different polymerisation techniques.[40]



Scheme 4 Simultaneous eROP of CL and ATRP of MMA in $scCO_2$. *Reaction conditions*: 0.4 g Novozym-435, 5 mL ε-CL, 3 mL MMA, 33 μL initiator, 32 mg $Cu^{I}Br$, 70 mg bpy, at 35 °C and 1500 psi (103 bar) for 20 h.

As mentioned above, we found that the use of ε-CL as a $scCO_2$ co-solvent for ATRP of vinyl monomers was crucial to allow the radical polymerisation to remain homogeneous and controlled. $scCO_2$ can plasticise and liquefy polymers such as PCL under very mild conditions and we reasoned that this

liquefied PCL may be able to act as the cosolvent for the ATRP polymerisation of MMA. Our results show that a combination of ε-CL monomer and PCL also has a very good effect as cosolvent for the ATRP polymerisation. We concluded that the presence of ε-CL monomer is advantageous for $scCO_2$ leading to plasticization and even dissolution of PCL. Moreover, both ε-CL monomer and the PCL acted together as co-solvent to solubilise the propagating PMMA chain in $scCO_2$. This is the determining factor that allows the two polymerisation techniques to be performed in a simultaneous one-pot synthesis.

The choice of the ATRP catalyst is crucial. For the eROP and ATRP to occur simultaneously, the ATRP catalyst is required to be compatible with the enzyme. Various metal complexes have been applied as ATRP catalysts. The compatibility of two systems (CuBr/bpy and $Ni(PPh)_3Br_2$) with eROP was investigated in organic solvents and in $scCO_2$. The results were similar in both media. Copper showed no influence, while nickel completely inhibited the enzyme activity. On the basis of this result, Cu^IBr/bpy was selected as the ATRP catalyst in our copolymerisations.

The ability to obtain absolute control over the propagation kinetics of each block individually in a one-step copolymerisation is very difficult. However, through delicate control of the parameters in the simultaneous copolymerisation, PCL-*b*-PMMA with various compositions can be synthesised. For the eROP of ε-CL, the molecular weight of PCL increases with higher enzyme/monomer ratio and higher monomer/initiator ratio. For the ATRP of MMA, the molecular weight of PMMA depends on the monomer/initiator ratio and the monomer conversion. By varying these parameters we have prepared copolymers with ratios of CL : MMA from 89 : 11 to 29 : 71. In addition, preliminary results suggested that lower levels of transesterification of MMA were observed in $scCO_2$ in comparison with conventional solvents. However, this aspect is currently under investigation. The copolymers were characterised completely by ^1H NMR, GPC and MALDI-TOF. To demonstrate copolymer formation, the final product was hydrolysed to remove the PCL. The removal of the PCL block was confirmed by ^1H NMR analysis and also by GPC, which revealed a peak at lower molecular weight with much narrower molecular weight distribution corresponding to the PMMA block (Fig. 2). The synthesis of the copolymer has been successfully conducted through both a two-step and a simultaneous single-step approach.

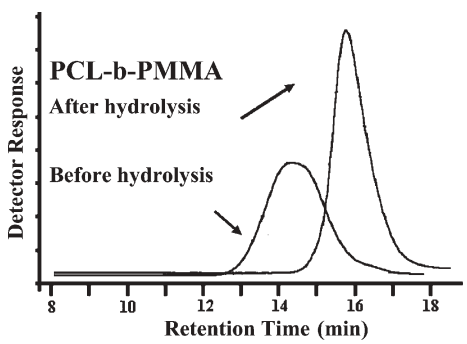


Fig. 2 GPC trace of copolymer before and after hydrolysis of the PCL block. (Modified with permission from ref. 40).

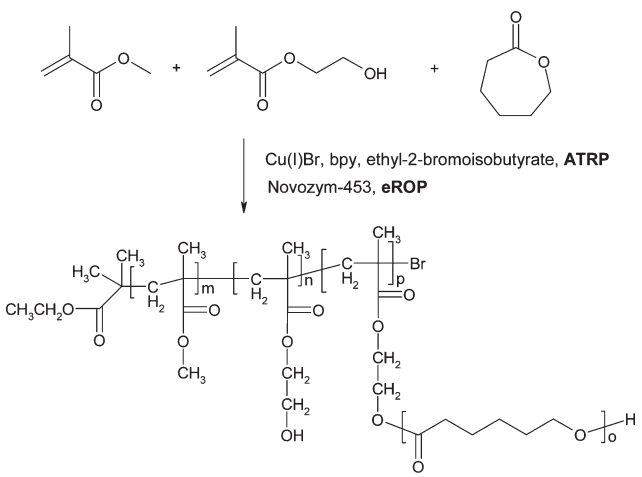
The unique ability of $scCO_2$ to solubilise highly fluorinated species was utilised by extending this methodology to the synthesis of novel diblock copolymers consisting of a semifluorinated block of poly(1*H*,1*H*,2*H*,2*H*-perfluorooctyl methacrylate) and a hydrocarbon block of polycaprolactone (PFOMA-*b*-PCL).[32] The synthesis of amphiphilic copolymers based on caprolactone units and fluorinated moieties is attractive for preparation of polymers with potential for biomedical applications or as emulsifiers and surfactants in $scCO_2$. Semifluorinated block and graft copolymers are of growing interest because of their unique properties, such as chemical inertness, solvent and high temperature resistance, and low surface tension. All of these could in principle be transferred to other polymeric materials by copolymerisation. The possibility of modifying the characteristics of polymeric materials by addition of fluorine-containing monomers represents a goal of increasing importance.[41] In addition, the covalent connection of thermodynamically incompatible segments in a block copolymer can lead to self-assembly of these components into ordered structures with periodicity or compositional heterogeneity on a nanometer length scale. The aforementioned unique properties of fluorine are predicted to have significant consequences on the self-assembly of block copolymers when contained in one or more of the segments.[42] For instance, comb-shaped copolymers synthesized from PCL macromonomer and a long perfluorinated acrylate have been shown to self-assemble at surfaces and interfaces. Since PCL is compatible with many other polymers, these copolymers could be used to modify the surface properties of PCL and many other polymers by macromolecular self-assembly of the surface-active fluorinated moiety at the air interface with the PCL side chain as an anchor into the bulk polymer.[43]

PFOMA-*b*-PCL copolymers were successfully synthesised by a two-step chemoenzymatic approach based on a sequential monomer addition technique using a bifunctional initiator. A PCL macroinitiator was obtained *via* eROP initiated by the hydroxyl moiety in the bifunctional initiator. It was then demonstrated that PCL macroinitiator polymerizes FOMA effectively by ATRP, leading to a PFOMA block with controlled molecular weight. Several block copolymers have been prepared with different PFOMA content showing different thermal behaviour. However, the one-pot concurrent synthesis in the presence of FOMA and ε-CL failed. The reaction was carried out in $scCO_2$ at 35 °C at 1700 psi (116.7 bar) using ε-CL and FOMA of varying initial feed ratios, the ATRP catalysts Cu^IBr/bipy and Novozym-435. However, no block copolymer was obtained. The resulting mixture contained homopolymers of both PCL and of PFOMA and a very small amount of block copolymer. The homopolymers could be separated by means of their different solubility in conventional solvents and NMR clearly identified both polymers. However, GPC analysis showed a bimodal distribution, indicating the presence of two homopolymers rather than a block copolymer structure.[32]

In addition, various experiments have been performed for polymerisation of the semifluorinated block copolymers in conventional solvents without success. We found that the

synthesis of the fluorine-containing block copolymers is limited by the solubility of these materials in common organic solvents and would normally require the use of fluorinated solvents (*e.g.*, trifluorotoluene or Freon 113). Once again, we believe that the combination of two different polymerisation techniques in $scCO_2$ opens up the synthesis of a wide range of novel block copolymers that up until now have not easily been accessible using conventional methods.

Achieving well-defined macromolecular architectures *via* controlled synthesis is a major challenge in polymer science. The purpose of our current research is to design exceptional polymer architectures which are not easily accessible by conventional polymerisation methodologies. Well-defined poly(alkyl methacrylate)-*graft*-polycaprolactones have been successfully synthesised in $scCO_2$ by the combination of ATRP and eROP. The goal of our most recent studies is the preparation of model linear polymer backbones with a defined number of hydroxy groups that will be used as initiator sites for enzymatic ring-opening polymerisation.[33] Using $scCO_2$ as the sole solvent, a one step synthetic approach was adopted to prepare methacrylate copolymer backbone *via* ATRP, and grafted chains are added *via* eROP of caprolactone (Scheme 5). A one-step approach means simultaneous radical copolymerisation of MMA and HEMA, initiated by ethyl 2-bromoisobutyrate and catalysed by copper(I) bromide, and the eROP of caprolactone initiated from the hydroxyl groups of the HEMA units. Essentially, all the reactive components are added to the autoclave at the same time. The graft copolymerisation was also performed *via* a sequential two-step approach. The copolymers were analysed by traditional techniques.



Scheme 5 One-pot synthesis of poly(alkyl methacrylate)-*graft*-polycaprolactone graft copolymer by the combination of eROP and ATRP in $scCO_2$. *Reaction conditions*: 0.264 g Novozym-435, 4 mL ε-CL, 1.0 mL MMA, 1.1 mL HEMA, 15 μL initiator, 15 mg $Cu^{I}Br$, 32 mg bpy, at 35 °C and 1500 psi (103 bar) for 20 h.

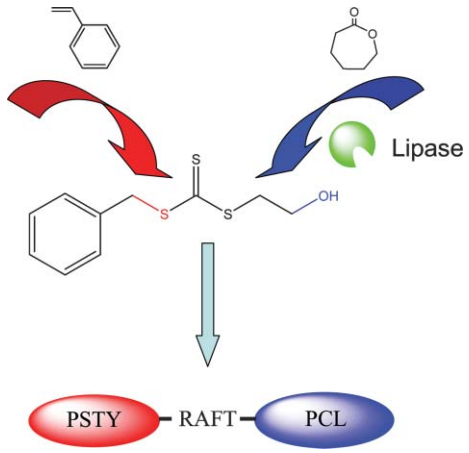
Exhaustive study of the enzymatic grafting efficiency showed that only 30–40% of the hydroxyl groups in the backbone initiated the polymerisation of CL.[33] Therefore, after the enzymatic grafting, the resulting structure contains unreacted hydroxyl groups in the polymer backbone, which are available for further chemical modification. Various experiments suggest that the limited amount of grafting is due to steric hindrance by the bulky side-chains (PCL graft chains), thus inhibiting access of the enzyme to all of the initiating hydroxyl groups. Also, if the OH groups are too close to each other along the poly(methacrylate) backbone, this could hinder access, preventing ROP from occurring at all possible sites. Indeed it is more favorable to promote the propagation of a pre-existing grafted PCL chain (on a neighbouring OH functionality) than to initiate a new chain from a new OH group. In order to determine if the limited grafting density is due to hindered accessibility to hydroxyl groups on the polymer backbone, grafting was carried out using a small molecule. Vinyl acetate was chosen as an end-capping reagent; only one molecule is added to the hydroxyl group as opposed to large number of CL units forming a PCL graft chain. Higher levels of grafting were obtained with the vinyl acetate (about 90%) compared with the maximum value of 40% for PCL grafting.[44] This experiment shows that the enzyme does indeed access all OH groups in the backbone. Therefore, we conclude that incomplete grafting is due to steric hindrance. This is in agreement with results obtained in conventional solvents.[45] However, these incompletely grafted copolymers have the unique feature of being doubly reactive since not only is each polyester graft end-capped by a hydroxyl end group, which can be easily derivatised into other organic functionalities, but also there are free hydroxyl groups in the backbone which could also be utilised. The free hydroxyl groups will be easily derivatised into other organic functionalities by conventional organic synthetic methods and they can also be used as initiators for conventional metal-catalysed ring opening polymerization (*i.e.* $Sn(Oct)_2$). These copolymers could thus be used as intermediates for the design of more complex macromolecular systems.

eROP-RAFT

RAFT-mediated controlled radical polymerisation can be also combined with other polymerisation techniques to prepare well-defined polymeric materials. Stenzel and co-workers synthesised a bifunctional RAFT agent, which initially acted as an initiator for the metal catalysed ROP of DL-lactide and then allowed RAFT-controlled polymerisation of *N*-isopropyl acrylamide.[46] This method involved purification and isolation of the RAFT terminated polylactide before the free radical step.

Recently, we reported 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.[34]

This strategy overcame many of the limitations present when using ATRP, *e.g.* removal of the copper catalyst from the polymer at the completion of the reaction and the inhibition effect that the ATRP catalysts can have on the enzyme activity. As with the ATRP reactions in $scCO_2$, we have demonstrated that RAFT-mediated free radical polymerisation occurs simultaneously with enzymatic ROP in $scCO_2$. Our approach uses a hydroxy-terminated trithiocarbonate (Scheme 6) which acts in the same way as the bifunctional initiators discussed previously. Experimentally, the high



Scheme 6 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 $scCO_2$. *Reaction conditions*: 0.3 g Novozym-435, 3 mL ε-CL, 3 mL styrene, 50 μL RAFT, 16.8 mg AIBN, at 65 °C and 4000 psi (274.7 bar) for 24 h. (Reproduced with permission from ref. 34).

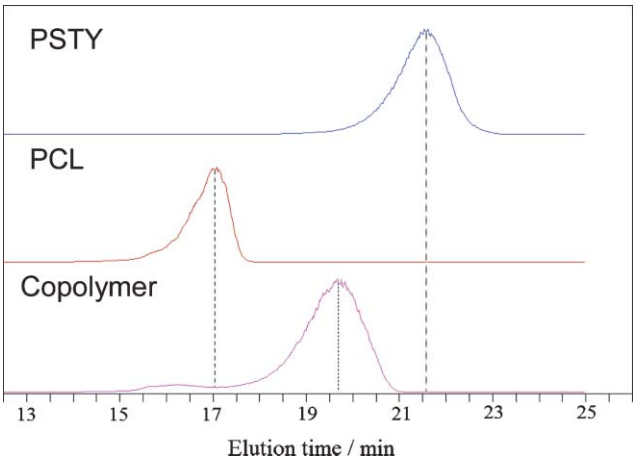


Fig. 3 GPEC traces of PSTY and PCL homopolymers and of the PCL-*b*-PSTY copolymer. The copolymer elutes at a time between the two homopolymers. (Reproduced with permission from ref. 34).

pressure vessel is loaded with the monomers (ε-caprolactone and styrene), the RAFT agent, the enzyme Novozym-435, and the free radical initiator AIBN. Once again, successful simultaneous polymerisation is facilitated by the excellent plasticising ability of $scCO_2$ in conjunction with the cosolvent effect of ε-caprolactone. The use of $scCO_2$ ensures the homogeneity of the mixture throughout the polymerisation reaction. In addition, very good control over the polydispersity of the free-radical polymerisation product is obtained. It has been demonstrated that control over the block lengths can also be achieved. The PCL block length can be controlled either by varying the RAFT agent concentration (*i.e.* initiating hydroxyl groups) or by varying the caprolactone monomer concentration. As expected, the molecular weight of the PCL block decreases when increasing the RAFT agent concentration because of the higher concentration of OH-initiating groups. On the other hand, as a result of decreasing the caprolactone feed, smaller PCL blocks lengths were observed in the copolymer. The length of the PSTY block is also affected by the changes in caprolactone monomer concentration. This clearly shows the extent to which variation in the reaction parameters can be utilised to control the copolymer properties.

The presence of the copolymer was proven using a number of different techniques (^{1}H NMR, GPC, DSC). In addition, we also analysed the copolymer by GPEC (gradient polymer elution chromatography). This technique has recently been developed as a tool for distinguishing between homopolymer blends and copolymers.[47] 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. Hence, the separation of block and homopolymers is solely based on their chemical nature. Using a solvent gradient from 100% methanol to 100% THF, good separation of the blend of homopolymers could be achieved. PCL was shown to elute first (retention time of 17 min) followed by PSTY (retention time of 21.3 min). It follows then that the copolymer elutes in the period between the two homopolymers centered at a retention time of 19.4 min, and is clearly separated from the traces of the two homopolymers (Fig. 3).

The use of a bifunctional RAFT agent in $scCO_2$ overcomes a major problem with the ATRP catalysts; purification of the final polymer product. In the case of RAFT, the final product can be purified at the completion of the reaction as previously described, by simply flushing with $scCO_2$ to remove residual monomer or oligomers. Furthermore, the reaction described[33] uses a RAFT agent that has been shown to have low cytotoxicity, hence its potential incorporation into biomedical polymers poses little problem.

Conclusions

We have presented the state-of-the-art concerning the synthesis of block and graft copolymer structures using dual/heterofunctional initiators, allowing the combination of mechanistically different polymerisation techniques in $scCO_2$. In particular we have highlighted the use of enzymes for the production of polyesters coupled with free radical polymerisation techniques such as ATRP and RAFT. The combination of these techniques with $scCO_2$ is attractive because of the elimination of the need for conventional solvents. Perhaps more importantly though, we have shown that $scCO_2$ offers far more than just an environmentally friendly solvent alternative, but rather that many synthetic processes work better in this solvent. Indeed, the simultaneous copolymerisation involving enzymatic catalysis presented here has, up till now, only worked successfully in $scCO_2$. We believe that these strategies offer new opportunities in the design and application of functional polymeric materials.

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