Journal of Controlled Release 319 (2020) 416–427

Contents lists available at ScienceDirect

Journal of Controlled Release

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

BEPO®: Bioresorbable diblock mPEG-PDLLA and triblock PDLLA-PEG-PDLLA based *in situ* forming depots with flexible drug delivery kinetics modulation

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ARTICLE INFO

Keywords:
PEG-PDLLA block copolymers
In situ forming depot
Bioresorbable
Long acting injectable

ABSTRACT

This article presents BEPO®, an *in situ* forming depot (ISFD) technology mediated by a solvent-exchange mechanism. The matrix of the *in situ* formed drug delivery depot is composed of the combination of a diblock (DB) and a triblock (TB) polyethylene glycol-polyester copolymer. This combination offers a broad capability to tune the release of a wide variety of drugs to the desired pharmacokinetics. The work described in the present article demonstrates that the delivery rate and profile can be adjusted by changing the composition of either TB or DB or the relative ratio between them, among other parameters. It has been shown that the polymeric composition of the formulation has a substantial impact on the solvent exchange rate between the organic solvent and the surrounding aqueous medium which subsequently determines the internal structure of the resulting depot and the delivery of the therapeutic cargo. This has been demonstrated studying the *in vitro* release of two model molecules: bupivacaine and ivermectin.

Formulations releasing these drugs have been administered to animal models to show the possibility of delivering therapeutics from weeks to months by using BEPO® technology.

1. Introduction

The interest around long acting injectables (LAI) for chronic or long-term treatments has grown exponentially during the last decades [1–3]. LAI present several significant clinical advantages compared to oral therapies such as enhanced compliance to treatment, avoidance of first pass metabolism, significantly higher bioavailability for poorly water-soluble drugs and, in some particular cases, improvement of functional outcomes [4–6]. Overall, this has been shown to impact positively the patients through improvement of quality of life. On top of it, LAI avoid the escape from the treatment by the patients, which is especially relevant in indications where the lack of adherence to the oral treatment may lead to incapacity and loss of autonomy [7]. Furthermore, contrary to non-bioresorbable releasing implants [8,9], bioresorbable LAI do not require a surgical excision to remove the drug releasing system once the delivery of the active pharmaceutical ingredient (API) is complete. These LAI utilize bioresorbable polymers to control the drug release after parenteral administration.

Commercially available LAI exist in many different therapeutic areas, involving a wide variety of controlled drug delivery technologies. Some are based on engineering the API physico-chemical characteristics by developing either poorly soluble salts of the API (*e.g.* olanzapine pamoate [10]) or making a poorly soluble prodrugs of the active substance (*e.g.* paliperidone palmitate [11], fluphenazine decanoate [12]). This technology may be combined with reducing crystal particle size to allow acceptable injectability while matching the desired release pattern. After parenteral administration, generally intramuscular, the release mechanism that governs the slow systemic absorption of the API is driven by either slow dissociation of the salt form or by the hydrolysis of the prodrug. While relatively simple and straightforward, these approaches are limited to a reduced number of molecules with the appropriate physicochemical characteristics compatible with this formulation procedure. In addition, regulatory challenges associated with the development of a prodrug, as a new chemical entity, are a limiting factor that make this approach riskier and more complex than reformulating existing APIs.

Other technologies rely on the encapsulation of the API within bioresorbable polymeric microspheres, typically made of aliphatic polyesters like the widely used poly lactic-*co*-glycolic acid (PLGA), which are then suspended into an aqueous vehicle prior to

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https://doi.org/10.1016/j.jconrel.2020.01.022
Received 17 September 2019; Received in revised form 28 December 2019; Accepted 9 January 2020
Available online 10 January 2020

administration (*e.g.* risperidone [13] or naltrexone [14] loaded microparticles). Particle size, polymer:API ratio, average molecular weight, lactic acid:glycolic acid (LA:GA) molar ratio and end chains functionality are parameters that can significantly impact the API release kinetics from the microspheres. However, the dose loading limitation, the need for reconstitution before administration or the manufacturing complexity including the use of large amounts of toxic solvents and subsequently high cost of production have prevented a widespread use of this technological approach [15]. Both slow dissolving salts and esters and polymeric microparticles can achieve very long-term release duration, up to several months, but the removal of the drug reservoir in case of emergency may not be possible [16,17].

Another promising technology relies on the utilization of crystalline lipid-based self-assembling gels [18], that allow a flexible design of the release profile by modulating several formulation parameters. Upon contact with physiological fluids, typically in the subcutaneous environment, the lipid solution spontaneously self-assembles into a gel-like depot, which effectively encapsulates the API. The drug is slowly released as the liquid crystalline matrix gradually degrades in the tissue. However, according to the literature, very long release durations have not been reported when using these lipidic formulations [19].

Another alternative route for the development of LAI is the formulation of solvent-exchange *in situ* forming depots (ISFD), also known as *in-situ* forming implants (ISFI) [20]. These systems are based on the incorporation of the API within an injectable bioresorbable matrix that will act as a drug reservoir upon parenteral administration [21–23]. Drug delivery systems based on solvent-exchange ISFD consist in either solubilizing or suspending the drug within an organic solution of a bioresorbable polymer using a biocompatible organic solvent. Upon contact with an aqueous medium, the solvent diffuses out and the polymer, designed to be insoluble in water, precipitates resulting in the physical entrapment of the API [24]. The drug is then released by diffusion and progressive degradation of the polymers. Based on this technological approach, drug products have been formulated using solutions of PLGA or amorphous poly-D,L-lactic acid (PDLLA) in N-Methyl-2-pyrrolidone (NMP) which yield polymeric depot drug reservoirs after injection [25,26]. This technology has been exploited clinically with a limited number of molecules, which might be explained by the relatively low number of variables that can be used to tune the release profile and duration of drugs. Additionally, PLGA based delivery systems are well known to generate acidic microenvironments due to the accumulation of polymeric degradation products within the polymeric matrix which may result into stability issues of the encapsulated therapeutic molecule [15,27]. Other polymers such as esterified sugar derivatives have also been used to formulate solvent exchange ISFD, but according to literature, drug loading is limited and very long-term release durations have not been published with the latter technology [28].

This article presents BEPO®, a solvent exchange ISFD based technology which overcomes the main limitations of commercially available LAI in terms of drug loading capability, release duration and control over the release kinetics. On top of it, it is possible to formulate ready-to-use products with BEPO® therefore avoiding complicated and difficult-to-reproduce reconstitution processes necessary for some of the technologies described above. BEPO®, developed and patented by MedinCell [29], utilizes the combination of (methoxy) polyethylene glycol ((m)PEG) –PDLLA block copolymers, specifically a diblock (DB) and a triblock (TB), for tuning the release characteristics of a wide variety of drugs, including macromolecules [30]. Several formulation parameters can be adjusted for achieving the target product profile, such as the composition and molecular weight of the DB and TB and their relative ratio, the weight fraction of PEG and PDLLA within each of the copolymers or the relative ratio between API, polymer and solvent. BEPO® is currently being used for formulating drug products with APIs with different physicochemical properties and is undergoing clinical trials for both systemic and targeted delivery of small therapeutic molecules. Its biocompatibility and bioresorbability in the subcutaneous and intraarticular environments have been demonstrated in several animal species during the preclinical studies allowing the initiation of clinical trials with this technology through these administration routes [31].

The work described in this article aims to highlight the flexibility and outstanding drug delivery capabilities of BEPO® in terms of control of the release profile and duration. Additionally, the physicochemical phenomena linked to the solvent exchange leading to different release kinetics during the formation of the depots have been explored. With this aim, two model APIs selected for their different aqueous solubility and lipophilicity, namely ivermectin and bupivacaine, have been formulated using different BEPO® formulations. Their *in vitro* release, together with the delivery of organic solvent, have been monitored. The structure of the resulting depots has been studied by X-ray microtomography and environmental scanning electron microscopy to investigate whether structural features may be related with the *in vitro* release profiles obtained. Eventually, formulations with these APIs have been tested *in vivo* by monitoring the pharmacokinetics of the drugs in animal models after subcutaneous administration.

2. Materials and methods

DB mPEG-PDLLA and TB PDLLA-PEG-PDLLA copolymers were either synthesized by MedinCell or by CM Biomaterials (Tucker, GA, USA). USP grade dimethyl sulfoxide (DMSO) was purchased from Gaylord Chemical (Los Angeles, CA, USA) and acetonitrile was acquired from Carlo Erba (Peypin, France). TB and DB used in this study are coded as TBm-n and DBs-t where m and s correspond to the molecular weight, in kDa, of the PEG and mPEG of TB and DB respectively and n and t correspond to the molecular weight, in kDa of the total PDLLA within the copolymer. For instance, TB2–11.5 stands for a triblock copolymer with 2 kDa PEG and 11.5 kDa PDDLA; DB0.35–4.85 stands for a diblock copolymer with 0.35 kDa PEG and 4.85 kDa PDDLA.

Ivermectin (Mw = 875.1 g/mol; log P = 4.4 [32]) and bupivacaine (Mw = 288.4 g/mol; logP = 3.41 [33]) were purchased from Interchim (Montluçon, France). The solubility at 37 °C of ivermectin in Krebbs-Ringer-Tris (KRT) buffer at pH = 7.4 containing 2% Tween 80 is 6.14 mg/mL and the solubility at 37 °C of bupivacaine in KRT buffer at pH = 7.4 is 260 μg/mL. The solubility in these buffers, used during the *in vitro* release tests, was determined in house by measuring the concentration of the drugs in the filtered supernatant of a saturated solution after 2 days under continuous agitation at 37 °C. The UPLC methods described in Supporting Information were used for carrying out these analyses.

All chemicals were used as received without further purification.

Formulations were coded "Fa: TBm-n DBs-t/R/z%" where a is the incremental number relative to the tested formulation; m, n, s and t correspond to the molecular weights in kDa of (m)PEG and PDDLA in the copolymers; z depicts the amount of copolymer in the formulation as % mass of the total formulation mass (*i.e.* polymer + API + solvent). R stands for the mean molar LA:EO ratio within the formulation and is calculated as follows:

$$\boldsymbol{R} = \frac{\frac{LA}{EO}TB * a + \frac{LA}{EO}DB * b}{a + b}$$

Where…

LA corresponds to the total number of lactoyl repeating units in the TB or DB;

EO corresponds to the total number of ethylene oxide repeating units in the TB or DB;

a and b correspond to the relative molar ratio of TB and DB in the polymer mixture respectively.

For example: F8, TB3–9.8 DB2–9.8/2.39/40% means that the formulation 8 was produced with a triblock with 3 kDa PEG and 9.8 kDa

Table 1
Compositions of formulations delivering ivermectin.

Formulation	Triblock	Diblock	Total polymer content (w %)	R	TB:DB weight ratio	API content (w%)	DMSO content (w%)
F1	2–11.5	2–9.8	40	3.3	5:3	7.5	52.5
F2	2–11.5	2–9.8	50	3.3	5:3	7.5	42.5
F3	2–11.5	2–9.8	30	3.3	5:3	7.5	62.5
F4	2–11.5	2–9.8	20	3.3	5:3	7.5	72.5
F5	2–11.5	0.35–4.85	40	6.54	5:3	7.5	52.5
F6	2–11.5	1–11.5	40	4.88	5:3	7.5	52.5
F7	1–9.8	2–9.8	40	4.94	5:3	7.5	52.5
F8	3–9.8	2–9.8	40	2.39	5:3	7.5	52.5
F15	2–11.5	2–9.8	40	3.3	5:3	5	55

Table 2
Compositions of formulations delivering bupivacaine.

Formulation	Triblock	Diblock	Total polymer content (w %)	R	TB:DB weight ratio	API content (w%)	DMSO content (w%)
F9	1–6.5	1–6.5	40	4	1:1	5	55
F10	1–6.5	1–6.5	40	4	1:4	5	55
F11	1–6.5	1–6.5	40	4	4:1	5	55
F12	2–11.5	2–9.8	40	3.3	5:3	5	55
F13	1–6.5	1–6.5	20	4	1:1	5	75
F14	1–6.5	1–6.5	50	4	1:1	5	45

PDDLA, a diblock with 2 kDa PEG and 9.8 kDa PDDLA; this mixture has a mean LA:EO molar ratio (R) of 2.39; the weight fraction of copolymers in the formulation is 40% *w*/w.

Tables 1 and 2 summarize the composition of ivermectin and bupivacaine formulations respectively tested in this study.

All tests described in this section were carried out in triplicate (n = 3) unless otherwise indicated. All results are shown as the average ± standard deviation unless otherwise indicated.

2.1. Preparation of bupivacaine and ivermectin formulations

The necessary amounts of TB and DB copolymers were weighed in a vial. The exact weight of DMSO was added to the copolymers. The vial was then closed and left under stirring on a roller-mixer at room temperature until complete dissolution of the copolymers.

In parallel, ivermectin or bupivacaine were mixed with DMSO on a roller-mixer at room temperature in a separate vial to prepare solutions of known concentrations.

Polymer and API solutions were mixed in a pre-determined ratio and left overnight on a roller-mixer to yield homogeneous formulations with the desired composition of copolymers, API and solvent. Formulations were kept at room temperature until utilization or analysis.

2.2. Bupivacaine and ivermectin in vitro release tests

In vitro release tests were carried out to determine the effect of changing several parameters of the formulations on the release kinetics from the resulting depots of two model molecules: bupivacaine and ivermectin.

0.3 mL of formulation was injected into half of a 000 gelatin capsule which was transferred into 40 mL of release buffer at pH = 7.4 KRT containing 2% Tween 80 for ivermectin and pH = 7.4 KRT for bupivacaine. Once the onset of polymer precipitation had occurred upon contact with the aqueous buffer (*ca.* 5 min), the depot together with the buffer were transferred into an Erlenmeyer with additional 160 mL of buffer. The closed Erlenmeyers were kept at 37 °C under continuous orbital shaking. The capsules, which dissolved after few minutes in the buffer, were used to minimize the variability of the morphology of the resulting depots upon copolymer precipitation.

At given timepoints, 2 mL of the release medium were withdrawn and kept for further analysis and the rest of the release medium was renewed with fresh buffer. Samples were kept at 4 °C until analysis.

Ivermectin could not be detected in pH = 7.4 KRT at 37 °C, without surfactant. Therefore, it was necessary to add Tween 80 to the medium for ivermectin *in vitro* release tests in order to ensure sink conditions, *i.e.* concentration of the drug in the release medium lower than one third of the saturation concentration according to the equilibrium solubility of each API in the buffer at 37 °C, during the full duration of the experiment. Sink conditions were also kept during *in vitro* release tests with bupivacaine.

The concentration of bupivacaine or ivermectin in the release medium was determined by RP-UPLC following the methods summarized in Supporting Information. Samples were filtered through a 0.2 μm hydrophilic filter prior to analysis.

In order to determine the amount of API remaining in the depot at the end of the *in vitro* release tests, depots were recovered from the medium and dissolved during one hour in a mix of acetonitrile and water (3:1 ratio for ivermectin and 3:7 for bupivacaine; v:v). Thereafter, solutions were filtered and analyzed by UPLC with the methods described in Supporting Information.

2.3. Quantification of DMSO in the release medium

DMSO was quantified in the release medium in order to investigate the effect of formulation parameters on the solvent exchange process, which leads to the formation of the depot upon injection in an aqueous environment.

Similar setup and procedure to those used for *in vitro* release tests were used. DMSO was quantified by RP-HPLC using the method detailed in Supporting Information.

2.4. Characterization of the depots

All depots used for characterization were prepared using 0.5 mL of formulation and the same procedure as that detailed above for *in vitro* release tests.

2.4.1. X-ray microtomography

X-ray microtomography (μCT) was used to analyze the internal structure of dry depots, in order to observe the pores within the bulk structure.

It was necessary to dry the depots for carrying out this characterization. For this purpose, at pre-determined timepoints, depots were transferred from the release medium to an empty glass vial and subsequently frozen at −80 °C for 12 h. The depots were then lyophilized for 48 h using a Cosmos-80 Lab Bench freeze-dryer (Cryotec, Saint-Gély-du-Fesc, France). Samples were kept at 4 °C until analysis.

Artifacts in the form of large cracks may be generated in the depots as a result of freezing and lyophilization. However, previous internal comparative analyses performed at MedinCell with non-dried and dried depots by ESEM and SEM respectively have demonstrated that the inner structure is not affected by the drying process.

Only one depot per composition was analyzed. Analyses were performed using an EasyTom 150 kV from RX Solutions. Depots were placed in specific tubes with appropriate diameter (2 or 3 cm) and analyzed. Main scanning parameters were:

Source voltage: 40 kV.

Frame-rate: 2.5 F/s for 2 cm tube / 3.5 F/s for 3 cm tube.

Intensity: 250 μA.

Final resolution (voxel size): 12 μm for 2 cm tube / 18 μm for 3 cm tube.

Focal spot size: 8 μm.

Filter: Carbon.

Xact software was used to reconstruct cross-section images from the cone-beam X-ray projections.

2.4.2. Environmental scanning electron microscopy

Environmental scanning electron microscopy (ESEM) was carried out to observe in detail the internal structure of the polymeric depots.

Only one depot per composition was analyzed. The surface and a cross section of wet depots were observed using a FEI Quanta 200 FEG Environmental Scanning Electron Microscope. Wet depots were fragmented by using a cutter blade, fragments with direct contact with the blade were discarded. One fragment was then placed on the Peltier module and recovered with a water drop prior to observation. Several areas of the samples were observed.

It must be noted that there was no drying step of the depots prior to the ESEM analysis.

2.5. In vivo studies

5.8 mL or 11.6 mL (delivered through 2 injections of 5.8 mL each) of F1 formulation containing ivermectin were injected subcutaneously in the upper shoulder areas of 4 young cows of *ca.* 250 kg each. At given timepoints, up to a year, 4 mL of blood were withdrawn from the jugular vein and transferred into tubes containing K_2EDTA. Samples were then centrifuged to recover the plasma which was stored in polypropylene tubes at −80 °C until analysis. The *in vivo* phase was performed in Cameroon by the Research Foundation in Tropical Disease and the Environment. Quantification of ivermectin in plasma was performed by Laboratorio de Análisis Echevarne, Spain.

1.3 mL of F9 formulation containing bupivacaine was injected subcutaneously in the interscapular area of 4 beagle dogs of *ca.* 10 kg each. At given timepoints, up to 14 days, 1.5 mL of blood was withdrawn from the jugular vein and transferred into tubes containing K_2EDTA. Samples were then centrifuged to recover the plasma which was stored in polypropylene tubes at −80 °C until analysis. The *in vivo* phase and the quantification of bupivacaine in plasma were performed by Amatsi group, France.

3. Results

The manufacturing process described in this article allowed to obtain copolymer based injectable ISFD solutions or suspensions of ivermectin and bupivacaine. A polymeric depot was formed upon contact of these solutions or suspensions with an aqueous medium due to the exchange of organic solvent (DMSO in this particular case) and water, which led to the precipitation of the copolymers. This process is illustrated in Fig. 1, which displays the formation of a depot from a solution of copolymers in DMSO upon injection in a buffered medium.

Fig. 1. Solutions of copolymers in DMSO (left) and depot formation upon injection in an aqueous buffered medium (right).

3.1. Bupivacaine and ivermectin in vitro release tests

The release of ivermectin and bupivacaine from depots of formulations with different copolymer content is shown in Fig. 2. Fig. 2A shows the release of ivermectin from depots of formulations with different copolymer content, while keeping the TB:DB ratio (5:3 w:w) and therefore the mean molar LA:EO ratio (3.3) constant; API content was the same in all formulations (7.5%). The higher the polymer content, the slower the release rate, which was more obvious during the first two weeks of delivery. Depots made from F2 released close to 50% of the initial dose whereas depots derived from F4 released almost the whole drug cargo (*ca.* 80%) after 14 days of release. A similar correlation between polymer content and release kinetics was obtained from depots of formulations with different polymer content delivering bupivacaine, as displayed in Fig. 2B: in this case too, the higher the polymer content, the longer the *in vitro* release.

The release of ivermectin from depots of formulations using different TB and DB copolymers is illustrated in Fig. 3. All formulations had the same copolymer content (40%) and a fixed TB:DB ratio (5:3 w:w) as well as the same API content (7.5%). Fig. 3A displays the influence of varying the DB copolymer on the cumulative release of the drug and Fig. 3B shows the specific influence of changing the TB copolymer composition. In both cases, the copolymer composition used in the TB:DB mixture affected the release kinetics of ivermectin. A rank order relationship in between R (mean LA:EO ratio in the formulation) and release kinetics ranking cannot be established; while a higher R corresponds with a slower release in formulations depicted in Fig. 3B, this is not the case in those of Fig. 3A.

Fig. 4 shows the release of bupivacaine from depots of formulations with the same TB and DB but different TB:DB ratios (4:1, 1:1 and 1:4 w:w for F11, F9 and F10 respectively). In this particular case, the TB and DB had the same (m)PEG and PDDLA molecular weights. Consequently, the R value of these formulations (*i.e.*, the mean molar LA:EO ratio) is constant and equals 4. Different release kinetics were obtained by combining different amounts of TB and DB, with slower delivery obtained with higher DB content. These results show that despite R value and total polymer content being constant, drug release rate could be controlled by varying the TB:DB ratio, which highlights the specific contribution of each block copolymer in the formulation.

Fig. 5 displays the delivery of bupivacaine (blue) and ivermectin (red) from BEPO® formulations having the same composition in terms of TB and DB type and ratio, polymer (40%) and API (5%) content; the same volume of formulation was used. As detailed above, the release media were different for bupivacaine and ivermectin for allowing sink conditions during the study. It can be observed that the release of bupivacaine is substantially quicker than that of ivermectin.

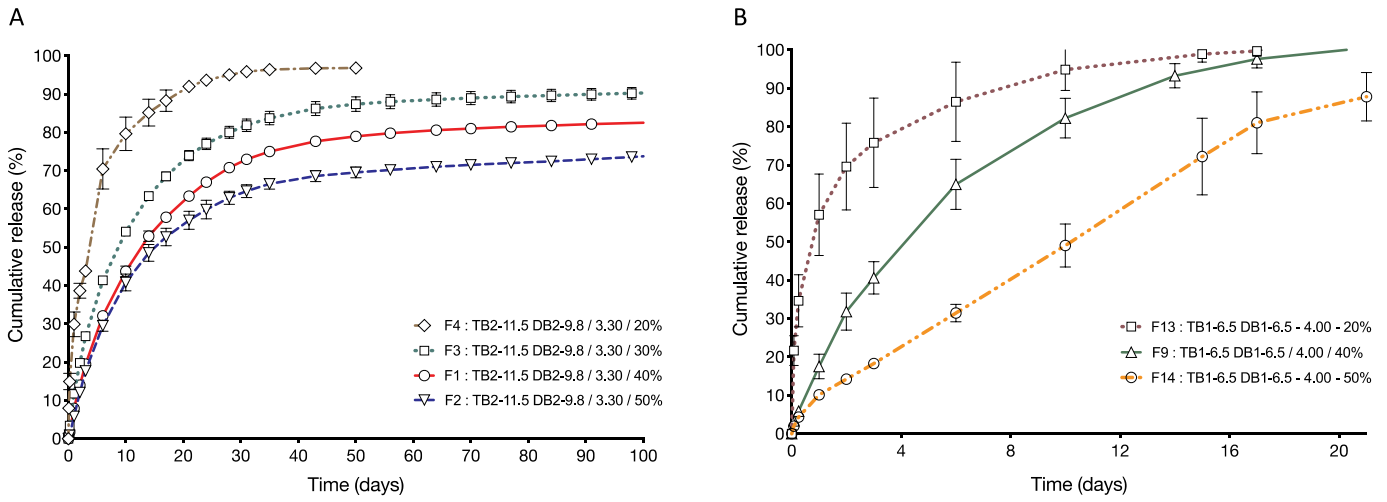

Fig. 2. Cumulative release of ivermectin (Fig. 2A) and bupivacaine (Fig. 2B) from depots of formulations with constant API content and TB:DB combination and ratio but different total copolymer content. Results are shown as average ± standard deviation (n = 3).

Ivermectin formulations with either different polymer content keeping the same DB and TB type (F1, 40% and F4, 20%) or the same polymer content (40%) and different TB or DB copolymers (F1, F6 and F8), were selected for further characterization. In all cases, TB:DB ratio and drug content were constant (5:3 w:w and 7.5% respectively). These formulations were selected as the resulting depots provide a wide range of release profiles *in vitro*, as displayed in Fig. 6. In order to explore the mechanisms driving the different release kinetics, additional characterization was performed to focus on the first two weeks of release; the difference on delivery rates was more evident during this period for all formulations.

3.2. Quantification of DMSO in the release medium

Fig. 7 displays the release rate of DMSO in the buffer medium after generating depots made from the four representative formulations depicted above. It can be observed that, the higher the R ratio at constant total polymer content (40%), which reflects the mean LA:EO ratio of the polymeric fraction of the tested formulation, the slower the release of DMSO. When comparing Figs. 6 and 7 a rank-order relationship between the release rate of DMSO in the buffer and the release rate of ivermectin in the first two weeks can be established. All DMSO had been released from F4 and F8 at the first sampling timepoint (1 day) and only a residual amount of DMSO remained in F1 and F6 after 3 days in the aqueous medium.

3.3. X-ray microtomography (μCT)

Fig. 8 shows three-dimensional reconstructions of dry depots from the model formulations after 3 or 14 days of immersion in a buffered solution. Representative sections of all analyzed depots can be found in Supporting Information. The depots present very different microstructures after 3 days of immersion in an aqueous environment. Reconstructions of depots made from F1 and F6 highlight the presence of a cavity in the core, surrounded by a matrix composed of precipitated copolymer. This cavity might be a result of an incomplete precipitation of the copolymers, which is in agreement with the presence of residual DMSO within the depots at that timepoint. The contrast is obvious with depots derived from F8, showing a homogenous structure composed of fully precipitated copolymer at 3 days, in line with the absence of DMSO in the bulk structure. Depots of F4, which has the lowest total copolymer content (20%), presented a less dense internal structure with large cracks and voids disseminated randomly in the bulk; it is important to stress out that all the DMSO had been released from this depot when the analysis was performed.

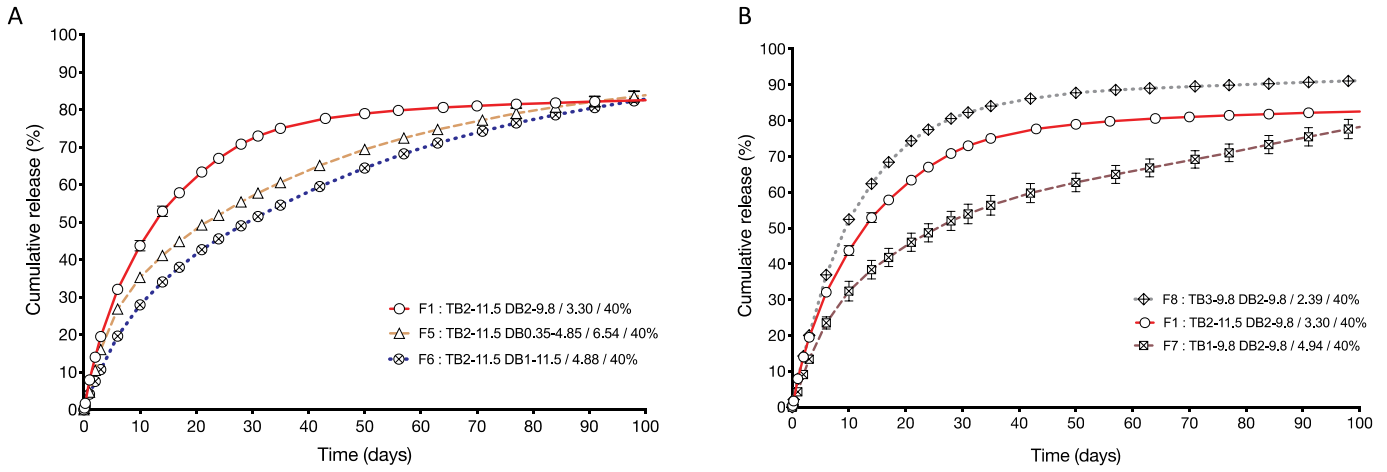

Fig. 3. Cumulative release of ivermectin from depots made with constant total API and copolymer content and TB:DB ratio but varying the diblock copolymer (A) and the triblock copolymer (B) compositions. Results are shown as average ± standard deviation (n = 3).

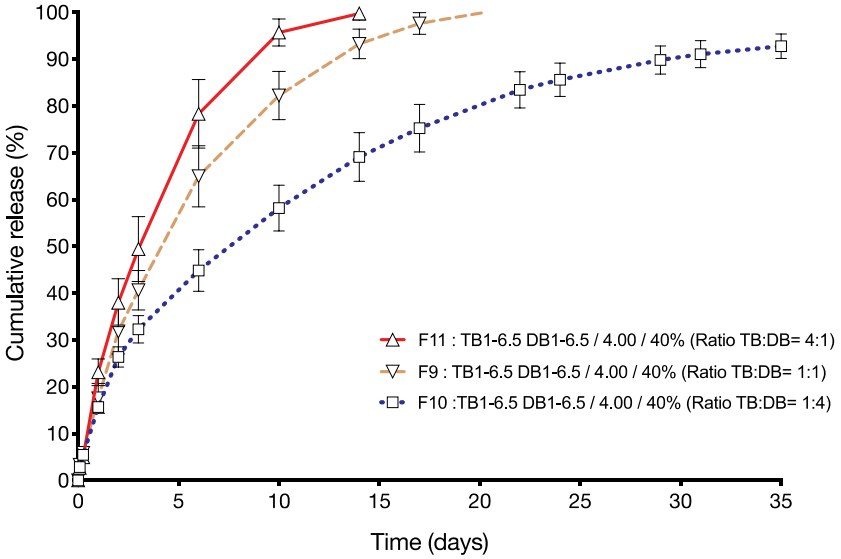

Fig. 4. Cumulative release of bupivacaine from depots made with the same combination of TB and DB and the same polymer (40%) and API (5%) contents but with different TB:DB ratio. Results are shown as average ± standard deviation (n = 3).

After 14 days of immersion in aqueous medium F1, F6 and F8 present an outer shell and a distinctly different microstructure from the interior domain. Also, the inner structure appears more or less homogeneous, the latter characteristic being largely influenced by the content of polymer (*i.e.* F4 with 20% polymer exhibits a relatively loose inner structure compared to other formulations). At this timepoint, DMSO had been completely exchanged with aqueous buffer in all depots.

3.4. Environmental scanning electron microscopy (ESEM)

Fig. 9 displays representative ESEM images showing both the surface (A) and the cross-section (B) of a depot of F1 after 14 days of immersion in an aqueous medium. F1 was selected with the aim of observing in detail the different porosities of the inner part and the outer shell revealed by μCT analysis of depots from formulations with 40% polymer content (F1, F6, F8).

ESEM analysis confirmed that two types of pores can be distinguished: finger-like, perpendicular pores to the surface in the outer layers and pores assembled as a honeycomb structure in the inner region of the depot. The range of pore sizes of the inner phase was estimated to be from the images in between 7 and 20 μm.

3.5. In vivo studies

Based on the *in vitro* release results, ivermectin-containing formulation F1 and bupivacaine-containing formulation F9 were selected to be tested *in vivo*, as they showed a limited initial burst and a release duration close to the targeted action duration.

Fig. 10 displays the plasma concentration-time profiles of ivermectin at 2 and 4 mg/kg dose in cattle after the subcutaneous administration of BEPO® F1 formulation. After an initial peak concentration (mean Cmax) of *ca.* 36.2 ng/mL for the highest dose of 4 mg/kg, mean plasma concentration declined to above 5 ng/mL, and remained at this level for a year, when the study was finished. The same pattern was obtained with the 2 mg/kg dose with a mean Cmax of *ca.* 16.3 ng/mL and a sustained concentration thereafter at *ca.* 2 ng/mL.

The pharmacokinetic profile of bupivacaine following the subcutaneous injection of BEPO® F9 formulation into beagle dogs is shown

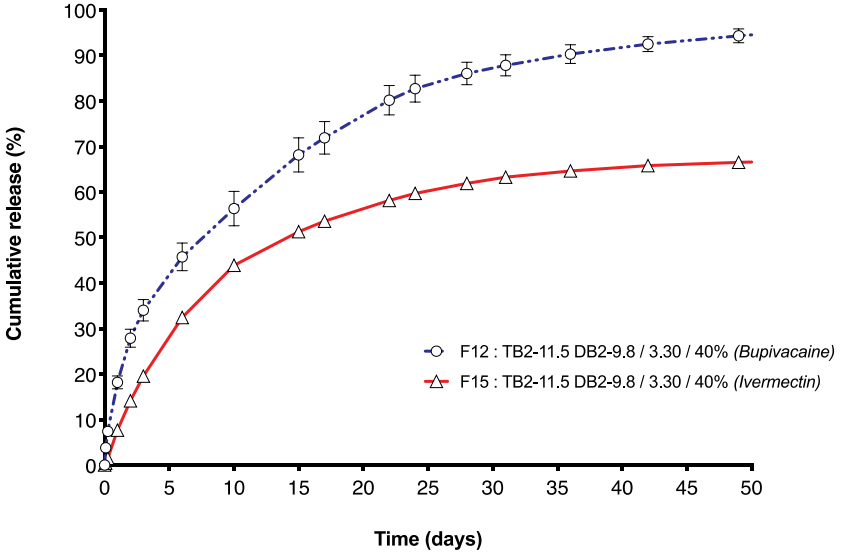

Fig. 5. Cumulative release of bupivacaine (blue) and ivermectin (red) from BEPO® formulations with the same composition. Results are shown as average ± standard deviation (n = 3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

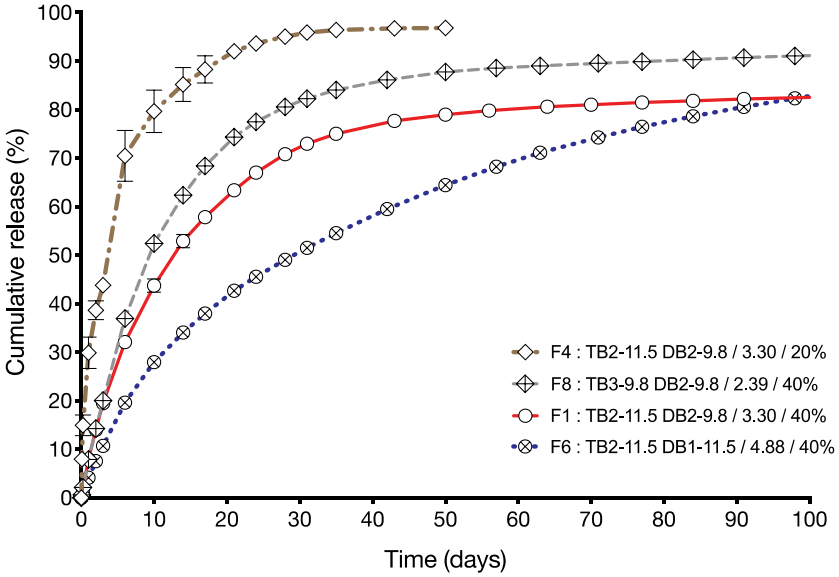

Fig. 6. Cumulative release of ivermectin from depots of BEPO® formulations with different compositions. Results are shown as average ± standard deviation ($n = 3$).

in Fig. 11. The plasma concentration-time profile shows a similar pattern to those observed for ivermectin, whereby an initial peak concentration of the drug occurs during the first days post-administration (*ca.* 150 ng/mL) associated with a rapid onset of absorption in the blood stream after subcutaneous injection. Subsequently, there is a progressive decline down to *ca.* 5 ng/mL at 14 days. Main pharmacokinetic parameters are summarized in Supporting Information.

4. Discussion

Block copolymers based on PEG-polyesters have been widely studied as functional excipients in the field of drug delivery for many different applications because of their biocompatibility, bioresorbability and relatively simple synthesis [34]. In this sense, micellar systems [35], polymersomes [36], nanoparticles [37] or thermogelling technologies [38] have been developed, with some of them reaching the clinical development. In all these systems, the average molecular weight of both PEG and polyester components as well as their relative ratio and architecture play a key role for tuning the physico-chemical properties of the resulting delivery systems and consequently for modulating the drug release kinetics. One of the aims of this study was to evaluate if, similarly to these technologies, a correlation between the hydrophobic:hydrophilic ratio within the polymeric matrix and the release kinetics could be observed in a more complex drug delivery system: BEPO®, a solvent-exchange based ISFD technology based on the combination of a DB and a TB (m)PEG- amorphous PDLLA copolymer. Additionally, the potential influence of varying several parameters correlated to the copolymers in the formulation, such as the total polymer content, the specific combination of TB and DB and the TB:DB ratio on the drug delivery kinetics was evaluated. To achieve this, the composition of BEPO® based formulations containing either bupivacaine or ivermectin was tuned and the resulting *in vitro* release of the API was monitored and evaluated.

From relatively short (*ca.* 10 days) to very long (up to 50 days with bupivacaine and over 100 days with ivermectin) *in vitro* delivery durations were obtained with the appropriate formulation composition and specific combinations of TB and DB. It is worth noticing that, for both tested drugs, the mass balance between the total released API

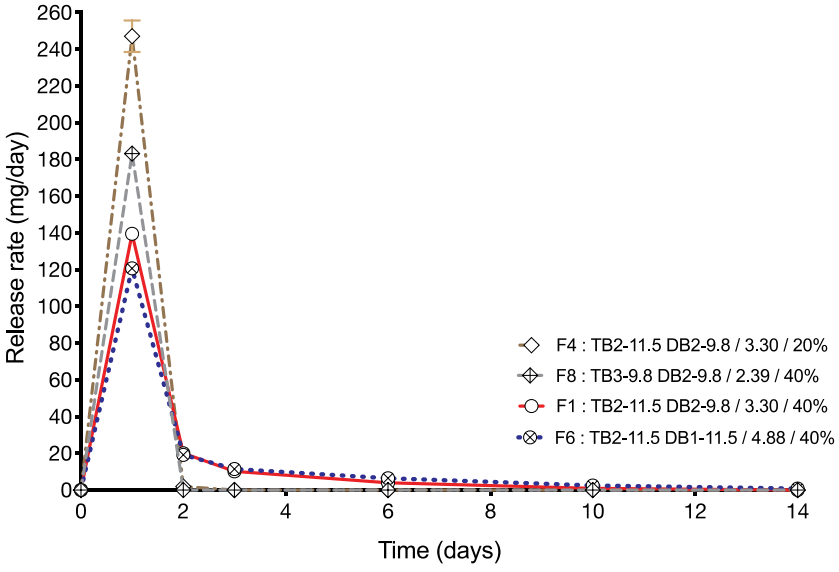

Fig. 7. Release rate of DMSO from representative depots of BEPO® formulations up to 14 days.

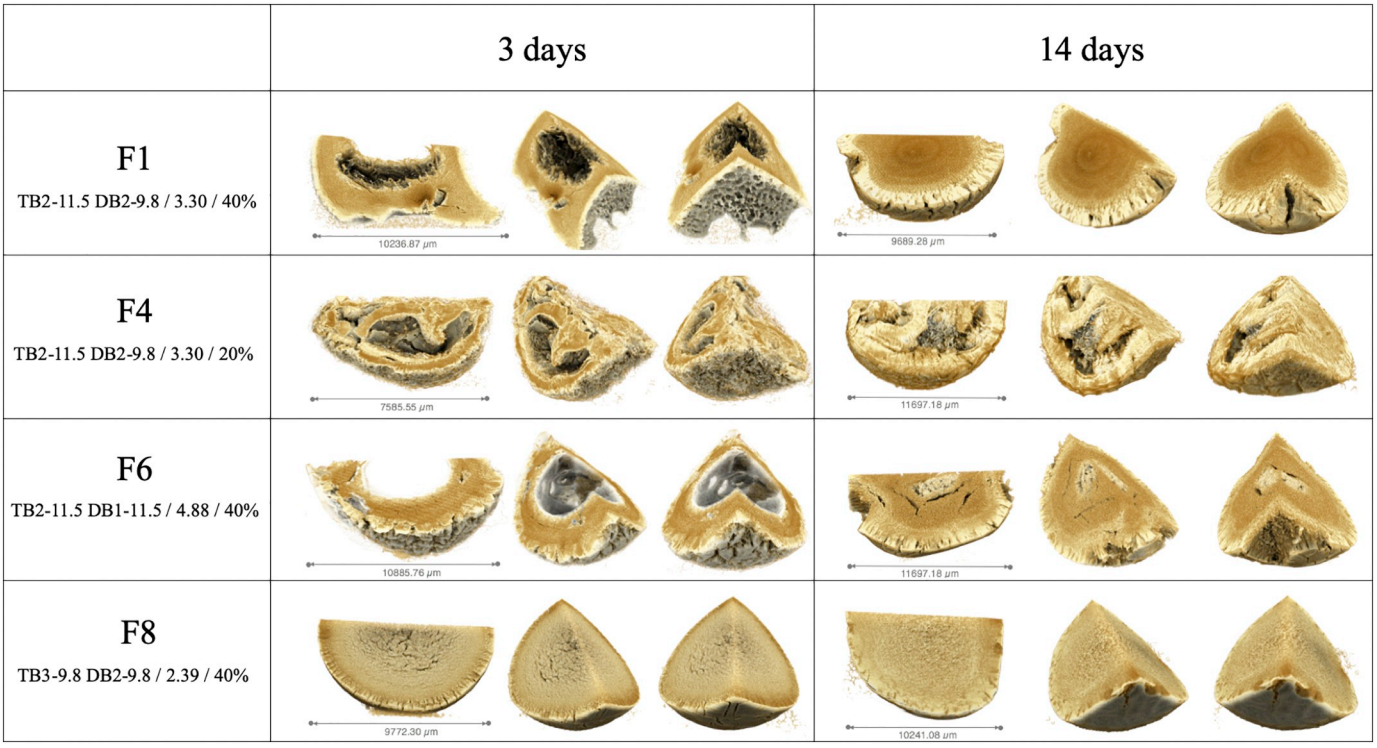

Fig. 8. X-Ray microtomography three-dimensional reconstructions of depots from representative BEPO® formulations after 3 and 14 days of immersion in a buffered solution.

added to the remaining quantity dosed in the depots at the end of the tests and the initial loaded amount of drug exceeds 92%; data can be found in Supporting Information. Specifically for ivermectin, no correlation can be observed between the release duration and the amount of recovered API at the end of the study, suggesting the long-term stability of the therapeutic molecule within the depot. For instance, 98.3% of ivermectin was recovered at the end of tests with F3 depots after 100 days of *in vitro* release. Therefore, the non-retrieved API does not seem to be related to ivermectin degradation within the depot but to losses associated to the experimental manipulations. Long-term *in vitro* release profiles of bupivacaine and ivermectin obtained with BEPO® formulations are similar to those described in the bibliography for fast-inverting ISFD technologies: an initial burst release of varying magnitude that is likely due to the release of solubilized molecules during the solvent exchange followed by a first delivery kinetics which is mainly driven by the diffusion of the drug through the forming polymeric matrix and another final kinetics further mediated mostly by the degradation of the copolymers, in this case by hydrolysis of the PDLLA within the TB and the DB [39].

An increase of the total polymer content in the formulation while keeping the rest of parameters (TB and DB type and ratio; API content) constant resulted in longer *in vitro* release duration (Fig. 2). This might be explained by the denser polymeric matrix within the resulting depot of formulations with higher polymer content. This difference in the inner structure is evident when comparing the µCT reconstructions of samples from F1 and F4 after 14 days of immersion in the release medium (Fig. 8). The denser matrix may hinder the influx of water within the depot, generate a more tortuous polymeric network and as a result of both hamper the drug diffusion. On top of it, lower water content in the inner phases of the matrix might result in a slower degradation by hydrolysis of the PDDLA blocks, which would extend the degradation-dependent late release stage [39]. *A priori* unexpected

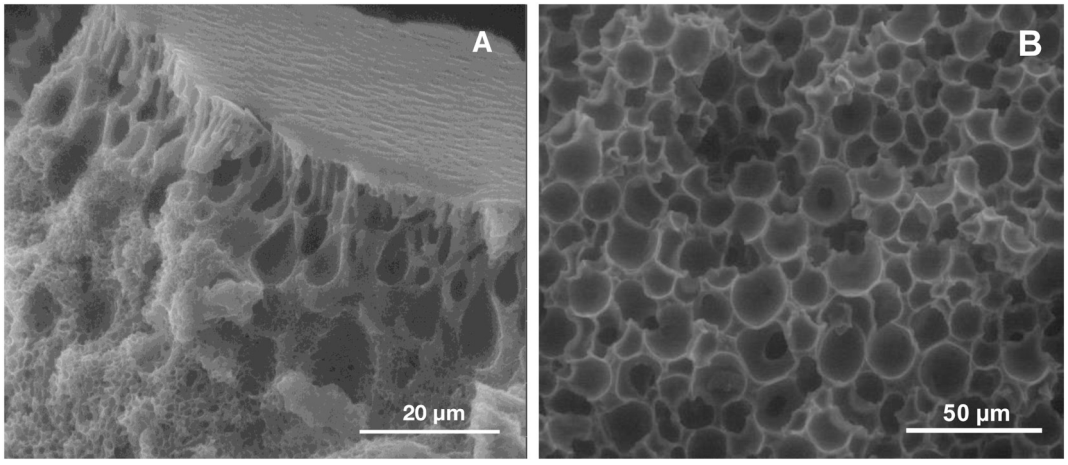

Fig. 9. ESEM images showing the surface (A) and the cross-section (B) of a depot of F1 after 14 days of immersion in a buffered medium.

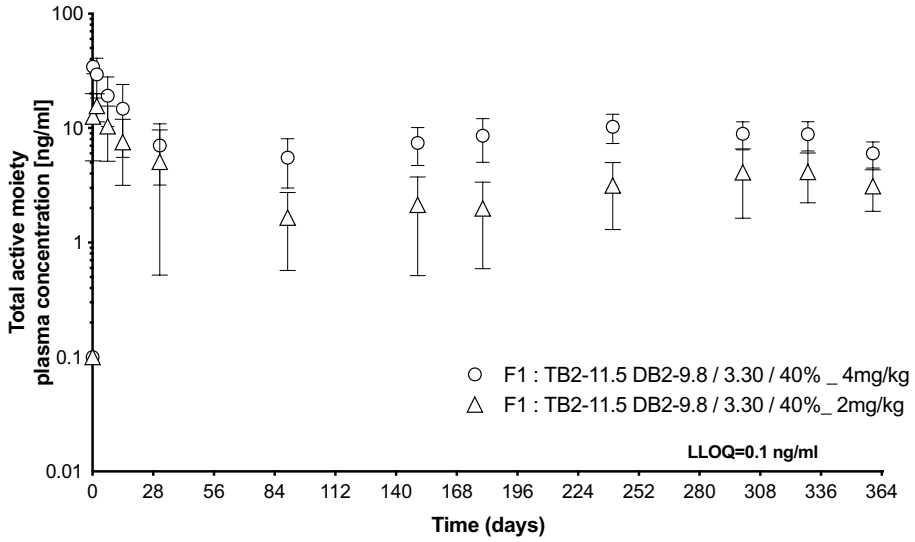

Fig. 10. Mean and standard error of the mean (SEM) plasma concentration-time profile of ivermectin in cattle (n = 4) after the subcutaneous administration of BEPO® F1 formulation at an ivermectin dose of either 4 mg/kg (11.6 mL) or 2 mg/kg (5.8 mL).

results were obtained when comparing the *in vitro* release of ivermectin from formulations with different combinations of TB and DB while keeping the rest of parameters (TB:DB ratio; polymer, API and DMSO content) constant (Fig. 3). Our results demonstrate that the utilization of formulations with a higher R, namely a higher proportion of hydrophobic LA within the polymeric matrix, does not consistently yield a slower drug release. Release kinetics of ivermectin from F5 (R = 6.54) is almost identical to that of F6 (R = 4.88). It could be expected that a polymeric network with a higher hydrophobic component would result in a slower solvent exchange, which subsequently would yield a slower precipitation of the copolymers and eventually depots with slower release kinetics [24,40,41], but this was not the case. F5 and F6 were formulated with the same TB (2 kDa PEG – 11.5 kDa PDLLA) but different DB. It was observed that the utilization of longer hydrophobic chains in the DB (11.5 *vs.* 4.85 kDa) resulted in a slightly slower release even if the hydrophilic chains were also longer (1 *vs.* 0.35 kDa). These results suggest that in BEPO® technology not only the ratio between hydrophobic and hydrophilic units within the polymeric matrix but also the length of the blocks within the copolymers will affect the delivery kinetics. This influence of the block chain length on the delivery was also observed when comparing the release of bupivacaine from formulations with different TB:DB ratio while keeping the rest of parameters (TB and DB type; polymer, API and DMSO content) constant (Fig. 4). In this particular comparative, the TB and DB of the formulations had the same (m)PEG and PDLLA molecular weight (1 and 6.5 kDa respectively) and therefore all depots had identical R (LA:EO molar ratio) = 4, independently of the TB:DB ratio. However, by changing the TB:DB ratio, different release kinetics of bupivacaine were obtained. The length of the PDLLA chains within the DB is double of that of TB, therefore it is obvious that the phase inversion and the drug delivery in BEPO® technology is influenced by additional physico-chemical parameters of the TB and DB copolymer mixture other than the mean LA:EO molar ratio within the polymeric component. Interestingly, the modulation observed in this particular test (longer

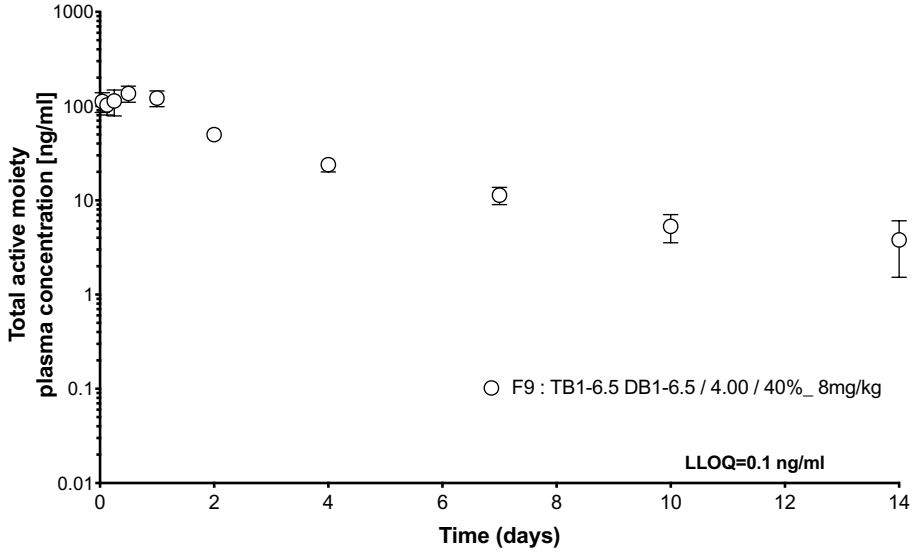

Fig. 11. Mean and standard error of the mean (SEM) plasma concentration-time profile of bupivacaine in beagle dogs (n = 4) after the subcutaneous administration of BEPO® F9 formulation at a bupivacaine dose of 8 mg/kg (1.3 mL).

hydrophobic chains slowing the release) is the opposite to another example described in the literature with PLGA in NMP based ISFD systems, where shorter PLGA chains induced a lower burst release of a model molecule [42]. On top of the characteristics of the copolymers, the inherent characteristics of the API such as its solubility in aqueous and organic media, its molar mass and its potential interactions with the copolymers are expected to have a major influence on the release kinetics. To support the latter, a comparative *in vitro* release test of bupivacaine and ivermectin from the same volume of formulations with the same composition (TB and DB type and ratio; polymer, API and DMSO content) was performed (Fig. 5). Bupivacaine was released quicker than ivermectin even if its solubility was lower than that of ivermectin in their respective release media (260 μg/mL and 6.14 mg/mL respectively). This difference in the release might be related to a number of factors: Ivermectin is more lipophilic than bupivacaine, as indicated by its higher log P, and thus it may be more prone to interact with PLA domains within the copolymers through short distance hydrophobic-hydrophobic interactions. Additionally, the presence of lipophilic ivermectin within the depot may hinder the influx of water, which may impact its delivery. Also, the larger molecular size of ivermectin (875.1 g/mol) compared to that of bupivacaine (288.4 g/mol) may lead to a slower diffusion of the drug through the resulting polymeric matrix. Based on all these observations, several factors including the optimal DB and TB combination and their relative ratio must be carefully determined for achieving the desired release profile and duration for each drug when using BEPO® technology. Additionally, other features not described in this article can be adjusted for achieving a desired drug release profile, such the type of solvent within the formulations [29,30,39].

Another aim of this study was exploring the factors that influence the first stages of the release from BEPO® technology depots, when the delivery is expected to be more dependent on the solvent exchange and the diffusion through the forming polymeric matrix rather than on the degradation of the depot. This was done by determining the DMSO exchange kinetics and analyzing the structure up to two weeks of depots delivering ivermectin with distinct release kinetics (Fig. 7). As expected, there is a direct rank correlation between DMSO and ivermectin delivery kinetics at the beginning of the study. A higher burst is observed in quicker phase exchange formulations as drug molecules are likely being delivered together with the organic solvent [43,44]. Results from this study suggest that, after the initial burst during the first 24 h, the diffusion-related drug release rate is linked to the structural properties of the polymeric depot, which are in turn correlated to the DMSO exchange kinetics. High polymer content BEPO® depots presented a heterogeneous pattern of pores, as evidenced by μCT and ESEM analyses after 14 days of immersion in the buffer: An outer layer with finger-like pores, perpendicular to the surface and an inner phase with interconnected, honeycomb-like pores (Figs. 8 and 9). This heterogeneous pore structure is known to be characteristic of fast phase inverting systems, which is expected to be the mechanism when using DMSO as a solvent once the formulation is injected in a water-rich environment. In the present study, formulations with a high polymer content and high R had residual DMSO amounts after 3 days of immersion in an aqueous medium. In these cases, the spontaneously solidified polymer layer in the outer shell forms a barrier that slows down the water-solvent exchange process in the sublayer structure [45]. It is hypothesized that this dense external polymeric layer hinders water influx into the depot which as a result also slows down the diffusion of ivermectin to the medium. Indeed, depots of F1 and F6, which presented a hollow structure after 3 days of immersion in aqueous buffer, were the ones with the slower *in vitro* release kinetics. This hollow core observed in some of the depots at early timepoints has already been described in PLGA-NMP based ISFD technologies [46] and is not present after 14 days in an aqueous medium, when the phase separation is complete as demonstrated by the DMSO quantification. The time required to complete the phase inversion process differed among the tested formulations, for which the selected combination of TB and DB and total polymer content influenced on the rate of water influx and subsequent polymer precipitation in the bulk. This behavior is similar to previously reported behaviors for solvent-exchange based ISFD; structural properties encompassing total porosity, mean pore size and size distribution, interconnectivity of the pores and presence of macrovoids, are known to be significantly influenced by the rate of solvent exchange leading to polymer precipitation [47]. After 14 days of immersion in aqueous medium the copolymer precipitation was complete and μCT and ESEM analyses suggest that depots are highly porous. Also, it was observed that the total copolymer content within the depot has a direct impact on its structural properties: lower polymer content results in less packed structures within the depots. Water influx and drug diffusion are eased in this type of structures and therefore shorter *in vitro* release durations are obtained.

According to the literature, the degradation rate of the polymeric depot will be the main factor determining the release kinetics of the drugs after the burst and the stage mainly driven by drug diffusion [39]. Very long-term release, up to several months, was aimed for formulations delivering ivermectin; therefore, depots with slow degradation kinetics were used for this particular API. Interestingly, a correlation in between the degradation kinetics of the polymeric matrix and the release of ivermectin was observed during the first 6 weeks of delivery for depots with similar polymer content (and therefore comparable internal porosity as shown in Fig. 8): The quicker the degradation of the polymeric matrix, the quicker the release of API (see Supporting Information). With BEPO®, the broad range of polymer degradation and depot erosion rates provides the possibility to formulate drug products with very different release durations. Degradation of the PEG-PDLLA copolymers by hydrolysis of the ester chains within the PDLLA can vary from several weeks to several months, depending on the composition of the formulations in terms of type of TB and DB and total polymer content. An illustrative example of this wide range of degradation kinetics can be found in Supporting Information.

F1 (delivering ivermectin) and F9 (delivering bupivacaine) formulations showed a limited burst and an appropriate delivery duration during *in vitro* release studies and therefore were selected to illustrate the flexibility of BEPO® technology in terms of drug delivery *in vivo*. A sustained release of at least two weeks was achieved for bupivacaine in dogs (Fig. 11). A very long release was achieved when administering ivermectin formulations to cattle; the concentration of the drug remained at therapeutic levels for one year after a single administration (Fig. 10). Interestingly, with ivermectin formulations, the release profile obtained at 4 mg/kg shows dose proportionality compared to the 2 mg/kg, meaning that the release rate of ivermectin was effectively controlled by the volume injected of the same formulation. The initial sampling time-points showed quantifiable concentrations of both APIs in plasma, highlighting the immediate onset of release and absorption within the blood following the subcutaneous injection. Also, an initial peak plasmatic concentration of the drug was observed, which is inherent to solvent-exchange ISFD technologies. As explained above, this phenomenon is likely associated with the delivery of solubilized molecules during the exchange of DMSO and aqueous solution from the environment, in this case the subcutaneous tissue. This might be an issue when delivering drugs with a narrow therapeutic window. However, it can be minimized by finely selecting appropriate formulation parameters such as the TB:DB couple and their relative ratio, the total polymer content or the type of solvent.

In summary, this study has demonstrated that BEPO® as a solvent-exchange ISFD technology allows the development of injectable drug products for the delivery of APIs from several days to months. The unprecedented flexibility of BEPO® is due to combination of a triblock and a diblock copolymer in the formulations, which enables the fine tuning of the depot characteristics and subsequently of its performance in terms of drug release kinetics and duration.

5. Conclusion

BEPO® is a versatile *in situ* forming depot-based long-acting injectable technology based on the combination of a diblock mPEG-PDLLA and a triblock PDLLA-PEG-PDLLA copolymer.

The present study illustrates that by varying several parameters of the formulations such as the type of triblock or diblock and their relative ratio or the total content of copolymers it is possible to fine tune the release kinetics. It has been shown that these parameters influence the solvent-aqueous medium exchange kinetics, which has a direct impact on the porous structure formed upon the precipitation of the copolymers and therefore on the drug delivery profile and duration.

The versatility of BEPO® technology has been demonstrated by providing examples of a short release of bupivacaine, limited to two weeks, and a very long release of ivermectin, up to one year, in different animal models.

Acknowledgements

3D data acquisitions were performed using the µ-CT facilities of the MRI platform member of the national infrastructure France-BioImaging supported by the French National Research Agency (ANR-10-INBS-04, «Investments for the future»), and of the Labex CEMEB (ANR-10-LABX-0004) and NUMEV (ANR-10-LABX-0020).

F. Fernandez, from the MEA Platform at *Université de Montpellier*, is acknowledged for his assistance with ESEM imaging.

P. Enyong, P. Chounna-Ndongmo, S. Pion, G. Gaudriault and M. Boussinesq are acknowledged for their assistance on cattle studies with the financial help of *Institut de Recherche pour le Développment* (IRD). The logistic help of Research Foundation for Tropical Diseases and the Environment (REFOTDE), Buea, Cameroon during the *in vivo* study is also acknowledged, specially Prof. S. Wanji and his team.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jconrel.2020.01.022.

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