Effect of Solvent on the Lipase-catalyzed Synthesis of Poly(octanedioladipate-*co*-octanediolmalate)

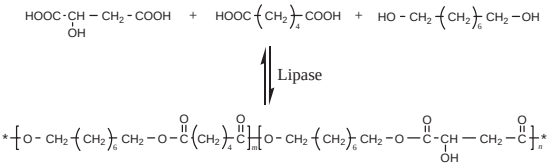
Dahu Yao,[1,3] Guangji Li,*[1] and Minhua Zong[2]
[1]*College of Material Science and Engineering, South China University of Technology, Guangzhou 510640, P. R. China*
[2]*Lab of Applied Biocatalysis, South China University of Technology, Guangzhou 510640, P. R. China*
[3]*College of Chemical Engineering and Pharmaceutics, Henan University of Science and Technology, Luoyang 471003, P. R. China*

(Received January 29, 2007; CL-070099; E-mail: gjli@scut.edu.cn)

Terpolymer of 1,8-octanediol, adipic acid, and L-malic acid was synthesized via a lipase-catalyzed direct polycondensation in different organic solvents. The results of GPC show that an organic solvent possessing low polarity is a better organic medium for the lipase-catalyzed polymerization. The ^{1}H NMR spectra of the obtained copolymer samples indicate that hydroxy groups of L-malic acid do not take part in the polymerization reaction.

As one of the most important classes of synthetic biodegradable polymers, aliphatic polyesters are generally considered to be well suited for temporary therapeutic applications as polymer-based biomaterials because of their good biocompatibility and biodegradability.[1] Most aliphatic polyesters have limited applications due to their hydrophobic property and the absence of functional groups on the polymer backbone; those groups could be used for tailoring physical properties and/or introducing bioactive substances. Poly(malic acid) and polyesters containing L-malic acid units have attracted much attention recently because there are many pendant carboxyl- or hydroxy-functional groups along the macromolecular chains of these polymers, which allows the introduction of a biologically active molecule and/or a targeting moiety by appropriate chemical modifications of these functions.[2,3] The conventional chemical methods for preparation of functional polyesters containing L-malic acid units include ring-opening polymerization (ROP) and condensation polymerization. The former requires lactone-like malolactonate as a monomer or comonomer, thus involving complicated preparing procedures and repeated purification steps in the reaction cycle;[4] while the latter requires protection–deprotection steps of hydroxy of L-malic acid for fear of crosslinking reaction.[5] Additionally, residual organometallic compounds used as catalysts often possess high toxicity, which is of concern in biomedical applications of these polymers.

In-vitro enzymatic synthesis of functional polyesters containing L-malic acid units may be a useful alternative to the conventional chemical methods. The lipase-catalyzed ring-opening polymerization of β-malolactonate has been reported.[6–8] However, The starting monomer β-malolactonate has to be synthesized through at least four steps, which contain repeated purification of intermediates. It should be a good way if L-malic acid can be used as a starting monomer to prepare polyesters having pendant functional group. In our previous work,[9] an investigation was made into the lipase-catalyzed direct polycondensation of L-malic acid (L-MA), adipic acid (ADA), and 1,8-octanediol (OC) for preparing polyesters containing L-MA units in a solvent-free system (Scheme 1). The purpose of this work is to study the effect of different solvents on the lipase-catalyzed direct polycondensation of comonomers L-MA, ADA, and OC.



Scheme 1.

The lipase-catalyzed direct polycondensation was conducted at a prescribed temperature in a sealed round flask containing 4 Å molecular sieves and different organic solvents with stirring, respectively. Novozyme 435 (immobilized lipase from *C. Antarctica*, type B, CAL-B, specific activity 10000 PLU/g) was used as a biocatalyst. The reaction was terminated by adding cold chloroform to the mixture, and the insoluble enzyme was removed by filtration. The polymeric product was obtained via evaporating under reduced pressure. The purified products were characterized by GPC and ^{1}H NMR.

The ^{1}H NMR spectroscopy of poly(octanediol adipiate) and copolyesters containing L-MA units synthesized in different organic media are shown in Figures 1 and 2. The resolved signals for the OC units protons C*H*$_2$–O(C=O), **1**, and C*H*$_2$–OH, **1′** can be observed at 4.07 and 3.65 ppm from the ^{1}H NMR spectrum of poly(octanediol adipiate), respectively. The signals attributed to the methylene protons C*H*$_2$–(C=O), **5**, and C*H*$_2$–(C=OOH), **5′**, of ADA units are not resolved and appear as a multiplet from 2.25 to 2.45 ppm. The signals attributed to protons C*H*$_2$CH$_2$–O(C=O), **2**, and C*H*$_2$CH$_2$–OH, **2′**, of OC units, and ADA units protons C*H*$_2$CH$_2$–(C=O), **6**, C*H*$_2$CH$_2$–(C=O)OH, **6′**, all appear as a broad multiplet at 1.57 to 1.78 ppm. Similarly, the signals corresponding to the methylene protons, C*H*$_2$CH$_2$CH$_2$–

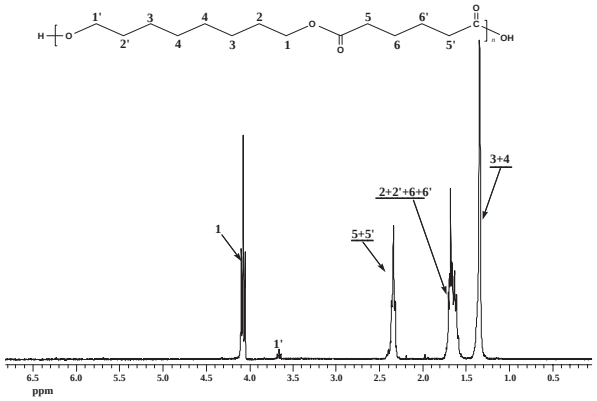


Figure 1. ^{1}H NMR spectrum of poly(octanedioladipate).

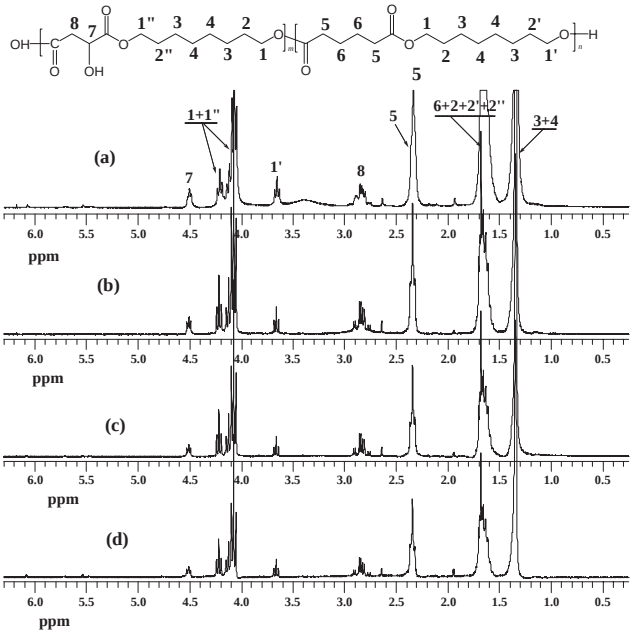


Figure 2. ^{1}H NMR spectrum of poly(octanediol adipate-*co*-octanediol malate) synthesized in different solvents. (a) *t*-BuOH, (b) toluene, (c) *n*-hexane, (d) isooctane.

O(C=O), **3**, and C*H*$_2$CH$_2$CH$_2$CH$_2$–O(C=O), **4**, of OC units are unresolved and found between 1.24 and 1.45 ppm.[10] The ^{1}H NMR spectrum of copolyesters containing L-MA units synthesized in different solvents (Figure 2) shows the signals corresponding to the L-MA units protons CHOH(C=O), **7**, and C*H*$_2$(C=O), **8**, at 4.5–4.6 ppm and 2.7–2.9 ppm.[5] And no reaction of the hydroxy groups of L-malic acid with the carboxyl groups of adipic acid or L-malic acid occurred according to the ^{1}H NMR. The malic acid units are incorporated into macromolecular chains exclusively through their carboxyl groups. It can be concluded that the obtained copolyesters in different solvents are poly(octanediol adipate-co-octanediol malate), and that by the Novozyme 435-catalyzed direct polycondensation, branching through esterification of L-MA hydroxy groups did not occur. Instead, the formed copolyesters containing L-MA units bear pendant hydroxy groups available for post-product modifications. The selectivity of Novozyme 435 did not change in different organic media.

The molecular weight M_w and its distribution M_w/M_n of the polymer samples prepared by the novozyme 435-catalyzed copolycondensation in various solvents are listed in Table 1. The results indicate that the organic solvent showed obvious effects on the molecular weight and polydispersity of the product. Generally speaking, a less polar organic solvent (such as isooctane, *n*-hexane or toluene) is a better organic media for lipase-catalyzed polymerization than a more polar one (such as *t*-BuOH, THF, or acetone). An enzyme has higher activity and stability in a hydrophobic solvent (log $P > 2$) due to its less effect on the essential water layer of the enzyme molecules, while a polar solvent usually strips off the essential water of the enzyme molecules, thereby inactivating the biocatalyst. Polydispersity of the sample with toluene as a solvent is lower than that with isooctane or *n*-hexane. This may attribute to the solubility of samples in different solvents (the copolymer is insoluble in isooctane and *n*-hexane, but partly soluble in toluene). The viscosity of the polymerization system in toluene is lower than that in isooctane or *n*-hexane, permitting transesterification and giving a narrower polydispersity.[11] Similarly, owing to the better solubility of the copolymer in *t*-BuOH, THF, or acetone, the polydispersity of the copolymer obtained with these solvents is narrow. Specially, log *P* of chloroform is similar to that of toluene, but the M_w of copolyester synthesized in chloroform is very low. Further analysis is now under study.

Table 1. Effect of solvent on the M_w and M_w/M_n[a]

Entry	Organic solvent	log *P*	Lipase /%	*T* /°C	M_w × 10^3	PDI	Yield /%
1[b]	—	—	10	70	11.2	2.44	98
2[b]	—	—	20	70	4.6	1.90	87
3	Isooctane	4.5	10	70	14.5	3.14	96
4	*n*-Hexane	3.5	10	70	17.8	3.42	97
5	Toluene	2.5	0	70	1.9	1.29	—
6	Toluene	2.5	10	70	12.4	2.15	96
7	Chloroform	2.0	10	55	2.7	1.55	—
8	*t*-BuOH	0.8	10	70	5.8	1.98	92
9	THF	0.49	10	55	5.4	1.95	94
10	Acetone	−0.23	10	55	5.4	1.98	90

[a]For a, feed molar ratio is OC:ADA:L-MA = 15:15:0, and for other entries, the feed molar ratio is OC:ADA:L-MA =15:9:6. [b]The reaction was carried out under reduced pressure (2000–6000 Pa). For all, the reaction time is 48 h. PDI is polydispersity index of polymer.

Linear copolymers with each L-malic acid repeating unit along the chain providing a hydroxy group, which could be converted via simple chemical transformations to many other functional copolymers or directly conjugate bioactive molecules, have been successfully prepared. In contrast with conventional methods of synthesizing linear copolymers containing L-MA units, the high selectivity of this biocatalytic process makes it possible to avoid a series of complicated protection–deprotection steps. The selectivity of Novozyme 435 does not change in different organic media. The polarity of the organic media has a great influence on the polymerization, and an organic solvent with a low polarity is better for the polymerization.

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Published on the web (Advance View) April 5, 2007; doi:10.1246/cl.2007.574