Polymer 49 (2008) 3444–3449

Contents lists available at ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

Chemo-enzymatic synthesis and sustained release of optically active polymeric prodrugs of chlorphenesin

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ARTICLE INFO

Article history:
Received 28 February 2008
Received in revised form 29 April 2008
Accepted 3 June 2008
Available online 12 June 2008

Keywords:
Copolymer
Lipase-catalyzed resolution
Optically active

ABSTRACT

Optically active polymeric prodrugs containing saccharides were prepared via a facile procedure combining enzymatic resolution with chemical polymerization. Polymerizable optically active chlorphenesin derivatives were obtained in excellent optically purity (ee > 99.9%) and high yield (~50%) in short time (~4 h) by lipase-catalyzed resolution after optimization of reaction conditions. Then, polymerizable optically active monomer was copolymerized with different polymerizable glycolipids using free radical polymerization method. The obtained optically active polymeric prodrugs bearing (*R*)-chlorphenesin residue were characterized by IR, NMR and GPC. *In vitro* released studies showed that the cumulative released optically pure chlorphenesin (ee = 88–92%) from the polymeric prodrug (VADG) was from 19.3% to 34.3% in pH 7.4, pH 5.4 and pH 1.2 after 7 days. The cumulative liberation rate of optically active polymeric prodrugs (ee = ~88%) with different glycolipids was from 26.4% to 38.5% in pH 1.2.

1. Introduction

In recent years, much attention has been focused on the design of polymeric prodrugs or polymer–drug conjugates because the drug released from the polymers increases the duration of drug activity and improves the targeting ability of polymeric prodrug in the body [1–7]. Several polymer-based pharmaceutical products have achieved both improved clinical outcomes and considerable market success [8]. However, there are few reports about the synthesis of optically active polymeric prodrugs starting from the *racemic* mixture of chiral drugs [9], though there are some researches on optical active polymers [10]. In particular, it has been an attractive and significative topic for the synthesis of polymeric prodrugs of optically active drugs with biocompatible moieties.

Biocompatibility is an important factor for application of macromolecular drugs [8,11]. Saccharides play a central role in living systems due to their biological recognition, functional biomolecular and excellent biocompatibility. The combinations of carbohydrates with other specific molecules provide much opportunity for developing new drug types and improving diseases therapy effect [12–18]. A great number of drugs in use today rely on carbohydrates as their important parts. As a result, the synthesis of polymeric prodrugs of optically active drugs containing saccharides is especially desirable [19].

Considerable efforts have been made to prepare optically active drugs by enzymatic resolution due to their high selectivity, mild reaction conditions and simplified downstream processing [20–27]. Our group has achieved successfully the enzymatic resolution of vinyl esters of non-steroidal anti-inflammatory drugs (NSAIDs), and prepared optical active polymeric prodrugs of NSAIDs with MMA [9,28]. In addition, a series of *racemic* polymerizable chlorphenesin monomers have been successfully synthesized [19,29]. However, the polymeric prodrugs of optically active drugs with saccharide branch and their *in vitro* sustained release haven't been investigated.

In this present work, polymerizable optically active chlorphenesin derivatives were obtained with excellent optical purity and in high yield in short time by lipase-catalyzed resolution. Then, we developed the efficient chemo-enzymatic method to prepare polymeric prodrugs of optically active chlorphenesin with glycolipids. Furthermore, the length of linker and pH value of incubation medium were found to have important influence on the release rate of chlorphenesin through *in vitro* release experiments. The investigation of the ee value of the residue chlorphenesin from polymeric prodrugs showed that the stereoselectivity of chlorphenesin remained after the sustained release of optically active polymeric prodrugs. This strategy can provide possibility to design other novel multifunctional polymeric prodrugs with optically active drugs.

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doi:10.1016/j.polymer.2008.06.009

2. Materials and methods

2.1. Materials

Lipozyme® (E.C. 3.1.1.1, an immobilized preparation of lipase from *Mucor miehei*), lipase from porcine pancreas (E.C. 3.1.1.3, Type II, powder), *Candida antarctica* lipase acrylic resin (E.C. 3.1.1.3) and lipase Type VII from *Candida rugosa* (E.C. 3.1.1.3, powder) were purchased from Sigma. Lipase PS "Amano" (E.C. 3.1.1.3, powder) and lipase PS–C "Amano" (E.C. 3.1.1.3, an immobilized preparation of lipase of *Burkholderia cepacia*) were purchased from Aldrich. Alkaline protease from *Bacillus subtilis* (E.C. 3.4.21.14, a crude preparation of the alkaline serine protease, powder) was purchased from Wuxi Enzyme Co. Ltd (Wuxi, P.R. China). Chlorphenesin was purchased from Alfa Aesar, a Johnson Matthey Company. All the enzymes were used directly in commercial preparations without further purification. 2,2′-Azoisobutyronitrile (AIBN) was purified by recrystallization with methanol and dried at room temperature under vacuum. All solvents were of analytical grade and were dried by storing over activated 3 Å molecular sieves before use. All other reagents were used as received.

2.2. Analytical methods

The process of reactions was monitored by TLC on silica. The ^{1}H NMR and ^{13}C NMR spectra were recorded with TMS as internal standard using a Bruker AMX-500 MHz spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively. Chemical shifts were expressed in ppm and coupling constants (J) in Hz. IR spectra were measured with a Nicolet Nexus FTIR 670 spectrophotometer. Analytical HPLC was performed using an Agilent 1100 series with a reversed-phase Shim-Pack VP-ODS column (150 × 4.6 mm) and a UV detector (275 nm). Methanol/water (80/20, v/v) was used as a mobile phase, while the flow rate was adjusted to 1 mL min^{-1}. GPC was performed with a system equipped with refractive-index detector (Waters 2410) and Waters Styragel GPC columns. The GPC columns were standardized with narrow dispersity polystyrene in molecular weights ranging from 4.7×10^6 to 2350. The mobile phase was tetrahydrofuran at a flow rate of 1.5 mL min^{-1}. The enantiomers of chlorphenesin were analyzed using an Agilent 1100 series with a chiral column (Chiral OD-H Column No. ODH0-EA031 (DAICEL CHEMICAL INDUSTRIES, LTD.)) and were detected at 275 nm. The mobile phase was *n*-hexane/isopropanol (85/15, v/v) with a flow rate of 0.5 mL min^{-1}.

2.3. Synthesis of (R,S)-chlorphenesin vinyl ester and D-glucose derivatives

Racemic polymerizable chlorphenesin derivatives were synthesized by enzymatic transesterification of chlorphenesin (**1**) and divinyl adipate using Lipozyme® as catalyst in anhydrous acetone at 50 °C under 250 rpm [29]. When alkaline protease from *B. subtilis* was employed as catalyst in pyridine, polymerizable sugar derivatives were prepared using the same method. By selective enzymatic synthesis, we prepared *racemic* polymerizable chlorphenesin derivative and three polymerizable glucose derivatives, 6-*O*-vinyladipyl-chlorphenesin (OVAC, **2**), 6-*O*-vinylsuccinyl-D-glucose (VSUG, **a**), 6-*O*-vinyladipyl-D-glucose (VADG, **b**) and 6-*O*-vinylsebacyl-D-glucose (VSEG, **c**) [30].

2.4. Synthesis of optically active polymerizable chlorphenesin vinyl ester

The synthesis of (*R*)-chlorphenesin vinyl ester ((*R*)-**2**) was catalyzed by PS–C in anhydrous MTBE. The reaction was initiated by adding 100 mg lipase to 10 mL solvent containing 1.0 g *racemic* chlorphenesin vinyl esters and vinyl acetate (1:2, molar ratio). The suspension was kept at 25 °C and shaken at 200 rpm. The reaction was detected by HPLC and terminated by filtering off the enzyme. The reaction mixture was concentrated under reduced pressure. The products (*S*)-**3** and (*R*)-**2** were separated by silica gel chromatography with a mobile phase consisting of petroleum ether/ethyl acetate (5:2, v/v) and petroleum ether/ethyl acetate (2:1, v/v), respectively. The product was analyzed by IR and NMR.

2.5. (R)-1-O-Vinyladipoyl-chlorphenesin ((R)-OVAC, (R)-**2**)

^{1}H NMR ($CDCl_3$): δ (ppm): 7.27 (dd, 1H, $J = 6.3$ Hz, $J = 14.1$ Hz, –CH═), 7.22 (d, 2H, Ar–H), 6.84 (d, 2H, Ar–H), 4.88 (dd, 1H, $J = 14.1$ Hz, $J = 6.3$ Hz, ═CH_2), 4.57 (dd, 1H, $J = 6.3$ Hz, $J = 1.5$ Hz, ═CH_2), 4.32–4.21 (m, 3H, –C*H*(OH)C*H*$_2$–), 3.98 (m, 2H, –OC*H*$_2$CH–), 3.05 (br, 1H, –CH(O*H*)–), 2.39 (m, 4H, 2-CH_2–), 1.68 (m, 4H, 2-CH_2–). ^{13}C NMR ($CDCl_3$): δ (ppm): 173.5, 170.6 (C═O), 157.1, 129.5, 129.5, 126.3, 116.0, 116.0 (Ar, chlorphenesin), 141.2 (–O–CH═), 98.0 (═CH_2), 69.2 (C–3, chlorphenesin), 68.5 (C–2, chlorphenesin), 65.4 (C–1, chlorphenesin), 33.8, 33.5, 24.3, 24.0 (–CH_2–). IR (KBr, film): ν (cm^{-1}): 3515 (OH), 1760, 1736 (O–C═O), 1647 (C═C), 1592, 1497, 836, 806 (Ar). ESI-MS (m/z): 379 $[M + Na]^+$.

2.6. 1-O-Vinyladipoyl-2-O-acetyl-chlorphenesin ((S)-**3**)

^{1}H NMR ($CDCl_3$): δ (ppm): 7.27 (dd, 1H, $J = 6.3$ Hz, $J = 14.1$ Hz, –CH═), 7.22 (m, 2H, Ar–H), 6.84 (m, 2H, Ar–H), 5.35 (m, 1H, –CH_2C*H*(OH)–), 4.88 (d, 2H, $J = 14.1$ Hz, ═CH_2), 4.57 (dd, 2H, $J = 6.3$ Hz, $J = 1.5$ Hz, ═CH_2), 4.44 (dd, 1H, $J = 4.0$ Hz, $J = 12.0$ Hz, –CHC*H*$_2$O–), 4.28 (dd, 1H, $J = 12.0$ Hz, $J = 6.0$ Hz, –CHC*H*$_2$O–), 4.08 (d, 2H, $J = 5.0$ Hz, –OC*H*$_2$CH–), 2.38 (m, 4H, 2-CH_2–), 2.10 (s, 3H, CH_3CO), 1.68 (m, 4H, 2-CH_2–). ^{13}C NMR ($CDCl_3$, δ, ppm): 173.0, 170.5, 170.4 (C═O), 157.1, 129.7, 129.7, 126.6, 116.2, 116.2, (Ar, chlorphensin), 141.3 (–O–CH═), 97.9 (═CH_2), 69.8 (C-2, chlorphensin), 66.6 (C-3, chlorphensin), 62.7 (C-1, chlorphensin), 33.8, 33.7, 24.4, 24.1 (–CH_2–), 29.9 (*C*H$_3$CO). IR (KBr, film): ν (cm^{-1}): 1758, 1734 (O–C═O), 1647 (C═C), 1597, 1495, 835, 804 (Ar).

2.7. Copolymerization of (R)-OVAC with 6-O-vinylsuccinyl-D-glucose (VSUG) (***4a***)

In a 25 mL sealed polymerization tube, a mixture containing OVAC (1 mmol, 0.36 g), VSUG (1 mmol, 0.31 g) with 2% AIBN (w/w) and DMF (0.3 mL) was maintained at 70 °C under nitrogen for 12 h after the solution was degassed. Precipitating the polymer in acetone terminated the reaction. Then the precipitated material was dried under reduced pressure. The light yellow solid was obtained in yield of 56.3%. Poly(OVAC-*co*-VSUG) has M_n of 1.86×10^4, and M_w/M_n of 2.36. IR (KBr, sheet): ν (cm^{-1}): 3419, 1167, 1056 (OH), 1732 (O–C═O), 1596, 1583, 1495, 825 (Ar). ^{1}H NMR (DMSO-d_6 + D_2O, 500 MHz): δ (ppm): 7.23 (Ar–H), 6.86 (Ar–H), 5.08, 4.54 (1-OH of glucose), 4.32–3.03 (–OC*H*$_2$C*H*(O*H*)C*H*$_2$– of chlorphenesin; 1-H, 2-H, 3-H, 4-H, 5-H, 6-H, 2-OH, 3-OH, and 4-OH of D-glucose), 2.53–1.68 (CH_2). ^{13}C NMR (DMSO-d_6 + D_2O): δ (ppm): 172.7, 172.1 (C═O), 157.9, 129.9, 129.9, 125.1, 116.9, 116.9 (Ar, chlorphenesin), 97.2 (C1 of β-D-glucose), 92.7 (C1 of α-D-glucose), 76.7 (C3 of β-D-glucose), 75.1 (C2 of β-D-glucose), 74.0 (C5 of β-D-glucose), 73.3 (C3 of α-D-glucose), 72.5 (C2 of α-D-glucose), 70.9 (C4 of α-D-glucose), 70.6 (C4 of β-D-glucose), 69.8 (C5 of α-D-glucose), 69.6 (C-3, chlorphenesin), 67.2 (C-2, chlorphenesin), 65.3 (C-1, chlorphenesin), 64.9 (C6 α, β of D-glucose), 36.6 ((–C*H*C*H*$_2$–)$_n$), 34.3, 34.1, 29.2, 29.1, 25.0, 25.0 (–CH_2–).

2.8. Copolymerization of (R)-OVAC with 6-O-vinyladipoly-D-glucose (VADG) (**4b**)

Poly-(OVAC-*co*-VADG) was synthesized using the same method as for poly-(OVAC-*co*-VSUG). The light yellow solid was obtained in yield of 59.5%. Poly(OVAC-*co*-VADG) has M_n of 1.05×10^4, and M_w/M_n of 1.35. IR (KBr, sheet): ν (cm^{-1}): 3420, 1168, 1055 (OH), 1734 (O–C═O), 1597, 1583, 1496, 826 (Ar). ^{1}H NMR (DMSO-d_6 + D_2O, 500 MHz): δ (ppm): 7.22 (Ar–H), 6.84 (Ar–H), 5.06, 4.53 (1-OH of glucose), 4.30–3.01 (–OCH_2CH(OH)CH_2– of chlorphenesin; 1-H, 2-H, 3-H, 4-H, 5-H, 6-H, 2-OH, 3-OH, and 4-OH of D-glucose), 2.39–1.66 (CH_2). ^{13}C NMR (DMSO-d_6 + D_2O): δ (ppm): 174.2, 173.1 (C═O), 158.4, 130.6, 130.4, 125.9, 117.5, 117.5 (Ar, chlorphenesin), 97.6 (C1 of β-D-glucose), 93.0 (C1 of α-D-glucose), 77.4 (C3 of β-D-glucose), 75.5 (C2 of β-D-glucose), 74.3 (C5 of β-D-glucose), 74.1 (C3 of α-D-glucose), 73.0 (C2 of α-D-glucose), 71.3 (C4 of α-D-glucose), 71.0 (C4 of β-D-glucose), 70.2 (C5 of α-D-glucose), 70.1 (C-3, chlorphenesin), 67.8 (C-2, chlorphenesin), 66.2 (C-1, chlorphenesin), 46.8 (C6 α, β of D-glucose), 34.0 ((–CHCH_2–)$_n$), 34.2, 29.7, 29.7, 25.0, 24.9, 24.9 (–CH_2–).

2.9. Copolymerization of (R)-OVAC with 6-O-vinylsebacyl-D-glucose (VSEG) (**4c**)

Poly-(OVAC-*co*-VSEG) was synthesized using the same method as for poly-(OVAC-*co*-VSUG). The light yellow solid was obtained in yield of 52.8%. Poly(OVAC-*co*-VSEG) has M_n of 2.65×10^4, and M_w/M_n of 2.95. IR (KBr, sheet): ν (cm^{-1}): 3420, 1168, 1055 (OH), 1734 (O–C═O), 1597, 1583, 1496, 826 (Ar). ^{1}H NMR (DMSO-d_6 + D_2O, 500 MHz): δ (ppm): 7.24 (2H, Ar–H), 6.86 (2H, Ar–H), 5.08, 4.54 (1-OH of glucose), 4.33–2.99 (–OCH_2CH(OH)CH_2– of chlorphenesin; 1-H, 2-H, 3-H, 4-H, 5-H, 6-H, 2-OH, 3-OH, and 4-OH of D-glucose), 2.38–1.22 (CH_2). ^{13}C NMR (DMSO-d_6 + D_2O): δ (ppm): 174.6, 174.6 (C═O), 158.5, 130.4, 130.4, 125.9, 117.4, 117.4 (Ar, chlorphenesin), 97.7 (C1 of β-D-glucose), 93.3 (C1 of α-D-glucose), 77.2 (C3 of β-D-glucose), 75.6 (C2 of β-D-glucose), 74.6 (C5 of β-D-glucose), 73.9 (C3 of α-D-glucose), 73.1 (C2 of α-D-glucose), 71.4 (C4 of α-D-glucose), 71.2 (C4 of β-D-glucose), 70.5 (C5 of α-D-glucose), 70.2 (C–3, chlorphenesin), 67.9 (C–2, chlorphenesin), 66.1 (C–1, chlorphenesin), 64.9 (C6 α, β of D-glucose), 34.7((–CHCH_2–)$_n$), 34.7, 31.9, 29.9, 29.8, 25.7, 25.7 (–CH_2–).

2.10. In vitro *hydrolysis of polymeric prodrugs*

Optically active polymeric prodrugs of chlorphenesin were prepared as described earlier and dried in vacuum at room temperature. *In vitro* drug release experiments were carried out as follows: the resultant polymeric prodrug (10 mg, (*R*)-**4a**, (*R*)-**4b** or (*R*)-**4c**) was added to 1 mL incubation medium (pH 7.4, 0.2 M Na_2HPO_4–$12H_2O$/NaH_2PO_4–$2H_2O$ buffer solution, pH 5.4, 0.2 M HAc/NaAc buffer solution, or pH 1.2, 0.1 M HCl/NaCl/glycine solution) and subsequently placed in a dialysis membrane (MWCO = 3500 Da) for release studies. The dialysis membrane was then placed in a 10 mL bottle with 5 mL corresponding incubation solution and the medium was stirred at 100 rpm at 37 °C. At set time intervals, the whole medium (5 mL) was taken and replaced with the same volume of fresh solution. The products released from the polymeric prodrugs were analyzed by HPLC with a UV–vis detector and a reversed-phase Shim-Pack VP-ODS column (150 × 4.6 mm). A mixture of methanol and water (v/v = 80/20) was employed as the mobile phase, and the flow rate was 1.0 mL min^{-1}. The detection wavelength was 275 nm. The concentration of the chlorphenesin released in incubation solution was determined by an Analytikjena SPECORD 200 UV–vis spectrophotometer at 275 nm. The ee values of released drugs from polymeric prodrugs were detected by HPLC with a chiral column.

Table 1
Influence of enzymes on lipase-catalyzed resolution of (*R*,*S*)-OVAC

Entry	Enzyme	Time (h)	Con. (%)	ee_s[a] (%)	E[b]	Config.[c]
1	No enzyme	24	–	3.6	–	–
2	Lipozyme®	6	40.1	32.5	4.0	*S*
3	CAL-B	24	67.9	34.3	1.8	*R*
4	PPL	24	21.0	5.1	1.6	*R*
5	CRL	36	49.2	16.7	1.7	*S*
6	Lipase PS	6	13.9	14.5	21.5	*R*
7	Lipase PS–C	6	54.5	>99.9	106.0	*R*

Reaction conditions: substrate 10 mg, enzyme 10 mg, MTBE (*tert*-butyl methyl ether) 1 mL, 25 °C, 200 rpm; Con.: the reaction conversion (%); Config.: the configuration of residual substrate.
[a] ee_s (%): $ee_s = ([R] - [S])/([R] + [S])$; [*R*]: the concentration of the *R* form of OVAC; [*S*]: the concentration of the *S* form of OVAC.
[b] *E*: the enantioselectivity, $E = \ln[(1 - c)(1 - ee_s)]/\ln[(1 - c)(1 + ee_s)]$.
[c] Ref. [34].

3. Results and discussion

3.1. Optimization for the synthesis of polymerizable optically active chlorphenesin

Optically active polymers can be prepared from optically active monomers [31–33]. Enzymatic resolution is a facile and useful approach to synthesize optically active compounds. In this study, six commercial enzymes were selected to prepare drug monomers with high ee value. There was no reaction without enzyme participation, and it was proved that the biocatalyst was the critical factor in this enzymatic resolution reaction. From Table 1, lipase PS showed moderate enantioselectivity ($E = 21.5$, C ~ 14%) at 6 h, and lipase PS–C was the most effective for enzymatic resolution in organic solvent, which showed highest enantioselectivity ($E > 100$). The conversion of reaction reached 54.5% and ee_s was more than 99.9% at 6 h. The catalytic activity and enantioselectivity were much better than previous experiment results [34]. It was proved that immobilized enzyme was much stable in the organic solvent and retained much more catalytic reactivity during the reaction procedure. In addition, the reactivity of *racemic* OVAC was higher due to the vinyl group of OVAC. The other lipases in this research showed poor catalytic reactivity and low enantionselectivity ($E < 4$).

As shown in Table 1, Lipozyme® and CRL selected *R* configuration of chlorphenesin vinyl ester (OVAC), while lipase PS–C, PS,

Table 2
Influence of solvents on lipase-catalyzed resolution of (*R*,*S*)-OVAC

Entry	Solvent	log *P*	Con. (%)	ee_s[a] (%)
1	THF	0.46	12.2	13.3
2	Toluene	2.2	27.5	38.3
3	IPE	1.9	61.5	96.1
4	Cyclohexane	3.4	42.0	90.3
5	Dixane	−0.5	8.5	5.0
6	MTBE	2.0	51.5	>99.9
7	*n*-Hexane	3.9	ns	>99.0
8	Isooctane	4.3	ns	91.6

Reaction conditions: substrate 10 mg, lipase PS–C 10 mg, solvent: 1 mL, 25 °C, 200 rpm, 4 h.
[a] Chiral OD–H column: hexane/isopropyl alcohol = 85:15.

Table 3
Effect of temperature on enzymatic resolution of (*R*,*S*)-OVAC

Entry	Temperature (°C)	Time (h)	Con. (%)	ee_s[a] (%)	*E*
1	5	4	35.5	54.5	352
2	15	4	41.0	68.5	287
3	25	4	51.5	>99.9	246
4	37	2	61.3	94.7	14
5	50	0.5	62.6	78.5	6

Condition: substrate 10 mg, lipase PS–C 10 mg, solvent: MTBE 1 mL, 200 rpm.
[a] Chiral OD–H column: hexane/isopropyl alcohol = 85:15.

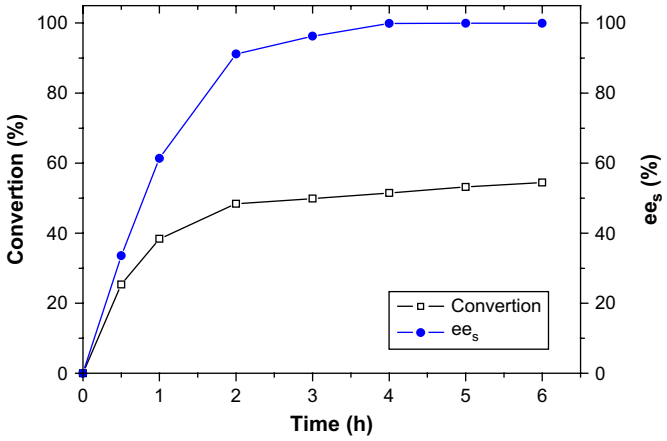


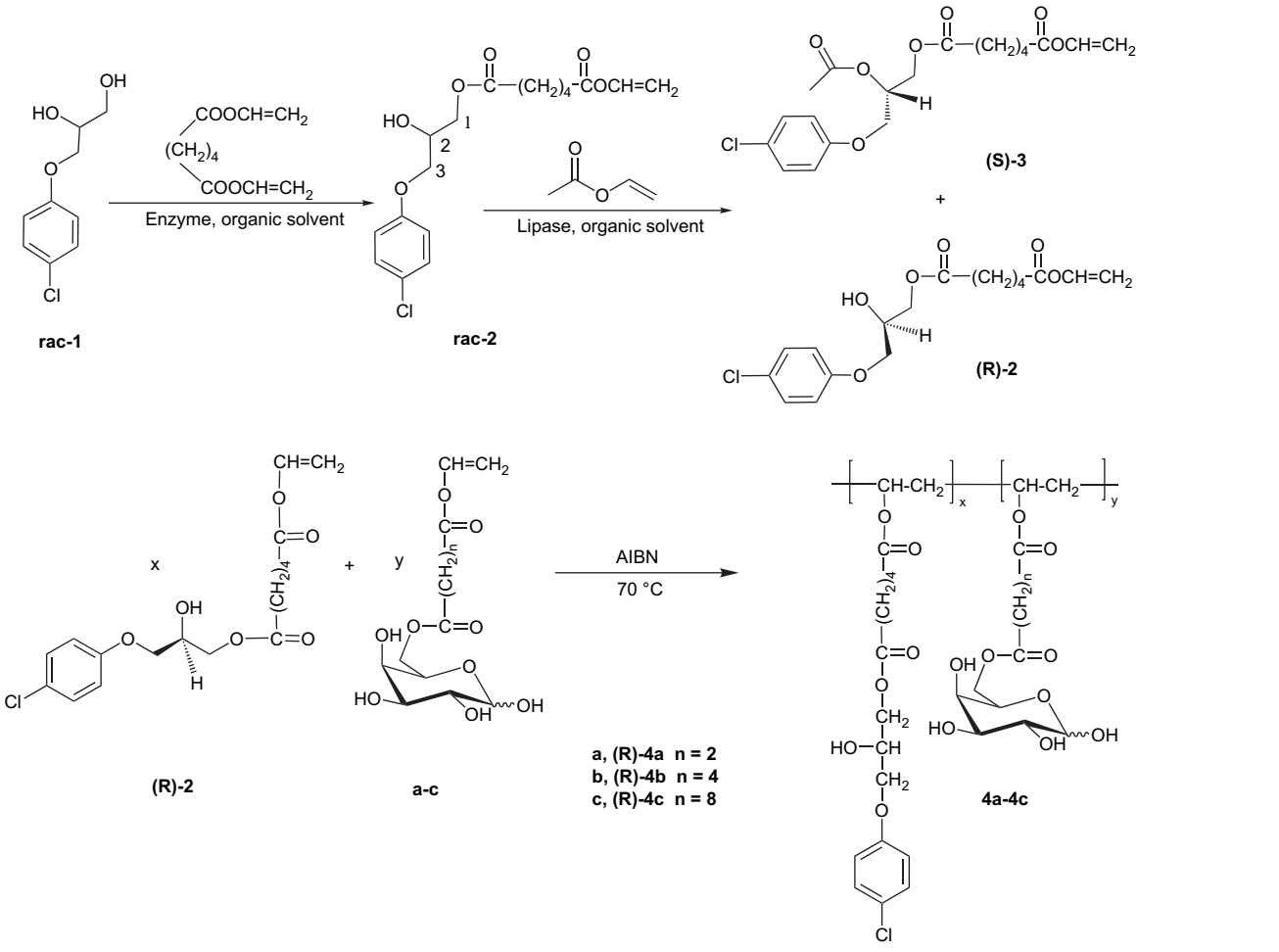
Fig. 1. Time-course analysis of the enzymatic resolution of (*R*,*S*)-OVAC.

CAL-B and PPL selected *S* configuration of substrate in the reaction, because enzymes derived from various sources showed different properties, such as activity and specificity. It gave the opportunity for the preparation of optically active monomers with different enantiomers. In this paper, lipase PS–C was chosen as the most efficient lipase for further investigation.

Improvement in the lipase catalytic activity and selectivity can be made by changing the solvents [35–37]. Eight organic solvents were investigated in order to prepare the optically active monomers efficiently and fast (Table 2). The lipase-catalyzed resolution of *racemic* OVAC is better in organic solvent with low polarity than in polar solvent. The ee values of (*R*)-OVAC were more than 99.9% in *n*-hexane and MTBE, and MTBE was selected for the lipase-catalyzed resolution of OVAC due to its well solubility for substrates and higher reaction rate.

The influence of the reaction temperature on the lipase-catalyzed resolution of OVAC in MTBE was investigated. As shown in Table 3, higher temperature caused lower enantiomeric excess value of OVAC but higher conversion. The *E*-value at 5 °C was the highest ($E > 300$) and that at 50 °C was only 6 as the enzyme was partly inactive in high temperature, while the reaction conversion at 5 °C was only about 35% after 4 h. Despite high enantiomeric excess observed at low temperature, the reaction rate was much slower than that at higher temperature. Considering the two factors, the optimal temperature in terms of enantioselectivity and conversion was 25 °C, which is close to ambient and thus environmentally benign.

Time process of lipase-catalyzed resolution of *rac*-**1** is shown in Fig. 1. The reaction conversion reached ~50% after 3 h and the enantioselectivity *E* was more than 200. The high catalytic reactivity and enantioselectivity was observed in lipase-catalyzed resolution of *racemic* OVAC in MTBE using lipase PS–C as biocatalyst in 4 h. The *E*-value decreased with the reaction proceeding after 4 h. The optimal reaction condition was 25 °C, lipase PS–C, MTBE, 4 h for the synthesis of optically active monomers.



Scheme 1. Chemo-enzymatic synthesis of optically active polymeric prodrugs of chlorphenesin with saccharides.

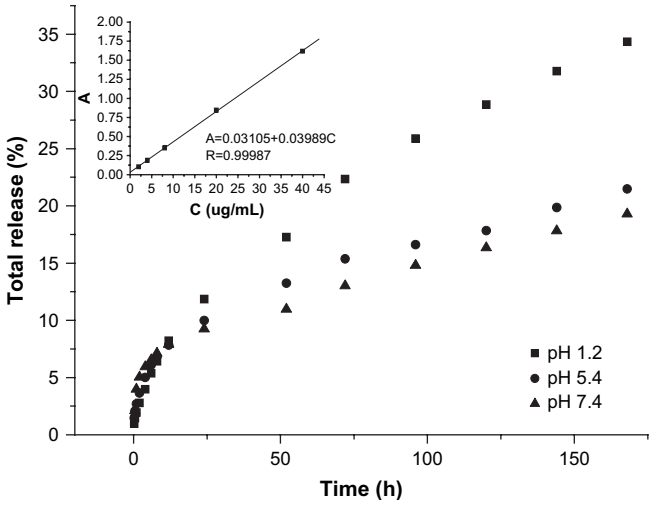


Fig. 2. Drug release of poly(OVAC-*co*-VADG) *in vitro* in different pH buffer solution.

3.2. Synthesis and characterization of polymer prodrugs with optically active chlorphenesin branches

Drug-containing monomer, optically active chlorphenesin, was copolymerized with a series of polymerizable glycolipids with different linkers in dried DMF solution by free radical technique at 70 °C using AIBN as initiator (Scheme 1). The structure of the optically active copolymers with different linker glycolipids was characterized with FT-IR, NMR and GPC. Taking poly-(OVAC-*co*-VADG) (**4b**) for example, in IR spectra, 3101 cm^{-1} and 1646 cm^{-1} assigned to the vibration bands of double bond in the optically active OVAC ((*R*)-**2**) disappeared, and the absorption at 1596 cm^{-1}, 1582 cm^{-1}, 827 cm^{-1} were assigned to the aromatic ring of the (*R*)-**2**. 3419 cm^{-1}, 1170 cm^{-1} and broad 1057 cm^{-1} were assigned to glycolipid (**b**). The results confirmed the structure of the synthesized polymers. NMR of **4b** revealed the disappearance of vinyl group (^{1}H NMR: δ 7.27, 4.88, 4.57; ^{13}C NMR: δ 141.2, 98.0) and existence of (*R*)-**2** (^{1}H NMR: δ 7.22, 6.84; ^{13}C NMR: δ157.1, 129.5, 129.5, 126.3, 116.0, 116.0, 70.1, 67.8, 66.2), D-glucose groups (^{1}H NMR: δ 5.03–3.45; ^{13}C NMR: δ 97.6, 93.0, 77.4, 75.5, 74.3, 74.1, 73.0, 71.3, 71.0, 70.2, 46.8) and poly(vinyl alcohol) main chain (^{1}H NMR: δ 2.39–1.66; ^{13}C NMR: δ 34.0). The polymeric prodrug **4a** has molecular weight with M_n of 1.86×10^4, and M_w/M_n of 2.36, **4b** has molecular weight with M_n of 1.05×10^4, and M_w/M_n of 1.35 and **4c** has molecular weight with M_n of 2.65×10^4, and M_w/M_n of 2.95. According to the calculation from the NMR spectra, the ratio of (*R*)-**2** to **a**–**c** monomers in copolymer was 0.88:1, 0.85:1 and 0.87:1. The loadup of (*R*)-**2** of the three optically active polymeric prodrugs was 26.8 wt% **4a**, 24.8 wt% **4b** and 23.6 wt% **4c**, respectively.

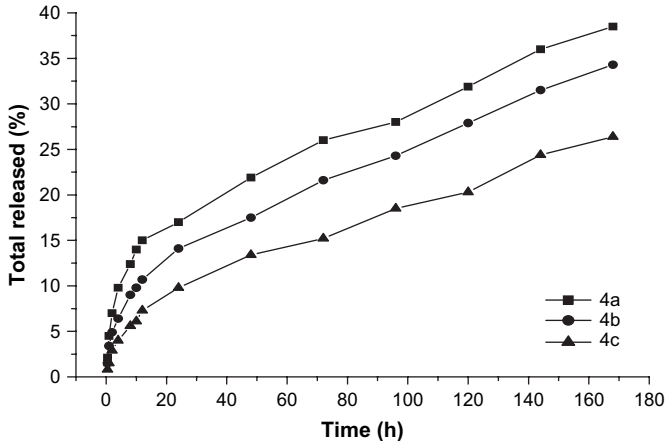


Fig. 3. *In vitro* release of (*R*)-**2** from the polymers with different linker glycolipids at pH 1.2.

3.3. In vitro *sustained release of polymeric prodrugs*

The side chain hydrolysis of polymers with drug-pendent depends on the strength and chemical nature of the drug–polymer chemical bonds and the surrounding conditions [38]. In this research, *in vitro* hydrolysis behavior of the optically active polymeric prodrugs was studied in three incubation media: pH 1.2 glycine solutions (simulated gastric juice), pH 5.4 acetate solutions and pH 7.4 phosphate solution (simulated extracellular fluids) at 37 °C. HPLC was employed for the qualitative analysis of the released product. The released drug of **4b** in different pH solutions passed through the dialysis membrane into the external buffer solution and was detected by UV spectrophotometer at 275 nm. The curves are shown in Fig. 2. It was found that *in vitro* release rate of **4b** was relative to the pH value of incubation solution. In pH 1.2 acidic solutions, the liberation rate of **4b** was faster than that in pH 5.4 acetate solution and pH 7.4 phosphate solutions. The cumulative release drug from **4b** in pH 1.2 solutions was 34.3% after 7 days, while that in pH 5.4 acetate solution and pH 7.4 phosphate solutions was 21.5% and 19.3%, respectively. It was because that the ester bond could more easily undergo hydrolysis in acidic medium. The product was confirmed as (*R*)-chlorphenesin with the ee value of 88–92% by HPLC with a chiral column after the product was dried in vacuum.

Drug liberation rate could be widely controlled by changing the relative length of linker between drug and polymer main chain [39]. Three optically active polymeric prodrugs bearing glycolipids with different linkers were selected to investigate the influence of linker length of monomers on *in vitro* release rate of drug. Fig. 3 shows the optically active polymer–chlorphenesin conjugates in pH 1.2 glycine solution after 7 days, the cumulative released (*R*)-chlorphenesin from polymeric prodrugs decreased from 38.5% to 26.4% with the increasing the length of glycolipid monomers. When the linker between polymer backbone and chlorphenesin branch was the same, the relative length of the linker between chlorphenesin and polymer main chain[1] was longer, the easier the ester bond connecting chlorphenesin to the linker was exposed to release buffer solution. Therefore, the above results suggested that the sustained-released of polymeric prodrugs could be effeciently controlled by the relative length of linker between drug and polymer main chain. The ee values of (*R*)-chlorphenesin released from three optically active polymeric prodrugs **4a**–**c** were ~88% detected by HPLC. The ee value of released drug little depended on the length of glycolipid monomers.

4. Conclusion

In this research, we developed a facile and efficient approach to synthesize optically active polymeric prodrugs of chlorphenesin with different linker glycolipids by combining lipase-catalyzed resolution with radical polymerization. High enantioselectivity (ee > 99.9%) and yields (~50%) of polymerizable chorphenesin monomer can be obtained in short reaction time (4 h) after optimization of enzymatic resolution condition. Then, optically active

[1] We use the letter "a" to denote the number of methylene units between chlorphenesin and polymer main chain, and "b" was used to denote that between D-glucose and polymer main chain. The value of "a/b" was used to represent the relative length of linkers between chlorphenesin and polymer main chain.

polymeric prodrugs bearing (*R*)-chlorphenesin and glycolipids with different linkers were obtained by free radical polymerization, which were characterized by IR, NMR and GPC. *In vitro* released studies showed that the cumulative released optically pure chlorphenesin (ee = 88–92%) from the polymer–chlorphenesin conjugate was 19.3%–34.3% in buffer solution with pH 7.4, 5.4 and 1.2 after 7 days. And the sustained release rate of optically active chlorphenesin decreased with increasing the length of glycolipid monomers. These results would be useful for the further research of other optically active polymeric prodrugs prepared from other functional comonomers.

Acknowledgements

The financial support from the National Natural Science Foundation of China (No. 20704037) and the Zhejing Provincial Science and Technology Council (Project No. 2006C11197) is gratefully acknowledged.

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