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Poly(2-alkyl-2-oxazoline)s: A polymer platform to sustain the release from tablets with a high drug loading

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ABSTRACT

Sustaining the release of highly dosed APIs from a matrix tablet is challenging. To address this challenge, this study evaluated the performance of thermoplastic poly (2-alkyl-2-oxazoline)s (PAOx) as matrix excipient to produce sustained-release tablets via three processing routes: (a) hot-melt extrusion (HME) combined with injection molding (IM), (b) HME combined with milling and compression and (c) direct compression (DC). Different PAOx (co-)polymers and polymer mixtures were processed with several active pharmaceutical ingredients having different aqueous solubilities and melting temperatures (metoprolol tartrate (MPT), metformin hydrochloride (MTF) and theophylline anhydrous (THA)). Different PAOx grades were synthesized and purified by the Supramolecular Chemistry Group, and the effect of PAOx grade and processing technique on the *in vitro* release kinetics was evaluated. Using the hydrophobic poly (2-*n*-propyl-2-oxazoline) (P^{n}PrOx) as a matrix excipient allowed to sustain the release of different APIs, even at a 70% (*w/w*) drug load. Whereas complete THA release was not achieved from the P^{n}PrOx matrix over 24 h regardless of the processing technique, adding 7.5% *w/w* of the hydrophilic poly (2-ethyl-2-oxazoline) to the hydrophobic P^{n}PrOx matrix significantly increased THA release, highlighting the relevance of mixing different PAOx grades. In addition, it was demonstrated that the release of THA was similar from co-polymer and polymer mixtures with the same polymer ratios. On the other hand, as the release of MTF from a P^{n}PrOx matrix was fast, the more hydrophobic poly (2-*sec*-butyl-2-oxazoline) (P^{sec}BuOx) was used to retard MTF release. In addition, a mixture between the hydrophilic PEtOx and the hydrophobic P^{sec}BuOx allowed accurate tuning of the release of MTF formulations. Finally, it was demonstrated that PAOx also showed a high ability to tune the *in vivo* release. IM tablets containing 70% MTF and 30% P^{sec}BuOx showed a lower *in vivo* bioavailability compared to IM tablets containing a low PEtOx concentration (7.5%, *w/w*) in combination with P^{sec}BuOx (22.5%, *w/w*). Importantly, the *in vivo* MTF blood level from the sustained release tablets correlated well with the *in vitro* release profiles. In general, this work demonstrates that PAOx polymers offer a versatile formulation platform to adjust the release rate of different APIs, enabling sustained release from tablets with up to 70% w/w drug loading.

1. Introduction

Oral solid dosage forms with sustained-release features are of high interest as they allow to maintain therapeutically optimal plasma drug concentrations for an extended time, therefore, decreasing the dosing frequency and improving patient compliance. Although sustained release dosage forms offer many advantages, the formulation of such products is mainly challenging for highly dosed and soluble active pharmaceutical

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ingredients (APIs) as the drug release is often too fast and/or shows a burst release [1,2].

Hot-melt extrusion (HME) is considered an essential drug formulation development technique in the pharmaceutical field. HME can be used to increase the bioavailability of poorly soluble APIs, mask the taste and control the release of specific APIs, develop enhanced drug delivery systems, and others. Consequently, therapeutic goals and patient compliance can be enhanced [3–6]. HME is also an eco-friendly technique that does not involve solvents. During HME, the API is embedded in a polymeric carrier under controlled conditions of elevated temperature and pressure. Subsequently, the material is forced through a well-defined die to form a uniform geometry and density product. After extrusion, extrudates can be processed into the desired dosage form (e.g. tablets, mini-matrices, granules, films, pellets, or others). The selection of the downstream approach is highly dependent on the intended application, the final dosage form's geometry, production cost, and material behaviour [7]. HME is an effective manufacturing technique to prepare sustained release dosage forms due to the intense mixing of crystalline drug particles with the release retarding matrix carriers [3,6]. However, there is only a limited number of thermoplastic pharmaceutical polymers with suitable physicochemical properties to allow successful HME: hydroxypropylmethylcellulose [8], xanthan gum [9], methacrylic acid co-polymers [10–12], ethylcellulose [13–17] and ethylene-vinyl acetate. However, commercially available polymers lack the possibility to tune their chemical structures as in most cases only the molecular weight is varied to obtain a polymer grade with different properties. Most polymers also require plasticizers to improve the processing conditions, and only a few allow to incorporate a drug load up to 50% w/w without processing issues or burst-release concerns [18]. Hence, expanding the range of polymers suitable for HME will support the development of alternative dosage forms.

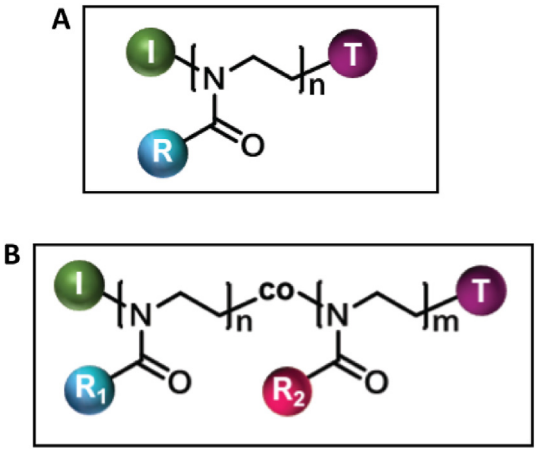

Fig. 1. General chemical structure of PAOx (A) homopolymers and (B) co-polymers, where R is the side chain group, while I and T are functionalities introduced during the initiation and termination steps, respectively.

Poly (2-alkyl-2-oxazoline)s (PAOx) are a polymer class comprising biocompatible, thermoresponsive, and amphiphilic polymers, depending on the side chain, that feature a tertiary amide group in the repeating units [19]. The synthesis of PAOx was developed by different research groups and is performed by cationic ring-opening polymerization (CROP) of 2-substituted 4,5-dihydrooxazoles, referred to as 2-oxazolines [20–22]. PAOx is an interesting polymer group due to its high tunability resulting from changing the alkyl substituent (R) that constitutes the polymer side chain (Fig. 1), affecting the overall hydrophilicity, thermal properties, and processability. The chain length of the functional group at (R) position highly affects the polymer physicochemical properties, which can be further fine-tuned by copolymerization of different 2-oxazoline monomers (Fig. 1). By increasing the length of the alkyl component on the 2-position, more hydrophobic polymers will be obtained (Fig. 2) leading to polymers with different water solubility and some of them exhibit lower critical solution temperature (LCST) behaviour [23–25]. Therefore, PAOx might be interesting for developing sustained-release dosage forms.

Recently, *Claeys* et al. proved the suitability of poly-2-ethyl-2-oxazoline (PEtOx) as a matrix excipient to produce controlled-release tablets using HME followed by injection molding [26]. Metoprolol tartrate and fenofibrate were used as water-soluble and poorly water-soluble APIs, respectively. HME of both formulations resulted in solid dispersions, and drug release showed slower MPT release from the PEtOx matrix compared to the pure API due to the slower dissolution rate of the polymeric matrix. However, the poorly water-soluble fenofibrate showed faster dissolution from the PEtOx matrix compared to the pure API. After this first report on using PAOx as excipient for drug formulation, various PAOx grades have been demonstrated for the efficient preparation of solid dosage forms [27–33]. However, to the best of our knowledge, PAOx have not been reported as excipient for sustained release formulations despite that they appear to be ideally suited for this purpose based on the tunability of their physical properties in combination with a good processability.

Therefore, in this work we studied the effectiveness of different PAOx polymers to sustain the release of highly dosed tablets. Poly (2-*n*-propyl-2-oxazoline) (P^{n}PrOx), poly (2-ethyl-2-oxazoline) (PEtOx), poly (2-*sec*-butyl-2-oxazoline) (P^{sec}BuOx), and poly (2-cyclopropyl-2-oxazoline) (P^{c}PrOx) were chosen based on their solubility behaviour ranging from water-soluble, thermoresponsive to almost water-insoluble (Fig. 2). These polymers were investigated as a formulation platform to control the sustained release behaviour of three APIs with different aqueous solubility: metoprolol tartrate (MPT), metformin hydrochloride (MTF)

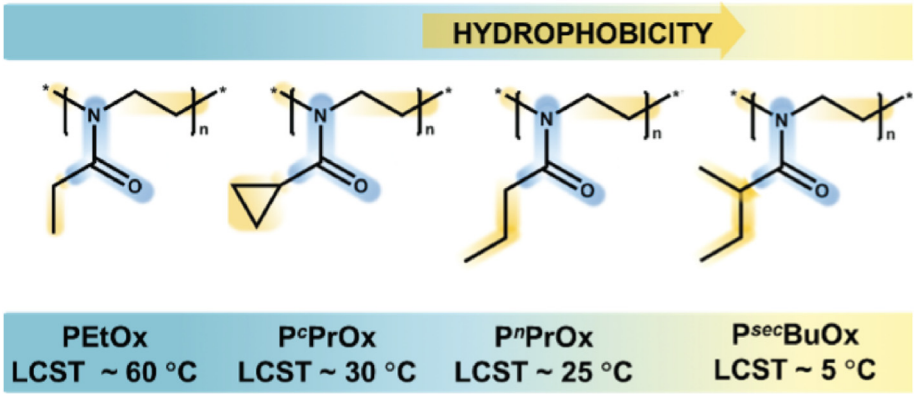

Fig. 2. *Different poly(2-alkyl-2-oxazoline)s that were investigated in this work, displaying their amphiphilic character and their lower critical solution temperatures. Adapted from Ref.* [24].

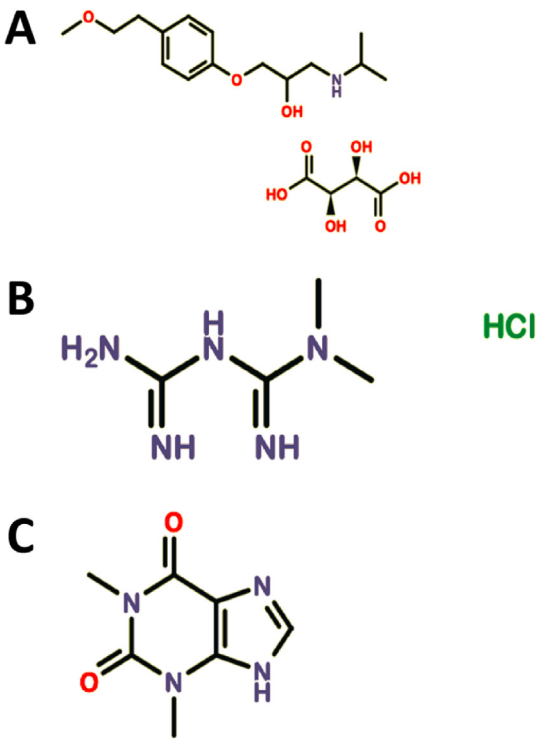

Fig. 3. The investigated drug structures (a) metoprolol tartrate (B) metformin hydrochloride and (C) theophylline anhydrous.

and theophylline anhydrous (THA) (Fig. 3). Several downstream processing techniques were used to prepare tablet-shaped dosage forms from the PAOx/API mixtures: (a) HME in combination with IM, (b) HME in combination with milling and compression, and (c) direct compression.

After establishing the optimal polymer-API combinations which allowed to sustain the *in vitro* release of tablets with 70% w/w API loading, the most promising formulations were used for *in vivo* studies using beagle dogs.

2. Experimental section

2.1. Materials

Model drugs with different aqueous solubility and melting temperature were used to examine their effect on the processability and the release kinetics. Metoprolol tartrate (MPT), metformin hydrochloride (MTF) and theophylline anhydrous (THA) were purchased from Utag (The Netherlands), Sigma-Aldrich (Germany) and Siegfried (Switzerland), respectively. The aqueous solubility at 25 °C is > 1000, 50 mg/mL and 8.3 mg/mL for MPT, MTF and THA, respectively, while the melting temperatures are 121, 231 and 273 °C for MPT, MTF and THA, respectively.

2.2. (Co-)polymer synthesis

Full experimental details are included in the supporting information. Five homopolymers and three co-polymers were synthesized for this study based on our recently published optimized protocol for preparing defined high molar mass PAOx (Table 1) [34]. The homopolymers P^{n}PrOx with 50 kg/mol, P^{n}PrOx with 80 kg/mol and P^{c}PrOx with 50 kg/mol were structurally similar. PEtOx with 50 kg/mol was also considered in this study due to its higher hydrophilicity, while a more hydrophobic polymer containing *sec*-butyl groups in the side chains was also included (P^{sec}BuOx with 50 kg/mol). Furthermore, three co-polymers containing different ratios of 2-ethyl-2-oxazoline and 2-*n*-propyl-2-oxazoline were synthesized. These co-polymers presented similar molar masses ~50 kg/mol (Table 1) with different hydrophobic properties depending on the ratio of the monomers. On the other hand, polymer blends were prepared by mixing P^{n}PrOx (50 kDa) and PEtOx using mortar and pestle. ^{1}H NMR spectroscopy, size exclusion chromatography, thermal gravimetrical analysis and differential scanning calorimetry of all polymers is presented in the supplementary data and is summarized in Table 1. After polymer synthesis, the (co-)polymers were cryo-milled using liquid nitrogen in a simple coffee blender to obtain a sufficiently small particle size in order to ensure good homogeneity of the drug/polymer mixtures.

Table 1
Composition, SEC, TGA and MDSC data of the (co-)polymers and polymer mixtures.

Polymer	Composition	SEC		TGA (°C)[c]	MDSC
		M_n[a] (kg/mol)	Đ[b]		Tg (°C)
Homopolymers					
P^{n}PrOx	H-P^{n}PrOx$_{443}$-OH	56.4	1.13	372	17.8
P^{n}PrOx	H-P^{n}PrOx$_{700}$-OH	78.5	1.25	395	20.9
P^{c}PrOx	Me–P^{c}PrOx$_{450}$-OH	51.3	1.77	377[c]	80.9
PEtOx	H-PEtOx$_{500}$-OH	50.7	1.12	375	62.2
P^{sec}BuOx	H-P^{sec}BuOx$_{430}$-OH	55	1.56	311	48.6
Co-polymers					
PEtOx:P^{n}PrOx (75:25%, *w/w*)	Me-PEtOx$_{379}$-*stat*-P^{n}PrOx$_{111}$-OH	42	1.18	336	24.5
PEtOx:P^{n}PrOx (50:50%, *w/w*)	Me-PEtOx$_{252}$-*stat*-P^{n}PrOx$_{221}$-OH	47.2	1.24	387	29.8
PEtOx:P^{n}PrOx (25:75% *w/w*)	Me-PEtOx$_{126}$-*stat*-P^{n}PrOx$_{332}$-OH	46	1.25	382	24.5
Polymer mixtures					
PEtOx:P^{n}PrOx (75:25%, *w/w*)					
PEtOx:P^{n}PrOx (50:50%, *w/w*)					
PEtOx:P^{n}PrOx (25:75% *w/w*)					

[a] Determined by SEC-MALS using DMA/LiCl as an eluent.
[b] Determined by SEC against PMMA standards.
[c] TGA measurements were determined at 5% weight loss for all polymers except for P^{c}PrOx (calculated at 10% weight loss).

2.3. Polymer characterization

2.3.1. Size exclusion chromatography

Size exclusion chromatography measurements were performed in an Agilent 1260- series equipped with an online degasser, an ISO-pump, an automatic liquid sampler, a thermostatted column compartment at 50 °C equipped with a precolumn and two PL gel 5 μm mixed-D columns in series, a 1260 diode array detector and a 1260 refractive index detector (RID). Measurements were performed in *N*,*N*-dimethylacetamide as an eluent containing 50 × 10^{-3} M LiCl to suppress interactions between the analyte and the packing material. The flow rate was set at 0.500 mL/min. To analyse the chromatograms, Agilent Chemstation software was used with a GPC add-on. Molar masses were calculated by light scattering detector, while dispersity values were calculated against poly (methylmethacrylate) (PMMA) standards.

Light scattering (LS) measurements are performed on a 3-angle static light scattering detector (miniDAWN TREOS, Wyatt Technology). The detector is coupled online to an Agilent 1260 infinity HPLC system (vide DMA-SEC) and used to determine the absolute molar mass of the polymer samples. The measurements are performed at ambient temperature, without a temperature control unit installed. The refractive index (RI) increment (dn/dc) values are either used as reported for certain polymers in *N*,*N*-dimethyl acetamide containing 50 × 10^{-3} M LiCl or determined via online size-exclusion chromatography (SEC) equipped with an RI detector, which measures the RI increase for a 1–10 mg/mL concentration series of the mentioned polymers. The LS results are further analyzed with the Astra 7 software from Wyatt Technology.

2.3.2. Thermal analysis

Thermogravimetric analysis (TGA) was performed using a TGA 2 (Mettler-Toledo, Switzerland) with a large furnace and an autosampler, using 70 μL alumina cubicles. Samples (5–10 mg) were heated at 10 °C/min from 25 to 800 °C under nitrogen atmosphere (80 mL/min). Evaluation was performed via the STARe software (Switzerland).

Modulated differential scanning calorimetry (MDSC) (Q2000 TA instruments, United Kingdom) was performed to study the physical state and the glass transition of all PAOx (co-) polymers using a heating rate of 2 °C/min. The modulation period and amplitude were set at 1 min and 0.32 °C, respectively. Samples of (5–10 mg) were placed in Tzero pans (TA instruments, Belgium) and heated from −10 to 120 °C. Dry nitrogen (50 mL/min) was used to purge the MDSC cell.

2.4. Preparation of extrudates by HME

HME was performed on selected PAOx in combination with different APIs (MPT, MTF and THA) using a co-rotating twin-screw extruder. Physical mixtures (70% or 80% drug load, *w/w*) were extruded using an Xplore micro-compounder (DSM, The Netherlands), operating at 100 rpm and using the processing temperatures that are listed in Table 2. Afterward, part of the extrudates was milled to prepare tablets by compression and the other part was used for injection molding.

2.5. Preparation of tablets

2.5.1. Injection molding

After HME, the extrudates were immediately processed into IM tablets via injection molding using a Haake MiniJet System (Thermo Electron, Germany) at a temperature in function of the formulation as shown in Table 2. During the IM process, an injection pressure of 800 bar for 10 s forces the material into the mold. A post-pressure of 400 bar for 5 s avoids expansion by relaxation of the polymer. Convex tablets were produced (mass: 410 ± 10 mg; diameter: 10 mm; height: 5 mm).

2.5.2. Compression

Both physical mixtures and milled extrudates were compressed using a STYL'One compaction simulator (Medelpharma, France) to produce DC and ME tablets, respectively. The compaction simulator was equipped with a single punch station. A 10 mm round punch set was used to compress convex tablets (350 ± 10 mg) at a compression force of 10 kN for all formulations. A dwell time of 100 ms was used without precompression.

2.6. Tablet characterization

2.6.1. Thermal analysis

DSC (Q2000 TA instruments, United Kingdom) was performed to evaluate the percentage of drug crystallinity after tablet preparation. Tzero pans (TA instruments, Belgium) were filled with approximately 5–10 mg sample and placed in the DSC equipment after being non-hermetically sealed with Tzero lids using a Tzero Press (TA instruments, United Kingdom). An empty Tzero pan was used as a reference. A single heating run was performed at a heating rate of 10 °C/min from 0 to 150, 260 and 280 °C for MPT, MTF and THA formulations, respectively. The DSC apparatus was equipped with a refrigerated cooling system and dry nitrogen at a flow rate of 50 mL/min. The percentage of drug crystallinity was calculated by means of Equation (1) using the melt enthalpy obtained in DSC experiments.

$$Xc = \frac{\Delta H1}{\Delta H2} \text{ x } 100 \quad (1)$$

Xc: the percentage of drug crystallinity (%)
Δ H_1: melt enthalpy of the drug in the tablet (J/g)
Δ H_2: melt enthalpy of the drug in the physical mixture (J/g)

2.6.2. Content uniformity

The content uniformity test was performed to check the degree of dose uniformity in the prepared tablets. A UV/VIS spectroscopy method was used for the determination of the API content in the prepared tablets. A pre-weighed tablet was crushed and transferred into a 100 mL volumetric flask containing simulated intestinal fluid without enzymes (SIF, pH 6.8). After shaking the flask for 48 h, the concentrate was filtered and diluted. The absorbance was measured using a UV-1650PC spectrophotometer (Shimadzu Benelux, Belgium) at a wavelength of 234, 223 and 273 nm for MTF, MPT and THA, respectively. The test was done in triplicate. Results were evaluated according to the European Pharmacopoeia [35].

2.6.3. Disintegration

Disintegration tests were performed per the USP standards [36] using a DIST-3 disintegration tester (Pharma Test, Germany) with discs. All experiments were conducted over 8 h in simulated intestinal fluid (SIF, pH 6.8) at a temperature of 37 °C. The disintegration time of 3 individual tablets was recorded. The time was recorded until no tablets were left on the mesh.

Table 2
Overview of the formulation composition, the extrusion (T_{ext}) and IM temperatures (T_{IM}) (°C). F, FM and FC stands for formulations prepared using homopolymer, polymer mixtures, and co-polymers, respectively.

	MPT	MTF	THA	P^{n}PrOx	P^{c}PrOx	P^{sec}BuOx	PEtOx	T_{ext} (°C)	T_{IM} (°C)
F1	70	–	–	30	–	–	–	110	130
F2	70	–	–	30[a]	–	–	–	110	130
F3	70	–	–	–	–	30	–	130	150
F4	–	70	–	30	–		–	130	160
F5	–	70	–	–	–	30	–	150	190
F6	–	70	–	–	–	24	6	150	200
F7	–	70	–	–	–	22.5	7.5	150	200
F8	–	–	70	30	–	–	–	130	150
F9	–	–	80	20	–	–	–	–	–
F10	–	–	70	–	30	–	–	–	–
FM1	–	–	70	7.5	–	–	22.5	160	180
FM2	–	–	70	15	–	–	15	150	170
FM3	–	–	70	22.5	–	–	7.5	140	160
FC1	–	–	70	7.5	–	–	22.5	160	180
FC2	–	–	70	15	–	–	15	150	170
FC3	–	–	70	22.5	–	–	7.5	140	160
FR	Glucophage™ SR 500 (1/2 tablet)								

[a] F2 was prepared using 80 kDa P^{n}PrOx while in all other formulations, 50 kDa P^{n}PrOx was used.

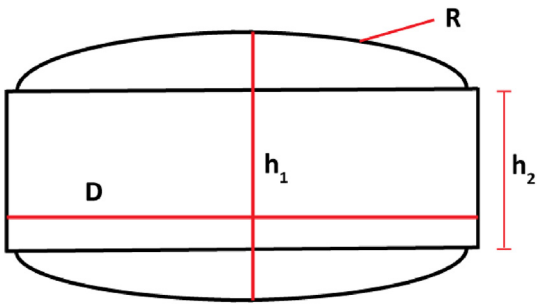

Fig. 4. The four dimensions used for calculation the tablet volume.

2.6.4. Porosity

The porosity of tablets (n = 3) was calculated based on Equation (2) by comparing the apparent density of the tablet calculated by dividing the mass by the volume of the tablet with the true density of tablets. The latter was measured using an AccuPyc 1330 helium pycnometer (Micrometrics, USA) at an equilibration rate of 0.0050 psig/min with the number of purges set to 10. The tablet volume (Equation (3)) was calculated by measuring four dimensions with a 96/0226 projection microscope (Reickert, Austria) as shown in Fig. 4.

$$P\,(\%) = 1 - \left(\frac{\rho\ app}{\rho\ true}\right) \times\ 100 \tag{2}$$

P: porosity (%)
ρ *true* : true density (g/mL)
ρ *app*: apparent density (g/mL)

$$Tv = \left(\pi \times \left(\frac{d}{2}\right)^2 \times h_2\right) + 2 \times \frac{1}{3}\pi \times \left(\frac{h_1 - h_2}{2}\right)^2 \times \left(3 \times R - \left(\frac{h_1 - h_2}{2}\right)\right) \tag{3}$$

2.6.5. In vitro dissolution

The impact of formulation composition and processing technique on the *in vitro* release was determined using USP apparatus II (paddle) on a VK 7010 dissolution system (VanKel Industries, USA). Drug release from all tablets (n = 3) was determined using the paddle method on a VK 7010 dissolution system (VanKel Industries, USA) with a speed of 100 rpm.

Simulated intestinal fluid (SIF, pH 6.8), simulated gastric fluid (SGF, pH 1.2) and SIF + ethanol (5, 10 and 20%, *v/v*) without enzymes were used as media. The temperature of the dissolution medium (900 mL) was maintained at 37 ± 0.5 °C. Samples of 5 mL were taken at ten time points (0.5, 1, 2, 4, 6, 8, 12, 16, 20 and 24 h) with a calibrated VK 8000 autosampler (VanKel Industries, USA). The absorbance of these samples was measured using a UV-1650PC spectrophotometer (Shimadzu Benelux, Belgium) at a wavelength of 234, 223 and 273 nm for MTF, MPT and THA respectively.

The similarity factor f_2 was used to measure the similarity between release profiles of two different formulations. As reported by *Shah* et al., the similarity factor can be calculated using Equation (4) [37]. Taking into consideration that only one sample point with a cumulative drug release higher than 85% can be included. Two release profiles are considered identical when $f_2 = 100$, while an average difference of 10% at all measured time points results in an f_2 value of 50. Dissolution profiles with f_2 values higher than 50 are considered similar.

$$f2 = 50\log_{10}\left\{\left(1 + \frac{1}{n}\sum_{t=1}^{n}(S_t - R_t)^2\right)^{-1/2} \times 100\right\} \tag{4}$$

$f2$: similarity factor
R_t: the cumulative percentage of drug released at each of the selected n time points from the reference
S_t: the cumulative percentage of drug released at each of the selected n time points from the sample

2.6.6. Micro-computed tomography analysis (μCT)

High resolution X-ray tomography (μCT) was used to study the effect of hydrophilic PEtOx on the pore distribution of IM tablets of F8 and FM3 before and after dissolution. Imaging was performed using the High Energy CT system optimized for research at the Ghent University Centre for X-ray Tomography (UGCT) [38] in which the source was operated at a voltage of 90 kV and a target power of 10 W. 2400 projections were taken with an exposure time of 1 s per image for a full 360° rotation. All scans were reconstructed using Octopus Reconstruction into a 3D volume (stored as a stack of 2D images) at a voxel size of 5.47^3 μm^3. At the given tube settings, the spatial resolution is almost not affected by the focal spot size. The in-house developed Octopus Analysis software package was used for 3D analysis of the reconstructed data to characterize the tablet porosity and pore distribution [39,40]. To segment the pore structure, thresholding was performed using the Octopus Analysis software. To identify the individual pores, labelling and watershed separation was performed. The total porosity was measured as the ratio of a tablet's pore volume to its total volume. To analyse the size of the pores the maximum opening and the equivalent diameter were used. The maximum opening and the equivalent diameter are the diameter of the largest sphere that fits in the pore space and the diameter of a sphere with the same volume as the pore space, respectively. Finally, the pores were classified based on their size (maximum opening) and VGStudio Max (Volume Graphics, Heidelberg, Germany) was used to visualize the virtual tablet in 3D.

2.7. In vivo experiments

In vivo studies were performed after the approval of the ethical committee of the Faculty of Veterinary Medicine (application ECD 2018–32).

Two IM tablets were studied to investigate the influence of the polymer grade on the *in vivo* release: P^{sec}BuOx:MTF (30:70%, *w/w*: F5) and P^{sec}BuOx:PEtOx:MTF (22.5:7.5:70%, *w/w*: F7). The commercially available Glucophage™ SR 500 mg (½ tablet) was previously tested by our research group and used as a sustained release reference formulation [41]. Tablets were administered orally with 20 mL water to beagle dogs after a wash-out period of 1 week. The dogs fasted for 12 h before the tablet administration with only access to water. Blank blood samples were collected before the tablet administration. Plasma samples were collected in dry heparinized tubes at 1, 2, 3, 4, 5, 6, 8, 12, 18 and 24 h post-administration. Afterward, blood was centrifuged for 10 min at 1500 g and frozen at −25 °C until analysis. Formulations based on PAOx were recovered from the faeces to determine the remaining amount of MTF. The gastro-intestinal residence time was also recorded.

2.7.1. Metformin hydrochloride assay

MTF plasma concentration was quantified based on the HPLC method developed and validated by *Verstraete* et al. [42]. Ranitidine (25 μL) was used as an internal standard and was mixed with 280 μL plasma supernatant after defreezing and centrifuging at 2300 g for 10 min. The first extraction involved mixing 50 μL 10 M sodium hydroxide solution and 3 mL organic phase (1-butanol/hexane, 50/50%, *v/v*) with the plasma samples. After centrifuging the tubes, the organic layer was used to perform back extraction by adding 1 mL 2 M HCl. Afterward, tubes were mixed and centrifuged. The organic layer was removed, 400 μL sodium hydroxide (10 M) and 2 mL organic phase (1-butanol/hexane, 50/50%, *v/v*) were added. The organic layer was transferred into a clean glass tube after mixing and centrifugation (10 min; 2300 g) and evaporated to dryness under a nitrogen stream.

HPLC (Merck-Hitachi, Germany) was used for the quantification of plasma concentration. Separation was performed using a reversed-phase column and pre-column (LiChroCart® 250-4 and LiChrospher® 100RP-18 5 μm, respectively) by injecting 100 μL sample. The flow rate of the

mobile phase (acetonitrile: potassium dihydrogen phosphate buffer pH 6.5 (34:66%, *v/v*) and 3 mM SDS) was set at 0.7 mL/min. The detection wavelength was 236 nm.

2.7.2. Data analysis

The chromatograms were recorded by the software package D-7000 HSM Chromatography Data Manager data collection and processing. The peak plasma concentration (C_{max}), time to reach C_{max} (T_{max}), half value duration ($HVD_{t50\%Cmax}$), and area under the curve ($AUC_{0\text{-}12h}$) were calculated from the plasma concentration curve. To compare the extent of sustained release between the tablets, the Rd ratio was calculated by dividing the $HVD_{t50\%Cmax}$ values of tested tablets over the $HVD_{t50\%Cmax}$ of an immediate-release formulation derived from the literature [43]. The half-value-duration (HVD) is defined as the total time in which the plasma concentration is above one-half of C_{max}. Low, intermediate and strong sustained release characteristics are defined as R_D ratios of 1.5, 2 and > 3, respectively.

Outcomes were statistically analyzed by repeated-measures ANOVA (univariate analysis) using SPSS 27 (SPSS, Chicago, USA). To compare the effects of the different formulations on the pharmacokinetic parameters, multiple comparisons among pairs of means were performed using a Bonferroni post-hoc test with $p < 0.05$ as the significance level.

3. Results and discussion

3.1. Polymer synthesis and characterization

The synthesis of pure and defined high molar mass PAOx is quite challenging, but has been recently achieved through careful optimization of the polymerization conditions [34]. These optimized conditions were here applied for the preparation of PEtOx, P^{n}PrOx, P^{c}PrOx and P^{sec}BuOx polymers as well as PEtOx-*stat*-P^{n}PrOx co-polymers with a targeted molar mass around 50 kg/mol as well as 80 kg/mol for P^{n}PrOx (see supporting information for full experimental details). The co-polymers were prepared to allow a direct comparison with polymer blends consisting of PEtOx and P^{n}PrOx co-polymers. The P^{sec}BuOx homopolymer was selected as the branched, racemic *sec*-butyl side-chain leads to an amorphous polymer with a higher glass transition temperature (T_g) than poly (2-*n*-butyl-2-oxazoline) that has a T_g of 25 °C and is semi-crystalline [44,45]. SEC analysis confirmed the relatively low dispersity for all synthesized polymers. P^{sec}BuOx was synthesized by bulk polymerization and had a somewhat higher dispersity of 1.56. The calculated molar masses for the synthesized polymers were all rather close to the targeted molar mass (Table 1). TGA data indicated that all polymers were stable up to at least 310 °C, confirming the high thermal stability of the PAOx used in this study. MDSC data showed no endothermic peaks, confirming the amorphous structure of these polymer grades with the glass transition reported in Table 1.

3.2. Processability of different PAOx grades

PAOx are highly stable polymers with physicochemical properties and solubilities that make them highly processable using diverse techniques as all formulations could be successfully processed via HME, IM and DC without the addition of plasticizers or other excipients. Different PAOx formulations could be processed with various drug loads during preliminary extrusion experiments, indicating that processing via HME was possible at 70% *w/w* drug load. Minimum processing temperatures were used to obtain good quality extrudates, keep the torque value below 80% of the maximum torque of the extruder (5000 N), and allow the melt to flow into the tablet molds during IM. Table 2 displays the extrusion and injection molding temperatures that were used.

Since the different PAOx have different T_g's (Table 1), the extrusion temperature was adjusted based on the PAOx grade. P^{sec}BuOx formulations were processed at a higher temperature than P^{n}PrOx due to the higher T_g for P^{sec}BuOx. Similarly, formulations containing more PEtOx required higher extrusion temperatures based on the higher T_g of PEtOx compared to P^{n}PrOx. The processing temperature also depended on the model drug: MTF and THA formulations were extruded at a higher temperature than the MPT-based formulations due to the higher melting temperature of MTF (231 °C) and THA (273 °C) compared to MPT (121 °C), requiring more energy to soften the high drug-loaded mixtures. Note that for the preparation of the sustained release formulations it was aimed to keep the processing temperature below the melting temperature of the API to retain the crystalline form of the drug in the final tablets.

3.3. Tablet quality

All tablets were successfully prepared via DC, HME/milling/compression and HME/IM to produce DC tablets, ME tablets and IM tablets, respectively. The drug content of each individual tablet was between 96% and 104% of the average content, which complies with the acceptance criteria of the European Pharmacopoeia. Good content uniformity was obtained due to adequate mixing before extrusion or compression, while HME provided additional intensive mixing due to the shear provided by the screws.

3.3.1. P^{n}PrOx matrix

During the first series of experiments, P^{n}PrOx (50 kg/mol) was chosen as a matrix excipient and processed with 70% w/w of MPT, MTF and THA. The drug release kinetics depended on the matrix composition, the manufacturing technique, and the model drug (Fig. 5). The release of MPT from the P^{n}PrOx matrix (F1) was complete within 1, 4, and 4 h for DC, ME, and IM tablets, respectively (Fig. 5A). It was previously reported by different research groups that tablets prepared by DC show faster release compared to tablets prepared by heat processing techniques such as HME and IM [13,15,46]. This is due to the densification during heat-involved processing, yielding tablets with fewer pores, less water penetration and slower drug release. The porosity of the F1 formulations was 17.0 ± 2.3%, 11.6 ± 2.2% and 2.2 ± 0.8% for DC, ME and IM tablets, respectively. Moreover, the extrusion process provides intensive mixing of crystalline drug particles with the release retarding matrix excipient, resulting in more sustained release profiles and reduced intergranular (pores between particles) and intragranular (pores within particles) porosity. In a study by *Crowley* et al., ethylcellulose was used as a matrix excipient in tablets containing 30% *w/w* of the highly water-soluble drug guaifenesin. The study revealed fewer pores and a smaller median pore radius for IM tablets than DC tablets regardless of the compression force used [15]. *Quinten* et al. indicated a faster burst drug release from DC formulations compared to IM formulations [13], due to the denser matrix obtained after injection molding. In another study, the release of caffeine from DC, IM and 3D printed tablets was compared. The formulation comprised 28.5% polyvinylpyrrolidone, 57% polycaprolactone, 9.5% polyethylene oxide and 5% *w/w* caffeine. Results revealed an immediate release behaviour from the DC tablets. However, the IM tablet showed sustained release over 48 h [47]. *Fuenmayora* et al. concluded that the processing technique affected the final tablet quality, including the release kinetics, and that tablets prepared by IM were densely packed, exhibiting more extended-release profiles compared to tablets prepared by DC. However, in the current study IM tablets of F1 had a similar release profile to the ME tablets (Fig. 5A). This was attributed to the loss of MPT crystallinity in the matrix after the second heat treatment during IM, while DC and ME tablets showed 100% MPT crystallinity. IM was performed at a temperature of 130 °C (Table 2) which was higher than the melting temperature of MPT, preventing a fraction (±15%) of the MPT content from recrystallizing upon cooling (Table 3).

To tune the release of MPT from the P^{n}PrOx matrix, a higher molar mass P^{n}PrOx (80 kg/mol) was also tested (F2) to study the effect of polymer molecular weight on the release kinetics. As shown in Fig. 5A, there was no significant difference (f2 value > 50) between tablets prepared from 50 or 80 kDa P^{n}PrOx, regardless of the processing technique.

On the other hand, the release of MTF from the P^{n}PrOx matrix (F4)

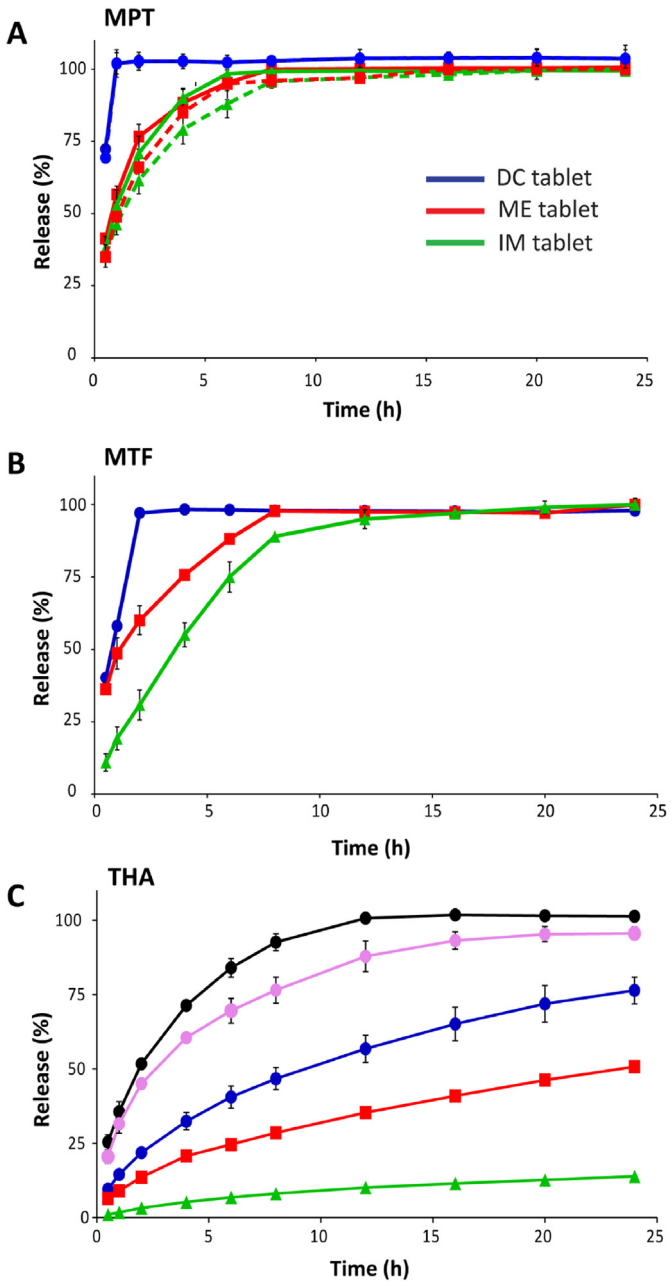

Fig. 5. *In vitro release kinetics of 70% w/w (A) MPT (B) MTF, and (C) THA from P^{n}PrOx* 50 kDa *(continuous line) and P^{n}PrOx* 80 kDa *(dotted line) for (●) DC, (■) ME and (▲) IM tablets. Graph (C) also shows the in vitro release of THA DC tablets from a P^{n}PrOx matrix with 80% (w/w) drug load (●) and from a P^{c}PrOx matrix with 70% (w/w) drug load (●).*

Table 3
Crystallinity (%) of IM tablets of different formulations.

IM tablet	Crystallinity (%)
F1	85.7 ± 3.2
F2	83.0 ± 4.1
F3	40.5 ± 5.4
F4	99.0 ± 1.3
F5	99.2 ± 2.1
F6	99.3 ± 0.9
F7	98.7 ± 2.4

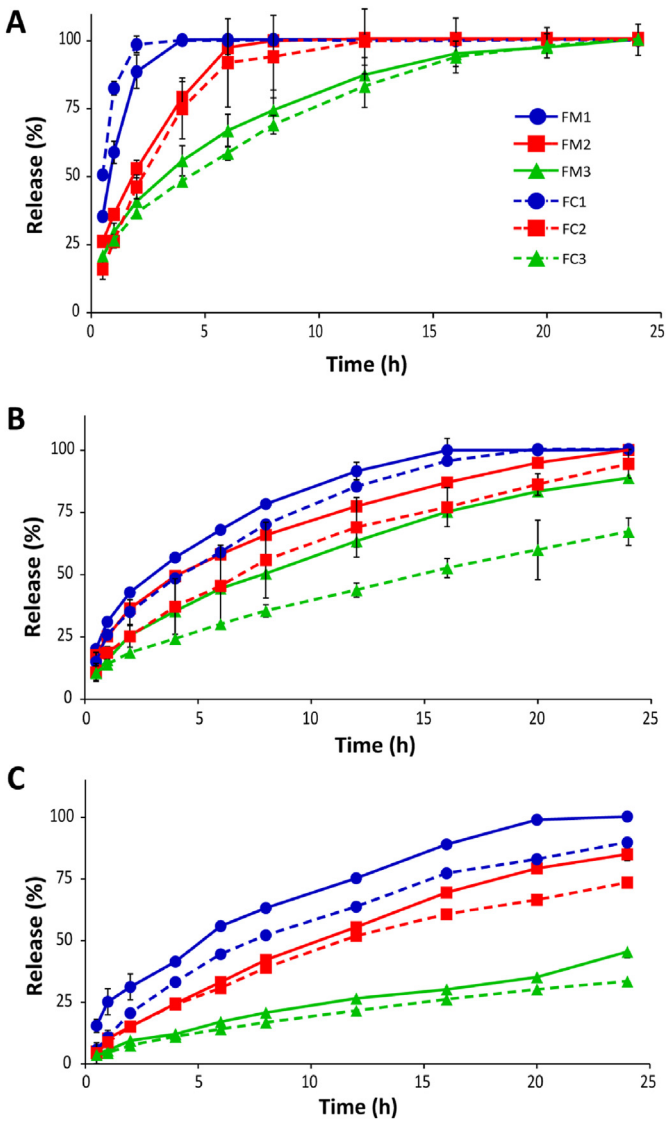

Fig. 6. Impact of formulation composition for polymer mixtures (continuous line) and co-polymers (dotted line) on the *in vitro* release of 70% w/w THA from (A) DC tablets, (B) ME tablets and (C) IM tablets.

displayed different release patterns with the highest release rate for the DC tablet, followed by the ME and IM tablet (Fig. 5B). IM tablets showed slower release due to matrix densification after tablet preparation using high temperature and pressure, leading to a lower porosity, tortuosity, and water penetration. The porosity of DC, ME and IM tablets was 19.0 ± 2.7%, 14.5 ± 1.9% and 3.2 ± 1.1%, respectively. As the processing temperature of MTF-based formulations was 30–70 °C below the melting temperature of MTF, this prevented crystallinity loss during heat processing.

DC tablets prepared using P^{n}PrOx matrix excipient showed a disintegration time of 25 ± 5 and 60 ± 15 min for MPT and MTF DC tablets, respectively. In contrast, ME and IM tablets did not disintegrate throughout the 12 h test period. DC tablet disintegration might be correlated with the low glass transition (17.8 °C) of P^{n}PrOx before heat processing, which is reflected by the first MDSC heating run (Figure S9), making P^{n}PrOx tablet rubbery at the temperature of the test medium (37 °C) and more prone to mechanical stress during disintegration testing. However, P^{n}PrOx showed a higher glass transition after heat treatments which is reflected by the second MDSC heating run, making the polymer

in the tablet core glassy at the disintegration test temperature and less affected by the mechanical stress.

The release of the more hydrophobic THA (F8) was significantly slower and also depended on the processing technique with 76, 51, and 14% THA released from the P^{n}PrOx matrix after 24 h from DC, ME, and IM tablets, respectively. However, complete THA release from the hydrophobic P^{n}PrOx matrix was not obtained, regardless of the processing technique. THA tablets prepared by DC showed a faster release behaviour due to their higher porosity (17.9 ± 1.9%) compared to tablets formed by IM (1.9 ± 0.9%). While sustained but incomplete THA release was achieved from a DC tablet, a higher drug load (i.e. 80% w/w) enhanced the drug release rate with complete THA release after 12 h (Fig. 5C). However, the noticeable burst release of the formulation with 80% w/w THA indicated that 70% w/w THA load was the maximum concentration resulting in sustained THA release, as a higher drug load increased the release rate due to the formation of more pores in the hydrophobic matrix. In addition, a lower fraction of hydrophobic polymer resulted in easier wetting of the tablet. Moreover, DSC data indicated that THA remained mainly crystalline after processing (the crystallinity varying between 97.3 and 99.1%), regardless of the processing technique and the formulation composition.

3.3.2. *Theophylline release from P^{c}PrOx matrix*

PAOx is tuneable by modifying the side chain at the 2-substituent of the 2-oxazoline monomer; this allows to control the hydrophilicity and lower critical solution temperature (LCST) [19]. This means that they are fully soluble at low temperatures and phase separate at temperatures beyond the LCST. The polymer chains are dehydrated, collapse and establish intramolecular hydrophobic interactions above the LCST. The higher hydrophilicity of P^{c}PrOx compared to P^{n}PrOx is reflected by the higher LCST (25 °C for P^{n}PrOx and 30 °C for P^{c}PrOx), which is a consequence of the more compact arrangement of the cyclic side chain (Fig. 2). A cyclic topology makes the cyclo-propyl group's rotation much more restricted than a linear propyl group [24,48]. A complete release of THA was not achieved using the P^{n}PrOx matrix. Therefore, P^{c}PrOx (F10) was evaluated as matrix excipient to tune the release by preparing DC tablets with 70% *w/w* THA. As shown in Fig. 5C, the release of THA from P^{c}PrOx tablets was significantly faster compared to P^{n}PrOx tablets, indicating the importance of minor changes in the polymer structure that influence the polymer hydrophilicity.

3.3.3. *Theophylline release from P^{n}PrOx and PEtOx polymer mixtures and co-polymers*

As the tablets consisting of P^{n}PrOx and THA did not reach full drug release, polymer blends and co-polymers consisting of PEtOx and P^{n}PrOx were investigated to tune the *in vitro* release of THA from the tablets by incorporation of the more hydrophilic PEtOx. Firstly, the hydrophobic P^{n}PrOx was mixed with the hydrophilic PEtOx in different ratios to enhance water penetration. To investigate the impact of having a physical mixture of two polymers versus the distribution of both ethyl and n-propyl units in a single chain, co-polymers of P^{n}PrOx and PEtOx were prepared. These co-polymers were synthesized as described in the supplementary data and were formulated with 70% *w/w* THA. Table 2 summarizes the different formulations used (FC1-FC3 indicating co-polymers and FM1-FM3 indicating polymer mixtures).

Introducing 7.5% PEtOx to the P^{n}PrOx release retarding matrix (FM3) resulted in a considerable increase in the drug release whereby 100, 88 and 45% THA was released from the DC, ME and IM tablets within 24 h, respectively. Moreover, the release of THA from FC3 was 100, 76 and 33% within 24 h from DC, ME and IM tablets, respectively (Fig. 6). Interestingly, the release of THA from DC, ME and IM tablets from the polymer mixtures and co-polymer formulations with the same polymer ratios was similar with f2 values > 50. These results indicate that the enhanced solvation of EtOx units will enhance water access to the tablet leading to faster release. The similar release kinetic for physical mixtures and co-polymers indicates that the small amount of PEtOx in the physical mixtures is most likely retained in the tablet as the co-polymers are not water-soluble at 37 °C.

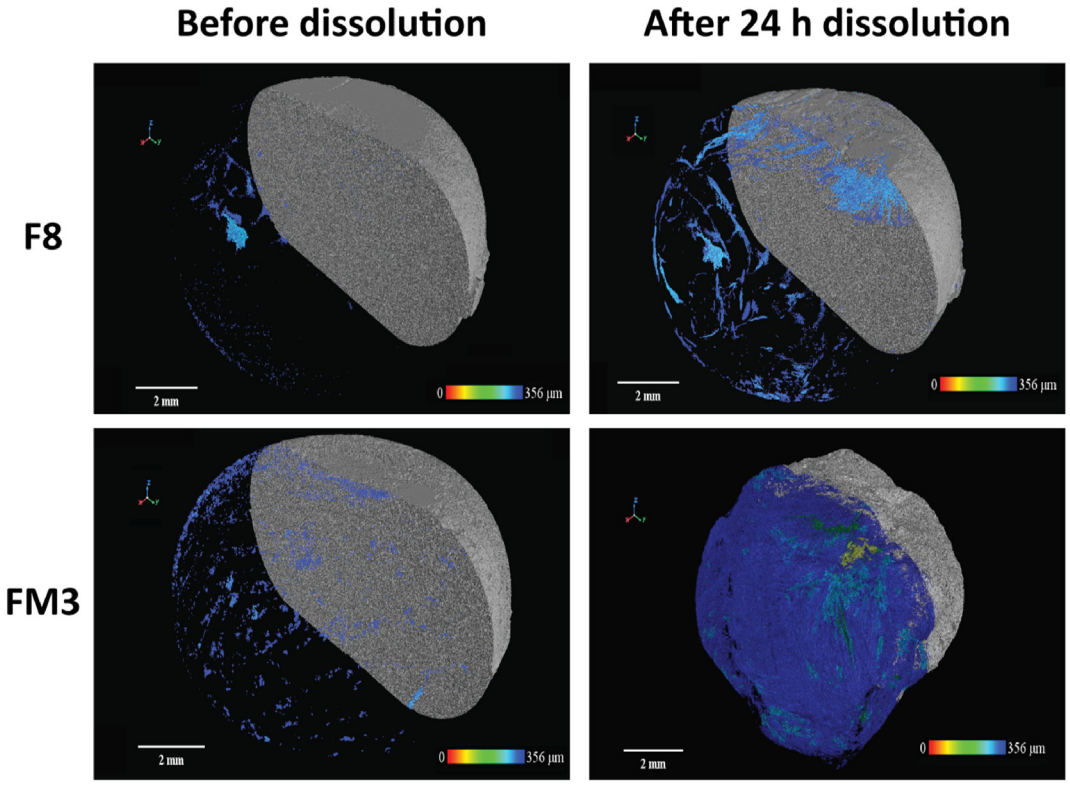

Fig. 7. µCT reconstruction of IM tablet of P^{n}PrOx:THA (30:70%, w/w) 'F8' and P^{n}PrOx:PEtOx:THA (7.5:22.5:70%, w/w) 'FM3' before and after dissolution. The grey part represents the tablet matrix, while the coloured part represents the pore distribution based on the maximum opening.

In general, THA release from formulations with higher PEtOx was faster regardless of the processing technique (Fig. 6) as the hydrophilic PEtOx enhanced hydration of the hydrophobic P^{n}PrOx matrix. PEtOx is first hydrated facilitating drug release that consequently leads to pore formation within the matrix. These channels increased the matrix permeability to the drug. This was supported by X-ray tomography images showing the pore distribution of IM tablets of F8 and FM3 before and after dissolution with a significant increase in the maximum pore opening of FM3 after 24 h dissolution (Fig. 7). The porosity increased from 0.08 to 0.27% and 0.09–11.48% for F8 and FM3, respectively.

3.3.4. P^{sec}BuOx matrix

The next series of experiments used P^{sec}BuOx as a matrix excipient to tune the release of the highly soluble APIs (MPT and MTF) that could not sufficiently be sustained with P^{n}PrOx. Since P^{sec}BuOx is more hydrophobic than P^{n}PrOx, as shown in Fig. 2, a more sustained release was anticipated for these more hydrophilic drugs. All P^{sec}BuOx-based formulations did not disintegrate over 12 h. This was due to the higher T_g (48.6 °C) compared to P^{n}PrOx (17.8 °C), making the polymer glassy at the test temperature and less prone to mechanical stress during disintegration. Firstly, the *in vitro* release of MPT from the P^{sec}BuOx matrix was found to be complete within 2, 12 and 0.5 h for DC, ME and IM tablets (Fig. 8A). Release from the P^{sec}BuOx matrix was slower from the DC and ME tablets compared to the P^{n}PrOx matrix due to the higher hydrophobicity of P^{sec}BuOx. However, the significantly faster release of the IM tablets could be ascribed to the loss of MPT crystallinity after the second heat treatment as P^{sec}BuOx had to be processed at higher temperatures compared to P^{n}PrOx-based formulation due to the higher glass transition (Table 1). IM for F3 was performed at 30 °C above the melting temperature of MPT (121 °C). As a result, the crystalline fraction of MPT significantly dropped to 40% (Table 3). On the other hand, P^{sec}BuOx exhibited an excellent ability to sustain the release of MTF (Fig. 8B) as no loss of crystallinity was observed after processing due to the higher melting temperature of MTF. The *in vitro* release of MTF was complete after 6 and 16 h for DC and ME tablets, respectively. However, a complete MTF release was not achieved within 24 h from the IM tablets. Subsequently, introducing a low concentration (6%, *w/w*) of the hydrophilic PEtOx (F6) significantly improved MTF release, as shown in Fig. 8B. Moreover, higher content (7.5%, *w/w*) of hydrophilic PEtOx (F7) was correlated with a faster MTF release from the IM tablet. This also indicated the ability to finely control the water penetration into the tablets by homogeneously blending the P^{sec}BuOx and PEtOx homopolymers, allowing fine control of the pore formation, presumably due to drug release in these high drug loading tablets. These results clearly demonstrate that the release kinetics of drugs with different aqueous solubilities can be easily steered by adjusting the ratio of physical mixtures of PAOx with different hydrophilicity in the formulation.

The IM tablets of F5 and F7 with 70% w/w MTF were selected as the most promising formulations for an *in vivo* study for which the influence of the pH change in the gastro-intestinal tract was first studied *in vitro* by evaluating the drug release in simulated gastric fluid for 2 h, followed by simulated intestinal fluid for 22 h. As demonstrated in Fig. 9, the drug release was pH-independent, and the release of MTF from and SIF was similar with an f2 value > 50.In addition, alcohol-induced dose dumping was evaluated as recommended by the EMA [49]. Co-ingesting alcoholic beverages with the medication might disrupt the sustained release mechanism of formulations and result in dose dumping and safety issues. Thus, a SIF medium containing 5, 10 and 20% (*v/v*) ethanol was used for testing the IM tablets of F5 and F7. The release of MTF in SIF with 5 and 10% (*v/v*) ethanol did not significantly differ from the release in non-alcoholic SIF media (f2 value > 50). However, dose dumping occurred using SIF with extremely high alcoholic concentration 20% (*v/v*), indicating a sharp solubility increase of P^{sec}BuOx at high ethanol concentrations. Similar findings were observed for F7 (data are not shown).

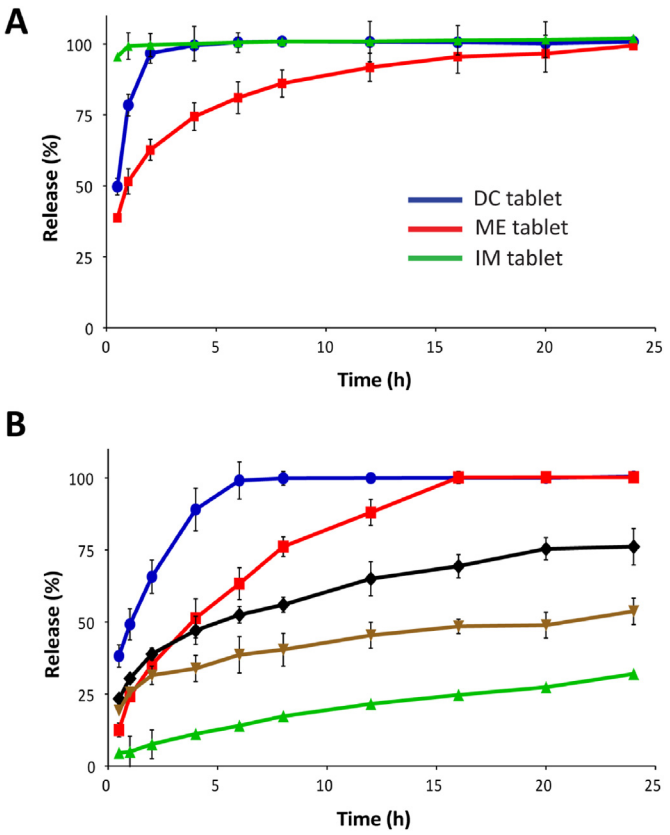

Fig. 8. *In vitro release kinetics of 70% w/w (A) MPT and (B) MTF from the P^{sec}BuOx matrix for (●) DC, (■) ME and (▲) IM tablets. The release of 70% w/w MTF from IM tablets prepared from polymer mixtures of (▼) P^{sec}BuOx:PEtOx (24:6%, w/w) 'F6' (◆) P^{sec}BuOx:PEtOx (22.5:7.5%, w/w) 'F7'.*

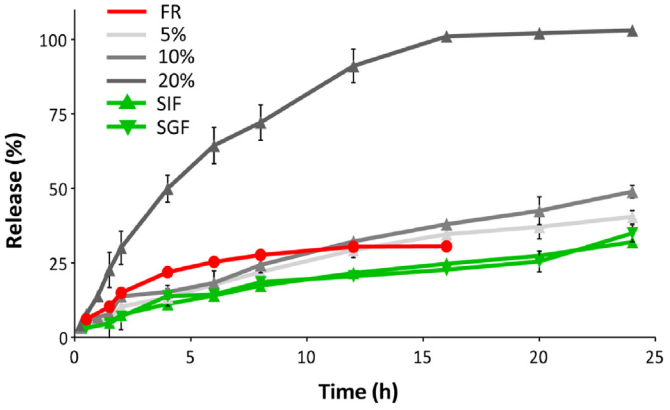

Fig. 9. *In vitro release of IM tablets of P^{sec}BuOx:MTF (30:70%,w/w/) 'F5' in (▲) SIF fluid, (▼) SGF followed by SIF, (▲)5%, (▲)10% and (▲) 20% (v/v) ethanol in SIF. In vitro release of (●)Glucophage™ SR 500 (1/2 tablet)'FR'.*

3.4. In vivo evaluation

The most promising IM tablets (Fig. 8B) were used to further investigate the *in vivo* performance of PAOx-based high drug-loading sustained release tablets. Thus, *in vivo* testing was performed on F5 and F7 containing 70% w/w MTF, using a commercially available Glucophage™ (FR) formulation previously tested by our research group as clinically approved reference [41]. The mean MTF plasma concentration-time profiles after oral administration of these formulations to dogs are illustrated in Fig. 10, while the mean pharmacokinetic parameters (AUC, C_{max}, T_{max}, $HVD_{t50\%Cmax}$ and R_D) are reported in Table 4.

Table 4
Pharmacokinetic parameters (mean ± SD, n = 3) after oral administration of F5, F7 and FR.

Formulation	C_{max} (μg/mL)	T_{max} (h)	AUC_{0-12h} (μg.h/mL)	$HVD_{t50\%\ Cmax}$ (h)	R_D (−)	Remaining MTF in tablet (%)
F5	1.3 ± 1.0[b]	5.3 ± 0.6[a]	1.0 ± 1.6[b]	10.2 ± 1.7[a]	3.2 ± 0.5[a]	55.6 ± 4.9[a]
F7	2.2 ± 1.0[a]	4.3 ± 0.6[a]	1.7 ± 1.0[a]	9.4 ± 0.9[a]	2.9 ± 0.3[a]	17.5 ± 2.8[b]
FR	2.4 ± 1.9[a]	2.8 ± 0.4[b]	1.5 ± 0.9[a]	5.6 ± 0.6[b]	1.7 ± 0.2[b]	–

[a,b] Means in the same column with different superscript are different at the 0.05 level of significance.

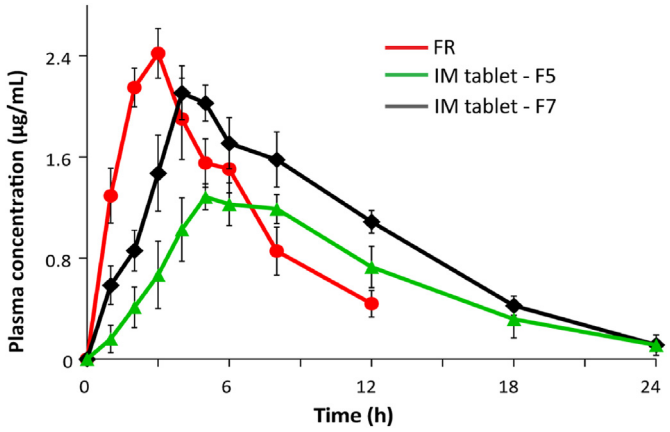

Fig. 10. *In vivo plasma concentration-time profiles after oral administration of MTF to dogs: (●) FR- Glucophage™ SR 500 (1/2 tablet), (▲) IM tablet of F5- P^{sec}BuOx:MTF (30:70%, w/w) and (◆) IM tablet of F7- P^{sec}BuOx:PEtOx:MTF (22.5:7.5:70%, w/w).*

Despite the slow *in vitro* dissolution rates of FR tablets, a faster *in vivo* drug release was observed (Fig. 10) with a mean C_{max} of 2.4 μg/mL after 3.0 h for FR. FR tablets form a surface gel layer in contact with water which has high sensitivity to the gastro-intestinal shear forces. The P^{sec}BuOx-based IM tablets (F5) revealed a C_{max} of 1.2 μg/mL after 5.3 h, whereby the slow *in vitro* release from P^{sec}BuOx formulation (F5) correlated with the low *in vivo* bioavailability, low C_{max} and incomplete MTF release. However, the addition of a small PEtOx fraction (F7) significantly increased C_{max} to 2.2 μg/mL after 4.2 h. The addition of PEtOx (7.5%, *w/w*) to the P^{sec}BuOx matrix enhanced the drug release from the PAOx matrix and significantly improved the *in vivo* bioavailability. The PAOx-based tablets were still intact after 24 h and could be recovered from the faeces, containing 56 and 18% of the MFT content in case of F5 and F7 IM tablets, respectively. In contrast, no FR tablets could be recovered from the faeces after oral administration, indicating that tablets were eroded by the gastro-intestinal motility. Besides, the gastro-intestinal residence time of F5 and F7 were 24 and 26 h, respectively.

The R_D values were calculated to indicate the extent of the sustained release based on the $HVD_{T50\%Cmax}$ value (3.2 h) of an immediate release formulation administrated to beagle dogs [43]. The R_D values of 1.7, 2.9 and 3.2 indicated low-intermediate, strong and strong sustained release properties of FR, F5 and F7, respectively.

4. Conclusion

PAOx are identified as promising excipient for preparing sustained-release matrix tablets of highly dosed (70% w/w drug), highly soluble model drugs, applying DC, HME, or IM as manufacturing techniques. Changing the alkyl group on the polymer side-chain to control the polymer solubility behaviour and using polymer mixtures or co-polymers significantly impacted the release rate, allowing its optimization in function of the application and the API. Polymer mixtures and co-polymers with the same polymer ratios showed similar release profiles, indicating that the PEtOx fraction did not dissolve from the physical mixture in the tablets. HME followed by IM was found to be a promising method to prepare sustained release dosage forms due to the intensive densification of the matrix. DC as a manufacturing technique also showed promising sustained-release results for the slightly soluble THA. The versatile potential of PAOx matrixes was also confirmed *in vivo,* where the sustained release properties of IM tablets were adjusted by mixing hydrophilic PEtOx with the thermoresponsive hydrophobic P^{sec}BuOx. Moreover, PAOx formulations showed superior sustained-release capacity compared to the commercially available FR sustained release formulation. We expect this system to be extended to different drugs and predict a dynamic future for using these polymers in sustained-release oral drug formulation.

Credit author statement

Aseel Samaro: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, **Maarten Vergaelen**: Conceptualization, Investigation, **Martin Purino**: Investigation, Writing – review & editing, **Ali Tigrine**: Investigation. **Victor R. de la Rosa**: Conceptualization, Writing – review & editing. **Niloofar Moazami Goudarzi**: Investigation, **Matthieu N. Boone**: Conceptualization, Writing – review & editing, **Valérie Vanhoorne**: Conceptualization, Writing – review & editing, Supervision, Funding acquisition, **Richard Hoogenboom**: Conceptualization, Writing – review & editing, Supervision, Funding acquisition, **Chris Vervaet**: Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Chris Vervaet has patent #US2021/015926A1 pending to Universiteit Gent.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mtbio.2022.100414.

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