Controlled release of ibuprofen from polymeric nanoparticles

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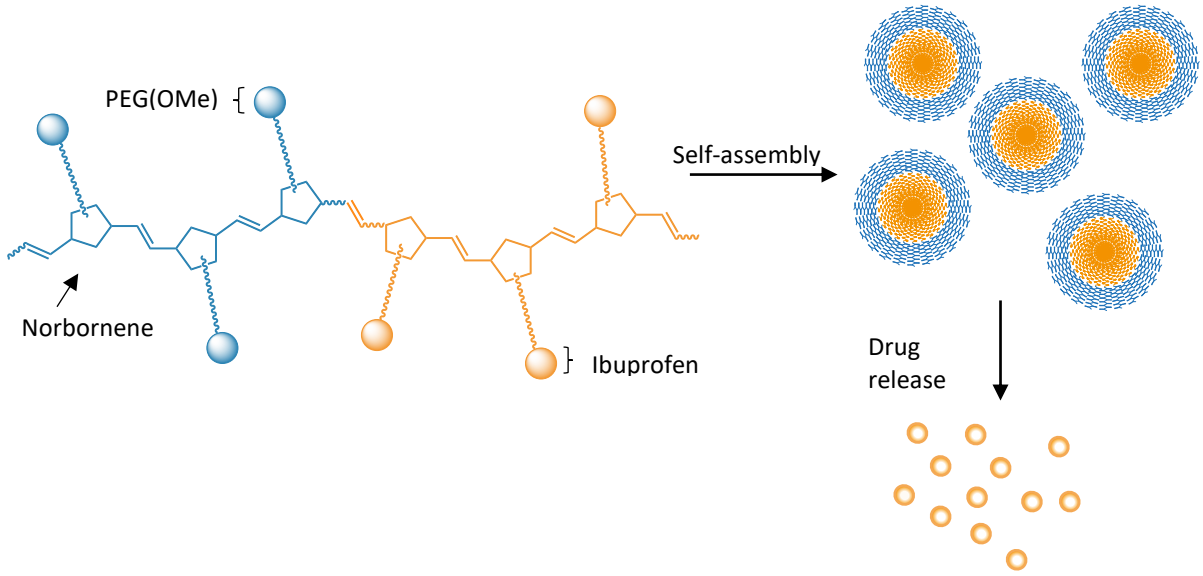
Smart polymeric systems are required that are able to release a therapeutic drug with controlled delivery. Herein we investigated the pH triggered release of ibuprofen from a polymeric nanoparticle system prepared using ring-opening metathesis polymerisation. The co-polymerisation of ibuprofen and poly(ethylene)glycol monomers followed by self-assembly produced a nanoparticle system that was shown to be stable at neutral pH but releases ibuprofen in alkaline conditions.



Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) possess analgesic, antipyretic and anti-inflammatory properties and are amongst the most widely prescribed drugs worldwide.[1] Pain relief is the primary clinical use for NSAIDs but the well-known association between inflammation and cancer has resulted in numerous investigations of NSAIDs for cancer prevention and treatment. Studies in various types of breast cancers, including prostrate[2], breast[3], colorectal[4,5] and ovarian[6] cancers indicate a positive effected linked to NSAID use. NSAIDs typically act by blocking the cyclooxygenase (COX) enzyme which is key in the synthesis of prostaglandins (PGs) which are required for the vasodilation associated with inflammation. There are however also epidemiological studies that contraindicate NSAID use which are associated with increased cancer risks, especially renal[7], although the mechanism of action is unclear[1]. Furthermore NSAIDs have been associated with unwanted nausea and dyspeptic symptoms including ulcers[1,8] and internal bleeding[9]. These latter complications are related to the oral ingestion of NSAIDs and we therefore wished to investigate a polymer approach for the delivery of these drugs[10] for tumour therapy.

The field of polymer therapeutics spans several decades and works on the development of polymer-drug systems that rely on a degradable or bio-degradable process to release a drug from a polymer[11]. There are several advantages in using these poly-prodrug systems, such as an increase in the drug water solubility, an enhancement of drug bioavailability, protection of the drug during its circulation to the site of action and an improvement in pharmacokinetics[12,13]. In cancer therapy the enhanced permeation and retention (EPR) effect is also a common property associated with therapeutic macromolecules[14,15] although this effect is questioned in human cell studies[16]. Having previously made a pure drug platform from salicylic acid[17], we were interested in utilising the ring opening metathesis polymerisation (ROMP) process as a means of approaching a controlled drug release polymer system. The exquisite control that ROMP affords in preparing well-controlled functionally dense polymers and copolymers[18,19] and their resulting self-assembly has led to several examples of bio-related and therapeutic ROMP polymers[20–26]. Chemically degradable ROMP polymers, in other words when the mechanism of drug release is a chemical process such as ester hydrolysis and not a biological process, is an area that is gaining more attention[27–29]. Previous work in our laboratories has shown that the copolymerisation of a polyethelene glycol (PEG) moiety in peptide derived ROMP polymer leads to self-assembled molecular architectures[30,31] and we were interested in investigating the stability and release of a non-steroidal anti-inflammatory drug (NSAID), namely ibuprofen, from a ROMP-PEG polymer systems. Nanoparticles derived from ROMP-PEG polymers have been shown to exhibit good stealth properties in tumour therapy studies[27] and the excellent living control polymerisation of ROMP allows for the post-released scaffold to be under 45 kDa, a requirement for renal excretion[32]. For this study we were considered four environments: aqueous, phosphate buffered saline (PBS), foetal bovine serum (FBS), pig liver esterase (PLE) and basic (2M NaOH in water).

Results and Discussion

Monomer synthesis

The monomers required for this investigation are not commercially available. Condensation reactions with the *exo*-carbic anhydride derivative **1** were chosen as these lead to symmetrical norbornene derivatives which minimise head to tail effects. The norbornene PEG-derivative **2** was prepared in a similar route to a previously reported methodology within the group[31], whereas the ibuprofen derivative **4** was prepared from *N*-(hydroxypentanyl)-cis-5-norbornene-*exo*-2,3-dicarboximide **3**[33], (Scheme 1).

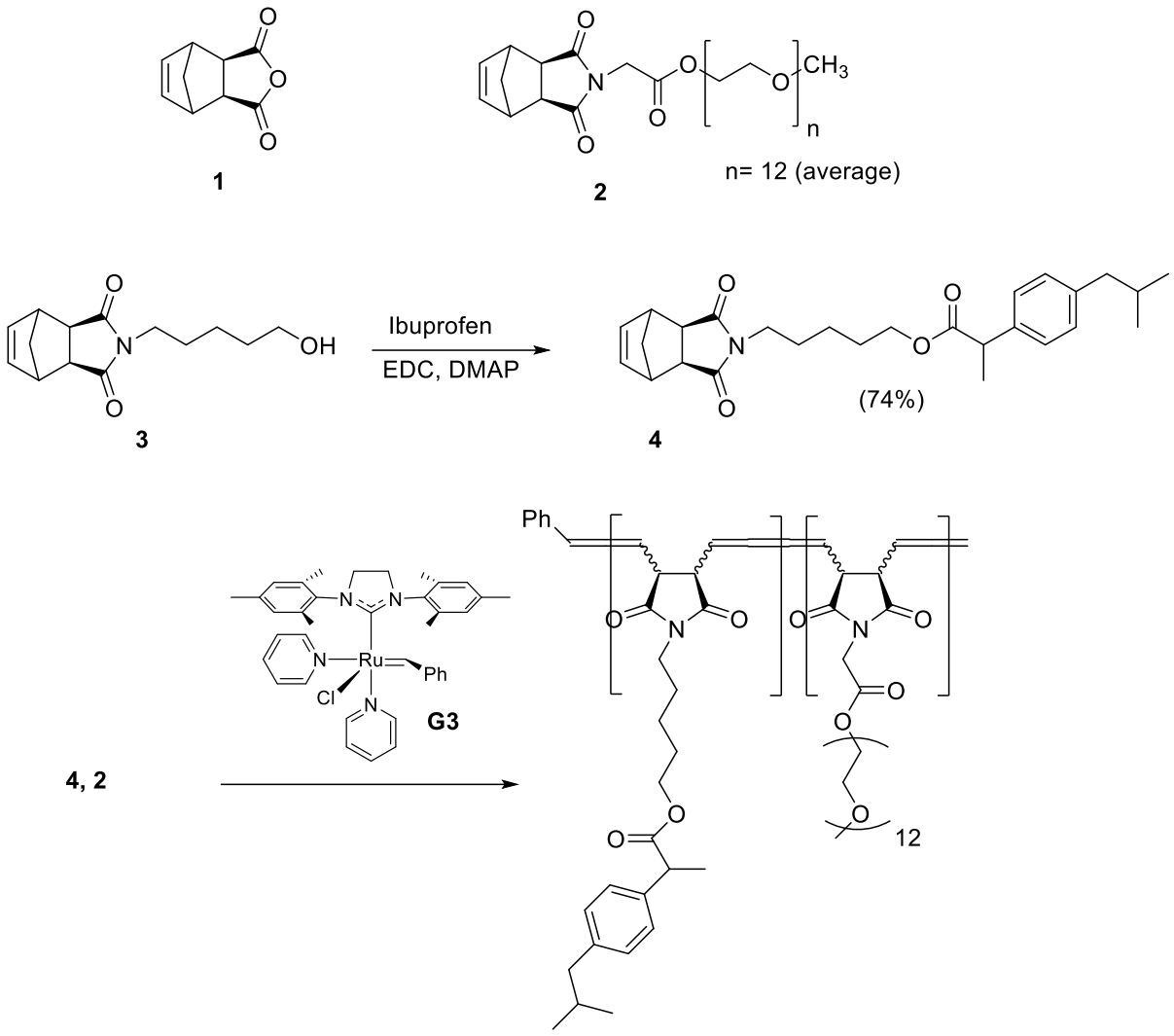


Figure 1 Synthesis of PEG-Ibuprofen copolymers

Synthesis of polymers

The monomers **2** and **4** were polymerised respectively, using the commercially available Grubbs G3 initiator[34] in THF at room temperature and were terminated with ethyl vinyl ether.

The individual homopolymers were readily formed and after isolation they were characterised by proton NMR and GPC (data presented in Table 1). The polydispersity of the PEG polymer poly-**2** was slightly higher than for poly-**4** and may be a reflection of the PEG chain length of the monomer which is itself an average distribution.

*Table 1: polymerisation characteristics for poly-**2** and poly-**4**.*

	Polymer	*Reaction time*	*Monomer conversion (%)*	*Yield (%)*	*M_n Theoretical*	*M_n ^{1}H NMR*	*M_n GPC*	*M_w GPC*	*Đ (M_w/M_n)*
G3 initiator	Poly-**2**	5 min	>99	92	14 852	16 947	10 984	14 229	1.36
	Poly-**4**	5 min	>99	98	8 752	8 755	11 116	13 640	1.27

To obtain the block co-polymer poly(**4-b-2**), the *exo*-norbornenyl ibuprofen monomer **4** was firstly polymerised using a ratio of monomer to G3 initiator of 20:1 and dry DCM as solvent. After 10 minutes an equimolar quantity of *exo*-norbornenyl PEGOMe monomer **2** was added to the reaction mixture giving an overall ratio of monomer to initiator of 40:1. Statistical copolymer poly(**4-co-2**) was synthesised by adding both of monomers (1:1 molar ratio) at the same time, into the G3 initiator solution. In each case the polymerisation was terminated by adding ethyl vinyl ether and the pure polymer was obtained by precipitation with diethyl ether (Figure 1).

It was found that both copolymers poly(**4-b-2**) and poly(**4-co-2**) possessed a polydispersity index lower than 1.4 and a number average molecular weight, M_n that is comparable with the theoretical one. For the block copolymer poly(**4-b-2**) it was also possible to estimate the average molecular weight from end group analysis from the proton NMR.

*Table 2: polymerisation characteristics of block and statistical copolymer derived from **2** and 4.*

Polymer	*Yield (%)*	*% PEG*	*% Ibuprofen*	*M_n Theoretic*	*M_n ^{1}H NMR*	*M_n GPC*	*M_w GPC*	*Đ (M_w/M_n)*
Poly(4-b-2)	78	36	64	21 895	24 632	19 318	24 905	1.29
Poly(4-co-2)	76	37	63	22 017	n.a.	19 263	26 191	1.36

Self-assembly of block and statistical copolymer

Self-assembly of the copolymers mentioned above were obtained by dissolving the polymer (< 10 mg) in 1 mL of acetone, and deionised water was added dropwise, over a prolonged time to the stirred solution to give a polymer with a final concentration of 1 mg/mL. The aggregate solution was subsequently transferred into a dialysis membrane, sealed and dialysed against distilled water for 24 hours to remove any traces of the organic solvent. The self-assembly was then analysed by DLS (Dynamic Light Scattering) and TEM (Transmission Electron Microscopy). DLS data was recorded using a polyphospholipid refractive index of 1.45.

Figure 2 shows the DLS particle distribution for the block copolymer poly(**4-b-2**) in acetone, and of the statistical copolymer poly(**4-co-2**) also in acetone. This latter copolymer presents, as expected, a different distribution of the particle size; the largest peak (67% by intensity) is for particles at 13nm. Because of the random distribution of the PEG and ibuprofen side chains tethered to the norbornene backbone, we interpret this as the polymer folding in on itself, forming single chain nanoparticles. A small amount of these nanoparticles (32%) form random aggregates of a bigger size (230 nm) that precipitate in solution. TEM analysis of poly(**4-co-2**) confirmed an absence of ordered self-assembly.

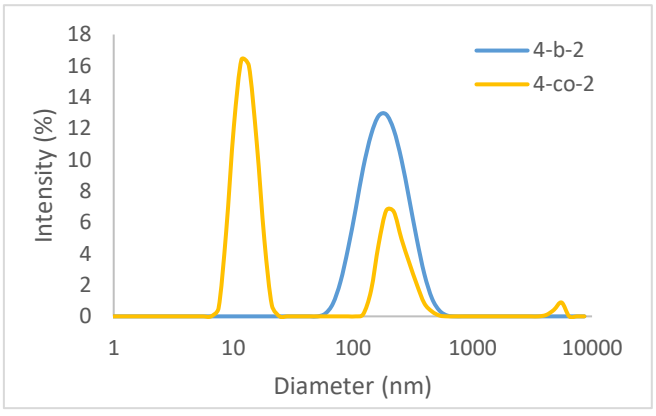


Figure 2: DLS particle size distributions of block and statistical copolymers.

The block copolymer, instead, behaves as a non-ionic amphiphilic polymer and in water forms particles in the size range of 50 - 600 nm as shown in Figure 6 with an average diameter of 196 nm in acetone.

The self-assembled morphologies of the copolymers were studied using TEM. Samples were analysed on Formvar coated copper grids, to which a negative stain of uranyl acetate was added, that allows for better contrasting of low molecular weight atoms (C, H, N) under the electron beam.

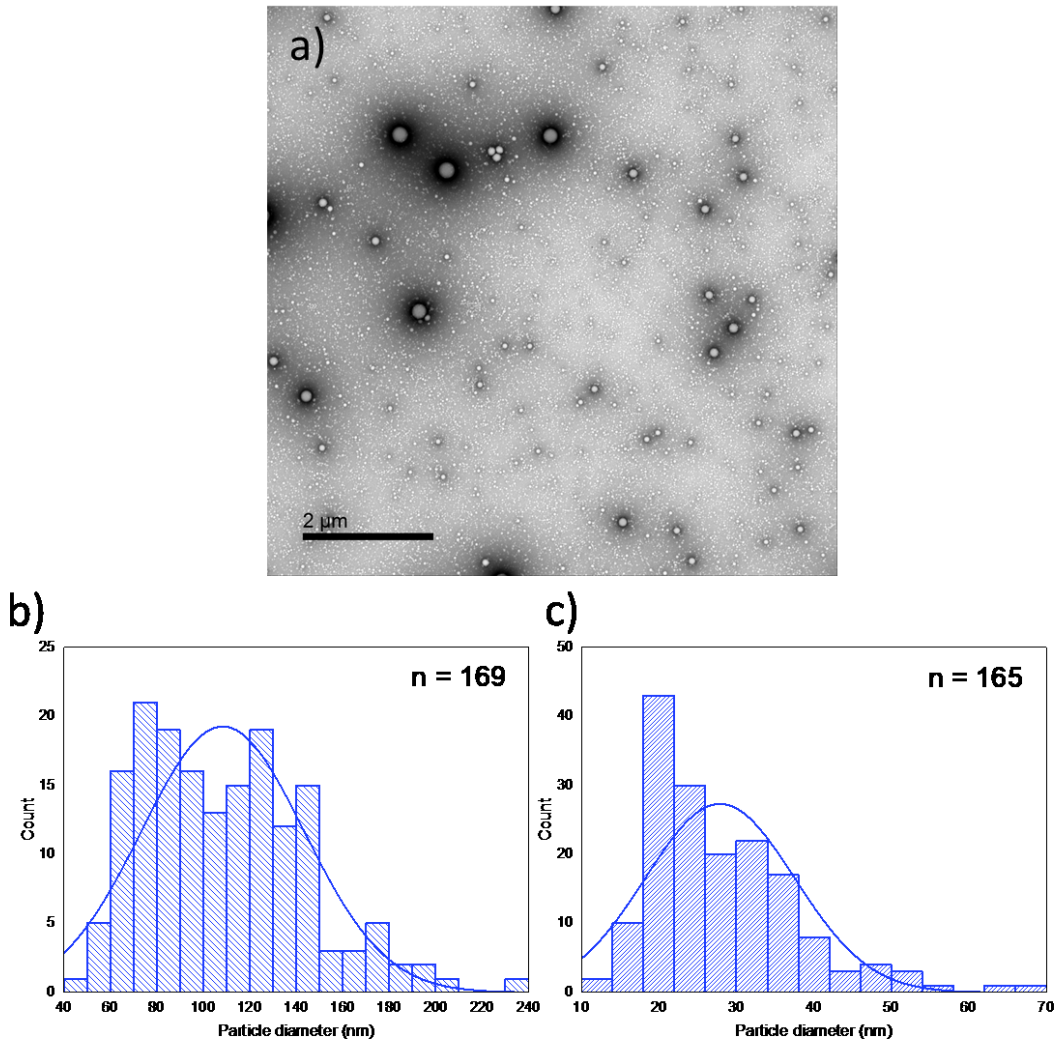


*Figure 3: a) TEM image of block copolymer poly(**4**-**b**-**2**) ; b) distribution of the larger particles of image (a); d) distribution of the smaller particles of image (a).*

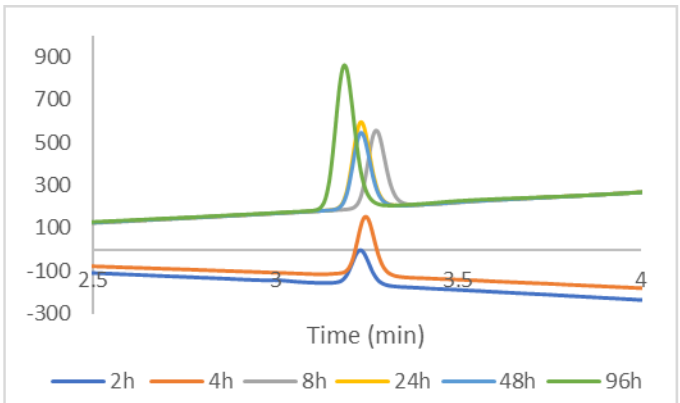
Figure 3a shows the TEM images obtained for the block copolymer poly(**4-b-2**). The image seems to reveal the presence of two different morphologies which can be considered vesicles and micelles. Figure 3b and 3c indicate that the copolymer poly(**4-b-2**) has a large distribution of particle size which ranges from 40 nm to 240 nm. Examining the histograms in more detail, it is possible to identify, for each plot, two different particle distributions. For example, in Figure 3b, there are two distributions centred at 70 nm and 120 nm respectively. These results do not entirely correspond to the DLS measurements, which provide a bigger average diameter, as is common due to the solvation sphere measured by DLS, and the compacting effect of the vacuum in TEM. Furthermore, Figures 3c indicates that the formation of spherical micelles is dominant, and they possess an average diameter of 30 nm. This result is in agreement with the calculations made using computational software, which afforded a

repeating unit length of 0.617 nm that multiplied by the degree of polymerisation (DP = 40) gave a predicted particle radius of 25 nm.

In vitro release studies

Block copolymer poly(**4-co-2**) (200 µg) was placed into vials and 200 µL solutions of 2M NaOH in water, phosphate buffered saline (PBS), foetal bovine serum (FBS), pig liver esterase (PLE) and water were added to different sets of vials. The samples were incubated at 40 degrees in a thermocycler. Each sample was removed at predefined time points (2h, 4h, 8h, 24h, 48h, 96h), frozen and analysed afterwards by HPLC. A gradient processing method was used, starting from 20% methanol in water with 0.1% of formic acid. Samples (10 µL) were run at 35 °C at a flow rate of 2 mL/min. Absorbance was monitored at λ = 225 nm. The instrument was calibrated using standard solutions of ibuprofen in methanol (50, 100, 150, 200, 250 ppm).

Figures 4a and 4b illustrate the release of ibuprofen using basic conditions: Figure 4a shows the hydrolysis of ibuprofen using NaOH in water. It is possible to distinguish the characteristic peak of ibuprofen at a retention time around 3 minutes and 20 seconds. It is evident from the graphs, that ibuprofen can be slowly released over an extended duration of at least 4 days. By a prior calibration of the instrument, it is also possible to quantify the concentration of the released drug (Tables 4) which after 96h is in agreement with the theoretically expected value for quantitative hydrolysis.



Time (hours)	2	4	8	24	48	96
Ibuprofen (ppm)	22	46	133	150	131	266

Figure 4: release study of ibuprofen with 2M NaOH in water. Chromatogram and the table including the concentration of ibuprofen released during time.

As mentioned above, the hydrolysis of ibuprofen from block copolymers was investigated also using media that can mimic physiological conditions, such as PBS, FBS and PLE. By HPLC analysis, it appears that the polymer conjugate is stable as none of these media release ibuprofen at a temperature of 40 °C. This suggests a polymer conformation which causes ibuprofen to be placed within the micelles where the proteins cannot hydrolyse the ester bond.

Conclusion

In summary we have shown that the block copolymerisation of norbornene monomers functionalised with polyethylene glycol and ibuprofen leads to the synthesis of a polymer which in an aqueous environment self-assembles to a nanoparticle system which in turn in an alkaline environment will release ibuprofen over a period of up to four days. Further work will explore different linkages between

the polymer backbone and the drug with the aim of inducing controlled release in the presence of specific physiological environments.

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Notes

The authors declare no competing financial interests. All authors have given approval to the final version of the manuscript.

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