High molecular weight bile acid and ricinoleic acid-based copolyesters *via* entropy-driven ring-opening metathesis polymerisation†

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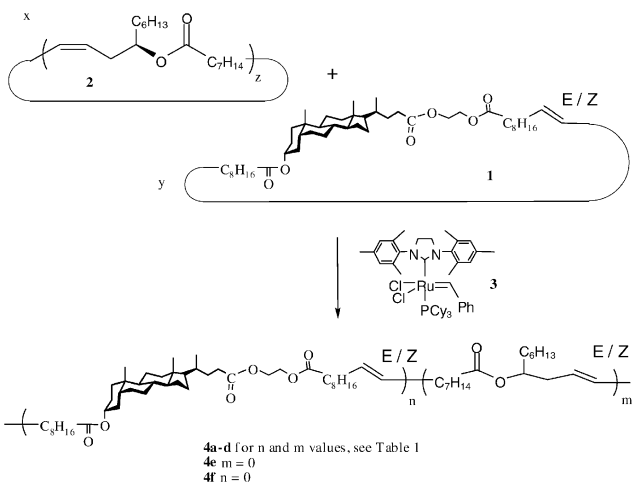
High molecular weight copolyesters based on bile acid and ricinoleic acid were synthesised *via* entropy-driven ring-opening metathesis polymerisations and were found to display tunable mechanical properties and heterogeneous degradation behaviours.

The rapid changes and evolution that characterise the field of biomedical applications require the design and synthesis of novel biomaterials. Most synthetic degradable polymers developed to date for this purpose are based on short chain aliphatic acids, such as lactic acid and glycolic acid.[1–5] In some cases, the properties of these polyesters are inadequate. For example, the substantial decrease in local pH that is typical of their degradation can elicit mild to severe inflammatory responses.[6] It is also difficult to tune the relatively hard mechanical properties of such polymers without affecting their degradation rate. For soft tissue engineering, a good mechanical matching between the tissue to be regenerated and the polymeric scaffold that supports it is essential to avoid necrosis and abnormal healing.[7,8] Cell substrate stiffness can control cell adhesion and motility[9,10] and also, more generally, activity and phenotype.[11,12] It is therefore important to design novel materials with controlled mechanical properties as well as degradation and drug release behaviour in order to develop new generations of scaffolds for tissue regeneration as well as implants for controlled release.

Bile acids, natural molecules existing in the body of all mammals in relatively large quantities, are useful building blocks for biomaterials design.[13–15] They are characterised by their facial amphiphilicity and their rigid steroidal backbone, which allow the tailoring of macromolecular architecture and macroscopic behaviour. In addition, the relatively high pK_a of bile acids[16] ensures a moderate variation of local pH upon hydrolytic degradation of these materials.

Recently we demonstrated that it is possible to incorporate these compounds into a polyester backbone using metathesis chemistry.[17] We used ring opening metathesis polymerisation[18] which, in contrast with acyclic diene metathesis, does not require the elimination of end groups from the reaction mixture (Scheme 1). Unlike the polymerisation of short cycles such as lactides, ε-caprolactone or norbornene where enthalpy and strain release are the driving force, ring opening of bile acid-based macrocycles is driven by entropy and the increase of conformational freedom experienced by each repeat unit.[19–24] We describe here the random copolymerisation of bile-acid macrocyclic monomer **1** with ricinoleide mixture **2**. The mechanical properties of the copolyesters obtained were studied by dynamic mechanical analysis and stress–strain experiments, which revealed a linear relationship between the chemical composition of the copolymers and their glass transition temperatures (T_g) as well as their Young's moduli (E). The degradation behaviour of the different materials was also investigated.

Monomers **1** and **2** were prepared as described in the literature.[17,25] Entropy-driven ring-opening metathesis polymerisation (ED-ROMP) of bile acid monomer **1** is known to afford high molecular weight (MW) polymer in high yields.[17] In contrast, ricinoleide monomer and oligomers **2** only afforded low MW homopolymers *via* a transesterification method.[26] These low MWs may be due to trace amounts of impurities as well as the modest polymerisability of the corresponding monomers, owing to the presence of a bulky alkyl substituent next to the reactive ester. However, treatment of the ricinoleide mixture **2** (which contains oligomers with n = 1 to 13, see Fig. S1†) with the metathesis catalyst **3** afforded high MW polymer **4f** within 15 min. Notably, the degree of polymerisation of the polymer formed goes through a maximum before decreasing again due to backbiting reactions (Fig. S2†). This phenomenon was also observed for bile



Scheme 1 Synthesis of polymers **4a–f**.

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acid-based monomer **1** (Fig. S2†). These results show that the low MWs obtained by Domb and Slivniak may not be due to the lack of strain of monomer **2**. Presumably the reduced crowding around the alkene bond compared to that near the ester function does not reduce the reactivity of the monomer to the same extent. Moreover, the high MWs obtained for ricinoleide mixtures by ED-ROMP contrast with those obtained for the opening of other olefin cyclic oligomer mixtures described in the literature.[20] This may be attributed to the absence of acyclic diene or other linear oligomers in the starting material, which are known to introduce end-groups and reduce MWs. Monomer **2** was obtained by macrolactonisation *via* an activated ester method in which any linear compound bearing a carboxylic acid end group can be easily separated by chromatography.

Therefore ricinoleide mixtures appear as ideal flexible co-monomers for tuning the mechanical properties of bile acid-based polyesters, which are known to display rubber-like elasticity.[17] A series of mixed copolyesters of lithocholic acid and ricinoleic acid were synthesised with varying co-monomer ratios. The effective co-monomer ratio in the final copolymer was determined *via* ^{1}H NMR spectroscopy (Table 1). Owing to the good reactivity of both co-monomers, high MW polymers were obtained with co-monomer ratios very close to the starting monomer mixture feed ratios. The expected similar reactivities of unstrained monomers may be an advantage for the synthesis of random copolymers with easily predictable compositions.

The mechanical properties of the different copolymer synthesised was then studied *via* dynamic mechanical analysis (DMA) and stress–strain experiments (Fig. 1). The T_gs and Young's moduli of the copolymers were both found to vary linearly as a function of the co-monomer composition in the polymers (Fig. 2). When the ricinoleide composition is varied between 7.4 and 46%, the T_g of bile acid polyesters decreases from 11.2 to −23.7 °C, whereas the Young's modulus at 37 °C decreases from 2.23 to 1.07 MPa. It was impossible to prepare samples of the pure homopolymer derived from ricinoleide to determine its T_g by DMA since it is a viscous oil at room temperature. However, the apparent linear relationship between copolymer composition and T_g indicates that the T_g of the pure poly(ricinoleic acid) homopolymer **4f** can be extrapolated from Fig. 2.[27] Such extrapolation leads to a T_g of −54.9 °C, which compares well to the T_g of −60.0 °C determined by DSC. It therefore appears that copolymerisation of a rigid bile acid-based monomer with a flexible ricinoleic acid co-monomer allows the fine tuning of the mechanical properties and especially the T_g of the corresponding polymers. Fig. 2 serves as an example of a general guide in the preparation of this kind of copolymers with a desired T_g to meet the requirements of a particular application. Changes in the structure of the bile acid moiety may alter the hydrophilicity, degradation and biocompatibility of these biomaterials whilst copolymerisation with ricinoleides enables them to maintain their T_g relatively constant. To some extent, tuning of the Young's modulus of the polymer is also possible (Fig. 1).

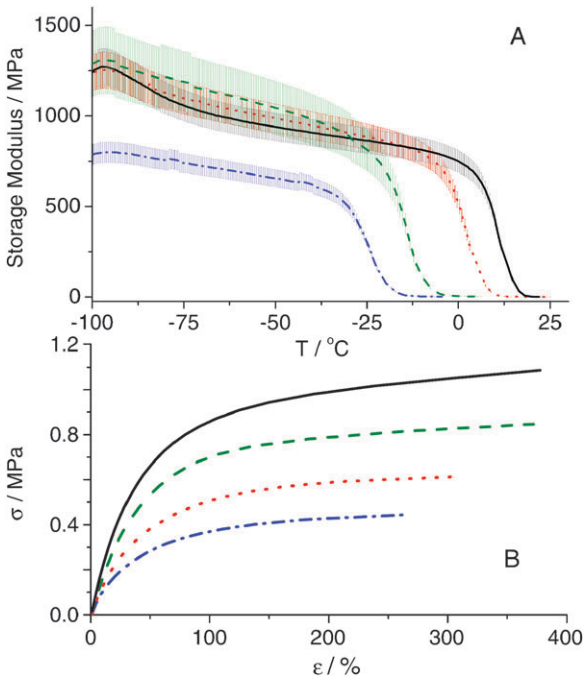

Fig. 1 DMA results obtained for films of polymers **4a** (black solid line), **4b** (red dots), **4c** (green dashes) and **4d** (blue dash–dots) cast in a mould from a DCM solution (100 g L^{-1}) and dried *in vacuo*. (A) Evolution of the storage modulus as a function of temperature; (B) stress–strain curves obtained at 37 °C.

The degradation of polymers **4a–d** was found to be slow, with 25% relative weight loss after 5 months (Fig. 3). It proceeds *via* a heterogeneous mechanism, as evidenced by the slow decrease of the bulk polymer MW over time (almost linear) and the lack of change in IR spectra (which should show the formation of carboxy- and hydroxyl-end groups). SEM images indicate that the surface of the films remains smooth with no apparent porosity over the whole period of time (Fig. S3†), which is typical of heterogeneous degradations. The polymers (**4a–d**) were found to degrade at similar rates, indicating that copolymerisation of monomer **1** with

Table 1 Physical properties and characteristics of polymers **4a–d** and **4f**

Polymer	Yield (%)[a]	Mol% RCA[b]	Mol% RCA in feed[c]	M_w[d] (kD)	PDI[d]	T_g[e]/°C	E at 37 °C[f]/MPa	Static contact angle/°
4a	84	7.4	10	528.3	2.34	11.2 ± 0.7	2.23 ± 0.11	81.2 ± 6.4
4b	77	19.4	20	462.0	2.43	0.8 ± 0.4	1.76 ± 0.11	77.3 ± 0.6
4c	75	40.5	40	443.1	2.33	−13.3 ± 0.6	1.5 ± 0.05	81.5 ± 2.7
4d	89	53.6	50	349.7	2.48	−23.7 ± 0.4	1.07 ± 0.11	83.8 ± 2.9
4f	90	100	100	140.3	2.05	−60.0 ± 1.2[g]	NA[h]	NA[h]

[a] After precipitation in methanol–hexane 1 : 2. [b] Ricinoleic acid; determined by ^{1}H NMR. [c] Mol% of ricinoleic acid introduced in the reaction mixture. [d] Polydispersity index; determined by SEC. [e] Obtained from DMA results. [f] Measured on stress–strain curves. [g] Determined by DSC. [h] Could not be measured for this sample. Polymerisation time was 15 min for all polymers.

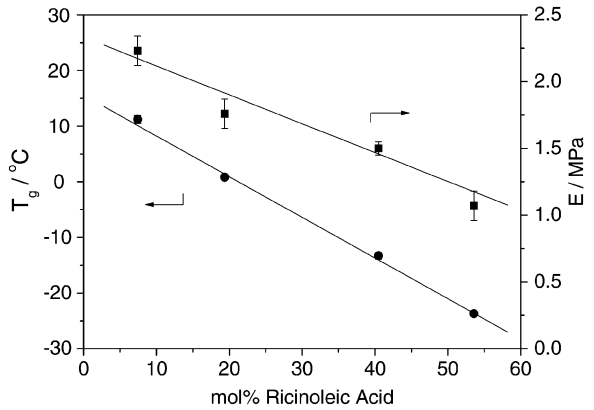

Fig. 2 Variation of the T_g (circles) and Young's modulus (squares) of polymers **4a–e** as a function of molar content of ricinoleic acid in the copolymer.

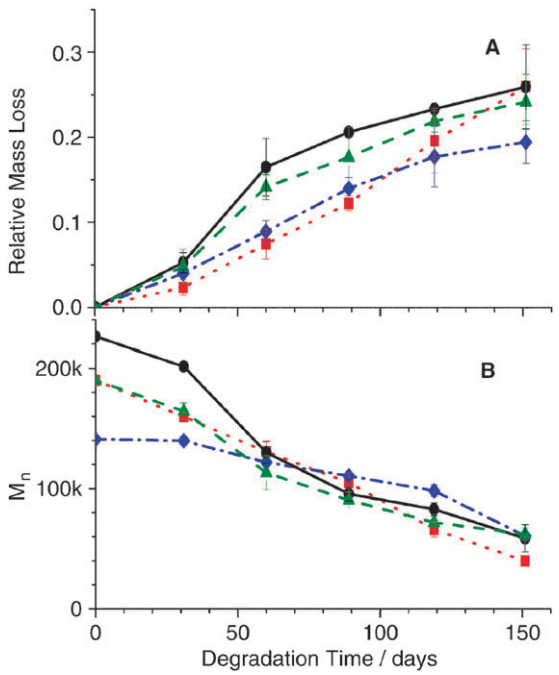

Fig. 3 Degradation of films of polymers **4a** (black circles), **4b** (red squares), **4c** (green triangles) and **4d** (blue diamonds) in PBS at 37 °C. (A) Relative mass loss; (B) M_n decrease.

varying amounts of ricinoleide **2** does not significantly change the degradation process. This is perhaps not so surprising given the relatively small change in chemical structure introduced (no new functionality), together with the fact that T_gs of polymers **4a–e** all lie well below the body temperature at which the experiment was carried out (Table 1). In addition, comparable static water contact angles (ranging from 77 to 83°) were measured for films of polymers **4a–e**. It may be possible to introduce more hydrophilic co-monomers to vary the degradation rate of the copolymers.

In conclusion, ED-ROMP is a useful tool for the synthesis of a new range of biopolymers incorporating naturally occurring compounds that may provide added functionality, responsiveness as well as a new handle for the design of biomaterials for specific applications. These materials are characterised by the relative ease in tuning the T_g and Young's modulus by the choice of the co-monomers and by variation of the composition of the copolymers. They may find a wide range of applications such as tissue regeneration and drug delivery. Only lithocholic acid and ricinoleic acid have been used in the examples shown in this preliminary study. It is certainly possible to use other bile acids and fatty acids for the preparation of such materials.

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