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ORIGINAL PAPER

Preparation and controlled release of degradable polymeric ketoprofen–saccharide conjugates

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Abstract A facile and efficient enzymatic and polymerization process was used to prepare polymeric prodrugs of ketoprofen with saccharide side chains. The chains included branches that included glucose, mannose, galactose, and lactose, and these were synthesized through free radical reaction. The prodrugs were characterized by FT-IR, NMR, and GPC and drug-loading capacity was influenced by varying the ratios of initiator and monomers (range 32.13 and 68.56% w/w). In vitro release characteristics of the polymeric drugs were systematically evaluated over the pH range 1.2–8.0 and the release profiles indicated that the hydrolytic nature of polymers were strongly depended on the variation in saccharide content, carbon chain length, and pH. The outcomes from this study demonstrate the importance of carbohydrate structures and how these are linked to drug release.

Keywords Ketoprofen · Polymeric prodrug · Copolymer · Enzymatic synthesis · Drug release · Polymer–drug conjugate

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Introduction

Studies on macromolecules-containing saccharide as selective drug delivery system have been developed for biomedicinal application [1, 2]. It has been reported that these systems, such as drug–saccharide complex [3–5] and polymeric micelle [6, 7], provide many advantages including prolonged drug release, changed biodistribution, reduced toxicity, and increased patient acceptance [8, 9]. Moreover, biological activities, environmental safety, and nontoxic of saccharide [10, 11] are also provided. In most cases, synthesis of a well-designed saccharide–macromolecular complex requires many reaction steps [4, 12], and it has been proved that drugs are rapidly released from these carriers (inclusion complexes or composite microcapsules) [13, 14]. Therefore, polymeric prodrugs with saccharide through chemical bond have been recently investigated as a potential strategy for overcoming such problems and merging the relative advantages of saccharide and macromolecules into a single system [15–17]. This system can be useful to both increase drug stability and avoid the rapid release. Thus, it is worthy of interest to evaluate sugar as a potential carrier for improving drug dissolution behavior.

Polymeric drug–saccharide conjugation has been attempted successfully and some prodrugs have shown promise [18], which offers a lot of opportunities for preparing drug derivatives with bioactive moieties and improving drug bioavailability. However, the further research, such as influence of factors in preparation progress (monomer concentration, solvent, and initiator, etc.) and effect of parameters to in vitro drug release (structure of linkers, solution property, etc.), is still a valuable and interesting topic which would provide available information to further investigate the therapeutic benefit of drugs and their derivatives. Unfortunately, there are few reports about these investigations of the polymeric drug–sugar single system.

Ketoprofen, a commonly drug used as non-steroidal anti-inflammatory drugs (NSAIDs), is widely applied to alleviate pain and inflammation associated with tissue injury [19]. However, ketoprofen is poorly soluble in aqueous media and causes irritant side effects especially on the gastro-enteric mucosal membranes [20, 21]. In order to reduce gastrointestinal side effects by controlling the release rate, duration, and site of release, many studies have been reported [22]. The inclusion of various modification stages to ketoprofen complicates the synthesis of ketoprofen structures [23]. Therefore, the development of easier protocols for the synthesis of polymeric prodrugs of ketoprofen with saccharide would be desirably.

The goals of this study were to control the release of ketoprofen through polymeric drug–saccharide conjugation and to systematically investigate the influence of various factors to preparation and drug release from polymeric prodrugs. In this work, first, a series of polymeric prodrugs of ketoprofen with different saccharide branches were synthesized and characterized by FT-IR, NMR, and GPC. And then, the effects of different saccharides with varying carbon chain lengths and at different pH values were systematically characterized in relation to in vitro drug release properties. Our studies showed that certain conjugates prolonged drug release.

Experimental

Materials

Alkaline protease, EC 3.4.21.62, was purchased from Wuxi Xue Mei Technological Co. Ltd. (Wuxi, China). Racemic ketoprofen (2-(3-benzoylphenyl) propionic acid) was supported by Zhejiang Jiuzhou Pharmaceutical Co. Ltd. (Taizhou, PR China). 2,2′-Azo-bis-iso-butyronitrile (AIBN) was purified by recrystallization with ethanol. All other chemicals used in this work were of analytical grade and were first dried over 4 Å molecular sieves for 24 h prior to use.

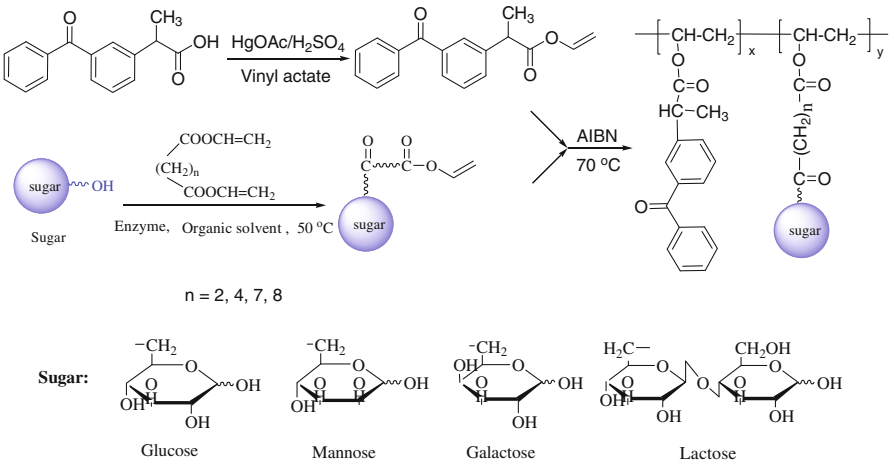
Synthesis of comonomers

Ketoprofen vinyl ester (KVE) was synthesized and purified as described by Cai et al. [24]. Polymerizable saccharide derivatives (SAD) were synthesized using alkaline protease as catalyst in anhydrous pyridine at 50 °C under 250 rpm for 4 days (Scheme 1). By selective enzymatic synthesis, seven monomers were obtained, including 6-*O*-vinylsuccinoyl-D-glucose (VSUGL), 6-*O*-vinyladipoyl-D-glucose (VADGL), 6-*O*-vinylazelaoyl-D-glucose (VAZGL), 6-*O*-vinylsebacoyl-D-glucose (VSEGL), 6-*O*-vinyladipoyl-D-mannose (VADMA), 6-*O*-vinyladipoyl-D-galactose (VADGA), 6-*O*-vinyladipoyl-D-lactose (VADLA).

Synthesis of polymeric prodrugs

*Preparation of homopolymeric prodrug (poly-KVE) (**3a**)*

Ketoprofen vinyl ester was placed in a 10-mL sealed polymerization tube and the initiator 2% AIBN (w/w) was added. The polymerization occurred under of N_2 at



Scheme 1 Synthesis of KVE, saccharide derivatives, and polymeric ketoprofen–saccharide conjugates

70 °C (Scheme 1) and the reaction was stopped by adding acetone that precipitated the polymeric prodrug. ^{1}H NMR ($CDCl_3$): δ (ppm): 7.35–7.81 (m, 9H, Ar–H), 4.74 (s, 1H, (–CHCH$_2$–)$_n$), 3.60 (s, 1H, –C$_6$H$_4$CH), 1.66–1.26 (m, 5H, (–CHCH$_2$–)$_n$, –CH$_3$). ^{13}C NMR ($CDCl_3$): δ (ppm): 196.1, 173.1 (C=O), 137.8, 137.5, 132.5, 132.4, 131.6, 130.0, 129.3, 129.2, 129.1, 128.7, 128.5, 128.3 (Ar, ketoprofen), 68.8 (C-3, ketoprofen), 68.6 (C-2, ketoprofen), 45.2 (–CHCH$_3$, ketoprofen), 29.5 ((–CHCH$_2$–)$_n$), 18.4 (–CHCH$_3$, ketoprofen). IR (KBr): ν (cm^{-1}): 1733 (O–C=O), 1659, 1596, 1580, 821, 721, 643 (Ar).

Copolymerization of KVE and saccharide derivatives

A mixture containing KVE (1 mmol), saccharide derivatives (1 mmol), AIBN (2%, w/w), and *N*,*N*-dimethylformamide (DMF) (600 μL) was again placed in a 10-mL sealed polymerization tube, stirred at 70 °C under N_2. The resulting product was repeatedly precipitated in methanol and then dried under reduced pressure. The reaction conditions were altered to include varying ratios of co-monomers (KVE/SAD: 1:1, 1:2, 2:1, 4:1, mol/mol) and initiator (1, 2, 4, w/w). The products were poly (KVE-*co*-VSUGL) (**3b**), poly (KVE-*co*-VADGL) (**3c**), poly (KVE-*co*-VAZGL) (**3d**), poly (KVE-*co*-VSEGL) (**3e**), poly (KVE-*co*-VADMA) (**3f**), poly (KVE-*co*-VADGA) (**3g**), poly (KVE-*co*-VADLA) (**3h**).

*Synthesis of poly (KVE-co-VSUGL) (**3b**)*

IR (KBr): ν (cm^{-1}): 3415, 1167, 1058 (OH), 1733 (O–C=O), 1659, 1597, 1448, 821, 722, 643 (Ar). ^{1}H NMR (DMSO-d_6): δ (ppm): 7.48–7.62 (d, 9H, Ar–H), 6.7 (br s, 0.58 H, β 1-OH of D-glucose), 6.33 (br s, 0.42H, α 1-OH of D-glucose), 5.06–4.42 (br m, other OH of D-glucose), 4.31 (m, 1.5 H, H-6 (1H) and β H-1 (0.5 H) of D-glucose), 3.99 (m, 1 H, H-6′ of D-glucose), 3.80 (m, 0.5 H, α H-5 of D-glucose), 3.45–3.34, 3.15, 3.05 (br m, other α or β H of D-glucose), 2.93 (m, 0.5 H, β H-2 of D-glucose), 2.67–2.51 (m, 4 H, –CH$_2$–CH$_2$– of butanedioyl part), 1.64–1.35 (–CHCH$_3$, ketoprofen, –CH$_2$–). ^{13}C NMR (DMSO-d_6): δ (ppm): 198.2, 174.6 (C=O), 139.9, 139.7, 135.4, 132.3, 131.2 (Ar, ketoprofen), 99.7 (C1 of β-D-glucose), 95.1 (C1 of α-D-glucose), 79.2 (C3 of β-D-glucose), 77.4 (C2 of β-D-glucose), 76.2 (C5 of β-D-glucose), 75.7 (C3 of α-D-glucose), 74.9 (C2 of α-D-glucose), 73.4 (C4 of α-D-glucose), 72.9 (C4 of β-D-glucose), 71.8 (C5 of α-D-glucose), 67.2 (C6 α, β-D-glucose), 47.2 (–CHCH$_3$, ketoprofen), 42.9, 42.7, 42.5, 42.3, 41.9, 41.7 (–CH$_2$–), 31.2 ((–CHCH$_2$–)$_n$), 21.2 (–CHCH$_3$, ketoprofen).

*Synthesis of poly (KVE-co-VADGL) (**3c**)*

IR (KBr): ν (cm^{-1}): 3306, 1178, 1076 (OH), 1733 (O–C=O), 1658, 1597, 1580, 821, 722, 643 (Ar). ^{1}H NMR (DMSO-d_6): δ (ppm): 7.45–7.61 (d, 9H, Ar–H), 6.33 (1-OH of D-glucose), 5.03 (d, 1 H, H-1 of α-D-glucose), 4.89 (m, 1 H, 4-OH of α-D-glucose), 4.73 (d, 1 H, 3-OH of α-D-glucose), 4.51 (d, 1 H, 2-OH of α-D-glucose), 4.27 (d, 1 H, H -6 of α-D-glucose), 3.99 (dd, 1 H, H-6′ of α-D-glucose), 3.77 (m, 1 H, H-5 of α-D-glucose), 3.41 (m, 1 H, H-3 of α-D-glucose), 3.13 (m, 1 H,

H-2 of α-D-glucose), 3 (m, 1 H, H-4 of α-D-glucose), 1.56 (m, 4 H, other 2–CH_2– of hexanedioyl part), 1.61–1.35 (–$CHCH_3$, ketoprofen, –CH_2–). ^{13}C NMR (DMSO-d_6): δ (ppm): 197.9, 175.4 (C=O), 139.7, 135.4, 134.4, 132.3, 131.1, 128.4 (Ar, ketoprofen), 99.8 (C1 of β-D-glucose), 95.2 (C1 of α-D-glucose), 79.3 (C3 of β-D-glucose), 77.7 (C2 of β-D-glucose), 76.5 (C5 of β-D-glucose), 75.6 (C3 of α-D-glucose), 75.0 (C2 of α-D-glucose), 73.4 (C4 of α-D-glucose), 73.0 (C4 of β-D-glucose), 72.0 (C5 of α-D-glucose), 69.5, 66.7 (C6 α, β-D-glucose), 47.2 (–$\underline{C}HCH_3$, ketoprofen), 43.1, 42.6, 42.5, 42.4, 42.1, 41.9, 41.7 (–CH_2–), 35.8 ((–$\underline{C}HCH_2$–)$_n$), 19.9 (–$CH\underline{C}H_3$, ketoprofen).

*Synthesis of poly (KVE-co-VAZGL) (**3d**)*

IR (KBr): ν (cm^{-1}): 3322, 1177, 1076 (OH), 1734 (O–C=O), 1659, 1598, 1582, 823, 722, 642 (Ar). ^{1}H NMR (DMSO-d_6): δ (ppm): 7.76–7.26 (d, 9H, Ar–H), 6.33 (1-OH of D-glucose), 4.96–3.07 ((–$CHCH_2$–)$_n$, –C_6H_4CH, 1-H, 2-H, 3-H, 4-H, 5-H, 6-H, 2-OH, 3-OH, and 4-OH of D-glucose), 2.52–2.20 (–CH_2–), 1.63–1.13 (–$CHCH_3$, ketoprofen, –CH_2–). ^{13}C NMR (DMSO-d_6): δ (ppm): 195.2, 172.7 (C=O), 137.0, 136.8, 132.5, 131.5, 129.4, 128.3 (Ar, ketoprofen), 96.8 (C1 of β-D-glucose), 92.2 (C1 of α-d-glucose), 76.3 (C3 of β-D-glucose), 74.6 (C2 of β-D-glucose), 73.4 (C5 of β-D-glucose), 72.8 (C3 of α-D-glucose), 72.1 (C2 of α-D-glucose), 70.5 (C4 of α-D-glucose), 70.1 (C4 of β-D-glucose), 69.0 (C5 of α-D-glucose), 63.8 (C6 α, β-D-glucose), 44.4 (–$\underline{C}HCH_3$, ketoprofen), 40.0, 39.9, 39.7, 39.5, 39.3, 39.1, 38.9 (–CH_2–), 33.3 ((–$\underline{C}HCH_2$–)$_n$), 18.2 (–$CH\underline{C}H_3$, ketoprofen).

*Synthesis of poly (KVE-co-VSEGL) (**3e**)*

IR (KBr): ν (cm^{-1}): 3285, 1178, 1058 (OH), 1735 (O–C=O), 1659, 1598, 1582, 821, 721, 644 (Ar). ^{1}H NMR (DMSO-d_6): δ (ppm): 7.45-7.61 (d, 9H, Ar–H), 6.32 (d, 1-OH of α-D-glucose), 5.01 (d, H-1 of α-D-glucose), 4.88 (m, 4-OH of α-D-glucose), 4.72 (d, 3-OH of α-D-glucose), 4.5 (d, 2-OH of α-D-glucose), 4.26 (d, H-6 of α-D-glucose), 3.98 (dd, H-6′ of α-D-glucose), 3.75 (m, H-5 of α-D-glucose), 3.42 (m, H-3 of α-D-glucose), 3.12 (m, H-2 of α-D-glucose), 3 (m, H-4 of α-D-glucose), 1.52, 1.25 (m, –CH_2– of decanedioyl part), 1.61–1.35 (–$CHCH_3$, ketoprofen, –CH_2–). ^{13}C NMR (DMSO-d_6): δ (ppm): 198.2, 175.7 (C=O), 139.9, 135.3, 134.3, 132.3, 131.2, 128.2 (Ar, ketoprofen), 99.7 (C1 of β-D-glucose), 95.1 (C1 of α-D-glucose), 79.2 (C3 of β-D-glucose), 77.5 (C2 of β-D-glucose), 76.3 (C5 of β-D-glucose), 75.7 (C3 of α-D-glucose), 75.0 (C2 of α-D-glucose), 73.4 (C4 of α-D-glucose), 73.0 (C4 of β-D-glucose), 72.0 (C5 of α-D-glucose), 69.5, 66.7 (C6 α, β-D-glucose), 47.2 (–$\underline{C}HCH_3$, ketoprofen), 43.0, 42.7, 42.5, 42.3, 42.1, 41.9, 41.7 (–CH_2–), 36.2 ((–$\underline{C}HCH_2$–)$_n$), 20.7 (–$CH\underline{C}H_3$, ketoprofen).

*Synthesis of poly (KVE-co-VADMA) (**3f**)*

IR (KBr): ν (cm^{-1}): 3306, 1178, 1072 (OH), 1733 (O–C=O), 1658, 1597, 1580, 823, 722, 643 (Ar). ^{1}H NMR (DMSO-d_6): δ (ppm): 7.45-7.61 (d, 9H, Ar–H), 6.33 (1-OH of D-mannose), 4.86–3.29 ((–$CHCH_2$–)$_n$, –C_6H_4CH, 1-H, 2-H, 3-H, 4-H, 5-H, 6-H,

2-OH, 3-OH, and 4-OH of D-mannose), 2.33–2.20 (–CH_2–), 1.61–1.35 (–$CHCH_3$, ketoprofen, –CH_2–). ^{13}C NMR (DMSO-d_6): δ (ppm): 197.3, 174.6 (C=O), 140.0, 135.3, 134.3, 132.3, 131.2, 128.3 (Ar, ketoprofen), 93.9 (C1 of D-mannose), 73.9, 73.4, 71.2, 70.3, 67.1 (C-2, C-3, C-4, and C-5 of D-mannose), 64.2 (C6 of D-mannose), 44.4 (–$\underline{C}HCH_3$, ketoprofen), 34.5 ((–$\underline{C}HCH_2$–)$_n$), 34.2, 32.9, 32.8, 25.0, 23.6, 23.6 (–CH_2–), 18.5 (–$CH\underline{C}H_3$, ketoprofen).

*Synthesis of poly (KVE-co-VADGA) (**3g**)*

IR (KBr): ν (cm^{-1}): 3306, 1178, 1075 (OH), 1733 (O–C=O), 1658, 1596, 1582, 822, 722, 643 (Ar). ^{1}H NMR (DMSO-d_6): δ (ppm): 7.45–7.61 (d, 9H, Ar–H), 6.33 (1-OH of D-galactose), 4.86–3.29 ((–$CHCH_2$–)$_n$, –C_6H_4CH, 1-H, 2-H, 3-H, 4-H, 5-H, 6-H, 2-OH, 3-OH, and 4-OH of D-galactose), 2.33–2.20 (–CH_2–), 1.61–1.35 (–$CHCH_3$, ketoprofen, –CH_2–). ^{13}C NMR (DMSO-d_6): δ (ppm): 195.3, 172.6 (C=O), 139.6, 135.1, 134.1, 132.2, 131.0, 128.2 (Ar, ketoprofen), 60.7–60.9 (C-6α and C-6β of D-galactose), 70.2 (C-5α of D-galactose), 75.5 (C-5β of D-galactose), 68.1 (C-2α of D-galactose), 68.5 (C-4β of D-galactose), 68.9 (C-3α of D-galactose), 71.6 (C-2β of D-galactose), 73.5 (C-3β of D-galactose), 93.0 (C-1α of D-galactose), 97.2 (C-1β of D-galactose), 44.4 (–$\underline{C}HCH_3$, ketoprofen), 35.1 ((–$\underline{C}HCH_2$–)$_n$), 34.4, 32.6, 32.5, 25.0, 23.5, 23.5 (–CH_2–), 18.4 (–$CH\underline{C}H_3$, ketoprofen).

*Synthesis of poly (KVE-co-VADLA) (**3h**)*

IR (KBr): ν (cm^{-1}): 3450, 1176, 1076 (OH), 1734 (O–C=O), 1659, 1598, 1582, 823, 722, 644 (Ar). ^{1}H NMR (DMSO-d_6): δ (ppm): 7.45-7.61 (d, 9H, Ar–H), 6.33 (1-OH of D-lactose), 4.86–3.29 ((–$CHCH_2$–)$_n$, –C_6H_4CH, 1-H, 2-H, 3-H, 4-H, 5-H, 6-H, 2-OH, 3-OH, and 4-OH of D-lactose), 2.33–2.20 (–CH_2–), 1.61–1.35 (–$CHCH_3$, ketoprofen, –CH_2–). ^{13}C NMR (DMSO-d_6): δ (ppm): 198.1, 175.6 (C=O), 140.3, 139.7, 135.3, 134.3, 132.3, 131.2 (Ar, ketoprofen), 103.8 (C-1′ of D-lactose), 92.7-96.6 (C-1α and C-1β of D-lactose), 79.4 (C-4α and C-4β of D-lactose), 75.6 (C-5β-D-lactose), 75.2 (C-3β-D-lactose), 74.7 (C-2β-D-lactose), 73.4-76.2 (C-3′ and C-5′ of D-lactose), 72.2 (C-3α and C-5α of D-lactose), 70.8 (C-2′ of D-lactose), 69.9 (C-2α of D-lactose), 69.4 (C-4′ of D-lactose), 61.9 (C-6′ of D-lactose), 61.0 (C-6α and C-6β of D-lactose), 44.2 (–$\underline{C}HCH_3$, ketoprofen), 34.8 ((–$\underline{C}HCH_2$–)$_n$), 34.3, 32.9, 32.8, 25.0, 23.3, 23.3 (–CH_2–), 18.3 (–$CH\underline{C}H_3$, ketoprofen).

Particle diameter measurement

The particle size of polymeric prodrugs was analyzed by an LS230 laser particle-size analyzer (LPA) from Beckman Coulter, Inc., USA.

Degree of substitution

The degree of substitution was determined with proton nuclear magnetic resonance (^{1}H NMR). It was achieved through the spectra according to a slight modification of the methods described by Fitzpatrick et al. [25].

In vitro release of polymeric prodrugs

All polymeric prodrugs were dried under vacuum at room temperature and information about particle size was given in Tables 1 and 2. Then, drug release profiles from polymers were investigated in buffer solutions. Poly (KVE-*co*-VAZGL) (**3d**) was also studied at different pH values (1.2, 5.8, 7.4, and 8.0). The products were placed in a dialysis membrane (MWCO = 3500 Da) adding with 1 mL medium, then dialyzed in a 25 mL bottle with 10 mL corresponding solution for 10 days at 37 °C stirring throughout (100 rpm). The 10 mL medium was replaced with the same volume of fresh solution at predetermined times. The released drug was determined by HPLC (mobile phase: methanol/water, 80/20, v/v; wavelength: 254 nm; flow velocity: 1 mL/min), and drug concentration was monitored by UV–vis spectrophotometer at 254 nm.

Table 1 Copolymerization of KVE with saccharide derivatives

Entry	Substrate		Ratio in the feed A:B (mol/mol)	M_n[a] × 10^{-4} (Da)	M_w/M_n[a]	Particle diameter (μm)	Drug content[b] (w/w %)	Degree of substitution
	A	B						
1 (**3a**)	KVE		1:0	1.709	1.341	3.58	90.71	1.00
2 (**3b**)	KVE	VSUGL	1:1	2.964	1.724	3.29	41.07	0.47
3 (**3c**)	KVE	VADGL	1:1	3.742	1.986	3.26	42.61	0.51
4 (**3d**)	KVE	VAZGL	1:1	4.238	2.579	3.21	44.88	0.56
5 (**3e**)	KVE	VSEGL	1:1	4.762	2.135	3.17	46.39	0.59
6 (**3f**)	KVE	VADMA	1:1	4.579	2.083	3.20	44.42	0.53
7 (**3g**)	KVE	VADGA	1:1	4.656	1.894	3.18	43.52	0.52
8 (**3h**)	KVE	VADLA	1:1	7.527	2.395	2.98	66.42	0.83

The ratio of AIBN in the reaction was 2% (w/w)

A KVE, *B* saccharide vinyl ester derivatives

[a] Determined by GPC analysis

[b] Determined by the integration ratio of ^{1}H NMR spectra

Table 2 Copolymerization in different conditions

Entry	KVE/VSEGL in the feed (mol/mol)	Ratio of AIBN (w/w %)	M_n[b] × 10^{-4} (Da)	M_w/M_n[b]	Particle diameter (μm)	Degree of substitution	Drug content[a] (w/w %)
1 (**3a**)	1/2	2	5.653	1.932	3.09	0.43	32.13
2 (**3b**)	1/1	1	4.135	1.824	3.22	0.57	44.54
3 (**3c**)	1/1	2	4.762	2.135	3.17	0.59	46.39
4 (**3d**)	1/1	4	3.827	2.859	3.31	0.60	47.32
5 (**3e**)	2/1	2	4.453	2.074	3.19	0.72	59.21
6 (**3f**)	4/1	2	4.221	1.876	3.26	0.81	68.56

[a] Determined by the integration ratio of ^{1}H NMR spectra

[b] Determined by GPC analysis

Results and discussion

Synthesis and characterization

For preparation of macromolecule prodrugs, ketoprofen and saccharide were firstly converted into polymerizable monomers (Scheme 1). Thereafter, a series of polymeric prodrugs of ketoprofen were synthesized and characterized. The structures of the polymeric prodrugs were confirmed by FT-IR and NMR. Taking poly (KVE-*co*-VAZGL) (**3d**) as an example, the IR spectrum demonstrated that the vinyl group absorption present in the KVE monomer disappeared (1647 cm^{-1}) in the corresponding polymer (Fig. 1a) and the characteristic absorption assigned to D-glucose moieties (3322, 1177, 1076 cm^{-1}) and ketoprofen moieties (1659, 1598, 1582, 823, 722, 642 cm^{-1}, Ar) were now apparent. Also, ^{1}H NMR (Fig. 1b) and ^{13}C NMR (Fig. 1c) of polymeric prodrugs revealed the absence of the vinyl group and existence of ketoprofen (δ 7.76–7.26 ppm) and saccharide groups (δ 4.96–3.80 ppm). Thus, IR and NMR spectra clearly demonstrated that the conjugate products were as expected. As determined from ^{1}H NMR data, the ratio of KVE to saccharide vinyl esters in polymeric prodrugs (AIBN: 2%, w/w, molar ratio of comonomers: 1:1, mol/mol) was 0.81:1 to 0.89:1.

Optimization of reaction conditions of polymeric ketoprofen–saccharide conjugates

To optimize the drug loading, conditions and molecular weight of polymeric ketoprofen–saccharide conjugates, a number of experiments including saccharide variation, ratio of monomers, and initiator mass were undertaken.

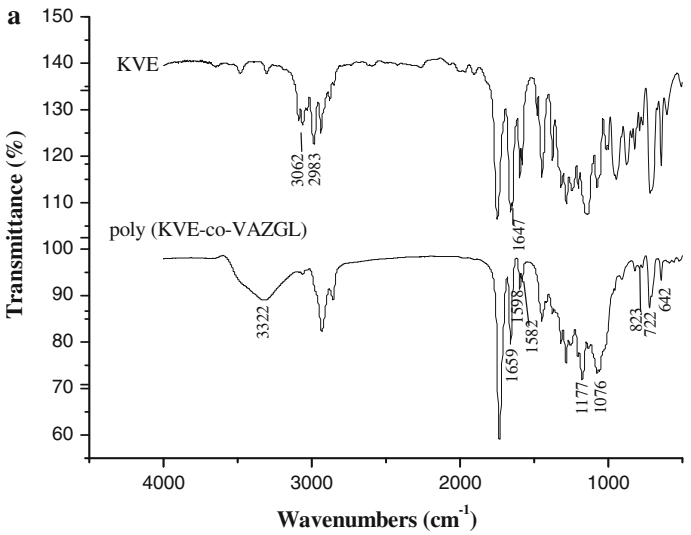


Fig. 1 IR and NMR spectra of KVE and poly (KVE-*co*-VAZGL) (**3d**). **a** IR spectra of **3d**, **b** ^{1}H NMR spectra of **3d**, **c** ^{13}C NMR spectra of **3d**

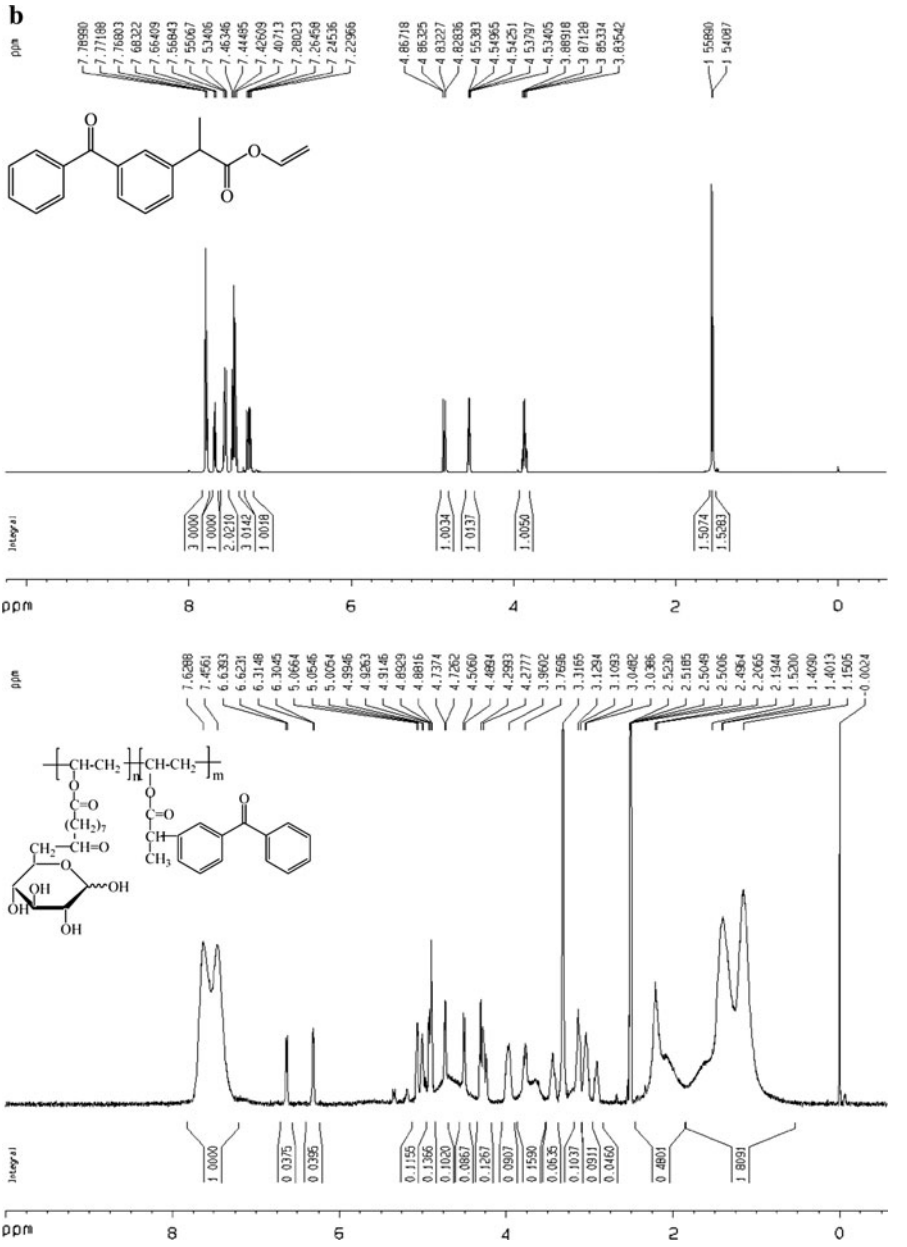


Fig. 1 continued

The molecular weights of ketoprofen containing varying saccharide branch chains (glucose, mannose, galactose, lactose) were determined with a BI-MwA GPC analysis instrument (Waters, LS: Brookhaven, USA) (mobile phase: DMF; run time:

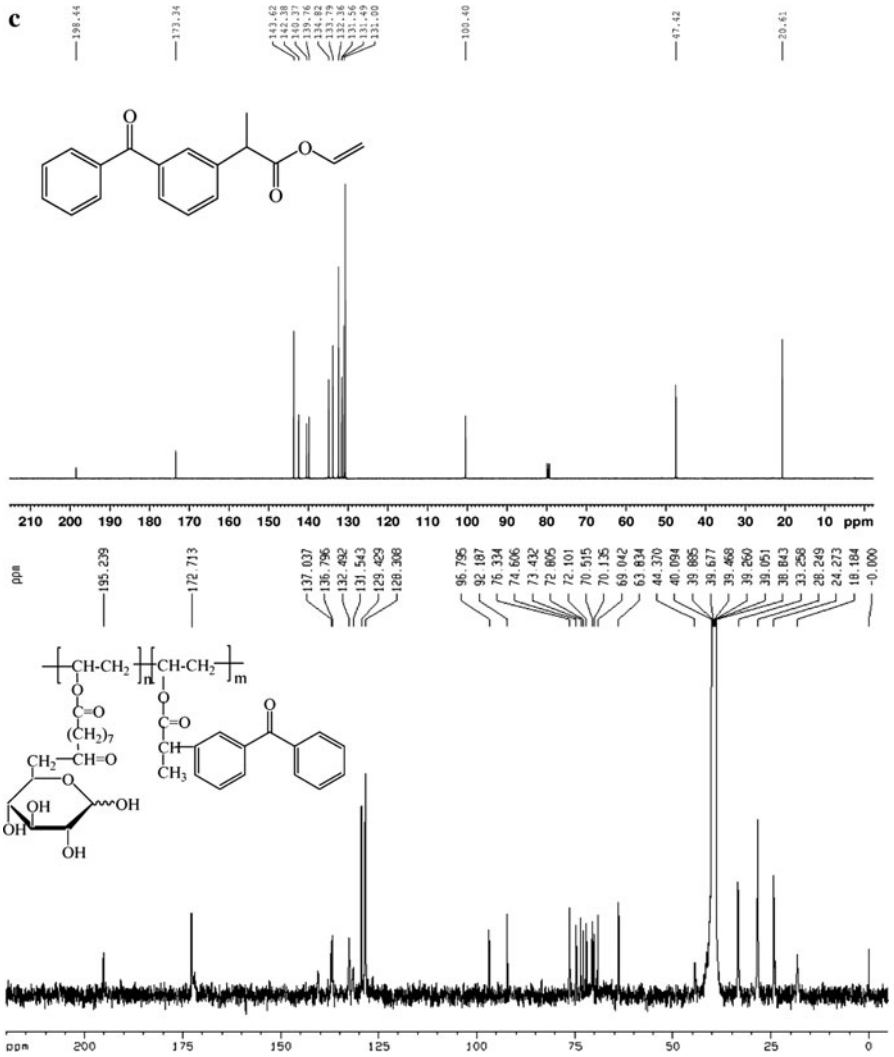


Fig. 1 continued

50 min; column temperature: 50 °C), and the data were given in Table 1. As demonstrated from the GPC profile, the polymeric prodrugs had moderately high molecular weight and good polydispersity, and the molecular weight was influenced by the saccharides variation. For example, KVE and lactose vinyl ester (1:1, mol/mol) were copolymerized using AIBN as an initiator (2%, w/w) to produce poly (KVE-*co*-VADLA) (**3h**), with M_n of 7.527×10^4 and M_w/M_n of 2.395. However, under similar conditions poly (KVE-*co*-VSUGL) (**3b**) had a M_n of 2.964×10^4 and M_w/M_n of 1.724, and poly-KVE (**3a**) M_n of 1.709×10^4 and M_w/M_n of 1.341.

As noted the radical copolymerization of KVE with saccharide derivatives provides considerable diversity especially in terms of composition. As a consequence,

the effect of molar ratio of KVE to saccharide derivatives was investigated, choosing poly (KVE-*co*-VSEGL) (**3e**) as a model system. Polymeric prodrugs with different compositions were generated with 80, 67, 50, and 33 mol% of KVE monomer (Table 2). By increasing saccharide derivatives in the feed, it caused a decrease in molecular weight of the polymeric prodrugs. For example, the loading capacity of ketoprofen in the polymeric prodrug was only 22.74% (w/w) when VSEGL was 67% (mol/mol) in the feed, while it reached 42.28% (w/w) when VSEGL was 20%. These results suggested that the loading capacity of ketoprofen in polymeric prodrugs could be controlled by altering the molar ratios of co-monomers.

The effects of monomer ratio and initiator mass were also studied. As noted in Table 2, the higher molecular weight poly (KVE-*co*-VSEGL) (**3e**) was obtained when the ratio of initiator was 2% (w/w) and M_n of 4.762×10^4 and M_w/M_n of 2.135 was determined. Interestingly, a reduction of molecular weight (M_n of 3.827×10^4 and M_w/M_n of 2.859) was noted by increasing the ratio of initiator (4%, w/w). These results suggested that the molecular weight and conversion of the polymeric prodrugs were greatly influenced by the concentration of initiator and the molar ratio of KVE to saccharide derivatives. Among the series of polymeric prodrugs, investigation of polymeric ketoprofen–disaccharide conjugates implied that it gave a high molecular weight.

In vitro release of polymeric prodrugs

The application and the synthesis of new drugs and their derivatives invariably influence drug behavior characteristics and their properties [26]. Here, the release of drugs from conjugates after incubating over various pH ranges was investigated. The hydrolysis of side chains depends on the strength and chemical nature of the chemical bonds and the surrounding conditions [27]. We used an in vitro hydrolysis system where polymeric prodrugs were subjected to different pH media (1.2, 5.8, 7.4, and 8.0) at 37 °C. HPLC was used to qualitatively analysis released hydrolyses products. The concentration of released ketoprofen was detected by UV spectrophotometer at 254 nm. We then compared the release curves of ketoprofen (raw drug) and poly (KVE-*co*-VADLA) (**3h**) at pH 7.4. As shown in Fig. 2, the raw drug was rapidly released and almost 100% total release was detected after 2.5 h, while over the same time interval only 5% of parent drug was released from poly (KVE-*co*-VADLA) (**3h**). This confirmed that polymeric ketoprofen–saccharide conjugates prolonged drug release effectively.

Influence of sugar variation

The cumulative in vitro release profile of ketoprofen from the polymeric prodrugs with different saccharide pendants was shown in Fig. 3. Compared with the parent drug, the polymeric prodrugs had prolonged the release effectively. Among the polymeric conjugates, ketoprofen from prodrug with disaccharide branch (poly (KVE-*co*-VADLA) (**3h**)) was released rapidly, reaching 60% after 10 days at pH 7.4, whereas drug released from poly-KVE at pH 7.4 was slower, 22% after

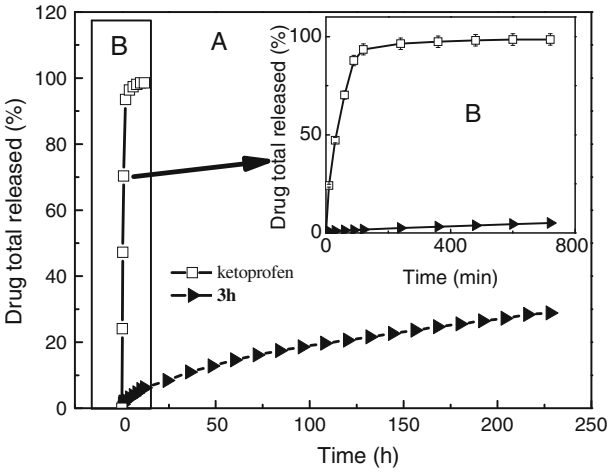


Fig. 2 The release curves of ketoprofen (parent drug) and poly (KVE-*co*-VADLA) (**3h**) in pH 7.4 buffer solution (37 °C)

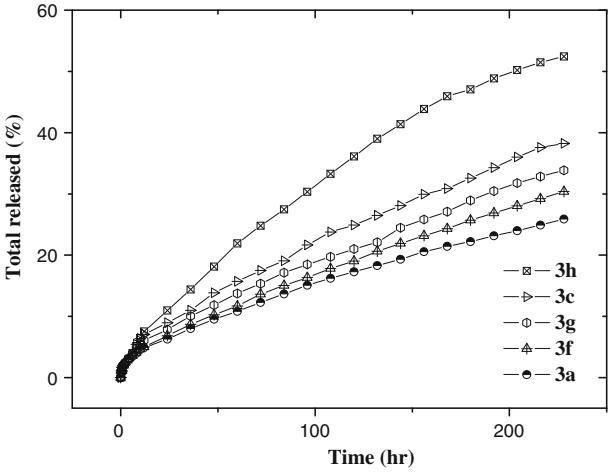


Fig. 3 In vitro release of polymeric prodrugs of ketoprofen with disaccharide and monosaccharide branches (pH 7.4, 37 °C)

10 days. This implies that the type of saccharide branch influences release capabilities of polymeric prodrugs.

Ketoprofen release behavior from polymeric prodrugs with monosaccharide attachments were also shown in Fig. 3. The curves implied that drug release from polymeric prodrugs with monosaccharide branches was higher than that of poly-KVE (**3a**). However, among the three types of polymeric prodrugs tested, release from polymeric prodrug with glucose branch (poly (KVE-*co*-VADGL) (**3c**)) was slightly higher than poly (KVE-*co*-VADMA) (**3f**) where optimal values was reached

39 and 31% after 10 days at pH 7.4, respectively. Thus, the structures of monosaccharides appeared having a little influence on ketoprofen release.

From the drug release profiles of **3a**, **3c**, and **3h**, it could be found that the particle diameter (Table 1) of polymeric prodrugs also affected the rate of hydrolysis. The rate of hydrolysis increased with a decrease of the particle diameter of polymeric prodrugs.

Influence of carbon chain length of saccharide derivatives

Drug liberation rate could be widely controlled by changing the relative length of linker between drug and polymer main chain. In this study, four types of polymeric prodrugs bearing glycolipids with different linkers were selected to investigate the influence of linker length on in vitro release rate of the drug (Fig. 4). In this experiment, the release of ketoprofen was seen to decrease along with increasing the length of carbon chains. In Fig. 4, poly (KVE-*co*-VSUGL) (**3b**), with the shortest carbon chain of saccharide vinyl ester was found to have the fastest release rate, reaching 41% after 10 days. This result suggested that the longer carbon chain associated with the vinyl esters restricted hydrolysis of the parent drug and this in turn affected drug release patterns. Furthermore, it was shown that drug liberation could be controlled by altering the relative length of the linker between drug and the main carbon chain.

Influence of pH

The release of the free parent drug from the carrier is an important factor when considering conjugate bearing drugs bound to the main chain through a labile bond. As in most cases, release of anti-inflammatory drugs from a polymer carrier is mediated by hydrolysis [27]. Similarly, we investigated drug release from the

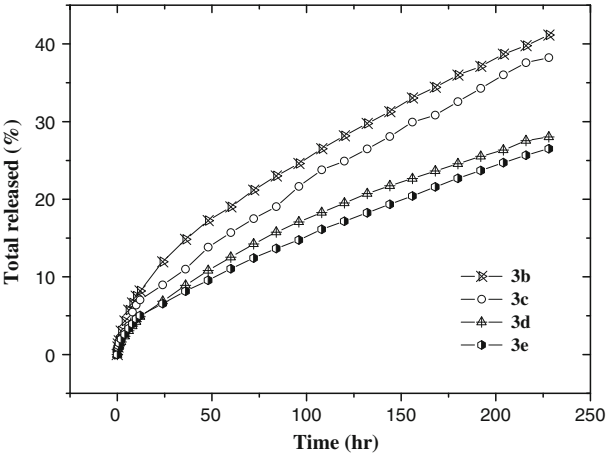


Fig. 4 In vitro release of polymeric prodrugs of ketoprofen with different carbon chain length of saccharides (pH 7.4, 37 °C)

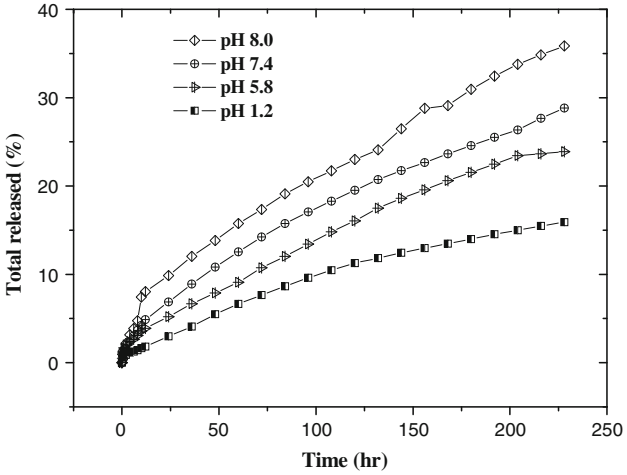


Fig. 5 In vitro release of poly (KVE-*co*-VAZGL) (**3d**) at different pH

polymeric prodrugs at different pH values and showed that poly (KVE-*co*-VAZGL) (**3d**) was useful to study this phenomenon. The profiles of the total amount of ketoprofen released when compared with pH are given in Fig. 5. Cumulative ketoprofen release reached 20% at pH 8.0, which was higher than that in pH 1.0. Thus, under alkali media was an influencing factor for ketoprofen release from conjugate structures. This might be explained on the basis of faster hydroxyl ions induced more rapidly hydrolysis of the ester bond between the drug and polymer chain. Another reason might be that carboxyl drugs tended to exist in a form of salt in alkaline solution which was beneficial to the drug release.

The investigation of other conjugates suggested that they had the same release behavior in different pH solution. Therefore, the release profiles indicated that the hydrolytic behavior of polymeric prodrugs is strongly depended on the pH.

Conclusions

A facile and efficient combined enzymatic synthesis and chemical polymerization strategy to prepare polymeric prodrugs of ketoprofen with saccharide branches was developed. The effects of different parameters to synthesis and drug release were systematically investigated and concluded.

In this study, 13 kinds of polymeric prodrugs of ketoprofen with saccharide branches were synthesized. The structures of products were confirmed by FT-IR, NMR and GPC, and degree of substitution and viscosities of the polymers were obtained. The loading capacity of ketoprofen in polymeric prodrugs could be easily controlled by changing the molar ratio of comonomers. In vitro release behaviors of polymeric prodrugs showed that the products could prolong drug release effectively. And sustained release rate of ketoprofen could be determined by composition and

variation of monosaccharide and pH. We believe that these results add further knowledge to the field of polymeric prodrugs and their use as drug delivery systems.

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