PHASE SEPERATION IN ARTIFICIAL CELLS OF POLY (2-OXAZOLINE) AND POLYLACTIDE COPOLYMERS

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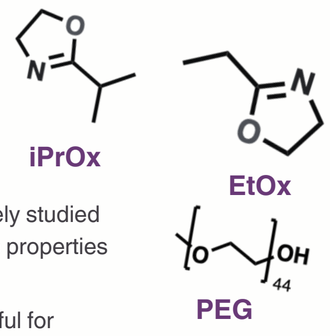
Introduction

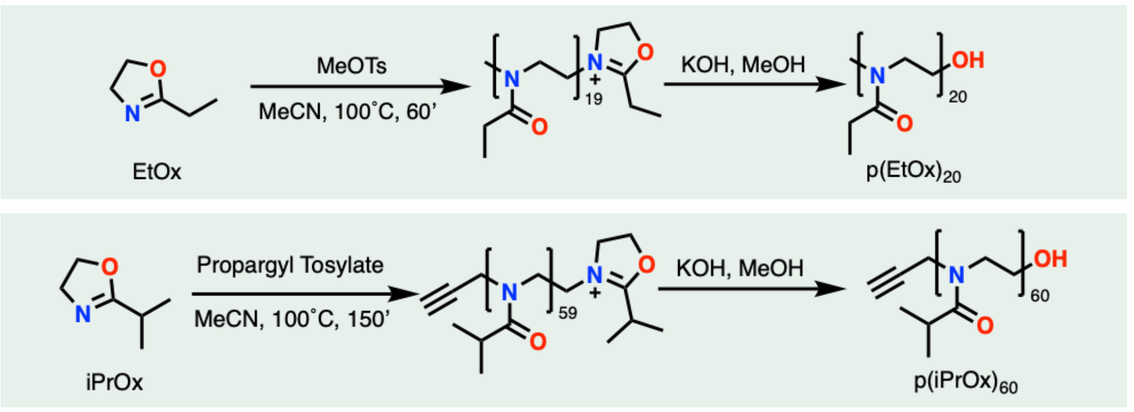


Phase-separated artificial cells can provide valuable insight into the fundamental processes of natural cells by mimicking their properties and functionalities.[1]

Poly(2-oxazolines) are a promising class of polymers that have been extensively studied due to their potential for a wide range of applications.[2] They possess desirable properties such as water solubility, low cytotoxicity and stealth effects which make them particularly useful for nanomedicines and drug delivery.[3] It is also known that poly D,L-lactides are highly biocompatible due to low toxicity and easy metabolization.[2] The synthesised amphiphilic block copolymers can be used as model membranes to explore the behaviours of biomolecules.[1] These polymers have gained particular interest in the biomedical field due to their ability to form nanostructures.[4]

The aim: Synthesising poly(2-ethyl-2-oxazoline)-*b*-PDLLA, poly(2-isopropyl-2-oxazoline)-*b*-PDLLA and PEG-*b*-PDLLA based block copolymers, using ring-opening polymerisation (ROP) to formulate phase-separated Giant Unilamellar Vesicles (GUVs).

Cationic Ring-Opening Polymerisation of Macroinitiators



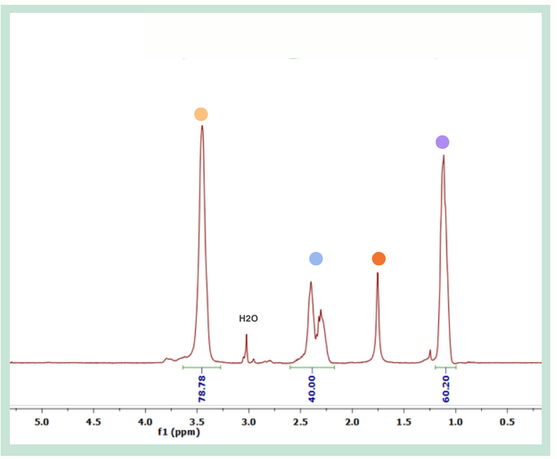
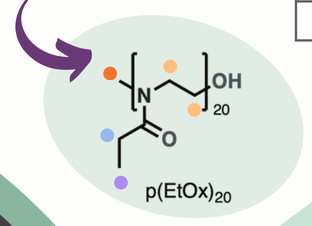


Figure 1. ^{1}H-NMR of $p(EtOx)_{20}$ in $CDCl_3$

- CROP was used to form the homo-polymers EtOx and iPrOx, varying the initiators (methyl or propargyl tosylate)
- All eight homopolymers had a polydispersity of Đ ≤ 1.21

Table 1. Molecular weight data of all synthesised homopolymers.

Polymer	$M_{n,\ NMR}$ (Da)	$M_{n,\ GPC}$ (Da)	Đ
Methyl-$EtOx_{20}$	1900	2000	1.12
Propargyl-$EtOx_{20}$	2000	1900	1.20
Methyl-$EtOx_{60}$	6000	5900	1.19
Propargyl-$EtOx_{60}$	6000	5800	1.21
Methyl-$iPrOx_{20}$	2100	2600	1.11
Propargyl-$iPrOx_{20}$	2000	2100	1.19
Propargyl-$iPrOx_{60}$	6000	5700	1.13
Methyl-$iPrOx_{60}$	5900	7300	1.12



Differential Scanning Calorimetery

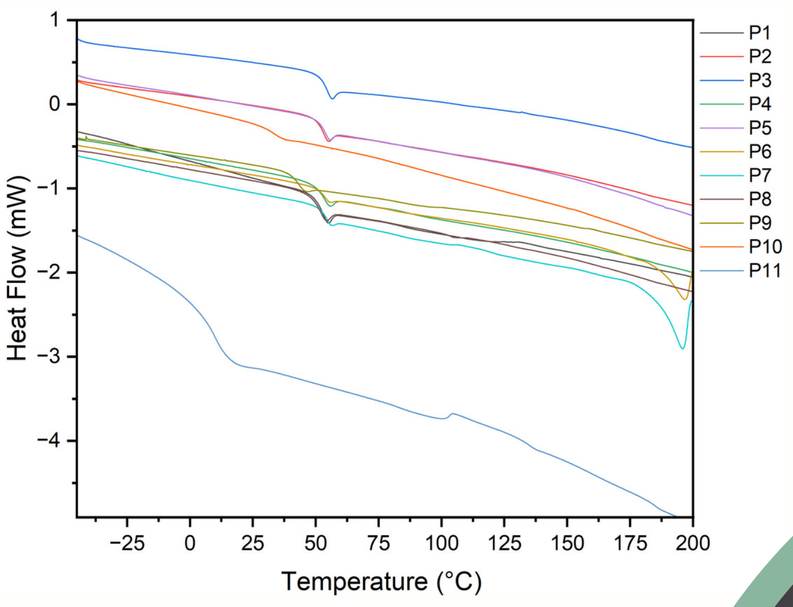


Figure 7. DSC curves of the second heating cycle of the copolymers P1 – P11. The curves were analysed with a Mettler-Toledo thermal analysis software to determine the glass transition temperatures of the synthesized polymers.

The T_gs ranged from **52 to 55˚C** (EtOx/iPrOx-*b*-PDLLA). The T_gs decreased as PEG length within the diblock increased (for P9 to P11 they were **43, 33 and 10˚C**, respectively).

Diblock copolymer synthesis

The synthesised polyoxazolines were the macroinitiators for ring-opening polymerisation (ROP) of D,L-lactide. The DP of the hydrophobic D,L-lactide block was targeted to be 120 (targeted Mn = 17 kDa).

Table 2. Molecular weight data of polymers P1-P11.

Polymer	Composition	$M_{n,\ NMR}$ (Da)	$M_{n,\ Theo}$ (Da)	$M_{n,\ GPC}$ (Da)	Đ
P1	$p(EtOx)_{19}$-b-$PDLLA_{123}$	17700	19600	14100	1.12
P2	$p(iPrOx)_{20}$-b-$PDLLA_{128}$	18500	20500	14500	1.26
P3	$p(iPrOx)_{21}$-b-$PDLLA_{124}$	17900	20000	12200	1.17
P4	$p(EtOx)_{60}$-b-$PDLLA_{115}$	16600	22600	12500	1.26
P5	$p(EtOx)_{20}$-b-$PDLLA_{131}$	18900	19500	15600	1.27
P6	$p(iPrOx)_{59}$-b-$PDLLA_{113}$	17500	23400	15500	1.27
P7	$p(iPrOx)_{60}$-b-$PDLLA_{121}$	16000	22000	20500	1.28
P8	$p(EtOx)_{60}$-b-$PDLLA_{111}$	17500	16200	13000	1.31
P9	PEG_{17}-b-$PDLLA_{121}$	17300	17400	13500	1.24
P10	PEG_{44}-b-$PDLLA_{120}$	16700	20500	21200	1.17
P11	PEG_{115}-b-$PDLLA_{116}$			17100	1.31

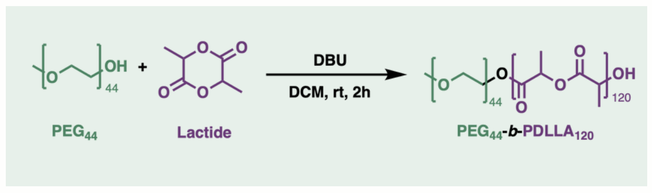
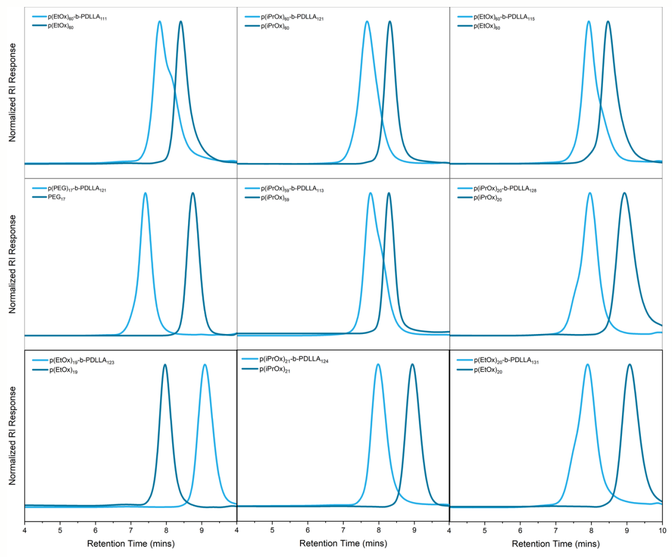


Figure 5. GPC traces of the homopolymer and resulting diblock copolymer. Measurements were performed using THF (2% TEA and 0.01% BHT) as the eluent. PMMA standards were used for the calibration.



- Copolymers have polydispersities of Đ ≤ 1.31
- Mn of lactide block calculated by ^{1}H-NMR (Table 2, $M_{n,NMR}$)

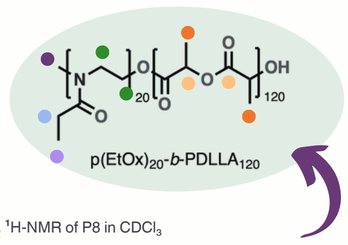
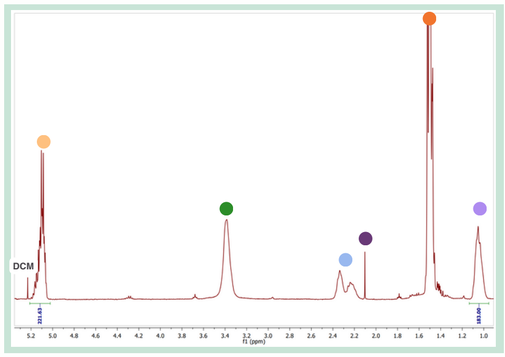


Figure 6. ^{1}H-NMR of P8 in $CDCl_3$



- ^{1}H-NMR analysis (Figure 6) shows expected product peaks for the ring opening of D,L-lactide with an oxazoline macroinitiator.
- The GPC shifts for the copolymers compared to the homopolymers (Figure 5) show the evolution of molecular weight.

Kinetics

Living polymerization was confirmed by kinetic analysis of the **homopolymerizations** (Figure 2,4), showing that each initiator initiated a monomer chain.

The trend in Figure 4 shows the GPC traces shift to the left as the homopolymerisation occurs, depicting a clear evolution of molecular weight.

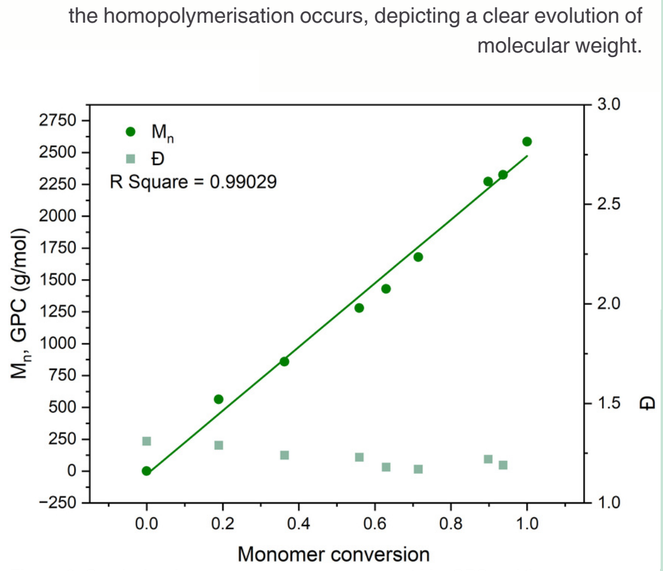


Figure 2. Graph showing monomer conversion against Mn, GPC and the polydispersity values.

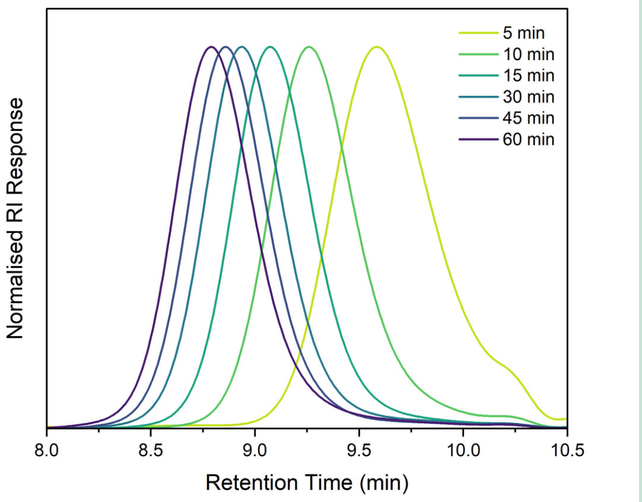


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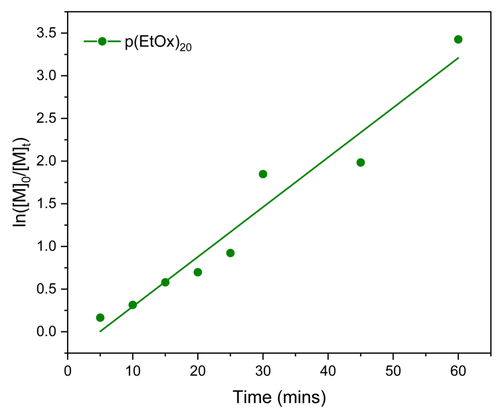
Figure 4. Graph showing monomer conversion against Mn, GPC and the polydispersity values.



Conclusion and future work

In summary, a series of the homopolymers 2-ethyl-2-oxazoline and 2-isopropyl-2-oxazoline followed by eleven diblock copolymers of D,L-lactide were synthesised. The ROP procedure yielded well-defined and narrowly dispersed copolymers.

In collaboration with Prof. Sébastien Lecommandoux's group at LCPO in Bordeaux, future work includes the study of phase-separated membranes by co-formulating Giant Unilamellar Vesicles (GUVs) by varying the ratio between the synthesised diblock copolymers. Localisation of alkyne groups would be proven by labelling the alkyne functionality groups with azide dyes.

References

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