Drug Development and Industrial Pharmacy, 34:877–884, 2008

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040801929075

informa
healthcare

Synthesis and Evaluation of Novel Polyester-Ibuprofen Conjugates for Modified Drug Release

C. J. Thompson, D. Hansford, and D. L. Munday
School of Pharmacy, The Robert Gordon University, Aberdeen, Scotland

S. Higgins, C. Rostron, and G. A. Hutcheon
School of Pharmacy and Chemistry, Liverpool John Moores University, Liverpool, England

Ibuprofen was conjugated at different levels to a novel polyester, poly(glycerol-adipate-co-ω-pentadecalactone) (PGA-co-PL), via an ester linkage to form a prodrug. The conjugates were characterized by differential scanning calorimetry (DSC), nuclear magnetic resonance (NMR), infrared (IR), gel permeation chromatography (GPC), ultraviolet (UV), and high-performance liquid chromatography (HPLC). The conjugates had a molecular weight between 18 and 24 kDa, and there was a suppression of the free hydroxyl groups within the conjugated polymer. DSC scans showed a lowering of the melting point (T_m) when compared with the polyester alone and a difference in the number and area of T_m peaks. Drug release studies showed an initial burst release (13–18%) followed thereafter by very slow release (maximum 35% after 18 days). Continuous work may produce ester-linked conjugates that are sufficiently labile to allow for complete release of ibuprofen over the time period studied.

Keywords polyesters; polymer degradation; modified release; conjugation; ibuprofen

INTRODUCTION

Over recent decades, polymeric microspheres have routinely been produced by the co-dissolution of the polymer and the drug followed by solvent removal (Edlund & Albertsson, 2002). However, these systems have tended to produce initial burst release of drug, and control of the level of drug loading can be difficult (Bodmeier & Chen, 1989; Flandroy, Grandfils, & Jerome, 1993; Jain, 2000; Oh, Nam, Lee, & Park, 1999; Zhu et al., 2005). In these delivery systems, the rate of release tends to be controlled by the level of drug loading, as well as a combination of the aqueous solubility and molecular weight of the drug, system morphology, and, to a lesser extent, the rate of polymer degradation (Fu, Shyu, Su, & Yu, 2002; Nam & Park, 1999; Perumal, Dangor, Alcock, Hurbans, & Moopanar, 1999; Tice & Cowsar, 1984). Therefore, conjugated systems have been developed in which the level of drug loading is built in during conjugation and the rate of drug release is governed by the rate of cleavage of the drug–polymer linker (Duncan, 2003; Khandare & Minko, 2006; Rimoli et al., 1999; Ustariz-Peyret, Coudane, Vert, Kaltsatos, & Boisrame, 2000; Yoo, Lee, Oh, & Park, 2000). Conjugation involves the chemical linking of the active compound to the backbone of a suitable polymer to control the rate and the site of drug release (Duncan, 2003; Khandare & Minko, 2006; Yoo et al., 2000).

Works involving the conjugation of drugs to hydrophobic biodegradable polymers have been limited. Different stereoisomers of poly(lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA) are among the polymers most commonly used in biodegradable delivery systems (Edlund & Albertsson, 2002). However, forms of these polymers having only two functional groups per polymer chain (uncapped carboxylic acid [–COOH] and hydroxyl [–OH] end groups) capable of forming covalent bonds with drugs have only been developed recently (Nam & Park, 1999; Oh et al., 1999; Rimoli et al., 1999; Ustariz-Peyret et al., 2000; Yoo et al., 2000). These uncapped functional groups allow for the formation of amide or ester linkages with drugs that have suitable functional groups. However, non-zero-order release due to lag times and incomplete release can occur with these systems. Zero-order release may not be achieved without enzymatic breakdown of the polymer–drug linker (Rimoli et al., 1999; Ustariz-Peyret et al., 2000). The lack of complete drug release could be due to unintended interactions between the polymer and the drug and/or the low lability of the type of bond used to link the polymer to the drug (Nam & Park, 1999; Rimoli et al., 1999).

A novel polyester, poly(glycerol-adipate-co-ω-pentadecalactone) (PGA-co-PL), was previously developed using a combination of enzyme-catalyzed polycondensation and ring-opening polymerization of equimolar quantities of the three monomers: divinyl adipate, glycerol, and ω-pentadecalactone. The regiospecificity of the chosen enzyme primarily yielded a

Address correspondence to D. L. Munday, Robert Gordon University, School of Pharmacy and Life Sciences, Schoolhill, Aberdeen, AB10 1FR. E-mail: d.munday@ rgu.ac.uk

linear polyester that was composed of a random mixture of the three monomers (Thompson et al., 2006). This aliphatic polyester contained a free –OH group in each repeat unit. In this work, attachment of this free –OH group to a model drug possessing a free –COOH group (ibuprofen) was performed. This conjugated form of the aliphatic polyester with an acidic drug may reduce the burst drug release as well as control the release more effectively by cleavage of the polymer–drug linker. To investigate this possibility, the polyester/drug conjugates were produced, evaluated, and the in vitro drug-release properties investigated.

MATERIALS AND METHODS

Materials

PGA-co-PL was prepared as outlined in previous work (Thompson et al., 2006). Novozyme 435, (±) ibuprofen, sodium phosphate, sodium acid phosphate, sodium chloride, anhydrous benzene, thionyl chloride, pyridine, ortho-phosphoric acid, and magnesium sulfate were purchased from Sigma-Aldrich Co. Ltd. (Gillingham, UK). Chloroform (CHL), dimethylformamide, acetonitrile, conc. HCl, tetrahydrofuran (THF), methanol (MeOH), and dichloromethane (DCM) (analytical grade) were all purchased from Fisher Chemicals (Fisher Scientific UK Ltd., Loughborough, UK).

Conjugate Synthesis

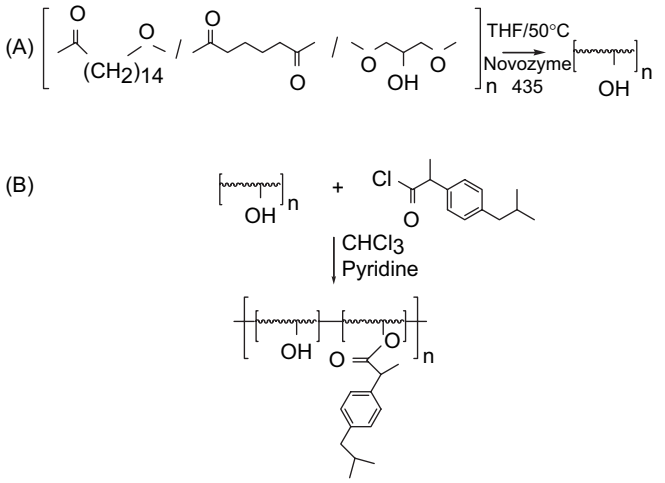
Conversion of Ibuprofen to the Acid Chloride Form

Scheme 1 details the synthesis of the reactive acid chloride of ibuprofen, which was prepared by refluxing, for 2 h, a benzene solution (75 mL) of ibuprofen (41.25 g and 200 mmol), excess thionyl chloride (50 mL), and a catalytic amount of dimethylformamide (0.5 mL). The unreacted thionyl chloride and benzene were removed by distillation at 80°C, and the acid chloride of ibuprofen was collected by vacuum distillation as a colorless liquid (yield = 39.39 g [87.9%], and boiling point of 66–68°C at 2.5×10^{-3} mbar).

Conjugation of Ibuprofen Acid Chloride to PGA-co-PL

Based on previous work (Thompson et al., 2006), it was assumed that the polymer was linear and composed of one pendant –OH group per repeat unit. Similarly, assuming that all three monomers were equally incorporated into the polymer structure, it is possible to predict the number of –OH groups per unit mass (Scheme 2). Thus, to a refluxing CHL solution

SCHEME 1. Conversion of ibuprofen to its acid chloride form.



SCHEME 2. Polymer synthesis (A) and covalent attachment of ibuprofen acid chloride to polymer (PGA-co-PL) (B).

(100 mL) of the copolymer, PGA-co-PA (10.00 g, 22.59 mmol), various amounts of the freshly distilled ibuprofen acid chloride were added (for 100% conjugation, 5.06 g [22.59 mmol]; for 50% conjugation, 2.53 g [11.29 mmol]; and for 25% conjugation, 1.27 g [5.67 mmol]). To each separate reaction mixture, 1 mL of pyridine was added to catalyze the conjugation reaction. The solutions were then refluxed further for 2 h before pouring into 200 mL dilute acid (2 mL of conc. HCl added to 198 mL water). The resultant aqueous layer was extracted twice with 25 mL CHL, the combined CHL extractions were washed twice with 200 mL water, dried over magnesium sulfate, and concentrated under vacuum at 60°C. The resultant white waxy solid was redissolved in 50 mL of warm DCM and poured onto 200 mL of MeOH. The mixture was stirred and heated to boiling point to dissolve the polymer and evaporate off most of the DCM. The resulting colorless solution was left to cool by stirring overnight at room temperature. The precipitated conjugate was then collected by filtration and washed further with 20 mL of ice-cold MeOH. The resultant solid was gently ground and stored in vacuum for 24 h at 40°C to remove any residual solvents.

Conjugate Characterization

Gel Permeation Chromatography

The polymer and conjugates were characterized by gel permeation chromatography (GPC) using a Viscotek system employing OmniSEC 3 software, TDA Model 300. The right-angle light scattering, refractive index, and viscometer detectors were all in a temperature-controlled oven set at 40°C coupled to a gpcMAX integrated solvent and sample delivery module (degasser, pump and autosampler). The system was fitted with two ViscoGEL GMHHR-N columns, also stored

in the detector oven at 40°C, at a flow rate of 1 mL/min, and using THF as the eluent. The detector alignment and instrument sensitivity parameters were previously calibrated using a low molecular weight narrow polystyrene standard (117 kDa).

Nuclear Magnetic Resonance Spectroscopy

The nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 300 MHz spectrometer operating via XWIN-NMR v3.5 and are expressed in parts per million (ppm) (δ) from internal tetramethylsilane. NMR samples were prepared using a saturated D-chloroform solution of the polymer and conjugates.

Infrared Spectroscopy

Polymer and conjugate samples were separately placed in a Nicolet Avatar 370DTGS spectroscope with a diamond (30,000–200 cm^{-1}) smart orbit attachment (Thermo Electron Corporation, Runcorn, England). Data were collected over 32 cycles. Wave numbers for any peak found were then determined using the attached software.

Differential Scanning Calorimetry

Conjugates and PGA-co-PL (5–7 mg) were placed in hermetically sealed aluminium pans. Thermal analysis was carried out in a Q100 differential scanning calorimeter (TA Instruments, New Castle, Delaware, USA). Samples were heated from 20to 90°C (at 20°C/min), cooled to –90°C (at 5°C/min), and then heated to 90°C (at 20°C/min). Thermal data were determined using the supplied software.

Drug Loading

Conjugate samples (10 mg) were dissolved in 10 mL of CHL. The ultraviolet (UV) absorbance of the solution was measured using a Biomate 5 UV spectrophotometer (Thermo Spectronic, Runcorn, England) at 273 nm. Drug loading was determined in duplicate for all batches, and values were expressed as a percentage (wt/wt).

In Vitro Ibuprofen Release

Conjugate samples (100 mg) were suspended in 20 mL of pH 7.4 phosphate buffer (37 ± 0.5°C) and agitated in a Grant OLS 200 shaking water bath (Grant Instruments, Cambridge, England) at 15.5 *g* in sealed glass jars. Samples were withdrawn at regular time intervals, filtered using a syringe and Millex GP 0.22-μm filter (Millipore, Billerica, Massachusetts, USA), and UV analyzed at 273 nm. The withdrawn volume was replaced to maintain constant volume.

High-Performance Liquid Chromatography

The high-performance liquid chromatography (HPLC) system was equipped with a Jasco PU-980 intelligent pump, a Jasco UV-975 UV-detector, and a Jasco AS-950 intelligent sampler (Jasco Corporation, Tokyo, Japan). Separations were achieved with a C_{18} column (Thermo Electron Corporation) using a mobile phase of 65% acetonitrile/35% water in 20 mM di-sodium hydrogen orthophosphate, adjusted to pH 2.5 with ortho-phosphoric acid for ibuprofen. Runs were carried out at 1 mL/min over 10 min at 273 nm. Samples from in vitro release studies were analyzed at 30 min and at 432 h. A 0.1% (wt/vol) solution of ibuprofen in pH 7.4 phosphate buffer and a 0.1% (wt/vol) solution of PGA-co-PL suspended in the same medium for 30 days were used as controls.

RESULTS AND DISCUSSION

Gel Permeation Chromatography

Contrary to expectations, GPC data suggest that the molecular weights of the conjugates decreased with ibuprofen substitution from 25 to 100%. PGA-co-PL had an average molecular weight of 24.9 kDa (± 75 Da), whereas the substituted polymers had average molecular weights of 21.8 kDa (± 532 Da), 18.1 kDa (± 274 Da), and 24.2 kDa (± 206 Da) for the 100, 50, and 25% conjugates, respectively (n = 2). The decrease in molecular weight and the increase in standard deviation with substitution indicate that the conjugation process may have caused some degradation of the polymer backbone resulting in the production of oligomers/monomers. The mean M_w/M_n values for PGA-co-PL and the conjugates were relatively constant at 2.98 (± 0.03), 3.44 (± 0.10), 3.00 (± 0.44), and 2.96 (± 0.43) for PGA-co-PL and the 100, 50, and 25% conjugates, respectively. The random nature of the polymer means that the molecular weight was expected to be highly variable. GPC therefore may not be the most appropriate method of confirming the degree of ibuprofen substitution.

Nuclear Magnetic Resonance

A comparison of NMR spectra (Figures 1 and 2) shows that new peaks appeared at approximately 0.80 and 7.10 ppm in the sample containing the 100% conjugate, which were not present in the sample containing PGA-co-PL, suggesting that conjugation was successful as these new peaks are associated with the conjugation of ibuprofen to the –OH group.

However, when the integration numbers of these spectra were used to calculate the percentage conjugation of –OH groups, it appears that conjugation was incomplete. The percentage of –OH groups conjugated was calculated by dividing the integration number of the peak at 0.80 ppm, corresponding to six protons in the conjugates, by the integration number of the peak at 2.25 ppm, corresponding to six protons in the polymer and conjugates. The percentage of –OH groups taken up were 28.5, 13.8, and 5.8% for the 100, 50, and 25% conjugates, respectively. These conjugation efficiencies are lower than those expected from theoretical considerations and may be due to degradation of the conjugates during synthesis. Alternatively, the acid chloride of the ibuprofen may have been impure, and therefore the expected number of acid groups were not present at the molarity used. Additionally, the concentration of

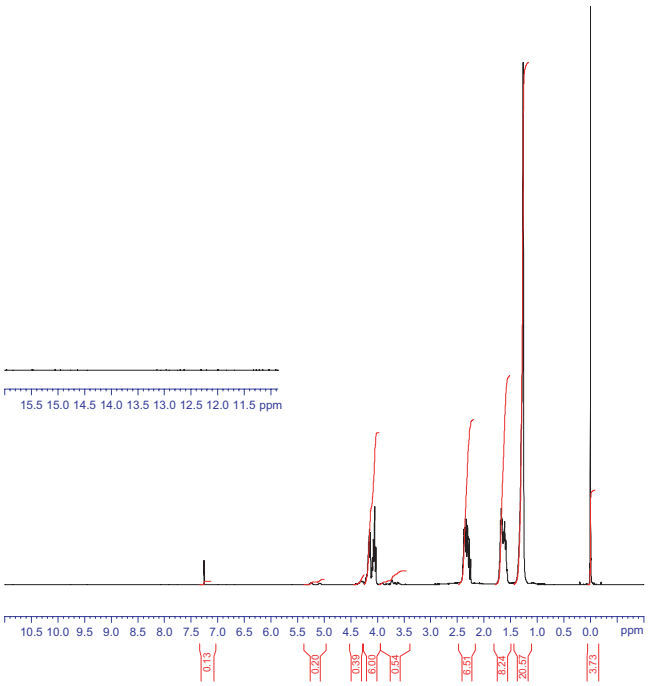


FIGURE 1. ^{1}H NMR spectra of PGA-co-PL.

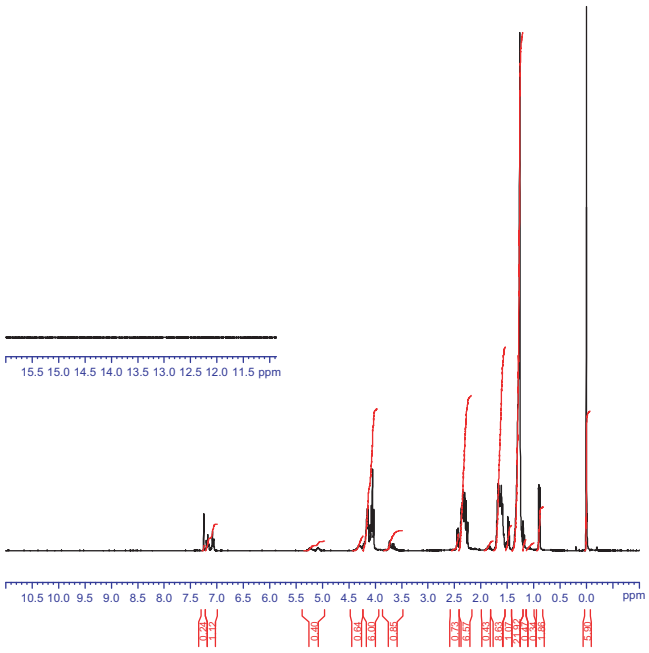


FIGURE 2. ^{1}H NMR spectra of 100% ibuprofen conjugate.

free –OH groups in the polymer may have been overestimated because of an incomplete enzyme polymerization process. Furthermore, the expected downfield shift for the resonances of the methine and methylene protons of the glycerol monomer of PGA-co-PL, because of the addition of the ester group from the ibuprofen conjugation, was not apparent from the NMR spectra, suggesting that conjugation may be at a level too low for detection.

Infrared Spectroscopy

Figure 3 provides a comparison of the infrared (IR) spectra of the backbone polymer with the 100% conjugate. All the major peaks of the polymer have been retained after conjugation to ibuprofen, indicating that the polymer retained its chemical identity after conjugation. However, there was a depression of the peak found between 3,400 and 3,500 cm^{-1}, suggesting that the free –OH group found in the polymer has been partially lost during conjugation. There were no apparent new peaks corresponding to ibuprofen within the spectra for the 100% conjugate. No unconjugated ibuprofen should be present in the sample and the low level of ibuprofen in the conjugate samples may preclude its detection (Rimoli et al., 1999). Using IR alone, it is not possible to confirm ester linkage between the ibuprofen and the polymer. The presence of the two peaks between 1,100 cm^{-1} and 1,300 cm^{-1} indicates ester bonds already present in PGA-co-PL and is not necessarily an indication of the formation of a PGA-co-PL-ibuprofen bond.

Differential Scanning Calorimetry

The T_m values of the conjugates were lower than the corresponding T_m values observed in previous work (Thompson et al., 2007) using solid dispersions of the drug in the polymer (e.g., microspheres) that contained unconjugated ibuprofen dispersed in the same polymer (Figure 4 and Table 1). The solid

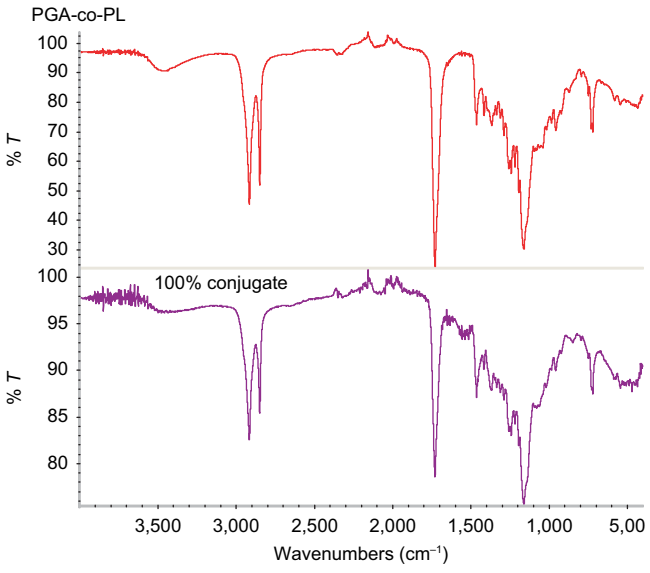


FIGURE 3. Infrared spectra of PGA-co-PL and 100% conjugate.

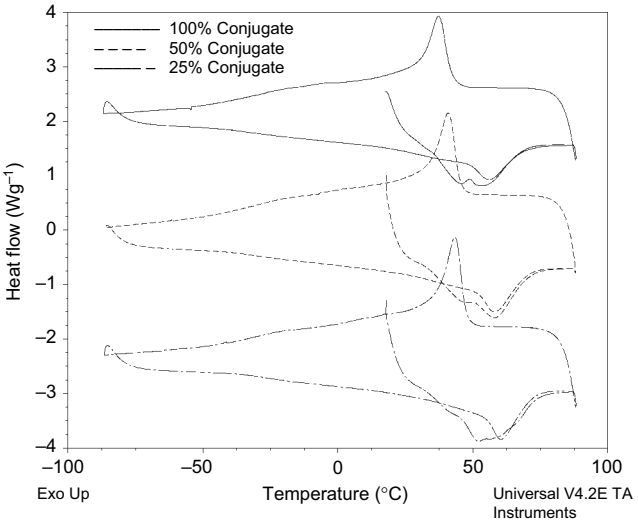


FIGURE 4. DSC scans of PGA-co-PL-ibuprofen conjugates.

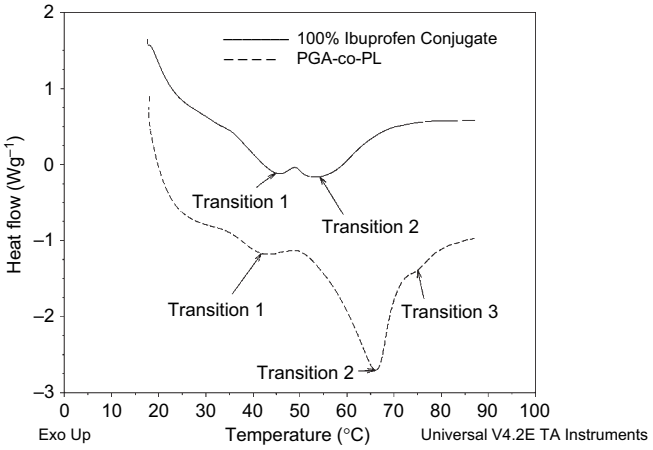


FIGURE 5. Comparison of thermal events of 100% conjugate and PGA-co-PL (first heating cycle).

TABLE 1
Thermal Data from the PGA-co-PL-ibuprofen Conjugate Particles

	Conjugates		
	100%	50%	25%
T_m (onset)(ºC)	31.5	32.8	38.9
T_m (peak) (ºC)	52.8	58.0	52.0
T_g (ºC)	–27.9	–28.8	–26.6
Peak area (J/g)	62.4	67.3	77.2

PGA-co-PL, poly(glycerol-adipate-co-ω-pentadecalactone).

dispersions had melting onsets from 37to 52°C and peaks at 62–68°C (Thompson et al., 2007). The lowering of the T_m could have been due to the presence of conjugated ibuprofen weakening the interchain forces (e.g., hydrogen bonding) of the polymer backbone, thus less heat energy was required to break these bonds. Moreover, a double peak was found in the 100% conjugate that became progressively less pronounced in the 50 and 25% conjugates. Changes in the percentage of –OH groups occupied by ibuprofen may have altered the interchain bonding in each conjugate. The presence of the double peak suggests two distinct regions of crystallinity within the 100% conjugate. As more –OH groups were free in the 50 and 25% conjugates, the bonding between chains could have been different from that found within the 100% conjugate. Consequently, the composition and number of crystalline domains may have been different from that found in the 100% conjugate.

Figure 5 shows that there is a definite change in the thermal profile of the polymer after conjugation with ibuprofen. Although there are three separate peaks produced during the melting of PGA-co-PL, there are only two for the 100% conjugate. This indicates a change in the nature and degree of crystallinity after conjugation.

The T_g values of the conjugates were similar to those of the polymer alone (–25°C) and the microspheres prepared from the polymer and unconjugated ibuprofen (–24 to –26°C). It seems that the conjugation of ibuprofen to the polymer does not have a plasticizing effect on the polymer, with no apparent effect on chain mobility and relaxation. It was also shown by Rimoli et al. (1999) that the addition of 5-iodo-2′-deoxyuridine, a much bulkier compound than ibuprofen, to poly(D,L-lactic acid) (molecular weight = 16,000 Da) also did not have an affect on the T_g.

Drug Loading

The levels of drug loading of the conjugated materials are shown in Table 2. The 25 and 50% conjugates had slightly higher levels of ibuprofen than expected compared with the 100% conjugate. A possible reason for this is that not all the –OH groups may be occupied by the drug in the 100% conjugate. The stereochemistry and/or conformation of the polymer could have prevented access to some of the –OH groups. However, NMR data shown previously suggested that instability of the conjugate or impurity of the ibuprofen acid chloride could be more plausible

TABLE 2
Drug Loading (%) of Conjugates (± *SD*; *n* = 3)

Conjugate	Drug Loading (%)
100%	12.6 (±0.05)
50%	6.8 (±0.05)
25%	3.4 (±0.01)

explanations for this phenomenon. Removal of unconjugated ibuprofen during purification may also have been more efficient in the 100% conjugate than in the 25 and 50% conjugates.

In Vitro Ibuprofen Release

Despite the covalent bonding between ibuprofen and the polymer, there was an initial burst of drug release during the first 30 min amounting to about 13% for the 100% conjugate and about 18% for the 25% conjugate. This was followed by very slow drug release over the remainder of the time period (Figure 6). The burst release may be due to the presence of unconjugated ibuprofen or to low molecular weight oligomers/monomers that were solubilized on contact with the buffer (Oh et al., 1999). However, the differential scanning calorimetry (DSC) scans showed no evidence of unconjugated drug (Figure 4). Perhaps any unconjugated ibuprofen was present in too small a quantity to be detected in the DSC sample. Conversely, the lack of proton shift shown in the NMR data suggested that a proportion of the ibuprofen may have been unconjugated. Incomplete purification of the conjugates using MeOH, leaving some residual unconjugated ibuprofen, could also have contributed to the burst release.

The 100% conjugate had the slowest rate and the least amount of ibuprofen release compared with the 25 and 50% conjugates. It may be that the 100% conjugate was the most hydrophobic and so the least wettable by occupation of all of the available –OH groups by ibuprofen. This may explain why the 25% conjugate had the greatest burst and the highest overall percentage release.

The slow and incomplete release following the initial burst maybe due to the chemical cleavage of the polymer backbone. Ester linkages in the polymer backbone may have been slowly hydrolyzed with possibly more oligomers/monomers being formed, which may then be solubilized when reaching a low enough molecular weight (Oh et al., 1999). Maximum drug release was only about 33% after 18 days (from the 25% conjugate) suggesting that the ester linkages between the polymer monomers and the polymer to ibuprofen linkages were very stable.

Other biodegradable polymers conjugated to drugs have also been shown to be very stable. The conjugation of cephradin to poly(D,L-lactic acid) oligomers (molecular weight = 900 Da) only allowed a very small amount of free cephradin release over 40 days (Ustariz-Peyret et al., 2000). However, the linkage between these oligomers and cephradin was an amide rather than an ester bond. Amide bonds are known to be more stable than ester linkages as they have considerably longer $t_{0.5}$ (83,000 years compared with 3.3 years) (Gopferich, 1996).

In other cases, the use of ester linkages produced near zero-order release (Oh et al., 1999; Yoo et al., 2000). Poly(D,L-lactic-co-glycolic acid) conjugates have been used to produce microparticles and nanoparticles with controlled release of *N*-(9-fluorenylmethoxycarbonyl-*N*-*tert*-butoxycarbonyl-L-tryptophan) and doxorubicin, respectively. The difference between these conjugates was the use of the less stable, amorphous, poly(D,L-lactic-co-glycolic acid) that contained lactide–glycolide and glycolide–glycolide linkages, which are more susceptible to cleavage than lactide–lactide linkages (Engelberg & Kohn, 1991; Jain, 2000; Park, 1995).

The ibuprofen/polymer conjugates produced in this work provided a much longer period of sustained release of ibuprofen than solid dispersions of the unconjugated drug in the polymers. Burst release was also less and occurred over a shorter time period. Solid dispersions produced burst release over about 4 h and released at least 60% of ibuprofen over that time period (Thompson et al., 2007). This was true even with batches that had no ibuprofen particles embedded on the surface of the dispersions and also those having a lower level of drug loading than the 100% conjugate. It appears therefore that conjugation of ibuprofen to this polymer reduced burst release and slowed the overall rate of release.

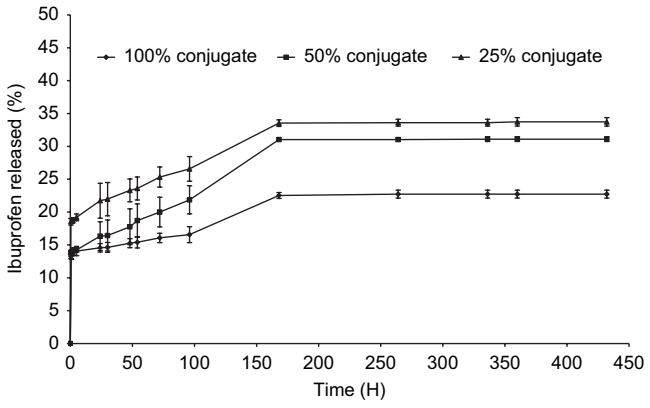


FIGURE 6. Release of ibuprofen from the PGA-co-PL-ibuprofen conjugates into pH 7.4 phosphate buffer at 37°C (±*SD*, *n* = 3).

High-Performance Liquid Chromatography

The chromatogram of the 100% conjugate showed multiple peaks (Figure 7). Ibuprofen dissolved in pH 7.4 buffer displayed only a single peak, whereas the polymer alone did not produce any peak (data not shown). The polymer had no UV absorbance at 273 nm and so any oligomers/monomers that were solubilized in the buffer would not appear on the chromatograph.

The largest peak in Figure 7 (3–4 min) was attributed to unconjugated ibuprofen. The smaller peaks may be attributed to ibuprofen bound to oligomers or monomers (Oh et al., 1999; Ustariz-Peyret et al., 2000; Yoo et al., 2000). These relatively small ibuprofen–oligomer/monomer molecules may also have been released on contact with the buffer and absorbed UV light. Therefore, they behave as unconjugated ibuprofen thereby contributing to the total absorbance measured. Therefore, burst

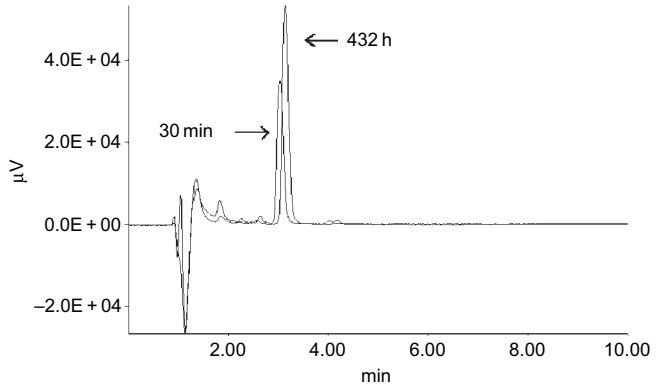


FIGURE 7. HPLC analysis on a sample of medium containing the 100% conjugate during *in vitro* release studies after 30 min and 432 h into pH 7.4 phosphate buffer.

release may have been mainly due to unconjugated ibuprofen and, to a lesser extent, short-chain ibuprofen conjugates.

The presence of multiple small peaks may suggest that more than one form of conjugate is present (Oh et al., 1999; Ustariz-Peyret et al., 2000). The polymer was randomly formed from three monomers (ω-pentadecalactone, divinyl adipate, and glycerol) which can join together in six different configurations (Thompson et al., 2006). Therefore, it is possible that the ibuprofen–oligomer/monomer conjugates present are constituted from a number of different combinations of these three monomers, for example, pentadecalactone–glycerol–ibuprofen, glycerol–ibuprofen, and so on. The shorter chain ibuprofen–oligomer/monomer conjugates would be eluted first, with higher molecular weight oligomers eluting later (Ustariz-Peyret et al., 2000). The low amount of drug release (Figure 6) could be due to certain bonds within the conjugate being more labile than others. As glycolide–lactide bonds are less stable than lactide–lactide bonds, it is possible, for example, that pentadecalactone–glycerol–ibuprofen conjugates are more stable than glycerol–ibuprofen conjugates. Further work involving the production of conjugates with specific, rather than random, arrangement of monomers would be required to test this hypothesis.

The very slow release rate after the first 30 min may have been due to the slow rate of polymer degradation. As stated above, ester linkages have a $t_{0.5}$ of 3.3 years (Gopferich, 1996). The slow rate of release and the little change in the chromatographic profile of these conjugates over time would seem to indicate that little or no degradation occurred. The small increase in elution time after 432 h could be attributed to a change in mobile phase polarity because of acetonitrile evaporation. If the small peaks found were due to ibuprofen–oligomer/monomer conjugates, then it appears that they did not degrade even after solubilization. Previously published work also indicates that solubilized conjugates do not necessarily degrade due to the stability of ester bonds (Yoo et al., 2000). The use of an alkaline pH was necessary to cleave the ester linkage between poly(D,L-lactic-co-glycolic acid) and *N*-(9-fluorenylmethoxycarbonyl-*N*-*tert*-butoxycarbonyl-L-tryptophan) (Oh et al., 1999).

There are several options available to increase the rate of breakdown of these conjugates. The use of acidic excipients, co-mixed or attached to the conjugates, could increase the rate of degradation by catalyzing the cleavage of the ester linkages (Gopferich, 1996). The presence of an esterase could also increase the rate of release by increasing the rate of ester cleavage (Rimoli et al., 1999). Alternatively, a more labile form of linkage could be investigated, for example, an anhydride (Edlund & Albertsson, 2002; Engelberg & Kohn, 1991; Gopferich, 1996).

CONCLUSIONS

Ibuprofen was conjugated to the free –OH groups of a novel polyester at different molar ratios of polymer : ibuprofen. Characterization of these conjugates demonstrated that the addition of ibuprofen to the polymer backbone altered the T_m, the number of distinct crystalline domains, and level of crystallinity of the polymer. The level of drug loading achieved for the 100% conjugate was not directly proportional to that in the 25 and 50% conjugates. GPC analysis indicated a nonlinear relationship between the level of conjugation and the molecular weight. NMR data suggested that conjugation was incomplete. Drug release from the conjugates was slower and burst release was less over a shorter time compared with unconjugated dispersions of the drug and the polymer. Burst release displayed by the conjugates may indicate that some of the ibuprofen was unconjugated. UV and chromatographic data suggest that the ester linkages between the polymer and the ibuprofen and within some domains of the polymer were too stable. The degree of initial burst drug release was less, and the rate of drug release was much slower from the conjugates compared with the unconjugated drug–polymer dispersions. Therefore, a more labile form of bonding may be required to produce a faster rate of drug release and a more zero-order release mechanism.

ACKNOWLEDGMENTS

This work was supported by the Royal Pharmaceutical Society of Great Britain (RPSGB) and the Biotechnology and Biological Sciences Research Council (BBSRC ref: GR42/13897).

REFERENCES

Bodmeier, R., & Chen, H. (1989). Preparation and characterization of microspheres containing the anti-inflammatory agents, indomethacin, ibuprofen, and ketoprofen. *J. Control. Release, 10*, 167–175.

Duncan, R. (2003). The dawning era of polymer therapeutics. *Nat. Rev., 2*, 347–360.

Edlund, U., & Albertsson, A.-C. (2002). Degradable polymer microspheres for controlled drug delivery. *Adv. Polym. Sci., 157*, 67–112.

Engelberg, I., & Kohn, J. (1991). Physico-mechanical properties of degradable polymers used in medical applications: A comparative study. *Biomaterials, 12*, 292–303.

Flandroy, P. M. J., Grandfils, C., & Jerome, R. (1993). Clinical applications of microspheres in embolization and chemoembolization: Comprehensive review and perspectives. In A. Rolland (Ed.), *Pharmaceutical particulate carriers: Therapeutic applications* (pp. 321–366). New York: Marcel Dekker Inc.

Fu, Y.-J., Shyu, S.-S., Su, F.-H., & Yu, P.-C. (2002). Development of biodegradable co-poly(D,L-lactic/glycolic acid) microspheres for the controlled release of 5-FU by the spray drying method. *Colloids Surf., B, 25*(4), 269–279.

Gopferich, A. (1996). Mechanisms of polymer degradation and erosion. *Biomaterials, 17*, 103–114.

Jain, J. A. (2000). The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. *Biomaterials, 21*, 2475–2490.

Khandare, J., & Minko, T. (2006). Polymer-drug conjugates: Progress in polymeric prodrugs. *Prog. Polym. Sci., 31*, 359–397.

Nam, Y. S., & Park, T. G. (1999). Protein loaded biodegradable microspheres based on PLGA-protein bioconjugates. *J. Microencapsulation, 16*(5), 625–637.

Oh, J. E., Nam, Y. S., Lee, K. H., & Park, T. G. (1999). Conjugation of drug to poly(D,L-lactic-co-glycolic acid) for controlled release from biodegradable microspheres. *J. Control. Release, 57*, 269–280.

Park, T. G. (1995). Degradation of poly(lactic-co-glycolic acid) microspheres: Effect of copolymer composition. *Biomaterials, 16*, 1123–1130.

Perumal, D., Dangor, C. M., Alcock, R. S., Hurbans, N., & Moopanar, K. R. (1999). Effect of formulation variables on in vitro drug release and micrometric properties of modified release ibuprofen microspheres. *J. Microencapsulation, 16*, 475–487.

Rimoli, M. G., Avallone, L., de Caprariis, P., Galeone, A., Forni, F., & Vandelli, M. A. (1999). Synthesis and characterisation of poly(D,L-lactic acid)-idoxuridine conjugate. *J. Control. Release, 58*, 61–68.

Thompson, C. J., Hansford, D., Higgins, S., Hutcheon, G. A., Rostron, C., & Munday, D. L. (2006). Enzymatic synthesis and evaluation of new novel ω–pentadecalactone polymers for the production of biodegradable microspheres. *J. Microencapsulation, 23*(2), 213–226.

Thompson, C. J., Hansford, D., Higgins, S., Rostron, C., Hutcheon, G. A., & Munday, D. L. (2007). Evaluation of ibuprofen-loaded microspheres prepared from novel copolyesters. *Int. J. Pharm., 329*, 53–61.

Tice, T. R., & Cowsar, D. R. (1984). Biodegradable controlled-release parenteral systems. *Pharm. Technol., 11*, 26–36.

Ustariz-Peyret, C., Coudane, J., Vert, M., Kaltsatos, V., & Boisrame, B. (2000). Labile conjugation of a hydrophilic drug to PLA oligomers to modify a drug delivery system: Cephradin in a PLAGA matrix. *J. Microencapsulation, 17*(5), 615–624.

Yoo, H. S., Lee, K. H., Oh, J. E., & Park, T. G. (2000). In vitro and in vivo anti-tumor activities of nanoparticles based on doxorubicin-PLGA conjugates. *J. Controlled Release, 68*, 419–431.

Zhu, K. J., Li, Y., Jiang, H. L., Yasuda, H., Ichimaru, A., Yamamoto, K., Lecomte, P., & Jerome, R. (2005). Preparation, characterization and in vitro release properties of ibuprofen-loaded microspheres based on polylactide, poly(ε-caprolactone) and their copolymers. *J. Microencapsulation, 22*, 25–36.