BUKTI SUBMIT MANUSKRIP

Universitas Hasanuddin

Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

Submission Confirmation for Poly(caprolactone)-based subcutaneous implant for sustained delivery of levothyroxine (adhm.202101047)

1 message

Advanced Healthcare Materials <em@editorialmanager.com> Fri, May 28, 2021 at 6:54 PM
Reply-To: Advanced Healthcare Materials <advhealthmat@wiley-vch.de>
To: Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

You are being blind carbon copied ("bcc:'d") on an e-mail "To" "Eneko Larrañeta" e.larraneta@qub.ac.uk

Dear Dr Larrañeta,

Your submission entitled "Poly(caprolactone)-based subcutaneous implant for sustained delivery of levothyroxine" has been received by journal Advanced Healthcare Materials. The manuscript number for your submission is adhm.202101047.

To view your submission, please login to https://www.editorialmanager.com/advhealthmat/ by entering your username (********) and password and selecting the "Author Login" option. Please note that the current status of your submission will remain "Under consideration" until an editorial decision is made, at which time you will be notified by e-mail.

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Kind regards,

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Please find a copy of the submission questions, which you answered during the submission, for your records:

Additional Information

12. Eneko Larrañeta, PhD

Question	Response
Please submit a plain text version of your cover letter here.	The Editor Advanced Healthcare Materials 27th May 2021 Dear Sir/Madam, I wish you to consider our manuscript entitled: “Poly(caprolactone)-based subcutaneous implant for sustained delivery of levothyroxine” for publication in your journal. This article describes the development of biodegradable subcutaneous implants for sustained levothyroxine release. The article follows our previous work developing new implantable systems for long acting drug delivery. In this case, we developed PCL-based implants containing levothyroxine for hypothyroidism treatment. By adapting the PCL composition of the implants we have been able to modify the release profile. This effect was observed no only during in vitro drug release experiments but also in vivo. These implants are biodegradable and do not require to be removed after depleting their drug cargo. This manuscript has not been previously published in any language anywhere and is not under simultaneous consideration by another journal. Should this manuscript be accepted for publication we transfer copyright to your publisher as appropriate. We hope that you consider this manuscript worthy of publication in your journal. Yours Sincerely, Dr. Eneko Larrañeta (on behalf of all authors)
Please confirm that the research and manuscript meet the ethical guidelines outlined in this journal's Author Guidelines, including adherence to the legal requirements of the study country.	Yes, I confirm
Does the research described in this manuscript include animal experiments?	Yes
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Does the research described in this manuscript include human research participants (including for experiments with sensors or wearable technologies) or tissue samples from human subjects (including blood or sweat)?	No
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Universitas Hasanuddin

Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

Fwd: Your Submission IJPHARM-D-21-01702

1 message

Andi Permana <apermana01@qub.ac.uk> Wed, Apr 5, 2023 at 4:00 PM
To: Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

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Subject: Fw: Your Submission IJPHARM-D-21-01702

Hello guys,

We have comments from IJP about the LEVO paper. We have a good few comments here but I will be taking care of them. The paper will be accepted.

Regards

Eneko

Dr Eneko Larrañeta

Lecturer in Pharmaceutical Sciences

School of Pharmacy, Queen's University Belfast

97 Lisburn Road, Belfast, BT9 7BL, UK
t: 028 90972360 **e**: e.larraneta@qub.ac.uk

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Ms. Ref. No.: IJPHARM-D-21-01702
Title: Poly(caprolactone)-based subcutaneous implant for sustained delivery of levothyroxine
International Journal of Pharmaceutics

Dear Dr. Larraneta,

Comments on your paper have now been received and are attached to this message. Please consider all the points made and upon returning your paper detail your responses and the actions taken.

Please submit the revised manuscript by Sep 05, 2021. Upon receipt of your revised paper, we will inform you of the outcome as soon as possible. Papers not received within that that time period will be considered to be withdrawn, you are welcome to resubmit your paper as a new submission at a later

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Yours sincerely,

Juergen Siepmann, PhD
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Reviewers' comments:

Reviewer #1: Comments on a manuscript entitled: "Poly(caprolactone)-based subcutaneous implant for sustained delivery of levothyroxine" by SA Stewart et al. and submitted for publication in IJP.

In this study, rod like implants were developed for slow release of levothyroxine. Normally, the drug is orally administered and in exceptional cases as an injection. One of the reasons to develop implants was to increase patient compliance. Unfortunately, the diameter of the rods is too large (2.5 mm) to fit in a needle (like Zoladex implants) and have to be administered after surgical incision to apply the rods subcutaneously, which not very patient friendly. Nevertheless, it is an interesting study, well written and the (most) conclusions are justified by the data. However, I have a major concern about the in vivo release rate when extrapolating rat data to the human situation (see below under discussion section), and some other comments:

Section 2.1.
Where was PEG 400 (or 4400?) used for?

Section 2.2.
I do not understand why the rods are circular, I would expect as they are solvent casted, one side should be flat. Furthermore, I miss some experimental details. How long was DCM allowed to evaporate, and at which temperature? Was residual DCM determined? I think residual DCM might affect the dissolution profile. Furthermore, as the authors correctly mention in the discussion section, the DCM levels should be below a "particular threshold" in the formulation.

Section 2.5.
Please mention aqueous solubility of levothyroxine (btw, BSA probably positively affects its solubility).

Section. 3.1.
It appears that levothyroxine only in the H35L30P30-20% formulation substantially degraded. This is the only formulation which contained PEG. So, apparently, PEG is responsible for degradation. Is there an explanation for this incompatibility? On the other hand, as the authors discontinued with this formulation, they could also simply remove it from the manuscript. I also do not see why PEG would prevent a burst release as the authors mention in the discussion section.
Would it be possible to calculate the degree of crystallinity of PCL in the formulations based on the heat of fusion in the DSC thermograms? If 100% crystallinity data are not available, the authors just could compare the heat of fusion of the different formulations.

Section 3.2.
In the dissolution profiles, I do not see any burst release (although the authors mention in the discussion section a short-lived burst release for the H100 formulation? And if so, why only this formulation? In other words why should L-PCL prevent this burst release as the authors mention in the discussion section). The SEM pictures clearly show that drug particles at the surface of the formulations, which should cause a burst release during the first day(s). Further, I am surprised that the drug crystals at the surface are still present after 105 days of release (Figure 5).

Discussion section:
The authors mention that the plasma levels for levothyroxine should be 50-200 microgram/day. I guess these levels refer to humans. In the current study, rat plasma levels were determined: 30-100 micron/day. As rats have about 200x less the amount of plasma than humans! So, how many implants should be given to humans?

The discussion section is very wordy and partially a repetition of the introduction. I could be substantially shortened.

Reviewer #2: This article focuses on the construction of PCL implants containing LEVO sodium for the treatment of hypothyroidism and evaluates their in vitro and in vivo activities. In general, this article has a realistic and practical subject, scientific and reasonable structure, and strict logic. The advantages are obvious, but this article also has the following problems:

1. In page 13
"This melting point reduces as the proportion of L-PCL is increased, as reported previously(S. A. Stewart et al., 2020)."
This cannot be said from the DSC thermograms, the reference given is by the author who submitted the article. This information cannot be proven in the reference either. Can the authors explain this?

2. In page 13
"For H100 formulations (20% w/w and 40% w/w LEVO sodium) the temperature at which the endothermic moisture loss peak occurred has increased, suggesting that H-PCL is making it more difficult for the water to be removed."
Can the authors explain this? This information may be valid for H100 20% but does not seem correct for H100 40%.
3. In page 13
"Formulation H35L35P30 does not show the characteristic LEVO sodium peaks. This, combined with the discolouration observed for this formulation and the change in drug structure visualised using SEM, may suggest that the LEVO sodium has degraded within this formulation"
This does not only indicate that the drug has degraded, but can also prove that the entire drug has been encapsulated into the implant. Drug peaks in DSC thermograms may also indicate the presence of free drug on the implant surface that is not loaded into the implant.

4. The authors need to explain in more detail why they expect the burst effect to increase with the addition of PEG. It should also be explained why the addition of PEG may have caused degradation.

5. When calculating the Levo content in in vitro cumulative release and in in vivo experiments, did the authors use the actual amount of drug in the implant using the measured content (%) in Table 3? Or did they use the theoretical amount of drug? It is not clear.

For in vivo experiments, the implants were used by cutting, it was not proven that the implant was homogeneous and exactly how much drug was in the cut part. Did the authors interpret the in vivo results according to the measured content?

Also why Figure 4 A-C has initial content (µg) on y-axis, while Figure 4 D-E has measured content (%) on y-axis.

6. In 2.7 in vivo experiment section, the authors wrote " The first and second groups were male rats and female rats, respectively, and were implanted with H100 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 H40L60 implants loaded with 40% w/w of LEVO", but Table 6 is called "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)."
Authors should check it.

7. In page 23

"DSC results indicated that LEVO sodium degraded at a lower temperature when incorporated within the implant when compared to pure LEVO sodium powder (Figure 3)"
Update this description by noting the degrees of the peaks on the DSC thermogram.

8. In page 24
"Other studies have reported 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"
The crossed out part should be deleted

9. In page 25
"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(S. A. Stewart et al., 2020). Therefore, these factors could explain the difference in the in vitro release experiments."
If the authors claim that the addition of L-PCL increases degradation, how can they explain the slower release of L-PCL in the H70L30 40% coded formulation compared to the H40L60 40% coded formulation in Figure 4E?

Reviewer #3:

The results are interesting, but the following major concerns must be addressed:

1) The proposed mathematical analysis must be omitted: It does not allow for reliable mechanistic conclusions in this case. It is well known that drug release from biodegradable implants can be much

more complex, involving polymer chain degradation, time- and position-dependent diffusion coefficients, pore formation and many other processes. Applying semi-empirical models can lead to misleading conclusions. The experimental results are sufficiently interesting to justify publication.

2) Figure 4 shows drug release in microgram, but drug release in percent is also very interesting. This should be added and discussed, especially in the light of my next comment.

3) The in vitro drug release goes on for at least 100 days, however, in vivo peaks are reached after already about 20 days. Looking at the in vivo data, a completely different in vitro release profile would be expected. This should be discussed. The conclusion might be that the in vitro data should be viewed with great caution.

4) The manuscript should be very carefully prepared, e.g. "Other studies have reported have reported a significant…".

Comment from the editor:

Please replace “6. Acknowledgment” by “Acknowledgment”, and “7. References” by “References”.

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BUKTI SUBMIT HASIL REVIEW

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

Sarah A. Stewart[1], Juan Domínguez-Robles[1], Emilia Utomo[1], Camila J. Picco[1], Francesca Corduas[2], Elena Mancuso[2], Muhammad Nur Amir[3], Muhammad Akbar Bahar[3], Sumarheni Sumarheni[4], Ryan F. Donnelly[1], Andi Dian Permana[5]*, Eneko Larrañeta[1]*

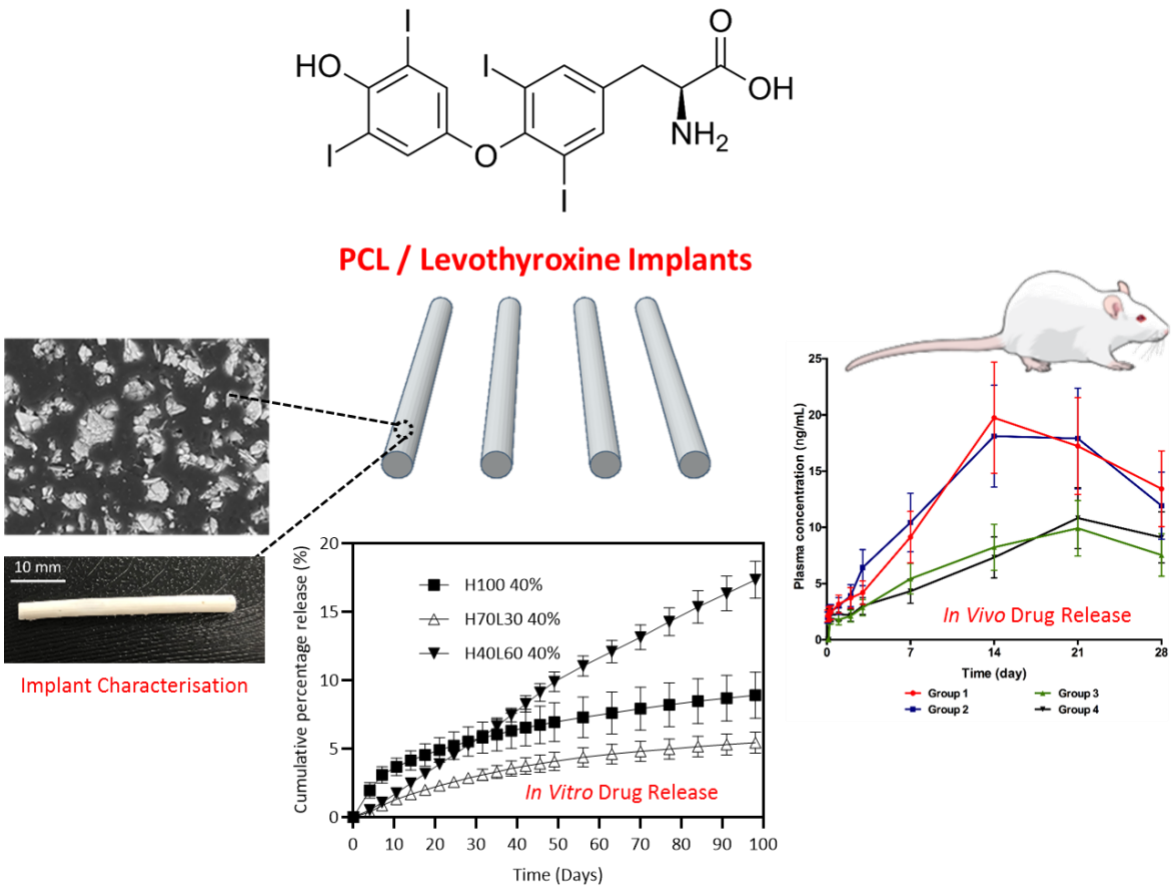
1. School of Pharmacy, Queen's University Belfast, Lisburn Road 97, Belfast BT9 7BL, UK
2. Nanotechnology and Integrated Bio-Engineering Centre (NIBEC), Ulster University, Jordanstown Campus, Newtownabbey, BT37 0QB, UK
3. Department of Pharmacology and Toxicology, Faculty of Pharmacy, Universitas Hasanuddin, Makassar 90245, Indonesia.
4. Department of Clinical Pharmacy, Faculty of Pharmacy, Universitas Hasanuddin, Makassar 90245, Indonesia
5. Department of Pharmaceutics, Faculty of Pharmacy, Universitas Hasanuddin, Makassar 90245, Indonesia

*Authors to whom correspondence should be addressed: e.larraneta@qub.ac.uk; andi.dian.permana@farmasi.unhas.ac.id

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 could 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.

Graphical Abstract



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[1]. 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[2–4]. The deficiency of thyroid hormones can result in debilitating symptoms, such as chronic fatigue, weight gain and cold intolerance[5,6]. In addition, long-term untreated hypothyroidism may result in myxoedema coma and premature death as a result of cardiovascular complications[7,8].

Levothyroxine sodium (LEVO sodium) is the treatment choice for the routine management of hypothyroidism[9]. LEVO sodium oral bioavailability is variable and can range from 40 – 80%. This is dependent mainly on the co-administration of food[1,10]. Therefore, LEVO sodium tablets should be taken at least 30 minutes before food, caffeine-containing drinks and other medication[11]. 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[11]. These additional administration instructions associated with oral LEVO sodium can often result in poor patient compliance[12]. 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[13].

In extreme cases of non-compliance, or in cases where sufficient absorption is not achieved despite good compliance, weekly intramuscular or subcutaneous injections of LEVO sodium have successfully restored thyroid levels[7,14–16]. This is due to the long half-life of thyroxine (7 days) and the slower conversion of LEVO to thyroxine at higher concentrations[17]. 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)[11].

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 Pharma-tech 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\,4}$400 and 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). Finally, 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[18,19]. 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 (Figure 1A). The resulting PLA and silicone moulds can be seen in Figure 1B.

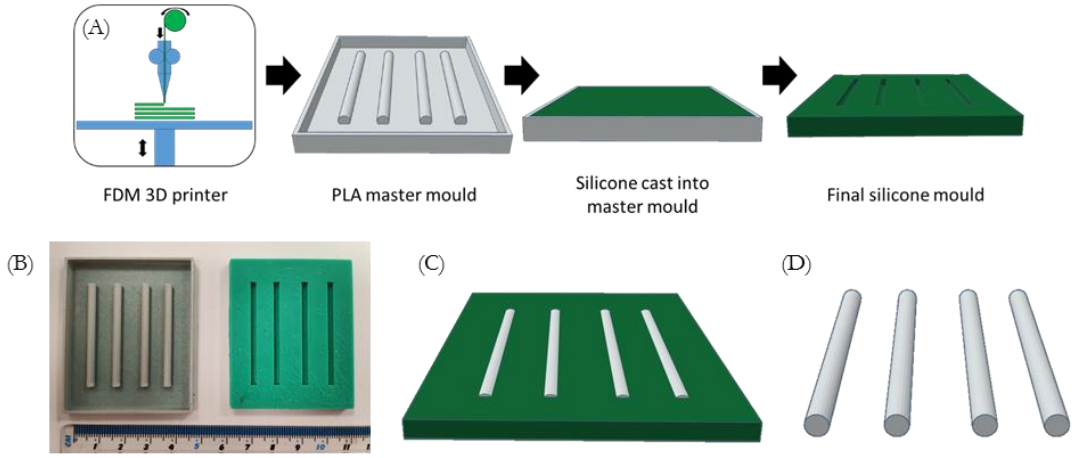


Figure 1. Schematic diagram of mould preparation (A). Image showing the 3D printed PLA mould (left) and the silicone mould (right) (B). Schematic diagram of (C) filled implant moulds and (D) implants after removal from the mould.

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 seconds x 4). This mixture was poured into the silicone moulds and DCM allowed to evaporate. Subsequently, the rod-shaped implants with dimensions of 2.5 x 40 mm could be removed from the mould (Figure 1C and 1D).

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

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 x 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 x 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. Sink conditions were defined as LEVO sodium concentration at or below 1/3 of its solubility[20]. 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 3.5 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.

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

Where, $\sum W_{0-t}$ is the sum of the weight of LEVO sodium release from t=0 until T=t and W_0 is the weight of drug in the implant at T=0.

2.6. Release data analysis

Various mathematical models were employed in this study to investigate the type of release from the implants: Korsmeyer-Peppas, zero-order model; Higuchi model; and Peppas-Sahlin model.

The Korsmeyer-Peppas model can be employed to ascertain the mechanism by which drug release is taking place. The Korsmeyer-Peppas model can be represented by Equation 2:

$$\frac{M_t}{M_\infty} = K_{KP.} t^n \qquad \text{Equation 2}$$

Where, M_t is the amount of drug release at time t, M_∞ is the amount of drug release after time ∞, n is the diffusional exponent and K_{KP} is the Korsmeyer rate constant. The value of n gives information about the type of diffusion that is occurring. For cylindrical drug delivery devices: $n \leq 0.45$ indicates that Fickian diffusion is taking place; $0.45 < n < 0.89$ indicates anomalous (non-Fickian) diffusion is taking place; and $n > 0.89$ indicates zero-order release[21,22].

Subsequently the zero-order and Higuchi models were applied to confirm the conclusion obtained from Korsmeyer-Peppas. The zero-order model can be represented by Equation 3:

$$C_t = C_0 + K_0.t \qquad \text{Equation 3}$$

Where, C_t is the amount of drug released at time t, C_0 is the initial concentration of drug at t=0 and K_0 is the zero-order rate constant. Zero-order kinetics defines the process of constant drug release from a delivery system[23]. The gradient of a graph of cumulative drug release *versus* time will give the zero-order release constant and the correlation of this graph will give information about whether the drug release follows zero-order kinetics or not.

The Higuchi model can be represented by Equation 4:

$$Q = K_H.t^{1/2} \qquad \text{Equation 4}$$

Where, Q is the cumulative drug released and K_H is the Higuchi dissolution constant. The gradient of a graph of drug release vs $t^{1/2}$ will equal K_H and, if the correlation is high, then it can be interpreted that diffusion is the main mechanism of drug release.

The Peppas-Sahlin model was applied to the formulations which showed a combination release mechanism. The Peppas-Sahlin model can be represented by Equation 5:

$$\frac{M_t}{M_\infty} = K_D.t^m + K_E.t^{2m} \qquad \text{Equation 5}$$

Where, K_D is the diffusional constant, K_E is the erosional constant and m is the release exponent[22,24]. The value of m for the implants was 0.45 as determined from the plot of aspect ratio (diameter/thickness) against m (m=0.45 when aspect ratio < 0.1)[22]. The percentage contribution of diffusion (D) and Erosion (E) were calculated using Equation 6 and 7, respectively[24].

$$D = \frac{1}{1+\frac{K_E}{K_D}.t^m} \qquad \text{Equation 6}$$

$$E = \frac{K_E}{K_D}.t^m \qquad \text{Equation 7}$$

2.7. 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 acclimatised 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 H100 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 H40L60 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 x 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 hours, 4 hours, 6 hours, 8 hours, 24 hours, 48 hours, 72 hours, 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 spun for 10 min at 4°C at 3000 x 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 x g. The supernatant containing LEVO was collected and placed in a glass vial. The glass vial was placed into a fume hood for 3 hours 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 x 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, 3.0 x 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 0.5 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).

2.8. 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 x 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.

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

SEM images (Figure 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 (Figure 2G) the crystalline form of the drug appears to have been altered.

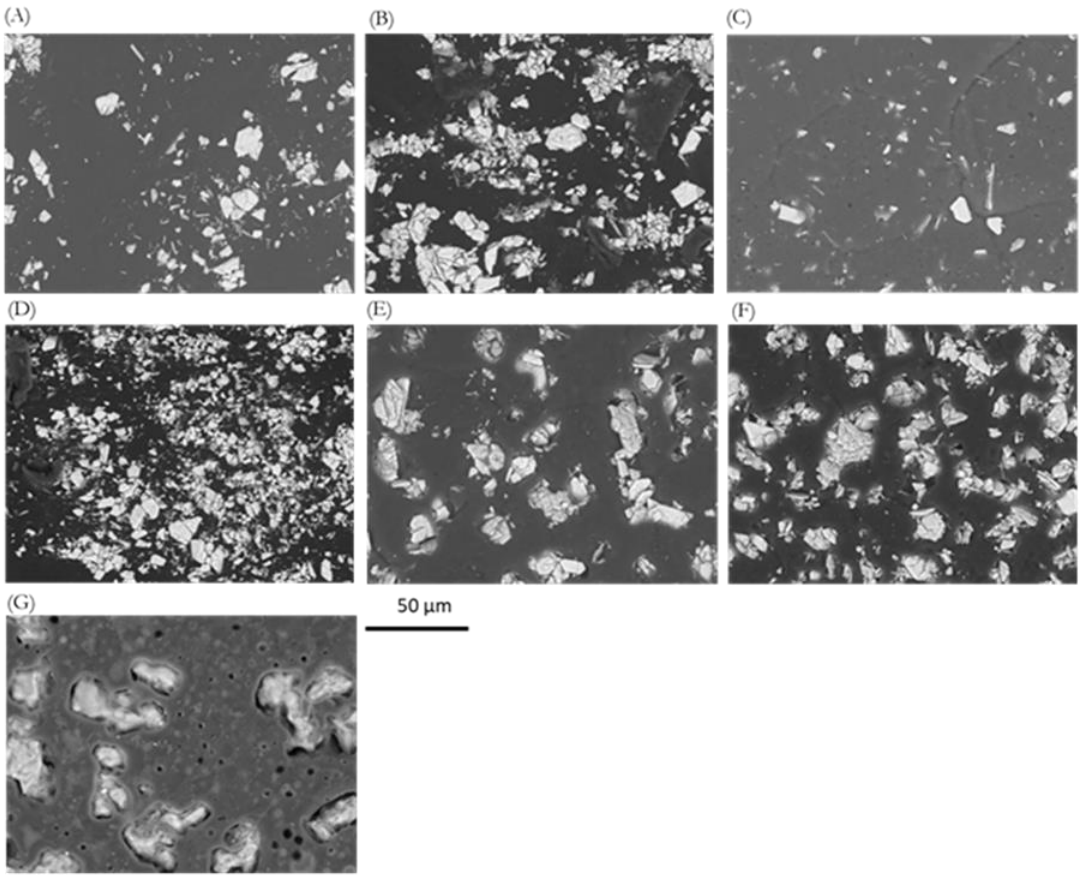


Figure 2. SEM images of (A) H100-20%; (B) H100-40%; (C) H70L30-20%; (D) H70L30-40%; (E) H40L60 -20%; (F) H40L60-40%; (G) H35L35P30-20%.

All DSC endotherms (Figure 3) showed the characteristic PCL melting endothermic peak around 60°C. This melting point reduces as the proportion of L-PCL is increased, as reported previously[25]. The LEVO sodium endotherm shows the characteristics of DSC endotherms previously reported: an endothermic peak around 90°C and 135°C (corresponding to moisture loss) and an exothermic peak at 200°C indicating decomposition[26]. 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. For H100 formulations (20% w/w and 40% w/w LEVO sodium) the temperature at which the endothermic moisture loss peak occurred has increased, suggesting that H-PCL is making it more difficult for the water to be removed. This effect is diminished in formulations containing L-PCL. Formulation H35L35P30 does not show the characteristic LEVO sodium peaks. This, combined with the discolouration observed for this formulation and the change in drug structure visualised using SEM, may suggest that the LEVO sodium has degraded within this formulation.

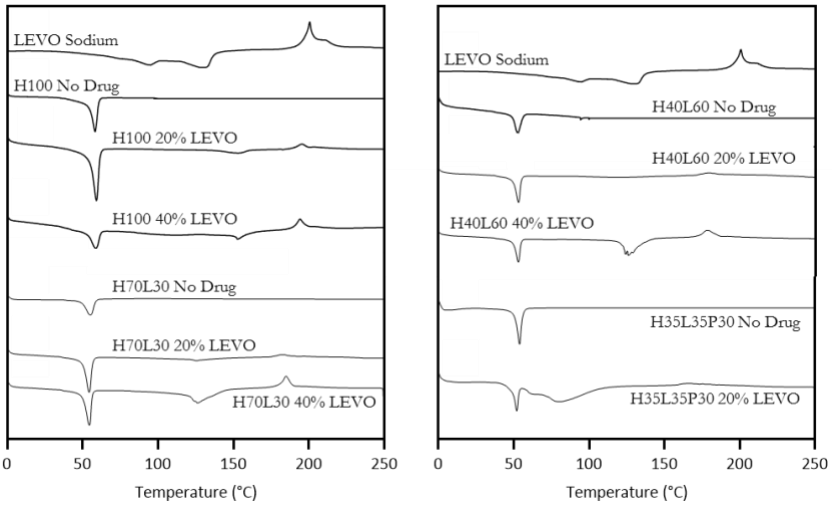


Figure 3. DSC thermograms of: LEVO sodium, H100, H100-20%, H100-40%, H70L30; H70L30-20%, H70L30-40%, H40L60, H40L60-20%, H40L60-40%, H35L35P30 and H35L35P30-20% (exo up).

FTIR analysis (supplementary material Figure S1-S4) 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[27,28]. 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 S4) 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.

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

3.2. In vitro LEVO release

In vitro release was conducted for H100, H70L30 and H40L60 for 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 (Figure 4).

Daily *in vitro* release rates were calculated from the linear section of each release profile (any burst release observed was removed from the calculation) (Table 4). Release rates ranging from 28.37 ± 1.19 – 98.92 ± 4.27 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. A significant difference ($p < 0.05$) in release rate was observed between H100-20% and 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.

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

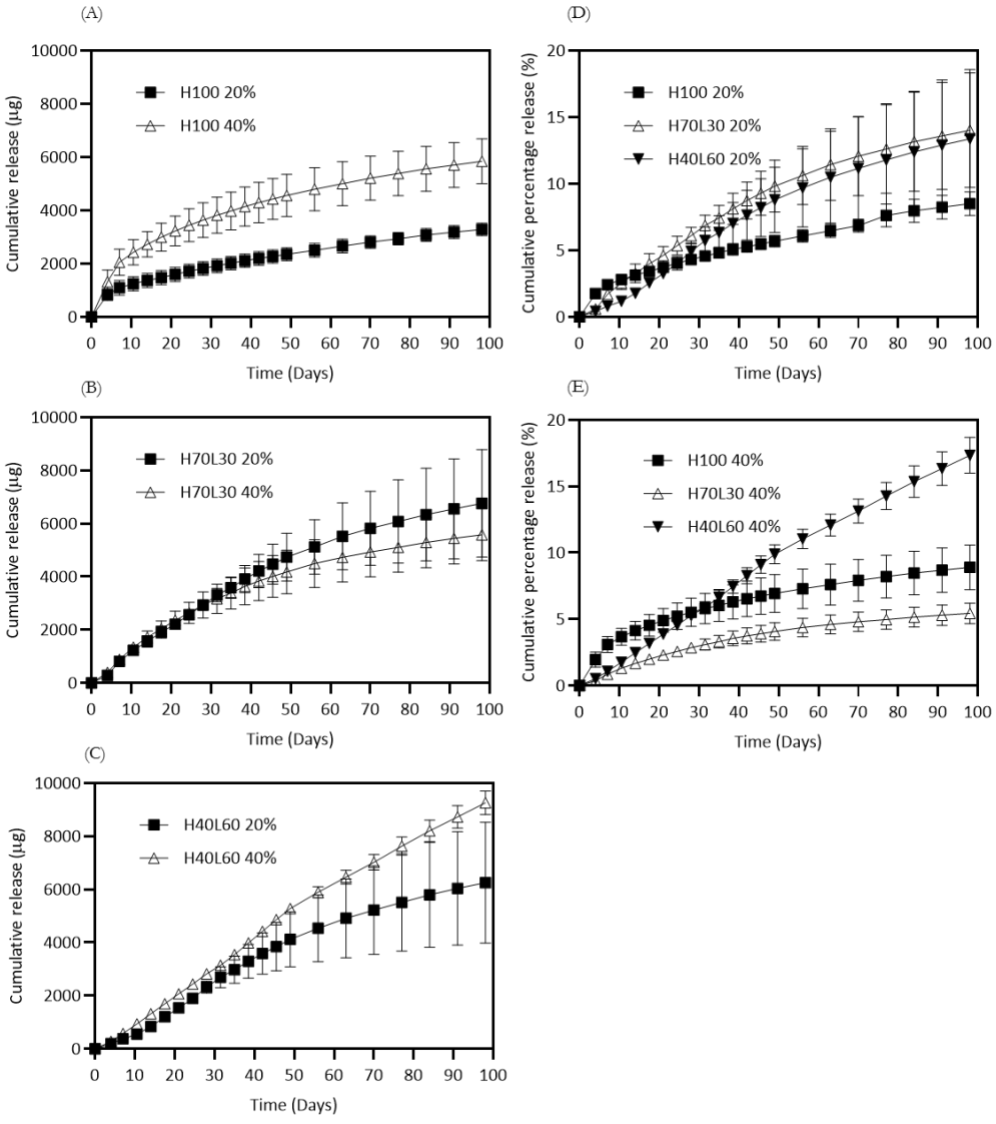


Figure 4. Cumulative *in vitro* release profiles for implants: (A) H100; (B) H70L30; and (C) H40L60. Cumulative percentage *in vitro* release profiles for implants containing: (D) 20% w/w LEVO sodium; and (E) 40% w/w LEVO sodium (means ±S.D., *n*=4).

Figure 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 (Figure 2). These pores are likely generated by the release of LEVO from the implant. Additionally, Figure S5 (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.

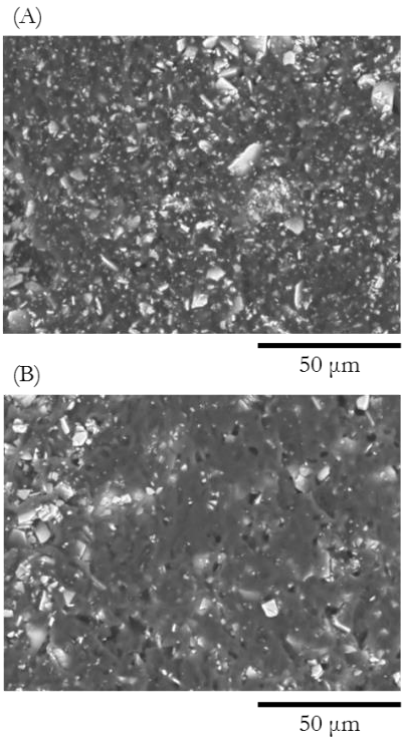


Figure 5. SEM images of: (A) H100-40% and (B) H40L60-40% after 105 days of release.

Modelling of the *in vitro* release data was based on Korsmeyer-Peppas, Higuchi, zero-order and Peppas-Sahlin models. The Korsmeyer-Peppas model was used to determine whether diffusion or degradation was the main mechanism occurring (Table 5). For cylindrical drug delivery devices: $n \leq 0.45$ indicates that Fickian diffusion is taking place; $0.45 < n < 0.89$ indicates anomalous (non-Fickian) diffusion is taking place; and $n > 0.89$ indicates zero-order release[21,22]. R^2 values for Higuchi and zero-order modelling were compared to confirm which model fitted best. For both drug loadings, H100 was found to follow a diffusion (Higuchi) release model, H70L30 followed a combination mechanism and H40L60 was found to follow a degradation (zero-order) model (Table 5). For those formulations which showed a combination release mechanism (H70L30-20% and H70L30-40%), Peppas-Sahlin modelling was carried out to evaluate the percentage contribution from diffusion and erosion as a function of time. K_D and K_E were calculated (Table 5). A graphical representation of the percentage contribution of each mechanism is shown in Figure 6. These show that for H70L30-20% erosion in the main mechanism, but when the drug loading for this formulation is increased to 40% w/w, diffusion becomes the dominant mode of release.

Table 5. Results of the fitting of release of LEVO sodium from different implant formulations to different kinetic equations.

Model		H100-20%	H70L30-20%	H40L60-20%	H100-40%	H70L30-40%	H40L60-40%
Korsmeyer-Peppas	**R^2**	0.999	0.996	0.992	0.999	0.999	0.998
	n	0.44	0.75	0.91	0.41	0.62	0.98
Higuchi	**R^2**	0.999	0.981	0.953	0.997	0.993	0.948
Zero-order	**R^2**	0.934	0.976	0.993	0.922	0.967	0.999
Peppas-Sahlin	**R^2**		0.994			0.991	
	K_D (min^{-m})	-	0.45	-	-	0.40	-
	K_E (min^{-2m})		0.20			0.05	

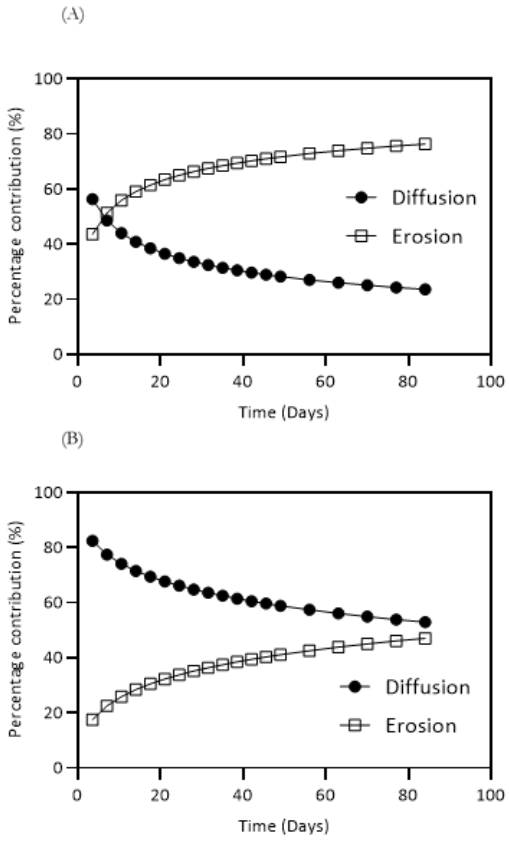


Figure 6. Percentage contribution of diffusion and erosion mechanisms of LEVO sodium release from (A) H70L30-20% and (B) H70L30-40%

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 6. Additionally, the curve describing the relationship between time profiles and mean plasma concentration is shown in Figure 7. It is important to note that normal LEVO plasma levels in rats (ca. 0.036 ng/mL)[29] are noticeable below the detection limit for the HPLC method used in this study.

Table 6. 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

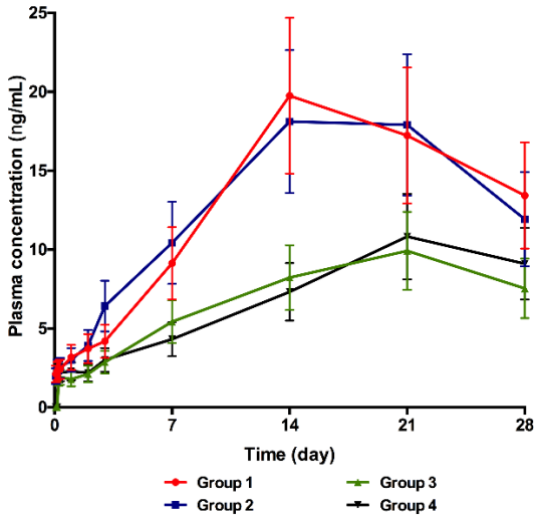


Figure 7. The mean plasma concentration and time profiles of LEVO sodium after subcutaneous administration of implant A in male (Group 1) and female (Group 2) Wistar rats, as well as implant B in male (Group 3) and female (Group 4) Wistar rats (means ± SD, n = 3 for each group).

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[11]. There has been 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.

As mentioned previously, the effect that non-compliance has on patient suffering from hypothyroidism could significantly alter their quality of life while contributing to increased treatment costs[13]. Supervised once weekly oral thyroxine dosing has been investigated as an alternative to daily oral LEVO sodium tablet administration, in patients who were thought to be non-compliant[12,17,30]. Thyroxine has a half-life of approximately seven days and, therefore, once weekly dosing should be feasible[1]. Weekly dosing was found to improve results for these patients and there was no indication of toxicity compared with daily therapy[12,17,30]. Weekly dosing could be an alternative for patients with compliance issues. However, oral LEVO sodium is known to have a number of drug and food interactions which are not overcome by oral once weekly dosing. A long-acting, non-oral drug delivery system that could deliver LEVO sodium in a way where its absorption was not affected by other medications would overcome many of these drug interactions and could further increase patient compliance. However, oral administration has some drawbacks as mentioned previously in terms of LEVO sodium absorption and interaction with food.

An alternative to 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[7,14–16]. Therefore, a long-acting polymeric subcutaneous implant could be promising for the treatment of this condition[31].

Both T_3 and LEVO (T_4) have been investigated as treatment options 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[9]. In addition, T_3 preparations result in a peak around 3 hours 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[32]. Multiple daily dosing may overcome this problem, but creates practicality issues, given the specific administration instructions associated with oral LEVO sodium[11,32]. However, currently Titan Pharmaceuticals (California, USA) is conducting studies for development of a subdermal implant to deliver T_3 for the treatment of hypothyroidism[33]. This device will utilise their ProNeura® drug delivery platform and aims to provide continuous delivery for 3 months to a year[32,33]. Research has been conducted into the use of combinations of T_3 and LEVO (T_4)[6,34]. However, the American thyroid association does not recommend the routine use of combination treatments (T_4 and T_3) for the treatment of hypothyroidism[32]. 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[25]. 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[35]. 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)[31,36–38]. PCL can be handled using a range of processing techniques such as 3D printing, melt or solvent casting or extrusion[31], which make it a flexible option when considering drugs with challenging stability issues, such as LEVO sodium[25]. 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[19,39–42]. 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[43,44]. 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[45,46]. Pharma applications of this solvent include tablet coating[45]. Accordingly, its use is accepted by the FDA when the levels of this solvent are below a particular threshold in the final product[47].

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 (Figure 2). DSC results indicated that LEVO sodium degraded at a lower temperature when incorporated within the implant when compared to pure LEVO sodium powder (Figure 3). FTIR confirmed that no new bonds were formed between the polymeric constituents and LEVO sodium (Figures S1-S4). Both FTIR and DSC confirmed that LEVO sodium was dispersed throughout, rather than dissolved in, the polymeric matrix. 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 short-lived initial burst release (where drug on the outer layer of the implant is dissolved and released) followed by a linear release phase. However, this burst release is below the reported maximum tolerated single dose of LEVO sodium for an adult and is, therefore, unlikely to cause toxicity *in vivo*[48]. Addition of L-PCL to the formulations prevented this burst release from occurring. Other studies have reported 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[35]. A PEG-containing formulation was included in initial investigations to prevent burst release. However, LEVO sodium was incompatible with PEG in the formulation and caused significant drug degradation. This 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[49]. The implants produced in this work resulted in extended release profiles in comparison to these *in situ* forming 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 pharmaceutical dose of LEVO sodium (50 – 200 μg/day)[11]. LEVO sodium has an oral bioavailability of 40 – 80%, therefore, if delivered subcutaneously lower doses may be required[1]. 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[50,51].

The release of hydrophobic and hydrophilic drugs from PCL based devices is primarily dominated by diffusion[35,52]. 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[52]. 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 Figure 5 shows the presence of pores created in the implant surface after 105 days of release. Moreover, this was confirmed by modelling which showed that, for both drug loadings, formulation H100 released by a diffusion mechanism and, for both drug loadings, H40L60 released by a degradation mechanism. The mechanism of release for H70L30 was dependent on the drug loading. This suggests that, as the proportion of L-PCL is increased degradation becomes the main mechanism for release from these implants. Increasing the amount of L-PCL in H-PCL/L-PCL mixtures has been previously reported to affect the crystallinity of the resulting material[25]. 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[25]. Therefore, these factors could explain the difference in the *in vitro* release experiments.

LEVO release was tested using an animal model. Figure 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 (Figure 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 x 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)[53,54]. 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)[55]. 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"[56–58]. 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 more than 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.

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7. References

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SUPPLEMENTARY MATERIAL

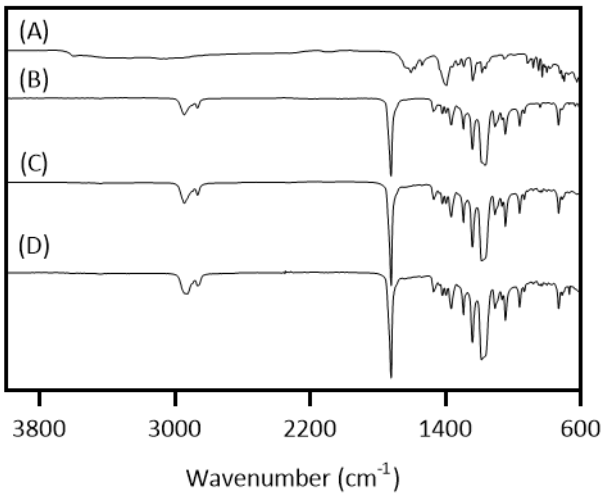

Figure S1. FTIR spectra of (A) LEVO sodium; (B) H100; (C) H100-20%; and (D) H100-40%.

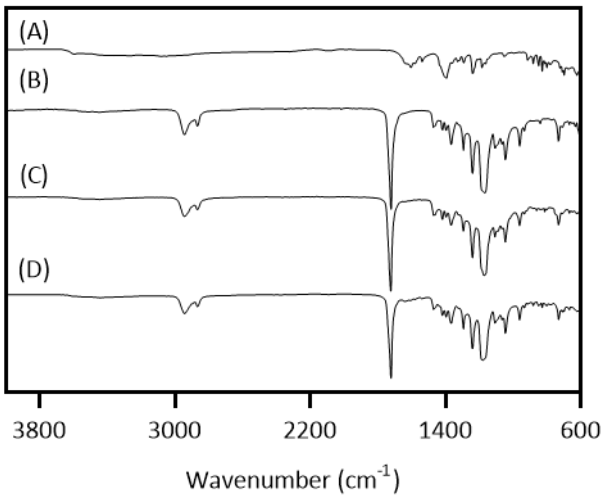

Figure S2. FTIR spectra of (A) LEVO sodium; (B) H70L30; (C) H70L30-20%; and (D) H70L30-40%.

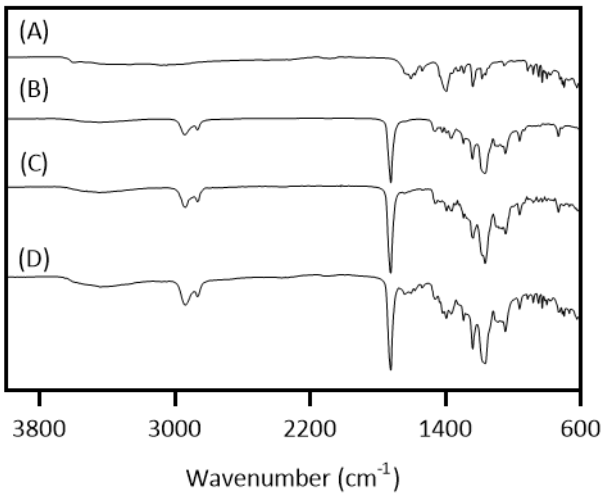

Figure S3. FTIR spectra of (A) LEVO sodium; (B) H40L60; (C) H40L60-20%; and (D) H40L60-40%.

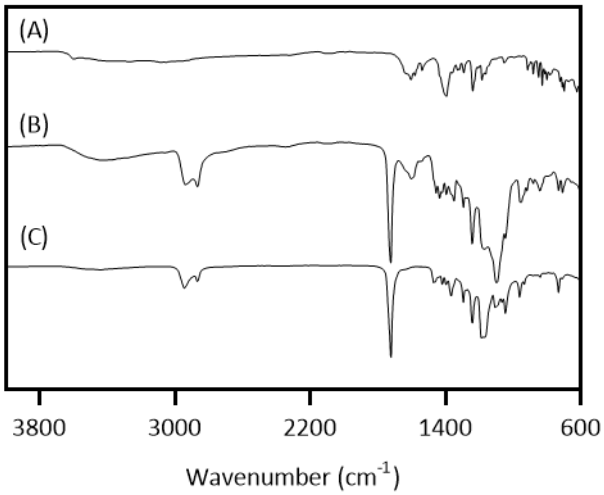

Figure S4. FTIR spectra of (A) LEVO sodium; (B) H35L35P30; and (C) H35L35P30-20%.

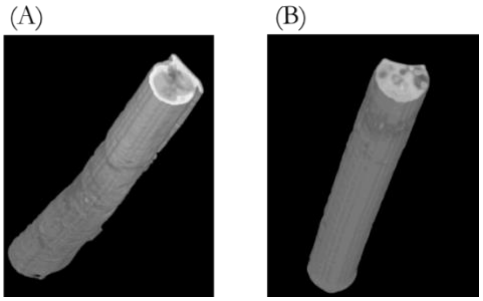

Figure S5. MicroCT images of (A) H100-40% and (B) H40L60-40% after 105 days of release. X-ray μ-CT scans were performed on the implants. The 3D reconstruction volumes and inner structures of the implants were observed by using a Skyscan 1275 system (Bruker, Billerica, MA, USA) with a Hamamatsu L11871 source. The microfocus of the X-ray source of the μ-CT scanner had maximum voltage of 80 kV and maximum current of 87 μA. No filter was applied for an exposure time of 49 ms. The data were then collected, and Data Viewer and CT-An software were used to analyze them. Finally, CTvol software was applied to generate 3D reconstruction images.

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