Poly(caprolactone)-based subcutaneous implant for sustained delivery of levothyroxine

by Andi Dian Permana

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Poly(caprolactone)-based subcutaneous implant for sustained delivery of levothyroxine

Sarah A. Stewart [a], Juan Domínguez-Robles [a], Emilia Utomo [a], Camila J. Picco [a], Francesca Corduas [b], Elena Mancuso [b], Muh. Nur Amir [c], Muh. Akbar Bahar [c], Sumarheni Sumarheni [d], Ryan F. Donnelly [a], Andi Dian Permana [e,*], Eneko Larrañeta [a,*]

[a] School of Pharmacy, Queen's University Belfast, Lisburn Road 97, Belfast BT9 7BL, UK
[b] Nanotechnology and Integrated Bio-Engineering Centre (NIBEC), Ulster University, Jordanstown Campus, Newtownabbey BT37 0QB, UK
[c] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Universitas Hasanuddin, Makassar 90245, Indonesia
[d] Department of Clinical Pharmacy, Faculty of Pharmacy, Universitas Hasanuddin, Makassar 90245, Indonesia
[e] Department of Pharmaceutics, Faculty of Pharmacy, Universitas Hasanuddin, Makassar 90245, Indonesia

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ABSTRACT

This work aimed to develop a subcutaneous implant for prolonged delivery of LEVO to treat hypothyroidism. This could overcome challenges with patient compliance and co-administration and would improve treatment of this condition. For this purpose, implants were produced by solvent casting mixtures of poly(caprolactone) (PCL), poly(ethylene glycol) (PEG) and LEVO sodium. These implants contained mixtures of PCL of differing molecular weight, PEG and different LEVO sodium loadings (20% or 40% w/w). SEM images confirmed that the drug was evenly dispersed throughout the implant. *In vitro* release rates ranging from 28.37 ± 1.19 – 78.21 ± 19.93 μg/day and 47.39 ± 8.76 – 98.92 ± 4.27 μg/day were achieved for formulations containing 20% and 40% w/w drug loading, respectively. Implants containing higher amounts of low molecular weight PCL and 40% w/w of LEVO showed release profiles governed by zero order kinetics. On the other hand, implants containing higher amounts of high molecular weight PCL showed a release mechanism governed by Fickian diffusion. Finally, two representative formulations were tested *in vivo*. These implants were capable of providing detectable LEVO levels in plasma during the entire duration of the experiments (4 weeks) with LEVO plasma levels ranging between 5 and 20 ng/mL.

1. Introduction

Thyroid hormones (thyroxine (T_4) and triiodothyronine (T_3) play an important role within the human body in the regulation of metabolic processes which are vital for normal growth and development(Colucci et al., 2010). The thyroid gland controls the synthesis, storage, and release of thyroid hormones through a negative feedback system. When this system fails, it can result in increased (hyperthyroidism) or decreased (hypothyroidism) levels of these hormones. Hypothyroidism, the deficiency of thyroid hormones, is the most common thyroid ailment, estimated to affect over 1.3 million people in the UK and the incidence is as high as 4.2% in other countries (National institute for health and care excellence, 2019; Taylor et al., 2018; Werhun and Hamilton, 2[illegible]). The deficiency of thyroid hormones can result in debilitating symptoms, such as chronic fatigue, weight gain and cold intolerance (Alomari et al., 2018; NHS, 2018). In addition, long-term untreated hypothyroidism may result in myxoedema coma and premature death as a result of cardiovascular complications (Taylor et al., 2015; Udovcic et al., 2017).

Levothyroxine sodium (LEVO sodium) is the treatment choice for the routine management of hypothyroidism(Singer, 1995). LEVO sodium oral bioavailability is variable and can range from 40 to 80%. This is dependent mainly on the co-administration of food (Balla et al., 2015; Colucci et al., 2010). Therefore, LEVO sodium tablets should be taken at least 30 min before food, caffeine-containing drinks and other medication (Joint formulary committee, 2020). LEVO sodium has a number of interactions with other medications, many of which are manifested by the effect they have on its absorption. This is managed in practice by separating the administration times of the medications, by at least four hours (Joint formulary committee, 2020). These additional

* Corresponding authors.
E-mail addresses: andi.dian.permana@farmasi.unhas.ac.id (A.D. Permana), e.larraneta@qub.ac.uk (E. Larrañeta).

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administration instructions associated with oral LEVO sodium can often result in poor patient compliance (Rajput and Pathak, 2017). Non-compliant patients are at risk of adverse health outcomes and there is an increased cost associated with poor compliance. Hepp *et al.* reported that compliant patients used significantly fewer resources than non-compliant patients, despite having a higher drug cost (Hepp et al., 2018).

In extreme cases of non-compliance, or in cases where sufficient absorption is not achieved despite good compliance, weekly intramuscular or subcutaneous injection of LEVO sodium have successfully restored thyroid levels(Groener et al., 2013; Kwek et al., 2018; Mikhail, 2020; Taylor et al., 2015). This is due to the long half-life of thyroxine (7 days) and the slower conversion of LEVO to thyroxine at higher concentrations (Rangan et al., 2007). This highlights the potential use of the intramuscular or subcutaneous route for the delivery of LEVO sodium. However, the requirement of weekly injections is not ideal, particularly because a healthcare professional would be required to administer the treatment. An implantable drug delivery device that could deliver LEVO sodium subcutaneously for prolonged periods of time could be a potential candidate to overcome many of these drug interactions and compliance challenges. However, this would not alleviate interactions which occur by other mechanisms. A good example of this is the interaction between LEVO sodium and amiodarone (an iodine rich drug that could affect the thyroid gland)(Joint formulary committee, 2020).

Currently, limited research has been conducted to develop long-acting treatments for hypothyroidism. However, a long-acting subcutaneous implant is a promising idea for the treatment of hypothyroidism. Weekly intramuscular injections present certain limitations as described previously. An implantable system capable of providing LEVO release over prolonged periods of time (months) after a single application could improve the management of hypothyroidism. Accordingly, in this work the development of an implantable device for the treatment of this condition has been explored.

2. Materials and methods

2.1. Materials

LEVO sodium pentahydrate ≥ 99% was obtained from Enke Pharmatech Co., Ltd, (Cangzhou, China). Trifluoroacetic acid (TFA) ≥ 99%, acetonitrile for HPLC, ethanol ≥ 99.8% for HPLC, bovine serum albumin (BSA) lyophilised powder ≥ 96%, poly(ethylene glycol (PEG) (M_W 1,000) were obtained from Sigma Aldrich (Dorset, UK). Sodium azide (SA) was purchased from Fluorochem Ltd (Hadfield, UK). Poly (caprolactone) (PCL) 6506 (M_W = 50,000 Da), henceforth referred to as H–PCL, and PCL 2054 (M_W = 550 Da), henceforth referred to as L-PCL were kindly donated by Perstorp (Malmö, Sweden). Poly(lactic acid) (PLA) filament was obtained from Ultimaker B.V. (Geldermalsen, Netherlands). Dichloromethane (DCM) was obtained from Merck (Darmstadt, Germany). Fisher, Silastic® S, a silicone rubber and curing agent mix were obtained from Thompson Bros. Ltd. (Newcastle Upon Tyne, UK).

2.2. Implant development

Master moulds were manufactured from PLA using fused deposition modelling (FDM) 3D printing (Ultimaker 3 (Ultimaker B.V., Geldermalsen, Netherlands). Firstly, the granulated PLA was extruded to obtain a 2.85 mm filament using a single screw extruder (3DEvo, Utretch, The Netherlands), as described previously(Dominguez-Robles et al., 2019; Stewart et al., 2020a). Subsequently, a mixture of Silastic® S and a curing agent, in a proportion of 10:1, was poured into the 3D printed master moulds and allowed to cure overnight to obtain silicone moulds (Fig. 1A). The resulting PLA and silicone moulds can be seen in Fig. 1B.

Implants were prepared from mixtures of H-PCL, L-PCL and PEG 1,000 with a LEVO sodium loading of 20% w/w or 40% w/w. Constituents (in the appropriate ratio) were weighed out (2 g) (Table 1) and dissolved/suspended in 5 mL of DCM. To ensure the mixture was homogenous, they were mixed using a SpeedMixer™ DAC 150.1 FVZ-K (Hauschild & Co. KG, Hamm, Germany) (3,000 rpm for 20 s × 4). This mixture was poured into the silicone moulds (size of the rods 2.5 × 40 mm) and DCM allowed to evaporate at room temperature overnight (ca. 12 h). Subsequently, the rod-shaped could be removed from the mould (Fig. 1C and 1D). Implants were left for at least 12 h before being used for any experiment. The resulting implants were not cylindrical. Due to the solvent casting method one of the sizes was flat on the top.

Table 1
Composition of each of the implant formulations with either a 20% w/w or 40% w/w LEVO sodium loading.

Formulation	Composition (%)		
	H-PCL	L-PCL	PEG 1,000
H100	100	0	0
H70L30	70	30	0
H40L60	40	60	0
H35L35P30	35	35	30

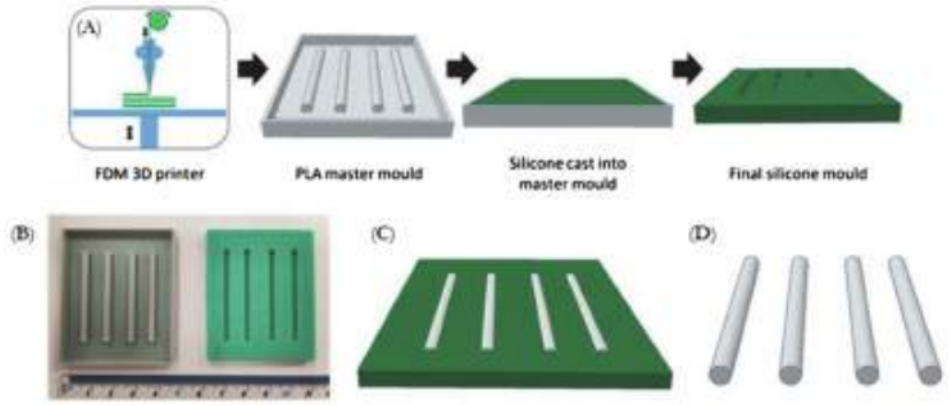


Fig. 1. Schematic diagram of mould preparation (A). Image showing the 3D printed PLA mould (left) and the silicone mould (right) (B). Schematic diagram of (C) filled implant moulds and (D) implants after removal from the mould.

2.3. Implant characterisation

Drug loading and distribution throughout the implants was visualised using SEM (Hitachi TM3030, Tokyo, Japan).

The thermal behaviours of LEVO sodium, PCL and drug loaded implants were investigated using differential scanning calorimetry (DSC). Analysis was carried out on samples of each implant formulation on a differential scanning calorimeter (DSC Q100) (TA Instruments, New Castle, USA). Samples of each formulation were heated from 0 to 250 °C at a rate of 10 °C/minute.

ATR-FTIR spectrometry was used to investigate any chemical interactions between the materials within each of the implants. A FTIR Accutrac FT/IR-4100 series (Jasco, Essex, UK) equipped with MIRacle™ diamond ATR was used at room temperature (20 °C). The IR transmission spectra were recorded between 600 and 4000 cm^{-1} with a resolution of 4.0 cm^{-1}. An average of 64 repeat scans were taken to obtain each spectrum.

LEVO sodium content of each of the implant formulations was tested. A known weight of implant was dissolved in 2 mL DCM and vortexed for 1 min. The resulting suspension was subsequently centrifuged at 10,000 rpm for 25 min. The supernatant was discarded, and the pellet was resuspended in 2 mL of ethanol. This solution was filtered and diluted as necessary and LEVO sodium content quantified using the method described in Section 2.4.

2.4. LEVO HPLC analysis.

LEVO sodium implant content analysis was carried out using RP-HPLC, on an Agilent 1220 Infinity II LC system (Agilent Technologies UK Ltd., Stockport, UK) equipped with a SphereClone™ ODS (1) C18 (5 µm particle size, 4.6 × 150 mm) (Phenomonex, California, USA). The system was equipped with a UV–visible detector. The analysis of LEVO sodium was carried out at 225 nm. A combination of acetonitrile and 0.1% TFA in water at a ratio of 65:35% v/v was used as a mobile phase. The mobile phase was degassed for 30 min using sonication. The flow rate was 0.9 mL/min and the injection volume was 50 µL. The column temperature was kept at 30 °C. Chromatogram analysis was performed using Agilent Chemstation® software version B.02.01.

LEVO sodium analysis was carried out on *in vitro* samples using RP-HPLC, on an Agilent 1220 Infinity II LC system (Agilent Technologies UK Ltd., Stockport, UK) equipped with a Zorbax Eclipse plus C_{18} column (95 Å pore size, 250 mm length × 4.6 mm internal diameter; 5 µm particle size) (Agilent Technologies UK Ltd., Stockport, UK) with guard column of matching chemistry. The system was equipped with a UV–visible detector. The analysis of LEVO sodium was carried out at 225 nm. A combination of acetonitrile and 0.1% TFA in water at a ratio of 50:50% v/v was used as a mobile phase. The mobile phase was degassed for 30 min using sonication. The flow rate was 0.6 mL/min and the injection volume was 50 µL. The column temperature was kept at 30 °C. Chromatogram analysis was performed using Agilent Chemstation® software version B.02.01.

2.5. In vitro release

In vitro release was carried out in 0.1% w/v BSA solution (with 0.05% w/v SA to prevent microbial growth) under sink conditions. BSA was added as it has been previously reported that prevents LEVO degradation in solution (Stewart et al., 2021). Sink conditions were defined as LEVO sodium concentration at or below 1/3 of its solubility (Barrett et al., 2018). The solubility of LEVO in 0.1% of BSA was measured at 37 °C: 1.66 ± 0.04 mg/mL. This value is in line with previously reported solubility data (Kaur and Suryanarayanan, 2021). Each implant was immersed in 50 mL of release medium in a watertight glass vial (37 °C and 40 rpm) for the first 49 days of release and every 3 days the release medium was analysed for LEVO sodium content (using the method described in Section 2.4) and replaced with fresh medium. After 49 days, sampling of the release medium was reduced to once weekly. Cumulative percentage drug release was calculated using Equation (1).

$$Cumulative release(\%) = \frac{\sum W_{0-t}}{W_0} \times 100 \quad (1)$$

Where, $\sum W$ is the sum of the weight of LEVO sodium release from t = 0 until T = t and W_0 is the calculated weight of drug in the implant at T = 0 according to initial drug loading.

2.6. In vivo experiments

The *in vivo* pharmacokinetic study of LEVO sodium from the implant formulations was approved by the Health Ethical Committee of the Faculty of Medicine, Hasanuddin University, Indonesia (License Number: UH20110635). Prior to the experiments, healthy male and female Wistar rats (average mass of 201 ± 8 g) were acclimated for 1-week period to the laboratory environment. The rats were divided into four groups (n = 3 per group). The first and second groups were male rats and female rats, respectively, and were implanted with H40L60 implants containing 40% w/w of LEVO. The rats in the third and fourth groups were male and female rats, respectively, and were implanted with H100 implants loaded with 40% w/w of LEVO. The implants used for the *in vivo* experiment had half of the length of the implants described before, 2.5 × 20 mm to allow an easier implantation into the animal. Therefore, LEVO loading in these implants was half of the that present in the full sized implants.

Initially, rats were sedated using ether and the hair from their dorsal was removed utilising hair removal cream. Subsequently, the hairless area was rubbed with an antiseptic solution and a 20 mm dorsal midline incision was created. Finally, the implants were inserted subcutaneously at the incised sides. To assess the plasma pharmacokinetics, blood samples were collected at 2 h, 4 h, 6 h, 8 h, 24 h, 48 h, 72 h, 1 week, 2 weeks, 3 weeks and 4 weeks after the implant administration. The blood obtained was collected into an Eppendorf tube containing 3.8% w/v of sodium citrate to prevent blood coagulation. The blood was then spun for 10 min at 4 °C at 3000 × g, obtaining the plasma samples. The plasma was stored at −20 °C prior to analysis.

LEVO was extracted from plasma samples with a simple one-step protein precipitation method using acetonitrile. In brief, 500 µL of acetonitrile was added to 100 µL plasma in an Eppendorf tube and the mixture was vortexed for 10 min. Afterwards, the samples were centrifuged for 15 min at 4 °C at 14,000 × g. The supernatant containing LEVO was collected and placed in a glass vial. The glass vial was placed into a fume hood for 3 h to evaporate the organic solvent, obtaining a dry excess. Subsequently, 100 µL of the mobile phase was added into the excess. The solution was vortexed for 10 min and centrifuged for 15 min at 14,000 × g. The supernatant obtained was injected into the HPLC columned and analysed using HPLC-UV as described in Section 2.4.

LEVO sodium analysis was carried out on *in vivo* plasma samples using an HPLC system (Shimadzu Prominence, Shimadzu, Kyoto, Japan) equipped with a Xselect CSH™ C_{18} column (Waters, 150 × 150 mm) with the particle size of 3.5 µm. The mobile phase used was the combination of 0.1% v/v of TFA in water and acetonitrile at a ratio of 65:35% v/v. The detection of LEVO was performed using a UV detector at 225 nm, with an injection volume of 20 µL and a flow rate of 0.6 mL/min at 25 °C. Chromatogram analysis was performed using with Shimadzu LC solution software (ver. 1.21 SP1).

PK Solver software was used to calculate pharmacokinetic profiles of LEVO from implant formulations, applying non-compartmental pharmacokinetic analysis In each case, the curve constructed from drug concentration in the plasma versus time of sampling was created. The pharmacokinetic profiles measured in this study were the maximum drug concentration (Cmax), the maximum concentration time (tmax), the drug concentration time curve from time zero (t = 0) to the last experimental time point (t = 4 weeks) (AUC0-4 week), the drug concentration time curve from time zero (t = 0) to infinity (AUC0-INF) and the mean half-life (t1/2).

2.7. Statistical analysis

Statistical analysis was performed using GraphPad Prism® version 8.0 (GraphPad Software Inc., San Diego, California, USA) and Microsoft® Excel 2016. Where appropriate all data were expressed as a mean ± standard deviation (S.D.) and compared using one-way analysis of variance (ANOVA) with Tukey's HSD *post-hoc*. In all cases, $p < 0.05$ was the minimum value considered acceptable for rejection of the null hypothesis.

3. Results

3.1. Implant manufacturing and characterisation

Rod shaped implants with dimensions of 2.5 × 40 mm were prepared using solvent moulding. Formulations H100, H70L30 and H40L60 formed opaque white implants which appeared homogenous (Table 2). However, formulation H35L30P30-20% showed an immediate discolouration.

SEM images (Fig. 2) showed that LEVO sodium was homogenously dispersed throughout all formulations and all formulations containing 40% w/w drug loading showed increased drug levels compared to their 20% w/w drug loading equivalent. Crystalline domains of drug are clearly visible throughout the implant matrix in formulations H100, H70L30 and H40L60, however, for formulation H35L35P30 (Fig. 2G) the crystalline form of the drug appears to have been altered.

All DSC endotherms (Fig. 3) showed the characteristic PCL melting endothermic peak around 50 °C. This melting point reduces as the proportion of L-PCL is increased (Fig. 3 and Figure S1 Supplementary Material), as reported previously (Stewart et al., 2020b). Additionally, the heat of fusion for this peak was measured (Fig. 3B). It can be seen that the heat of fusion obtained for samples containing L-PCL is lower than the one obtained for H100. Additionally, the heat of fusion for this peak decreases as LEVO sodium loadings increase. On the other hand, LEVO sodium DSC curves shows peaks that have been previously reported: an endothermic peak around 90 °C and 135 °C (corresponding to water loss) and an exothermic peak at 200 °C indicating decomposition (Hamad et al., 2015). No melting peak is seen for LEVO sodium because it degrades at a lower temperature than its melting point. For formulations H100, H70L30 and H40L60 containing 20% w/w and 40% w/w LEVO sodium the exothermic decomposition peak has reduced in comparison to pure LEVO sodium (LEVO sodium: 200.7 °C; H100 20% LEVO: 198.7 °C; H100 40% LEVO 195.2 °C; H70L30 20% LEVO: 182.8 °C; H70L30 40% LEVO: 184.7 °C; H40L60 20% LEVO: 179.9 °C; H40L60 20% LEVO: 178.8 °C). For H100 formulations (20% w/w and 40% w/w LEVO sodium) the temperature at which the endothermic moisture loss peak occurred has shifted to higher temperatures (ca. 150 °C). This suggest that that H-PCL is making it more difficult for the water to be eliminated from the drug as it is trapped inside a polymeric matrix. This effect is diminished in formulations containing L-PCL. Formulation H35L35P30 does not show the characteristic LEVO sodium peaks. As mentioned before, this implants showed a discolouration and the drug loaded in the surface of the implants showed a different structure. These evidences suggest that the drug has been degraded and that is why LEVO characteristic peaks cannot be seen in the DSC curves. Therefore, drug content analysis was performed to ascertain the degradation of the drug. Interestingly, H35L35P30 shows a broad desolvation peak between 50 and 100 °C suggesting the presence of humidity in these implants.

FTIR analysis (supplementary material Figure S2-S5) showed typical PCL absorption bands at ~ 1295 cm^{-1} (C—O and C—C stretching), ~1730 cm^{-1} (C═O), ~2940 cm^{-1} and ~ 2860 cm^{-1} (C—H) present in all formulations(Kmiec et al., 2013; Zehnder et al., 2016). The characteristic peaks of LEVO sodium are difficult to distinguish in these graphs. This is most likely because LEVO sodium is dispersed throughout, rather than dissolved in, the polymer. No new peaks were observed for formulations H100, H70L30 and H40L60 when compared to the equivalent non-drug containing implants or LEVO sodium. This suggests that no new chemical bonds have been formed between LEVO sodium and the constituents of the implants and that the drug is simply dispersed throughout the matrix. However, the FTIR spectra for formulation H35L35P30 (Supplementary material Figure S5) shows some changes in the peaks present. These new peaks are most likely as a result of degradation of the LEVO sodium within these formulations.

Implant LEVO content was investigated (Table 3) to ensure that LEVO sodium remained stable during the implant manufacturing process. Implants prepared using H100 formulation were lighter than the other formulations. Therefore, they contained lower total amounts of LEVO. After analysing LEVO content within the implants, all formulations were found to contain over 85% of their expected drug content, except for H35L35P30-20% which contained 12.27 ± 2.46% of its expected content. This confirmed that LEVO sodium had degraded within formulation H35L35P30, as was suspected from the discolouration and DSC and FTIR investigation.

3.2. *In vitro* LEVO release

In vitro release was conducted for H100, H70L30 and H40L60 for

Table 2
Images of each of the implant formulations prepared.

Formulation	20% w/w LEVO sodium	40% w/w LEVO sodium
H100		
H70L30		
H40L60		
H35L35P30		N/A

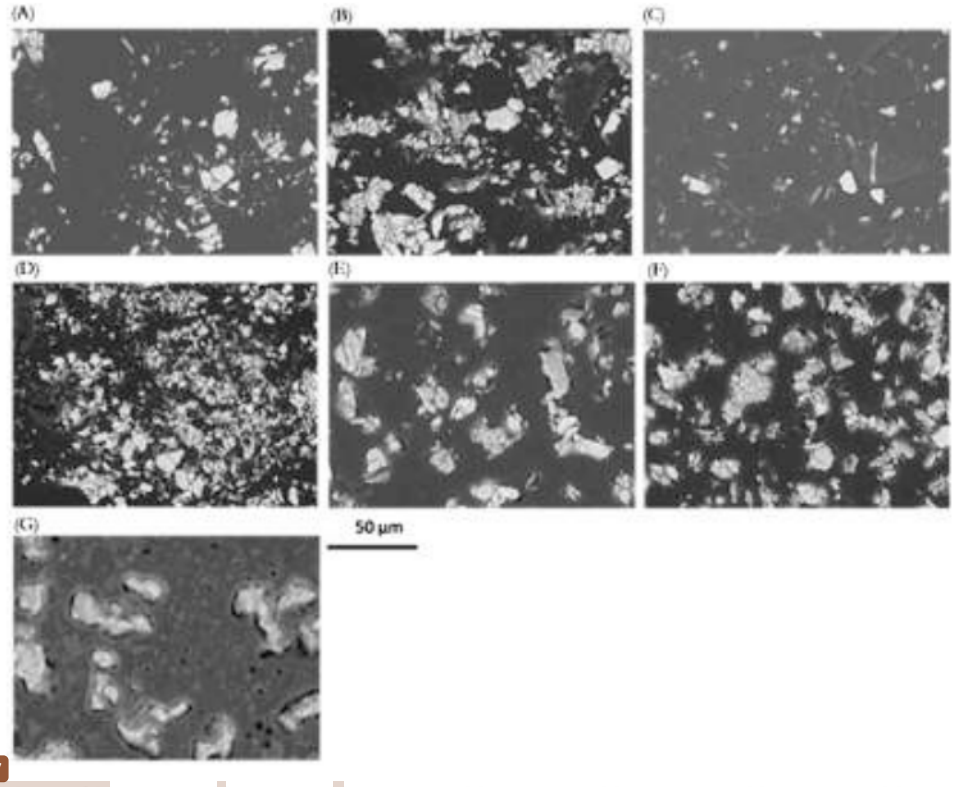
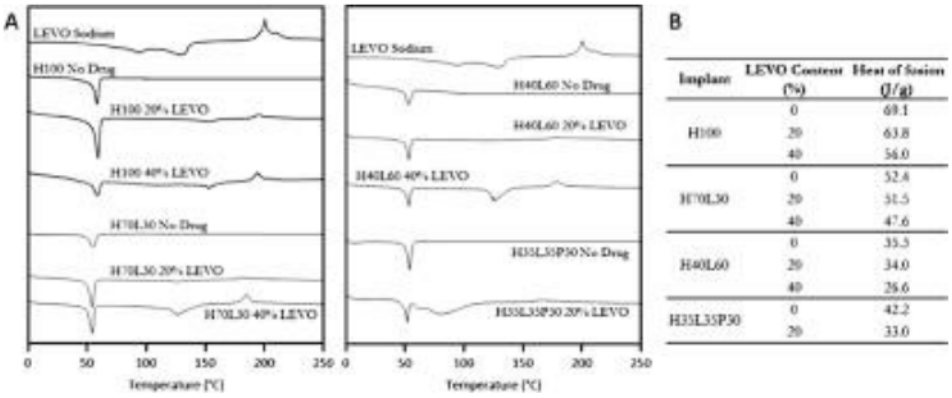


Fig. 2. SEM images of (A) H100-20%; (B) H100-40%; (C) H70L30-20%; (D) H70L30-40%; (E) H40L60 −20%; (F) H40L60-40%; (G) H35L35P30-20%.



Implant	LEVO Content (%)	Heat of fusion (J/g)
H100	0	69.1
	20	63.8
	40	56.0
H70L30	0	52.6
	20	51.5
	40	47.6
H40L60	0	35.3
	20	34.0
	40	26.6
H35L35P30	0	42.2
	20	33.0

Fig. 3. DSC thermograms of: LEVO sodium, H100, H100-20%, H100-40%, H70L30; H70L30-20%, H70L30-40%, H40L60, H40L60-20%, H40L60-40%, H35L35P30 and H35L35P30-20% (A) (exo up). PCL heat of fusion for H100, H100-20%, H100-40%, H70L30; H70L30-20%, H70L30-40%, H40L60, H40L60-20%, H40L60-40%, H35L35P30 and H35L35P30-20% (B).

both drug loadings (20% w/w and 40% w/w). Formulation H35L35P30 was not included in further testing because of the degradation observed. Cumulative percentage release and cumulative release were calculated (Fig. 4).

Daily *in vitro* release rates were calculated from the linear section of each release profile (Table 4). Release rates ranging from 28.37 ± 1.19 – 98.92 ± 4.17 μg/day were achieved. For 20% w/w LEVO sodium loaded implants, significant differences ($p < 0.05$) in release rate were observed between H100 and H70L30 and between H100 and H40L60, however, no significant difference ($p > 0.05$) was observed between H70L30 and H40L60. For 40% w/w LEVO sodium loaded implants, significant differences ($p > 0.05$) in release rate were observed between all formulations. Interestingly, H100 40% showed a higher drug release than H70L30 40% when the release curves are plotted using the percentage of LEVO sodium released instead of the amount. This is due to the lower total drug loading measured for H100 implants, as these implants were lighter than implants containing L-PCL (Table 3). A significant difference ($p < 0.05$) in release rate was observed between H100-20% and

Table 3
Content analysis results for implants (means ± S.D., $n = 3$).

Formulation	Initial Content (mg)	Initial Content (mg)	Measured Content (%)	
	20% w/w LEVO sodium	40% w/w LEVO sodium	20% w/w LEVO sodium	40% w/w LEVO sodium
H100	39 ± 3	67 ± 8	95 ± 6	88 ± 9
H70L30	48 ± 4	102 ± 8	95 ± 5	97 ± 3
H40L60	47 ± 2	107 ± 5	85 ± 2	97 ± 3

H100-40%. No significant differences ($p > 0.05$) were observed as a result of changing the drug loading of any of the other formulations. This suggests that when the drug loading is higher, the implant formulation plays a more important role in controlling the release rate.

Fig. 5 shows representative SEM images of H100 and H40L60 loaded with 40% w/w of LEVO after 105 days of release. These images showed that both implants are still showing drug crystals on their surfaces. H100 presented a higher number of drug crystals on the surface, as these implants released lower amounts of LEVO during the *in vitro* release experiment. Moreover, H40L60 showed the presence of pores that were not seen before the release process took place (Fig. 2). These pores are likely generated by the release of LEVO from the implant. Additionally, Figure S6 (Supplementary Material) showed a X-ray MicroCT images of these implants. However, these images did not clearly show any pores in the implant surface, due to the limited resolution of the technique.

3.3. *In vivo* LEVO release

In this study, the plasma pharmacokinetic profiles of LEVO sodium after subcutaneous administration of implant formulations were evaluated. Two different formulations were selected from the *in vitro* study results. H40L60 containing 40% w/w of drug showed the most promising *in vitro* LEVO release profiles. Therefore, this type of implant was selected for further *in vivo* testing. Additionally, H100 containing 40% w/w of drug was tested *in vivo* to evaluate if the composition of the implant influenced *in vivo* drug release. Additionally, the effect of sex on *in vivo* pharmacokinetic profiles of LEVO sodium from implants was also investigated, using male and female Wistar rats. The profiles of the pharmacokinetics of LEVO sodium following implant administration are depicted in Table 5. Additionally, the curve describing relationship between time profiles and mean plasma concentration is shown in Fig. 7. It is important to note that normal LEVO plasma levels in rats (ca. 0.036 ng/mL)(De Sibio et al., 2013) are noticeable below the detection limit for the HPLC method used in this study.

The pharmacokinetic analysis showed that the C_{max} values of LEVO from animals implanted with H40L60 were found after 14 days of implantation in both male and female rats, with concentrations of 19.75 ± 1.98 ng/mL for the male group and 18.12 ± 2.31 ng/mL for the female group. There was no significant difference ($p > 0.05$) between pharmacokinetic profiles of LEVO from H40L60 implants between male and female groups, suggesting that their sex did not influence the pharmacokinetics of LEVO sodium. On the other hand C_{max} values of 9.92 ± 1.81 ng/mL and 10.83 ± 1.73 ng/mL were found for animals implanted using H100. Moreover, animals implanted with this type of devices showed longer T_{max} when compared to animals implanted with H40L60 (21 vs. 14 days). Similar to implant H40L60, the pharmacokinetic profiles of LEVO were not statistically affected ($p > 0.05$) by sex. Interestingly, when analysed statistically, C_{max}, T_{max} and AUC values of LEVO from implant H40L60 were statistically greater than those values from implant H100. The results found in this study showed the possibility of a sustained-release formulation of LEVO in implant formulations to control *in vivo* delivery over a 4 week period.

4. Discussion

The present work is focussed on development of a subcutaneous implant for the treatment of hypothyroidism. Currently, tablets, capsules and oral solutions are the formulations available for the treatment of hypothyroidism (Joint formulary committee, 2020). There has been

Table 4
Daily *in vitro* release rate (means ± S.D., $n = 4$).

Formulation	Daily release rate (μg/day)	
	20% w/w LEVO sodium	40% w/w LEVO sodium
H100	28.37 ± 1.19	47.39 ± 8.76
H70L30	78.21 ± 19.93	66.40 ± 12.21
H40L60	72.32 ± 25.60	98.92 ± 4.27

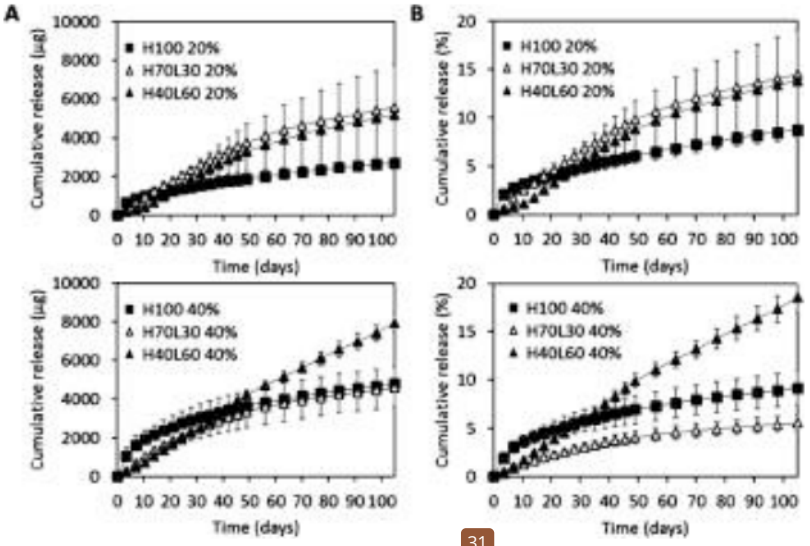


Fig. 4. Cumulative *in vitro* release profiles for H100, H70L30 and H40L60 containing 20 and 40% (w/w) LEVO sodium (A). Cumulative percentage *in vitro* release profiles for H100, H70L30 and H40L60 containing 20 and 40% (w/w) LEVO sodium (B) (means ± S.D., $n = 4$).

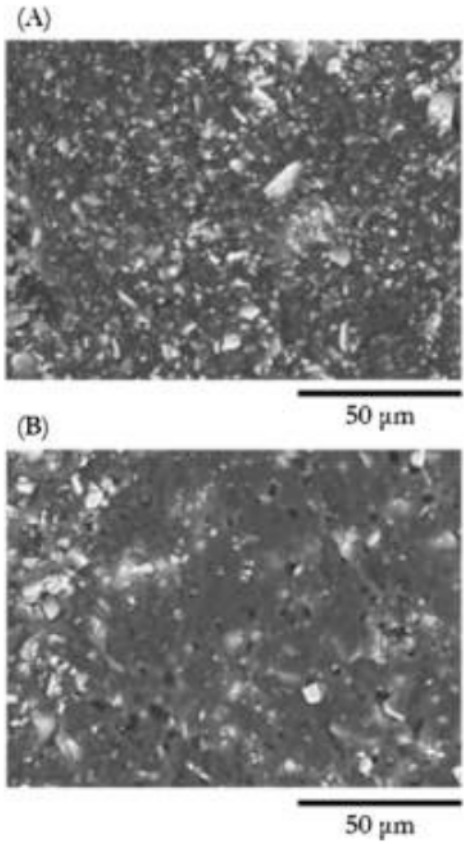


Fig. 5. SEM images of: (A) H100-40% and (B) H40L60-40% after 105 days of release.

Table 5
Pharmacokinetic parameters of LEVO sodium after subcutaneous administration of H40L60 implants loaded with 40% w/w LEVO sodium in male (Group 1) and female (Group 2). Wistar rats, as well as H100 implants loaded with 40% LEVO sodium in male (Group 3) and female (Group 4) Wistar rats (means ± SD, n = 3 for each group).

Parameters	Group 1	Group 2	Group 3	Group 4
C_{max} (ng/mL)	19.75 ± 1.98	18.12 ± 2.31	9.92 ± 1.81	10.83 ± 1.73
T_{max} (day)	14	14	21	21
AUC_{0-t} (ng/mL. day)	374.45 ± 65.32	332.32 ± 57.11	202.57 ± 39.21	184.54 ± 27.38
$T_{1/2}$ (day)	25.16 ± 4.32	23.22 ± 3.91	19.09 ± 3.87	21.23 ± 2.98

limited research conducted to investigate a long-acting formulation for this condition. However, a long-acting formulation could improve patient compliance and reduce drug interactions with food and other drugs and improve the treatment of this condition. An alternative to conventional oral LEVO sodium administration is the use of subcutaneous or intramuscular injections. Limited case studies in non-compliant patients have shown that subcutaneous and intramuscular injections were successful at restoring normal thyroid levels, and these routes could be promising for the delivery of LEVO sodium(Groener et al., 2013; Kwek et al., 2018; Mikhail, 2020; Taylor et al., 2015). Therefore, a long-acting polymeric subcutaneous implant could be promising for the treatment of this condition(Stewart et al., 2018).

Both T_3 and LEVO (T_4) have been investigated as treatment options

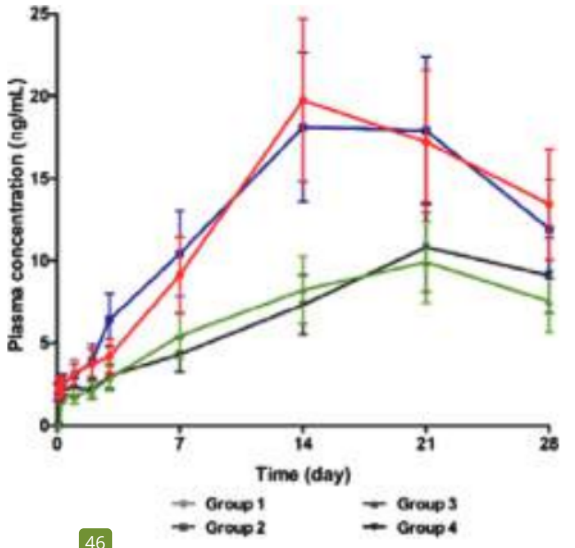


Fig. 7. The mean plasma concentration and time profiles of LEVO sodium after subcutaneous administration of implant A in male (Group 1) and female (Group 2) Wistar rats, as well as implant B in male (Group 3) and female (Group 4) Wistar rats (means ± SD, n = 3 for each group).

for hypothyroidism. Despite being the more active form of the hormone, the use of T_3 is not recommended because of the increased risk of iatrogenic hyperthyroidism associated with it (Singer, 1995). In addition, T_3 preparations result in a peak around 3 h after dosing followed by a rapid decline in T_3 levels. The high peak observed is not physiologically representative and has given cause for concern (Idrees et al., 2019). Multiple daily dosing may overcome this problem, but creates practicality issues, given the specific administration instructions associated with oral LEVO sodium (Idrees et al., 2019; Joint formulary committee, 2020). However, currently Titan Pharmaceuticals (California, USA) is conducting studies for development of a subdermal implant to deliver T_3 for the treatment of hypothyroidism (Titan Pharmaceuticals, 2020a). This device will utilise their ProNeura® drug delivery platform and aims to provide continuous delivery for 3 months to a year (Idrees et al., 2019; Titan Pharmaceuticals, 2020a). Research has been conducted into the use of combinations of T_3 and LEVO (T_4) (Alomari et al., 2018; Wartofsky, 2013). However, the American thyroid association does not recommend the routine use of combination treatments (T_4 and T_3) for the treatment of hypothyroidism (Idrees et al., 2019). Given the shortcomings associated with T_3, this work is focussed on developing an implantable device for the delivery of LEVO sodium (T_4).

PCL blends were chosen as the basis of the polymeric matrix formulation due to the promising results that these mixtures demonstrated in previously published studies as a polymeric coating to prolong the release of hydrophilic compounds from reservoir-type implants (Stewart et al., 2020b). PCL is a US Food and Drug Administration (FDA) approved material and its long degradation time and low cost when compared to poly(lactic-glycolic acid) (PLGA) make it a promising choice for long-acting delivery formulations (Cheng et al., 2010). Additionally, as PCL is biodegradable, implants prepared using this material do not require to be extracted after depleting their drug cargo. This is an advantage over other polymers approved for biomedical applications that are non biodegradable such as thermoplastic polyurethane, poly(propylene) or poly(ethylene vinyl acetate) (Maitz, 2015; Mathew et al., 2019; Osman et al., 2015; Stewart et al., 2018). PCL can be handled using a range of processing techniques such as 3D printing, melt or solvent casting or extrusion (Stewart et al., 2018), which make it a flexible option when considering drugs with challenging stability

issues, such as LEVO sodium (Stewart et al., 2020b). Solvent casting into a mould was chosen as the method of fabrication of the implants. This method was chosen to minimise the risk of drug degradation as a result of heat exposure used during other methods such as injection moulding or 3D printing (Domínguez-Robles et al., 2020; Farmer et al., 2020; Martin et al., 2021; Mathew et al., 2020; Stewart et al., 2020a). Other methods such as 3D printing or extrusion rely on heat to melt the polymer and, therefore, would have been unsuitable for use with LEVO sodium. Although PCL has a low melting temperature (around 60 °C), previous work has extruded PCL at 100 °C and this temperature has been shown to degrade LEVO sodium (Collier et al., 2010; Zhang et al., 2016). In addition to heat avoidance, the choice of solvent used was a factor that could influence stability. Therefore, DCM was chosen as the solvent for PCL dissolution to minimise LEVO sodium degradation in the solvent. PCL is soluble in DCM, however LEVO sodium is insoluble in DCM. This allowed LEVO sodium to stay in the solid state throughout implant manufacture and minimised possible drug degradation. High concentrations of DCM in the final product can be problematic in terms of toxicity issues. However, this solvent has been used previously in the manufacture of foods and pharmaceutical/cosmetical products (Final report on the safety assessment of methylene chloride, 1988; Sohi et al., 2004). Pharma applications of this solvent include tablet coating(Sohi et al., 2004). Accordingly, its use is accepted by the FDA when the levels of this solvent are below a defined threshold in the final product (FDA, 2017).

Implants were characterised using SEM, DSC and FTIR. On visual inspection, implants were opaque and drug dispersion appeared homogenous (Table 2). This was confirmed by SEM (Fig. 2). DSC results indicated that LEVO sodium degraded at a lower temperature when incorporated within the implant when compared to pure LEVO sodium powder (ca 200 °C for pure drug and 178–198 °C for drug containing implants) (Fig. 3). FTIR confirmed that no new bonds were formed between the polymeric constituents and LEVO sodium (Figures S1-S4). PCL heat of fusion was measured (Fig. 3B). These results showed that PCL heat of fusion on samples containing L-PCL was lower than the one obtained for H100. This is consistent with previous findings suggesting that the crystallinity (measured using X-Ray Diffraction) of PCL-based materials is influenced by L-PCL content (Stewart et al., 2020b). Additionally, increasing LEVO sodium cargo inside the implants reduce PCL heat of fusion. This is expected as in these cases the percentage of PCL in the implants is lower. However, the reduction in the heat of fusion for lower drug concentrations are not proportional to the drug loading. The heat of fusion obtained for samples containing 20% LEVO sodium is similar to the one obtained for implants containing no drug. Implants containing 20 and 40% of LEVO sodium should show a reduction in the heat of fusion of 20 and 40% respectively. The obtained heats of fusion are higher than expected suggesting an increase in the crystallinity of PCL. This suggest that LEVO sodium has a nucleation effect on crystallisation of PCL. This phenomenon has been previously reported when combining PCL with other substances such as drugs, nalidixic acid, (Douglas et al., 2016) and other pharmaceutical excipients, cellulose (Lv et al., 2017). LEVO sodium content analysis confirmed that all implants (except for H35L35P30) contained over 85% of their expected content (Table 3). No additional peaks for LEVO sodium appeared in any of the HPLC chromatograms and the retention time remained unchanged. This confirmed that LEVO sodium was unchanged by the implant fabrication process.

After completing the characterisation of LEVO containing implants. *In vitro* drug release was evaluated. H100 implant formulations showed a quick release after 24 h followed by a linear release phase. Addition of L-PCL to the formulations prevented the fast release over the first 24 h. Other studies have reported a significant fall in drug release rate after an initial burst release from PCL implants, however, this was not observed in the first 100 days of *in vitro* release in this work (Cheng et al., 2010). Implants containing PEG were included to evaluate if the presence of a hydrophilic excipient can alter the release profile of LEVO. However, LEVO sodium was incompatible with PEG in the formulation and caused significant drug degradation. DSC results suggest that the combination of PEG and LEVO makes implants hygroscopic and the presence of humidity will promote the degradation of LEVO (Collier et al., 2010). This is not surprising considering the hygroscopic nature of both, PEG and LEVO (Kolská et al., 2019; Rostami et al., 2014). Similar behaviour has been reported for the formulation of LEVO with alternative hygroscopic excipients such as povidone or sodium lauryl sulfate (Kaur and Suryanarayanan, 2021). The degradation of the drug was confirmed by the visual discolouration observed and by content analysis.

All formulations showed a cumulative percentage release of between 8 and 20% after 98 days. If release from these implants continued in the same linear manner, these formulations could be estimated to deliver LEVO sodium for approximately 1.3 – 3.4 years. Kamali *et al.* investigated the use of an *in situ* forming LEVO sodium implant and reported 100% *in vitro* drug release after 35 days(Kamali et al., 2020). The implants produced in this work resulted in extended release profiles in comparison to these *in situ* forming implants. It is important to note that H40L60 showed more sustained drug release profiles. This can be due to the presence of higher amounts of L-PCL in the implant. It was demonstrated that LEVO is partially soluble in L-PCL as opposed to pure DCM. Accordingly, the presence of L-PCL can lead to a better integration of the drug with the polymer due to the dissolution of part of the LEVO sodium content during implant preparation. This could explain the sustained drug release profile obtained for this type of implants.

Daily *in vitro* release rates were calculated (Table 4) and *in vitro* release rates ranging from 28.37 ± 1.19 – 98.92 ± 4.27 μg/day were achieved. The release rates achieved were promising and are close to the oral dose of LEVO sodium (50 – 200 μg/day) (Joint formulary committee, 2020). LEVO sodium has an oral bioavailability of 40 – 80%, therefore, if delivered subcutaneously lower doses may be required (Colucci et al., 2010). For those implants that delivered LEVO sodium at a rate lower than the recommended daily dose, multiple implants could be inserted into the patient to achieve the desired dose. This is the case for Probuphine®, a buprenorphine containing implant which consists of four rod shaped implants (Titan Pharmaceuticals, 2020b; WebMD, 2020). However, *in vitro* release rates cannot be directly extrapolated to *in vivo* release rates. Accordingly, *in vivo* animal experiments were conducted.

The release of hydrophobic and hydrophilic drugs from PCL based devices is primarily dominated by diffusion (Cheng et al., 2010; Rosenberg et al., 2007). The semi-crystalline nature of PCL is thought to inhibit matrix collapse after drug release. This results in voids through which water can diffuse and drug can be released (Rosenberg et al., 2007). The high drug content of these implants is likely to contribute to the formation of voids and channels throughout the implant which would facilitate drug dissolution and diffusion. This is consistent with the results obtained, as Fig. 5 shows the presence of pores created in the implant surface after 105 days release. Increasing the amount of L-PCL in H-PCL/L-PCL mixtures has been previously reported to affect the crystallinity of the resulting material (Stewart et al., 2020b). Moreover, the same work report that the degradation kinetics of the materials containing L-PCL combined with H-PCL is faster than the degradation of pure H-PCL (Stewart et al., 2020b). Therefore, these factors could explain the difference in the *in vitro* release experiments.

LEVO release was tested using an animal model. Fig. 7 shows *in vivo* drug release from H100 and H40L60 implants containing 40% w/w of LEVO. The obtained results suggested that both systems were capable of providing drug release over a period of at least 4 weeks. Interestingly, H40L60 provided higher levels of drug in plasma. These results are consistent with the results obtained during *in vitro* drug release testing (Fig. 4). This experiment was designed to evaluate if these implants are capable of providing *in vivo* LEVO release. The release of drug over 4 weeks showed high plasma LEVO levels. It was anticipated from the *in vitro* release results that humans will require an implant similar to the one presented in this manuscript (2.5 × 40 mm). However, the obtained

plasma levels for rats implanted with an implant half that size were up to 2220 times the lowest plasma levels required for healthy humans (0.009 – 0.018 ng/mL)(Kang et al., 2017; Pantalone et al., 2015). Moreover, the average weight of an average human is approximately 300 times the weight of a Wistar rat. Accordingly, it can be estimated that the implant sizes required to provide therapeutically relevant LEVO doses should be between 5 and 14 times smaller than the original implant presented in this work. In reality, the size of the implants can be even smaller, considering that LEVO elimination in rats is quicker than in humans (LEVO half life 0.5–1 days vs 5–6 days)(Lewandowski et al., 2004). Considering the size reduction this might open the possibility of using novel drug delivery systems that will improve implant insertion, reducing (or even eliminating) the pain associated with implant insertion, such as "bioneedles" or "microneedles"(Donnelly and Larrañeta, 2019; Donnelly and Larrañeta, 2018; Hirschberg et al., 2008). These types of devices have been used to administer miniaturised implants in a painless way. Moreover, these devices do not need healthcare professionals to be inserted they can be self-administered.

5. Conclusions

Hypothyroidism is a chronic condition effecting>1.3 million people in the UK. A long-acting formulation of LEVO sodium could improve treatment for these patients by reducing the burden on patient compliance and minimising drug and food interactions. This work describes a range of subcutaneous polymeric implants which delivered LEVO sodium *in vitro* at rates ranging from 28.37 ± 1.19 – 98.92 ± 4.27 μg/day. All implant formulations (except the PEG containing formulation) retained over 85% of their expected drug content after implant production. Moreover, preliminary *in vivo* results suggest that this type of devices are capable of providing sustained *in vivo* LEVO release. The obtained LEVO plasma levels ranged between 5 and 20 ng/mL. Future work should aim to study the applications of these implants for long term LEVO release adjusting the size of the implant to achieve drug doses within the therapeutic window.

CRediT authorship contribution statement

Sarah A. Stewart: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Juan Domínguez-Robles:** Supervision, Writing – review & editing. **Emilia Utomo:** . **Camila J. Picco:** . **Francesca Corduas:** . **Elena Mancuso:** . **Muhammad [illegible] Amir:** . **Muhammad Akbar Bahar:** . **Sumarheni Sumarheni:** . **Ryan F. Donnelly:** Supervision, Writing – review & editing. **Andi Dian Permana:** Writing – review & editing. **Ileko Larrañeta:** Supervision, Conceptualization, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2021.121011.

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