Optimization and in Vitro Evaluation of Injectable Sustained-Release of Levothyroxine Using PLGA-PEG-PLGA

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Abstract

Purpose In situ-forming gels (semi-solid state) (ISFGs) are widely used as sustained drug delivery, but they show a high burst release as well. The purpose of the current study is to make triblock that can make a quick gel on injection with a minimum burst release.

Methods In this study, to control the release of levothyroxine from ISFG, PLGA-PEG-PLGA (triblock) polymer was used. The melting method was employed to synthesize the triblock via ring-opening polymerization (ROP). Different weight percentages of triblock in the formulation were investigated to reach the minimum initial burst release of levothyroxine from ISFGs. Furthermore, the results of the in-situ forming implant (solid-state) (ISFI) of levothyroxine prepared from PLGA 504 H polymers were compared with ISFG.

Results The melting method employed in this study showed a successful ROP of the triblock. As the % triblock concentration was increased from 30 to 50%, the initial burst release decreased significantly. The initial burst release levothyroxine from ISFG ($6.52 \pm 0.30\%$) was much lower than the amount of levothyroxine released from ISFI ($14.15 \pm 0.79\%$). No cytotoxicity was observed for the sustained-release formulation containing ISFG 50% according to the MTT assay.

Conclusion The results indicated that this formulation was safe to be administered subcutaneously. As the synthesized triblock has thermosensitive properties, and also has the hydrogen bonding between the N-methyl pyrrolidone molecules and PEG, therefore, these properties make ISFG formulation to have a smaller initial burst release compared to ISFI formulation.

Keywords Levothyroxine · Initial burst release · In situ-forming implant · In situ-forming gel

Introduction

In hypothyroidism disease, the primary function of the human thyroid, endogenous production of thyroxine (T4), and triiodothyronine (T3) is below normal. Enough secretion of these thyroid hormones is essential to maintain cardiovascular, physical, and mental health, as well as the growth and development of children which is done via the regulation of gene transcription [1, 2]. Levothyroxine (T4), as a thyroid-stimulating hormone, is primarily used in the treatment of hypothyroidism.

Levothyroxine sodium is administered orally as a thyroid hormone [3, 4]. The oral bioavailability of levothyroxine is 65%, which is achieved if consumed 30 min to 1 h before breakfast on an empty stomach with a full glass of water [5, 6] because of complex formation of levothyroxine with soybean products, sodium, calcium, and other minerals [7]. Furthermore, levothyroxine should be taken daily, and this

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requires the patient's full cooperation with physicians and pharmacists. Therefore, sustained-release formulation of levothyroxine can be a good choice in hypothyroidism for patients who have poor compliance [8].

Recently, in situ-forming implants (solid-state) (ISFI) are one of the popular sustained drug delivery systems containing poly D,L-lactic-co-glycolic acid (PLGA) as a water-insoluble biocompatible and biodegradable polymer, and N-methyl pyrrolidone (NMP) as a physiologically compatible and water-miscible organic solvent [9]. After injection of the formulation into an aqueous environment, the NMP diffuses into the release medium, while the water diffuses into the polymer matrix (phase inversion mechanism); hence, the polymer precipitates as soon as it contacts water leading to the formation in the solid polymeric implant [10, 11]. NMP is an organic solvent and used in some of the products in the market such as Doxirobe® (doxycycline hyclate using PLA), Eligard® (leuporelin acetate using PLGA) and Nuflor® (Florfenicol using PEG) [12, 13]. However, the high-burst release in the first 24 h (15–80% of the total drug contents) is a drawback of these kinds of ISFIs [14, 15]. This initial burst release occurs by emerging NMP with the drug into the water upon injection that subsequently could cause systemic toxicity and tissue inflammation [16].

ISFI presents several significant advantages such as the avoidance of the first-pass metabolism, higher bioavailability for poorly water-soluble drugs, avoiding the escape of the treatment by the patients, which is especially relevant in indications where the lack of adherence to oral treatment may lead to incapacity and loss of autonomy [17–19]. Furthermore, ISFI utilizes bioresorbable polymers to control the drug release after parenteral administration. So, contrary to non-bioresorbable, releasing implants do not require surgical excision to remove the drug releasing system once the drug delivery is complete.

In this study, the use of triblock (PLGA-PEG-PLGA) instead of PLGA was suggested to prevent initial burst release. It seemed that the hydrogen bonding between the NMP molecules and PEG prevented the rapid diffusion of NMP into the release medium. Furthermore, the thermosensitive properties of PLGA-PEG-PLGA triblock could be helpful in matrix formation and decrease the initial burst release. Triblock should be liquid at room temperature and quickly convert into a gel at the physiological temperature (37 °C) [20, 21]. Therefore, there is no need for any surgery for the placement of this injectable triblock formulation [22]. The main drawback is that, generally, triblock is not stable in the presence of water. If water has to be used to make gels for mixing the triblock and drug powder (each in a separate vial), ultrasonication and a bath shaker are needed to be used for about 24 h to prepare the gel (semi-solid state). This makes it clinically impossible to use. To solve this problem, NMP was used to dissolve the triblock.

In the current research, to prepare ISFI formulations, only two syringes or vials were filled by the polymeric solution (polymer + NMP), and the drug in powder form is mixed together before injection and no need to use water (Fig. 1). The prepared formulation was administered at the injection site, and the gel is formed through two mechanisms: (a) phase inversion and (b) thermosensitive response of the triblock. The benefit of this formulation containing triblock is to have faster dissolution in NMP for preparing formulation and also making a quick gel on injection. Moreover, in the current study, to prevent the burst release of levothyroxine the application of the different weight ratios of triblock rather than PLGA is suggested. In situ-forming gel of levothyroxine has also been not formulated in other studies. It is hypothesized that the diffusion of NMP into the dissolution medium could be decreased by the hydrogen bonding between the PEG of triblock and NMP, and also with the thermo-sensitive characteristics of the triblock.

Material and Methods

Materials

For the ROP, PEG 1500, D,L-Lactide, glycolide, and stannous octoate ($\text{Sn}(\text{Oct})_2$) were used (Merck, Germany). Levothyroxine sodium was supplied from Iran Hormone Pharmaceutical Co. (Iran). PLGA RG 504H (50:50, M_w 38,000–54,000 Da) and levothyroxine were purchased from Sigma (USA) as the standard materials. 3-(4,5-dimethylthiazol-2-yl)-2, diphenyltetrazolium bromide (MTT) and N-methyl pyrrolidone (NMP) were also provided by Merck, Germany. Roswell Park Memorial Institute (RPMI) 1640 culture medium, fetal bovine sera (FBS), penicillin-streptomycin, and trypsin were purchased from Gibco, Germany. Mouse fibroblast L929 cell lines were donated from the Pharmaceutical Technology Institute of Mashhad University of Medical Science (Iran).

Methods

The Application of Melting Method (Three-Necked Flask) for the ROP of Triblock

The melting method using a three-necked flask was employed to synthesize the triblock copolymer. The ROP scheme was shown in Fig. 2a [23, 24]. Five grams of PEG was loaded into a vacuum three-necked flask under continuous stirring using a magnetic stirrer for 0.5 h to evaporate the absorbed water ($T = 100\text{ }^\circ\text{C}$) from PEG. Next, the catalyst $\text{Sn}(\text{Oct})_2$ (20 μL), D,L-lactide (9.45 g), and glycolide (2.54 g) (LA:GA molar ratio (3:1)) were added into the polymeric solution under vacuum condition for 24 h ($T = 140\text{ }^\circ\text{C}$). After the completion of

Fig. 1 Preparation of ISFI or ISFG using two coupled syringes [21]

polymerization, the copolymer was dissolved in water (5–8 °C) and precipitated at 60 °C for purification. The unreacted monomer, low-molecular-weight, and water-soluble polymer in the supernatant were eliminated. Finally, the precipitate was collected and freeze-dried at a temperature of –20 °C at a pressure of 103.2446 Pa to remove the residual water [25, 26]. The purification process was repeated three times to obtain the purified triblock [27].

Characterization of Triblock

¹H-NMR To evaluate the structure of triblock (PLGA-PEG-PLGA) and LA:GA molar ratio of the triblock copolymer, the ¹H-NMR spectrum of the copolymer was obtained by Bruker FT-500 and 300 MHz instruments (USA) in CDCl₃ at room temperature [28, 29]. The LA:GA ratio was determined by integration of the signals pertaining to each

Fig. 2 ROP scheme (a) [21], ¹H-NMR (b), and FTIR spectrum (c) of triblock

monomer, such as the peaks from the CH and CH₃ groups of LA and the CH₂ groups of GA.

Fourier Transform Infrared Analysis The triblock was mixed with KBr, and then its structure was also confirmed by the Fourier transform infrared analysis (FTIR) (vertex 70, Bruker, Germany) [23, 30].

Polydispersity Index To determine the polydispersity index (PDI), average molecular weight (M_w), and the number average molecular weight (M_n) of the synthesized triblock, a GPC-Addon apparatus with Plgel® columns (Agilent, USA) was used. In the GPC technique, tetrahydrofuran as eluent at a flow rate of 1 mL/min was employed, and polystyrene was used as a standard [23].

Differential Scanning Calorimetry To identify the glass transition and melting temperatures of triblock, differential scanning calorimetry (DSC) was conducted by a DSC822 (Mettler Toledo, Switzerland). Approximately, a few mg of samples (3–4 mg) were accurately weighed into aluminum pans and then sealed. The pans were cooled down to –60 °C followed by heating the samples to 60 °C at a scanning rate of 5 °C/min under nitrogen atmosphere.

Sol-Gel Transition Temperature To determine the sol-gel transition temperature of the synthesized triblock, different weight % of triblock ranging from 10 to 50 w/w% was prepared in PBS (pH 7.4), and the temperature of the prepared samples was elevated from 0 to 60 °C at a scanning rate of 0.5 °C/min. The sol-gel transition temperature was recorded when the magnetic flea in the solution stopped spinning [31, 32].

Scanning Electron Microscopy To evaluate cross-sections morphology of the matrix, the gel which formed after 3 days was freeze-dried and analyzed using SEM (LEO1450 vp, Zeiss Company, Germany) [24]. Scanning electron microscopy (SEM, Tescan, model Vega II, Czech) was equipped with an energy dispersive X-ray analyzer system (EDXA, Oxford Instrument, INCA, England). Samples were freeze-dried and placed on the specimen holder using double-sided carbon conductive tape and coated with gold to render them electrically conductive.

Preparation of Liquid Formulations

PLGA 504H (ISFI, 33% w/w) and the triblock (ISFG, 30, 40, and 50% w/w) were dissolved in NMP and sonicated at room temperature for 2 h [33, 34]. To sterilize the formulations, an autoclave was used for 15 min at 121 °C at 3 bars. To complete the formulation, 3 mg of levothyroxine sodium was added to the autoclaved polymeric solution followed by sonication of the sample to achieve a homogeneous solution. The

formulations were passed through a 20-gauge syringe at 25 °C at a steady shear rate of 75 s^{–1} to investigate the syringability of each sample. A cone/plate viscometer (Brookfield, Germany) at a shear rate of 0.3 r.p.m. was used to measure the viscosities of ISFG formulations [35].

In Vitro Release Study

To obtain dissolution profiles for the prepared formulations, the dissolution test was performed. Phosphate buffer with pH 7.4 (50 mL) was used as a dissolution medium, and the medium was kept at 37 ± 0.5 °C with a rotational speed of 35 rpm. One milliliter of the formulation was injected directly into each vial containing dissolution media (100 mL PBS) by a 20-gauge needle. The solution turned into a gel or an implant after injection to the medium. The vials were kept in a reciprocal shaking water bath during the entire release study. The test was followed by withdrawing manually 3 mL from the dissolution medium at predetermined time intervals up to 35 days (2, 4, 6, 8, 12, 18, and 24 h; 2, 3, 4, 5, 7, 10, 14, 16, 18, 21, 28, and 35 days). Following the withdrawal of the samples, 3 mL of fresh PBS was transferred to the dissolution medium to keep the volume of the dissolution medium constant, and also to maintain a sink condition [36, 37]. The withdrawn samples were subsequently analyzed by HPLC to determine the concentrations of levothyroxine from the calibration curve (0.007–10 µg/mL). The samples (20 µL) were analyzed through HPLC (Shimadzu, Japan) with an LC-6 AD pump linked to a diode array detector (DAD) at 225 nm with a C18 column (25 cm × 4.6 mm, pore size 100 Å). A mixture of double-distilled water (59.5% v/v), acetonitrile (40% v/v), phosphoric acid (0.5% v/v) (isocratic) with a flow rate of 1.2 mL/min was used as eluent. The NMP was also detected in the release medium at 220 nm with double-distilled water (68% v/v) containing trifluoroacetic acid (0.1% v/v) and acetonitrile (32% v/v) (isocratic) as eluent with a flow rate 0.5 mL/min [38, 39].

The weight of remained matrix (implant or gel) was determined at 1, 2, 3, 5, 7, 14, 21, 28, and 35 days in the release medium (deionized water) after freeze-drying to monitor its degradation percentage using Eq. 1.

$$\text{Degradation\%} = \frac{(W_0 - W_d)}{W_0} \times 100 \quad (1)$$

where w_0 and w_d are the initial and secondary weight of gel or implant.

In Vitro Cellular Cytotoxicity

The RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated FBS, 100 IU/mL of penicillin, and 100 mg/mL of streptomycin containing the mouse L929 fibroblast cell line

Fig. 3 GPC chromatogram of triblock

was incubated in a humidified incubator at 5% CO₂ at 37 °C for 1 week [11]. The cells were seeded at a 96-well plate at a density of 5×10^4 cells/well. The plates were divided into 6 groups ($n = 3$ per group): group I: control (containing cells and culture medium without sample); group II: levothyroxine solution in NMP (3 mg levothyroxine in 670 mg NMP); group III: ISFI; group IV: ISFG 30%; group VI: ISFG 40%; and group VII: ISFG 50%. Firstly, a 50 mL PBS (pH 7.4, 37 °C)

for each group was prepared and incubated for 24 h. Then, a sterile membrane (pore size 0.22 μm) was employed to filter 2 mL of this solution, and subsequently, 20 μL of the filtered solution was directly added into the center of each well and incubated for 24 h. Then, the culture medium was discarded, and the cell viability % was determined by adding 500 μL of MTT solution (0.5 mg/mL in PBS). After 4 h, 100 μL of DMSO was replaced with the culture medium, then the plate

Fig. 4 DSC spectrum of triblock

Fig. 5 Phase diagram of triblock (Sol to gel and gel to precipitate) (Mean $\pm$ SD, $n = 3$)

was agitated for 1 h. Finally, the absorbance was measured by a microplate reader (VMax®, Canada) at 570 nm (sample) and a reference wavelength was set up at 630 nm [39].

Statistical Analysis

Data reported in the current research were expressed as means and standard deviations. To show the significance of the data,

one-way ANOVA was performed followed by *t* test using linear regression where $p < 0.05$ was quoted as significant.

Results and Discussion

ROP and Characterization of Triblock Copolymer

In this study, the melting method was successfully employed to ROP of the triblock. A typical $^1\text{H-NMR}$ spectrum of the triblock was shown in Fig. 2b. The formation of a terminal CH_2 of PEG, the CH of LA, the CH_3 of LA, and the CH_2 of GA were identified by signals appearing at 4.253, 3.115, 5.131, 1.427, and 4.763 ppm, respectively. The structure of copolymer was also investigated using FTIR spectroscopy [40]. The FTIR characteristic peaks appearing at 3508.2, 1268.1, 2875.1, 1755, and 842.8 cm^{-1} represented the terminal OH of PEG, C–O of PEG, CH_2 of GA, and PEG, C=O of LA and GA and the CH_3 of LA, respectively [41] (Fig. 2c). The results obtained by FTIR and $^1\text{H-NMR}$ were in agreement with each other. The unimodal trace obtained by GPC and also a low polydispersity observed in GPC chromatogram of triblock were an indication of a successful synthesis and purification of the triblock (Fig. 3).

According to the DSC thermogram shown in Fig. 4, the melting temperatures (T_m) and the glass transition temperature (T_g) of triblock were about 27.46 and -5.19 °C, respectively. The results indicated that the state of the synthesized triblock was semisolid. The phase diagram of triblock with different concentrations of copolymers was shown in Fig. 5. The results

Fig. 6 Chromatogram of HPLC analysis for pure levothyroxine (a) and NMP (b) in the PBS (10 $\mu\text{g/mL}$), a sample of in vitro release medium from ISFG formulation after 24 h for levothyroxine (c) and NMP (d)

presented in Fig. 5 exhibited that an increase in the concentration of the copolymer from 30 to 50% (w/v) resulted in an increase in the precipitation temperature and also caused a reduction in the sol to gel transition temperature. The aggregation of the micelles was accelerated when the concentration of the copolymer was increased, this, in turn, could cause an enhancement in the micelle concentrations, and therefore, the transition of sol to gel occurred at a lower temperature, and the precipitation temperature decreased.

In Vitro Release Evaluation

HPLC chromatograms of pure levothyroxine, NMP in PBS, and levothyroxine and NMP for the ISFG formulation in PBS at 37 °C after 24 h were shown in Fig. 6a–d, respectively. The HPLC thermograms showed retention times of 2.56 ± 0.11 min and 5.62 ± 0.28 min for levothyroxine and NMP, respectively. Sharp peaks from levothyroxine and NMP showed that the HPLC was able to separate the two substances from the release medium and be detectable. Also, the limit of quantification (LOQ) and the limit of detection (LOD) were 15.9 ng/mL and 5.3 ng/mL for levothyroxine and 3.9 µg/mL and 1.3 µg/mL for NMP respectively.

It was observed that all formulations were easily injectable through a 20-gauge syringe at 25 °C. Figure 7 showed the viscosity of formulations and the effects of triblock concentration in NMP on the viscosity [42]. The figure showed a nonlinear increase in the viscosity of PLGA-PEG-PLGA polymer solution in NMP with a sharp change at the concentration of 50% which could be due to more hydrophobic lactate concentration leading to the aggregation of triblock.

The gel or implant was formed immediately after the injection of the formulation into the release medium. The cumulative in vitro release of levothyroxine and NMP from ISFG (using different concentrations of triblock %) and ISFI using PLGA were shown in Fig. 8a, b, respectively. The initial burst release of levothyroxine from ISFG 30%, ISFG 40%, and ISFG 50% were $17.30 \pm 0.90\%$, $9.57 \pm 0.28\%$, and $6.52 \pm$

Fig. 7 The effect of viscosity vs. concentration of triblock solution in the NMP

Fig. 8 Cumulative in vitro release of levothyroxine from formulations (a), cumulative in vitro release of NMP from formulations (b), and in vitro degradation of formulations without levothyroxine (c). (Mean $\pm$ SD, $n = 3$)

0.30%, respectively. Furthermore, the initial burst release of NMP from ISFG 30%, ISFG 40%, and ISFG50% were $34.06 \pm 1.81\%$, $19.15 \pm 0.57\%$, and $13.05 \pm 0.59\%$, respectively. An increase in weight percentage of triblock from 30 to 50% caused an increase in the cross-links between the copolymer molecules which led to an increase in the tortuosity and the viscosity of the hydrogel. Subsequently, the initial burst

Fig. 9 Cross-section morphologies of the implant or gel from the formulations using SEM (scale bar: 100 μ m): ISFI (a), ISFG 30% (b), ISFG 40% (c), and ISFG 50% (d)

release significantly reduced as a result of the increased in hydrogel network porosity, and a reduction in drug and NMP diffusion rate [21, 23, 43]. The early burst release of levothyroxine from the ISFI formulation ($14.15 \pm 0.79\%$) was significantly ($p < 0.05$) higher than the ISFG 50% ($6.52 \pm 0.30\%$). Furthermore, NMP showed an early burst release of 28.31% from the ISFI formulation which was remarkably higher than the ISFG 50% which showed 13.05% initial drug release ($p < 0.05$). A lower burst release of NMP observed for the ISFG formulation compared to ISFI could be related to the

formation of hydrogen bonding between terminal hydroxyl groups of triblock and NMPs carbonyl group.

Based on the loss of weight, in vitro degradation results for ISFI and ISFG formulations without levothyroxine in the PBS were shown in Fig. 8c. The faster degradation rate of ISFG compared to ISFI formulation could be attributed to the existence of PEG in the triblock structure which caused an increase in the penetration of more water into the matrix [44, 45]. The faster degradation of triblock in comparison to PLGA 504H caused a complete drug release from the ISFG within

Table 1 Regression coefficient (R^2) and “ n ” of predicted in vitro release models

Model	Equations	ISFG 30%		ISFG 40%		ISFG 50%		ISFI	
		R^2	n	R^2	n	R^2	n	R^2	n
Higuchi (diffusion)	$Q_t = K_H \times \sqrt{t}$	0.9108	—	0.8430	—	0.8178	—	0.932	—
Zero-order (degradation)	$Q_t - Q_0 = K_0 \times t$	0.9228	—	0.9854	—	0.9872	—	0.939	—
Korsmeyer-Peppas	$M_t/M_\infty = Kt^n$	0.952	0.726	0.9867	1.075	0.9940	1.178	0.974	0.721

Q_t , drug release at time t ; Q_0 , the initial drug at time zero; K_0 , zero-order constant; K_H , Higuchi constant; M_t , the drug released at time t ; M_∞ , the drug release at t , infinite; k , power-law constant; n , diffusion exponent [Fickian diffusion (less than 0.43), non-Fickian (between 0.43–0.89), zero-order (greater than 0.89)]

21 days, while the complete drug release occurred for ISFI after 28 days. The in vitro degradation results and NMP release profiles both were confirmed the in vitro drug release results (Fig. 8a–c).

The morphology of ISFG with different weight% of triblock (ranging from 30 to 50 w/w%) in NMP and ISFI formulations were shown in Fig. 9. The images showed that ISFI formulation was spongy and highly porous (Fig. 9a), whereas the ISFG gels containing 30% (Fig. 9b), 40% (Fig. 9c), and 50% (Fig. 9d) of triblock showed low porosity and more packed morphology due to the decreased rate of NMP exchange with water that subsequently prevented the formation of a highly porous, spongy, and finger-like structure [46].

To evaluate the kinetics and mechanisms of drug release, the release data were fitted into various release models [47, 48]. The models used were zero-order, Higuchi, and Korsmeyer-Peppas (Ritger–Peppas) models which were listed in Table 1.

Table 1 shows the modeling of cumulative in vitro release experimental data based on zero-order, Higuchi, and Korsmeyer-Peppas models. The determination coefficient (R^2) of the zero-order model was higher than the Higuchi model for four formulations (ISFG 30%, 40%, 50%, and ISFI), which indicated that the release data followed well the zero-order model (Table 1). The result indicated that the main mechanism of the drug release followed the zero-order model (degradation). Furthermore, according to the Korsmeyer-Peppas model, the n value for ISFG 40% and ISFG 50% was greater than 0.43 which indicated the drug release mechanism for these two formulations was also the degradation model. The n value for ISFG 30% and ISFI was $0.43 \leq n < 0.89$ which represented a non-Fickian transport. But, since the determination coefficient (R^2) for the zero-order model of ISFG 30% and ISFI was greater than the Higuchi model, this indicated that the experimental data followed the zero-order model (Table 1). Therefore, the modeling of four formulations proved that the dominant mechanism of drug release was degradation (zero-order model) [49].

In Vitro Compatibility Evaluation

For any subcutaneously administered formulation, it is vital to investigate its side effects on the skin [50]. To this end, the biocompatibility projections of ISFI formulation including their cytotoxic effects were carried out via mouse L929 cell line (fibroblast cells) that has been initiated from adipose tissue with fibroblast morphology [11, 39]. The cell viability after 24 h was investigated by employing MTT assay [51] and the results were shown in Fig. 10. Rahimi et al. evaluated the cytotoxicity effects of ISFI formulation on fibroblast cells after 24 h and reported cell viability values from 89 to 100% [39]. The cell viability of the ISFG 50% ($96.17 \pm 1.15\%$) was higher than that of the ISFG 30% ($86.37 \pm 0.76\%$), ISFG 40%

Fig. 10 Cell viability of L929 mouse fibroblast cells in contact with release media after 24 h using MTT assay. (mean $\pm$ SD, $n = 3$). **** $p < 0.0001$

(90.27 ± 2.01), and ISFI ($91.70 \pm 2.17\%$) as shown in Fig. 10. The higher cell viability could be due to the lower early release of NMP and levothyroxine from this formulation which was confirmed by the in vitro release evaluation. Based on these results, it can be concluded that the formulation containing ISFG 50% showed good biocompatibility; therefore, it could be administered subcutaneously.

Conclusion

One of the main problems of sustained-release formulations is the initial burst release of a drug at the beginning of the therapy during the first 24 h. For levothyroxine depot forms, the high initial drug release increases the serum concentration of the drug which may cause serious side effects such as tachycardia and fatigue. In this work, PLGA-PEG-PLGA copolymer was used for ISFG preparation to overcome this phenomenon (controlling the early drug release), because the hydrogen bonding between the NMP molecules and the PEG chains in ISFG formulation prevented the rapid diffusion of NMP into the release medium and showed a lower initial drug release. Our results were indicated that the copolymer and solvent NMP were biodegradable, biocompatible, and enable to deliver the drug for a long time (21 days in vitro drug release) with the acceptable unwanted initial release than ISFI using PLGA 504H which has not yet been reported in other studies so far.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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