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ORIGINAL ARTICLE / ÖZGÜN MAKALE

FORMULATION AND EVALUATION OF PARENTERAL *IN-SITU* FORMING BIODEGRADABLE IMPLANT FOR CONTROLLED RELEASE OF LEVOTHYROXINE SODIUM

LEVOTİROKSİN SODYUMUN KONTROLLÜ SALIMI İÇİN IN-SITU PARENTERAL BİYOBOZUNUR İMPLANT FORMÜLASYONU VE DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: *The objective of present research work is formulation and evaluation of parenteral in-situ forming biodegradable implant for controlled release of levothyroxine sodium.*
Material and Method: *The present study used N-Methyl pyrrolidone (NMP) and triacetin as solvents and PLGA as a biodegradable polymer to manufacture two biodegradable polymeric drug delivery systems, in-situ forming implant (ISFI) and in-situ micro particles (ISM). Other evaluation tests, such as sterility, percent drug entrapment capacity, and so on, were also carried out. ISFI and ISM were tested for up to one month at three different temperatures (4ºC, 25ºC, and 40ºC).*
Result and Discussions: *The drug release from both systems was compared. In batch F4, burst release was 10.72%, while in batch EP8, it was 8.16%. F4 was released 94.54% in roughly 30 days and EP8 was released 95.72%. The polymer content, type of solvent (hydrophilic or hydrophobic), and implant morphology all contributed to increased burst release in the ISFI formulation. Burst release was decreased using a combination of hydrophilic and hydrophobic solvents (NMP and Triacetin). When compared to other formulations, ISM had the lowest burst release. Both the ISFI and ISM formulations might deliver medications for up to 30 days. Both formulation show good*

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drug entrapment efficiency F4 (87.74%) and EP8 (90.37%) respectively. Both formulations passed all their physicochemical proprieties included visual examination, pH, and injectability respectively. No visible growth of microorganisms was seen in growth media treated with both formulations after 30 days. The injection site (on the skin) and adjacent muscles showed no symptoms of irritation. It was confirmed when the results were compared to those of the control group. There was no hyperemia, discoloration, or necrosis at the site and no sign of irritation by both formulations. In the case of ISM (EP8), drug release follows zero order kinetics with an R^2 value of 0.9814 and ISFI (F4) follows Korsmeyer peppas and both transport drug through Fickian diffusion mechanism. Both formulations were found to, be stable. Hence, Long-acting Levothyroxine sodium formulations (ISFI & ISM) may be a superior option for hypothyroidism treatment.
Keywords: *Biodegradable polymers, ISFI, ISM, in-situ implant, solvent exchange technique.*

ÖZ

Amaç: *Mevcut araştırmanın amacı, kontrollü levotiroksin sodyum salımı için parenteral in-situ oluşan biyobozunur implantın formülasyonu ve değerlendirilmesidir.*
Gereç ve Yöntem: *Bu çalışmada, in-situ implant (ISFI) ve in-situ mikro parçacıkları (ISM) şeklindeki iki biyobozunur polimerik ilaç taşıyıcı sistemi hazırlamak için çözücü olarak N-Metil pirolidon (NMP) ile triasetin ve biyobozunur polimer olarak da PLGA kullanılmıştır. Sterilite, etken madde yükleme kapasitesi yüzdesi ve benzeri diğer değerlendirme testleri de gerçekleştirilmiştir. ISFI ve ISM, bir aya kadar üç farklı sıcaklık (4ºC, 25ºC ve 40ºC) değerinde test edilmiştir.*
Sonuç ve Tartışma: *Her iki sistemden etken madde salımı karşılaştırıldı. F4 kodlu formülasyonda başlangıç doz boşalması %10.72 iken, EP8 kodlu da %8.16'di. Etken madde F4 koldu formülasyondan yaklaşık 30 günde %94.54 ve EP8 kodlu formülasyondan %95.72 düzeyinde açığa çıkmıştır. Polimer içeriği, çözücü tipi (hidrofilik veya hidrofobik) ve implant morfolojisi olmak üzere hepsi, ISFI formülasyonundan başlangıç doz boşalmasının artmasına katkıda bulunmuştur. Başlangıç doz boşalması, hidrofilik ve hidrofobik çözücülerin (NMP ve Triasetin) kombinasyonu kullanıldığında azaltılmıştır. Diğer formülasyonlarla karşılaştırıldığında, ISM en başlangıç doz boşalmasına sahiptir. Hem ISFI hem de ISM formülasyonları, etken maddeleri 30 güne kadar verebilmektedir. Her iki formülasyon da sırasıyla iyi etken madde yükleme etkinliği F4 (%87.74) ve EP8 (%90.37) göstermektedir. Her iki formülasyon da sırasıyla görsel inceleme, pH ve enjekte edilebilirlik dahil tüm fizikokimyasal özelliklerinden geçmiştir. 30 gün sonra her iki formülasyonla işleme tabi tutulan büyüme ortamındaki mikroorganizmaların gözle görülür bir büyümesi görülmedi. Enjeksiyon bölgesi (cilt üzerinde) ve bitişik kaslar hiçbir irritasyon belirtisi göstermedi. Sonuçlar kontrol grubu ile karşılaştırıldığında doğrulanmıştır. Her iki formülasyonda da irritasyon belirtisi ve enjeksiyon bölgesinde hiperemi, renk değişikliği veya nekroz yoktu. Her iki formülasyonun da stabil olduğu bulunmuştur. Bu nedenle, uzun etkili Levotiroksin sodyum formülasyonları (ISFI ve ISM), hipotiroidizm tedavisi için üstün bir seçenek olabilir.*
Anahtar Kelimeler: *Biyobozunur polimerler, ISFI, ISM, in situ implant, solvent değişim tekniği.*

INTRODUCTION

Hypothyroidism is a disease in which the thyroid gland fails to produce enough thyroid hormone to meet the body's metabolic needs [1-3]. Dyslipidemia, infertility, cognitive impairment, hypertension, neuromuscular dysfunction, and other symptoms of untreated hypothyroidism might occur. There is no cure for it, and the patient must supplement with Thyroxin from tablets (Levothyroxine Sodium Tablets) to maintain normal TSH levels [4]. Levothyroxine (T4) and Liothyronine (T3) are the most often used supplements in treatment. Levothyroxine is the more often utilized of the two. Levothyroxine Sodium is a synthetic version of the main thyroid hormone that is used to treat hypothyroidism, as well as Myxedema coma and other thyroid problems [5].

The main disadvantage of this treatment (oral) is that patients must take tablets every day, and the overall effectiveness of treatment reduces as a result of patient noncompliance, such as drug-food interactions, irregular administration, or missing doses [6-8]. Parenteral thyroxin is required in some circumstances, such as severe malabsorption of thyroxin via the oral route, and typical parenteral treatment involves frequent dosage, which is uncomfortable [8-10]. As a result, a long-acting formulation of Levothyroxine sodium is needed, one that can release the hormone in a controlled manner

over a longer period of time. This will eventually improve patient compliance and aid in achieving a more consistent hormone plasma concentration in the body [10].

This type of new drug delivery method is well suited for PLGA-based long-acting in situ biodegradable implant formulation [11,12]. Biodegradable injectable implant drug delivery devices represent a novel physical method to improving drug pharmacokinetics and pharmacodynamics. Other benefits include medication distribution at a controlled rate into the systemic circulation for a longer period of time, less frequent administration maintaining a better therapeutic level of drug, elimination of patient-related errors (Medication non-adherence), and lower total treatment costs. As a result, the focus of the research is on the development and characterization of such formulations [13,14].

In the market, only oral pills and traditional parenteral solutions are available. There is no controlled-release formulation available. For the need of long-term controlled release of Levothyroxine Sodium, In-Situ Implant (ISFI) or In-Situ Micro particles (ISM) may be the ideal drug delivery technology. The goal of this research is to develop a stable ISFI or ISM-based drug delivery system for the long-term controlled release of Levothyroxine Sodium for hypothyroidism treatment.

MATERIAL AND METHOD

Materials

Levothyroxine Sodium Purchased from Taj Mahal VisionChemicals Pvt. Ltd. Mumbai, India. PLGA has obtained a Gift sample from Evonik, Mumbai. N-Methyl 2-Pyrrolidone and Triacetin were obtained Gift Sample from Research Lab FineChemicals, Ankleshwar. Tween 80, Span 80, Sodium Azide, Potassium DihydrogenPhosphate, Potassium Chloride, Sodium Chloride, and Disodium HydrogenPhosphatewas purchased from Merck Private Ltd, Mumbai. All other materials were used of analytical grade.

Methods

Determination of Absorption Maxima (λ max) of Levothyroxine Sodium

A 1 ml standard stock solution of Levothyroxine Sodium (100µg/ml) was transferred to a 10 ml volumetric flask and the volume was adjusted to 10 ml with phosphate buffer pH 7.4 before the absorbance of the solution was scanned in the range of 200 to 400 nm using a double beam UV-visible Spectrophotometer. The absorbance maxima were determined by scanning a 10µg/ml solution [15-17].

Methodology for Preparation of ISFI & ISM

Figure 1&2 explained in detail the preparation of ISFI and ISM [19].

Evaluation of ISFI and ISM

Physicochemical Properties of ISFI and ISM

Visual examination, pH of the formulation was all performed according to the protocol [20].

Syringeability & Injectability

Syringe ability (ease of withdrawal from vial to syringe) and Inject ability (formulation performance during injection) are two very important parameters in handling & delivering formulation safely to patient. Syringeability of formulation was evaluated by transferring formulation into the vial using different gauge of needle & Injectability was evaluated by Injecting from the different gauges of needles. The ease of injection was observed [20].

Drug Entrapment Efficiency

To find entrapment of drug during solidification process (Implant formation) 0.5 ml of formulation (polymeric solution (ISFI)) was injected into 50 ml of phosphate buffer & the formation of Implant was occurred. After formation of it (after 1 minute) 2 ml of phosphate buffer was taken & evaluated for drug content using HPLC. The released drug after 1 minute was subtracted from the total

drug loaded. That will directly give the amount of drug entrapped inside formed Implant [20]. The entrapment efficiency calculated using following formula:

Drug entrapment efficiency = Total Drug loaded – Drug released just after implant formation / Total drug loaded*100 -------- (1)

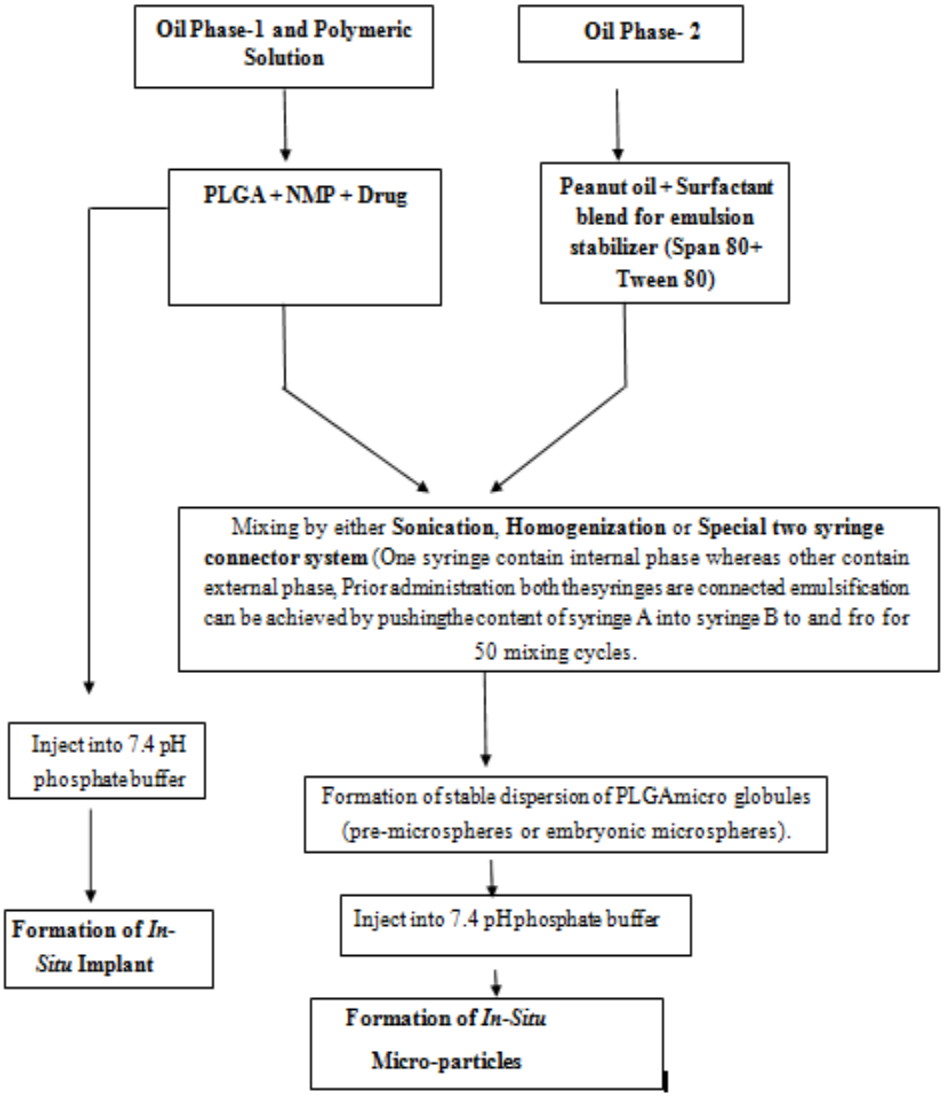

Figure 1. Method of preparation of ISFI & ISM

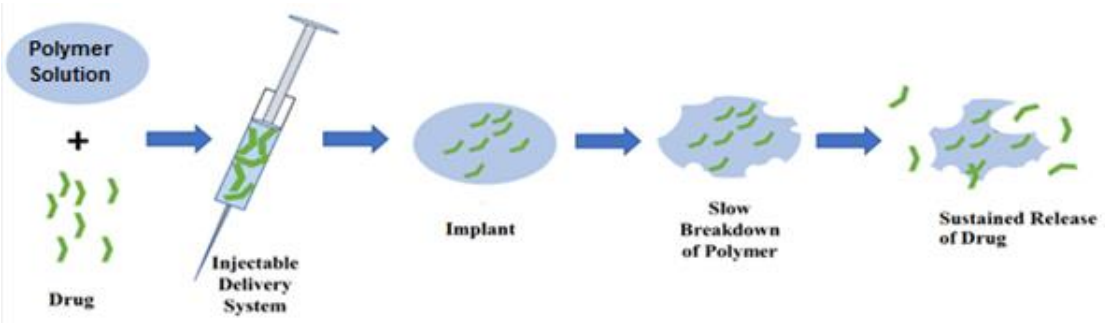

Figure 2. Graphical presentation of preparation of implant

Morphology Study of ISFI & ISM

The 0.5 ml formulation was injected into phosphate-buffered saline pH 7.4 at 37°C, and the morphology of the implant formed was evaluated until the polymer matrix was entirely dissolved by hydrolytic cleavage. After injection, the in-situ micro particles were filtered and dried for ISM formulation. The sample was subsequently taken to the facility for SEM analysis [20,21].

***Ex-vivo* Formation of ISFI & ISM**

Monitoring the growth of muscle implants 0.5 ml of the formulation was injected into the chicken leg muscle using 21 gauge needles. To make it easier to visualize how the implants formed, patent blue dye was added to the mixture. After 15 minutes, dissecting the chicken leg muscle revealed the formation of an Implant (at the injection site). After injecting 0.9 ml formulation using a 21 gauge syringe, a small tissue section was taken from the injection site and the development of In-situ micro particles was studied under an optical microscope [22,23].

Sterility Testing

Sterility testing is essential since ISFI and ISM are parenteral formulations. The samples were evaluated for sterility using the "Direct Inoculation Method" under aseptic circumstances. Both ISFI and ISM were made in an aseptic atmosphere and then filtered with a 0.22 micron syringe filter. The formula was kept in sterile containers. Finally, for microbial growth testing, sterile SCDM (Soybean Casein Digestive Media) was utilized to inoculate both ISFI and ISM. Inoculation procedures were placed in a LAF chamber. Microbial growth was assessed using visual examinations [24,25].

***In-vitro* Drug Release**

For ISFI

ISFI was tested *in-vitro* by injecting 0.5 ml of the formulation into 50 ml of PBS pH 7.4 in an incubator shaker bath. The temperature was kept constant at 37ºC. Sampling was done after 1 hour, 3 hours, 6 hours, 12 hours, 1 day, 3 days, 7 days, 12 days, 19 days, 25 days, and 30 days. The sample analysis was carried out using HPLC [26].

For ISM

The *in-vitro* drug release experiment from ISM was investigated using dialysis. The LA 390-5 MT dialysis membrane was cut into 1.2-inch lengths and submerged overnight in phosphate buffer saline pH 7.4. 1 ml of EP8 formulation was injected onto a dialysis membrane (tube) that had been pre-loaded with 2 ml of phosphate buffer saline (PBS). The membrane's ends were stitched together to prevent leakage. In a beaker containing 50 ml of release media, the entire system (dialysis tube filled with generated micro-particles) was incubated at 37°C with light agitation (PBS). Sampling was done after 1 hour, 3 hours, 6 hours, 12 hours, 1 day, 3 days, 7 days, 12 days, 19 days, 24 days, and 30 days. HPLC was used to analyze the samples [23].

Post Injection Tissue Irritation Test

Because the formulation was designed for intramuscular administration, skin irritancy should be evaluated. Skin irritation experiments on Albino Wister male rats (age, 10 to 16 wk; weight, 17 to 30 g; 6 male (3 control and 3 treated) were used to measure irritancy after a single application of ISFI and ISM. The test solutions (ISFI & ISM) were injected intramuscularly into the rat's M. vastus medialis as follows: the rat was securely placed in a supine position without anesthesia. One of the back legs was linked and stacked on top of the other. This method made it easy to inject the test solutions into the muscle's core. The fur from the injection site was removed with an electric trimmer. With a 1-ml syringe, a 21-gauge needle was inserted into the skin of the thigh at an angle of around 30 degrees to the center of the muscle. The test solution was then injected gently into a 0.5 ml volume [24-26]. The ethical committee approval number SDPC/AFC12017/12.

Kinetic Study and Mechanism of Drug Release

To determine the drug's release mechanism, data from the ISFI and ISM release studies were statistically analyzed using the Zero order, First order, Higuchi, and Korsmeyer Peppas equations.

Stability Study

To assess formulation stability, final formulations were subjected to an ICH guideline Q1C stability analysis. The ISFI (F4) and ISM (EP8) formulations were stored at 40±2°C& 75%±5%RH relative humidity. The pH and medication content were measured for up to one month. The pH was obtained by placing the electrode directly into the formula using a pH meter. An aliquot of each sample was taken, mixed with a predetermined volume of methanol, centrifuged at 5000 RPM, filtered through a 0.22µ filter, and HPLC was used to evaluate the resulting solution. The ISM method only utilized the methanol component (at the top) for drug analysis, while the fraction separated at the bottom (oil part) was discarded. ISM formulations' pH and pharmacological concentration were also measured [24-27].

RESULT AND DISCUSSION

Absorption Maxima (λmax) of Metronidazole

Identification and Confirmation of the drug were carried out by UV. From the UV spectroscopic analysis in (Figure 3), the maximum wavelength is found at 225.00 nm & the standard reported value is also 225.00 nm. Hence, 225.00 nm is taken as a maximum wavelength.

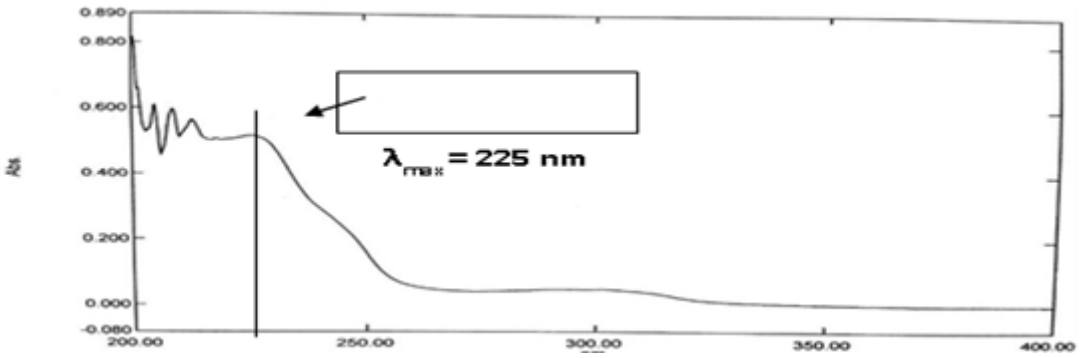

Figure 3. λmax of levothyroxine sodium in phosphate buffer pH 7.4

Preparation of ISFI & ISM

The formulation batches of ISFI and ISM was explained in (Table 1&2).

Table 1. Formulation of ISFI.

***In-situ* Implant formulation**		
Formulation Code	**PLGA 50:50 (%w/v)**	**Solvent**
F1	20%	0.5 ml NMP
F2	30%	0.5 ml NMP
F3	40%	0.5 ml NMP
Optimization of Polymer concentration Optimization of Burst release using a solvent combination		
	Optimized polymer Concentration (%w/v)	**NMP: TA**
F4	30%	90:10
F5	30%	70:30
F6	30%	50:50
NMP: N –Methyl 2- Pyrrolidone TA: Triacetin		

Table 2. Formulation of ISM

Internal Phase		
1	Levothyroxine Sodium	3 mg
2	N-Methyl 2-Pyrrolidone	0.3 ml
	PLGA	30 % w/v (of Internal phase Volume)
External Phase		
1	Peanut Oil	0.6 ml
	Surfactant blend (Span80 & Tween80)	10% V/V(of total formulation volume)

Evaluation of ISFI and ISM

Physicochemical Properties of ISFI and ISM

Table 3. Visual appearance of polymeric solutions

Formulation Code	**Visual Inspection**	**Inference**
***In-Situ* Forming Implant (ISFI)**		
F1	Clear, colorless, less viscous liquid	Due to low polymer concentration(20%) & freely solubility of the drug in NMP
F2	Clear, colorless, Slightly viscous liquid	Due to the higher polymer concentration (30%) &freely solubility of a drug in NMP
F3	Clear, colorless, highly viscous liquid	Due to higher polymer concentration (40%) & freely solubility of a drug in NMP
F4	Cloudy, colorless, highly viscous liquid	Cloudiness because of partial solubility of drug in Triacetin (NMP: TA50:50)
F5	Clear, colorless, slightly viscous liquid	Because of a higher amount of NMP than TA (70:30) aids drug to get solubilized.
F6	Clear, colorless, Slightly viscous liquid	Because of a higher amount of NMP than TA (90:10) aids drug to get solubilized.
***In-Situ* Forming Microparticles (ISM)**		
EP8	Clear, yellowish, less viscous liquid	Complete Miscibility of both Internal & external phases

Table 4. Physicochemical properties of ISFI and ISM

Formulation Code	**pH**	**24 Gauge syringe**	**22 Gauge syringe**	**21 Gauge syringe**
***In-Situ* Implant (ISFI)**				
F1	7.95 ± 0.054	Fail	Pass	Pass
F2	7.85 ± 0.036	Fail	Fail	Pass
F3	7.91 ± 0.147	Fail	Fail	Pass
F4	7.98 ± 0.164	Fail	Fail	Pass
F5	7.99 ± 0.156	Fail	Fail	Pass
F6	8.09 ± 0.110	Fail	Fail	Pass
***In-Situ* Microparticles (ISM)**				
EP8	8.11 ± 0.077	Fail	Pass	Pass

The formulation's syringe-ability was tested by injecting it with different syringe gauges, as shown in (Table 3). All other formulation-related physicochemical characteristics are listed in (Table 4). It was remarked how simple it was to deliver shots. The higher gauge (24G, 23G, and 22G) syringes have insufficient Injectability due to the increasing viscosity of the polymeric solution (in the case of ISFI). As a result, a lower gauge (larger needles) is necessary to easily inject formulation. However, there was a higher amount of Injectability in the case of ISM [19].

% Drug Entrapment Efficiency

The drug entrapment efficiency of different formulations is dependent on the type of solvent (hydrophilic or hydrophobic), the rate of solvent exchange, and the rate of implant formation, as shown in (Figure 4 and Table 5). In formulation F4, just NMP was used as a solvent, and because it is particularly hydrophilic, it is swiftly exchanged with the physiological fluid. The drug that has been solubilized in NMP but has not yet solidified into an implant is lost during this phase due to fluid exchange at the injection site. The implant solidifies soon after insertion due to the high polymer concentration in formulation F3. As a result, it has a high trapping efficiency. The formulations F4 and F5 are made up of a mix of hydrophilic and hydrophobic solvents (NMP and Triacetin), which results in a slower rate of solvent exchange. In the long run, it leads to better drug entrapment. The ISM formulation (EP8) also had high entrapment efficiency. The PLGA micro-globules in the emulsion harden and PLGA micro particles form as a result of the solvent exchange. Polymer concentration and syringe gauge were two more factors that influenced entrapment efficiency [22-24].

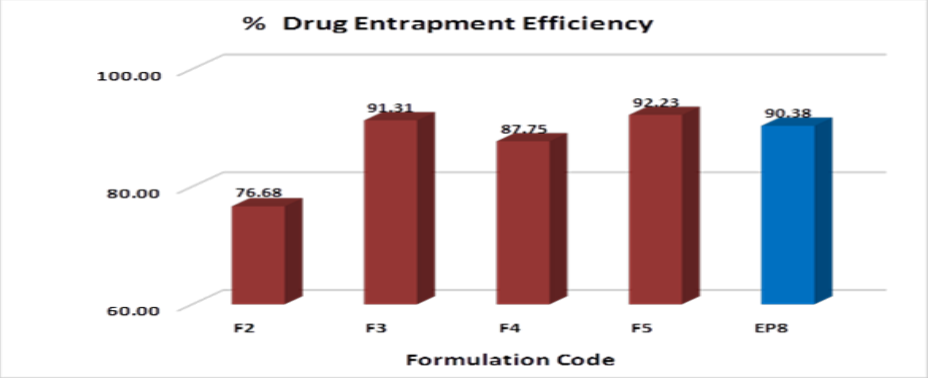

Figure 4. % Drug entrapment efficiency

Table 5. % Drug Entrapment Efficiency

Formulation Code	% Drug Entrapment Efficiency
***In-Situ* Forming Implant (ISFI)**	
F2	76.67±1.487
F3	91.30 ± 0.804
F4	87.74 ± 1.357
F5	92.23 ± 1.385
***In-Situ* Micro-particles (ISM)**	
EP8	90.37 ± 1.174

Morphology Study of ISFI & ISM

The morphology of the implant was investigated. Using a 0.5 ml formulation injected in phosphate-buffered saline pH 7.4 at 37°C, the morphology of produced Implants was examined up till complete breakdown of polymer matrix by hydrolytic cleavage. Due of bulk and surface erosion, the hydrolysable backbone of PLGA is prone to hydrolysis or enzymatic degradation in the cell environment. Water penetration and slow scissions of long polymer chains occur across the cross-

section in the former, whereas the latter is a surface occurrence in the later. The area exposed to the hydrolytic environment determines surface erosion, while the crystalline structure and porosity of the polymer matrix determine bulk erosion. (Figures 5,6,7, and 8) shows the ISFI morphological study and a SEM image of the ISM formulation, respectively [23]. Figure 8 i.e. SEM image of ISM (EP8) show crystalline structure and porosity of polymer matrix and show spherical shape of microparticles as shown in Figure 7.

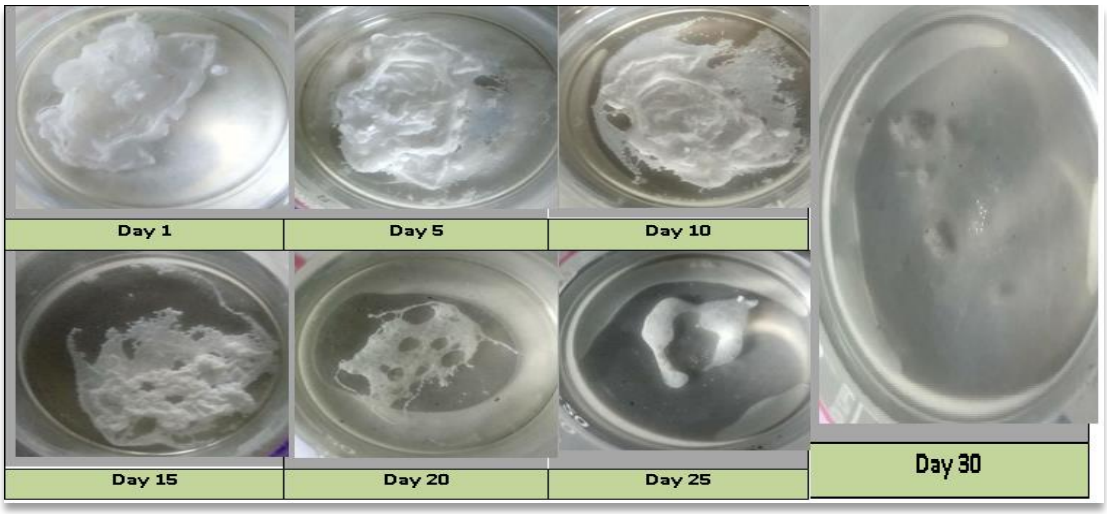

Figure 5. Morphology study of ISFI (F4)

Figure 6. *In-vitro* formation of in situ microparticles (EP8)

Ex-vivo Formation of ISFI & ISM

The chicken leg muscle was injected with 0.5 ml of the formulation via 21 gauge needles. To make it easier to visualize how the implants formed, patent blue dye was added to the mixture. After 30 minutes, dissecting the chicken leg muscle revealed the formation of an Implant (at the injection site). It formed a solid implant at the injection location, as seen in (Figure 9). The development of In situ hybridization was studied using a small tissue segment taken from the injection site. After injecting 0.5ml of formulation with a 21 gauge syringe, microparticles were inspected under an optical microscope. The formation of microparticles is depicted in (Figure 10) [25,26].

Sterility Testing

On sterility testing, both ISFI and ISM formulations showed microbe growth in SCDM growing media at regular intervals (filtered through 0.22-micron syringe filter). Throughout the 30-day

experiment, no visible growth of microorganisms was seen in growth media treated with formulations, as shown in (Figure 11).

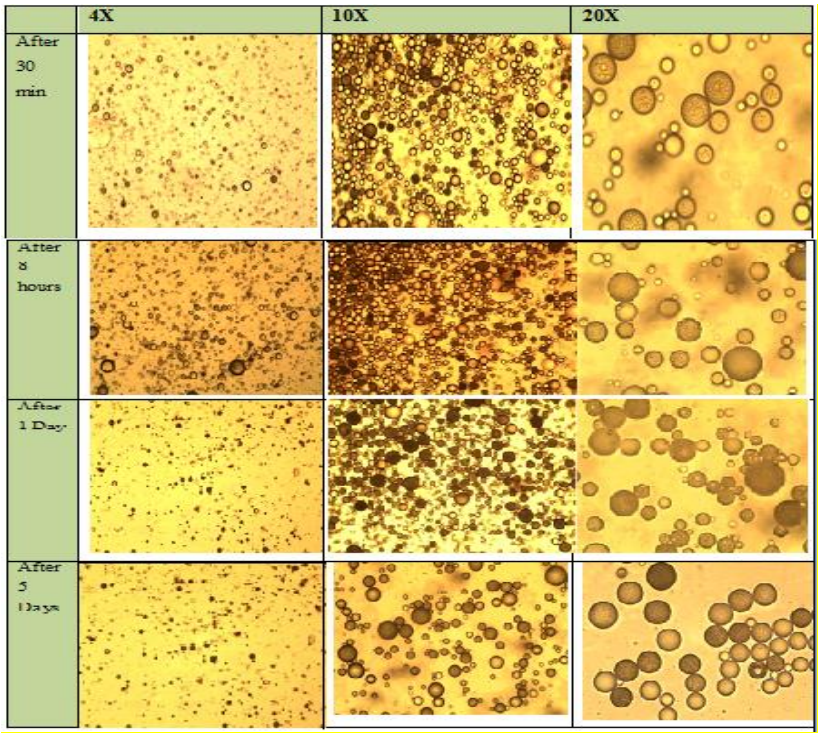

Figure 7. Images of ISM formation under microspcope (EP8)

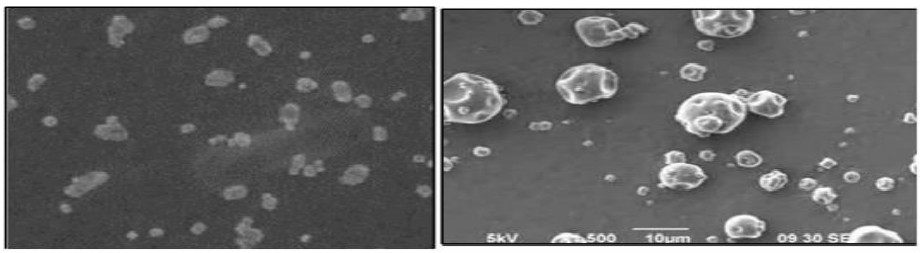

Figure 8. SEM images of ISM (EP8)

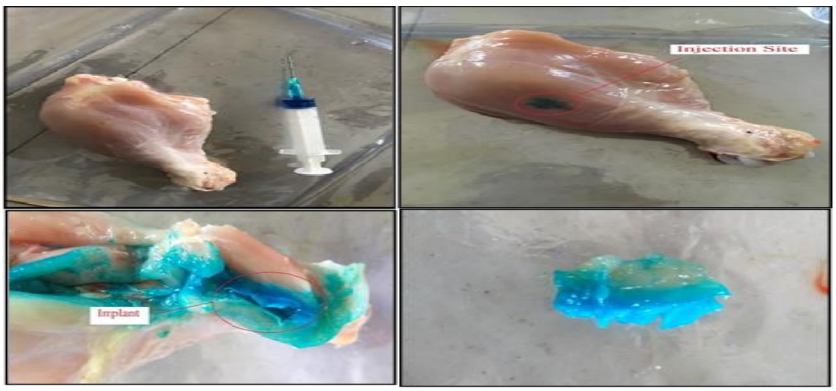

Figure 9. *Ex-Vivo* formation of ISFI

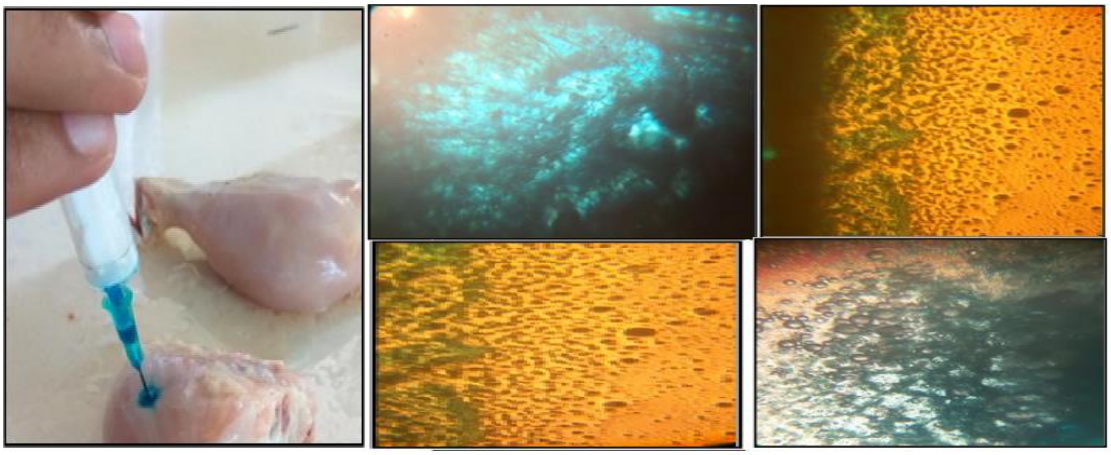

Figure 10. *Ex-Vivo* formation of ISM

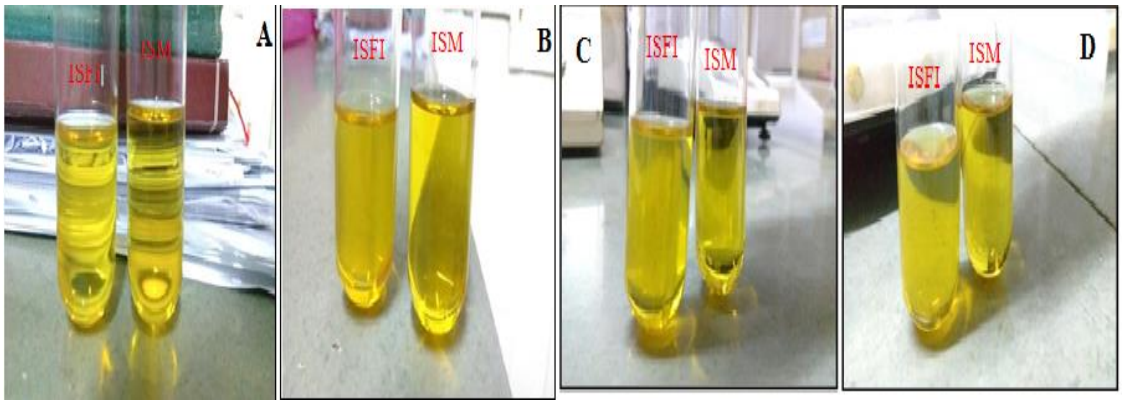

Figure 11. Sterility testing of formulations (A) Day 1, (B) Day 10, (C) Day 20, (D) Day 30

In-vitro Drug Release Study of ISFI and ISM

The gel or implant was generated promptly after injecting the formulation into the release medium stated in (Table 6). The entire in-vitro release of levothyroxine sodium from ISFI and ISM using PLGA is shown in (Figure 12). The primary disadvantage of ISFI systems is "initial burst release." This is the most prevalent occurrence when a hydrophilic solvent is used in the formulation. There are several options for dealing with this. One of these procedures was used on the formulation F2: "Combination of Hydrophobic Solvent with Hydrophilic Solvent." More batches F4, F5, and F6 were made using the solvent mixture (NMP & Triacetin in different ratios). It's also critical to choose a high-concentration hydrophobic solvent because it impacts the implant's breakdown period in addition to managing burst release. Higher doses slow decomposition, and it's not acceptable if it lasts more than 30 days. As a result, the last batch must be selected. *In-vitro* release testing was performed on batches F2, F4, and F5. (Batch F6 was rejected due to drug precipitation during storage.) In an *in-vitro* release investigation, Batch F2 exhibited the highest burst release (21%) and practically all medications were released within 25 days. In batch F4, burst release was 10.72 percent, while in batch F5, it was 9.44%. F4 was released in roughly 30 days, but F3 was released in about 60 days. Drug release in batch F5 remained incomplete (69.77%) after 30 days, showing that drug release in that formulation was extremely sluggish.

Out of the F2, F4, and F5 formulas, F4 was picked as the best batch. Another way for managing burst release is In-Situ micro particles (ISM), which scatter the polymeric phase in an external oil phase. It forms an emulsion with PLGA micro-globules that hardens when it comes into touch with physiological fluid. A vital stage in the procedure is locating RHLB of oil to stabilize ISM formulation (to prevent phase separation in the emulsion). There were 11 found here for peanut oil. This HLB created

the final ISM formulation, which was compared to the ISFI formulation in terms of *in-vitro* drug release. When compared to ISFI formulations F4 and F5, the ISM formulation EP8 showed less burst release (8.16%).

Table 6. *In-vitro* drug release study of ISFI and ISM

% Cumulative *in-vitro* drug release study of ISFI and ISM				
Time (Hours)	**F2**	**F4**	**F5**	**EP8**
1	21.33	10.72	9.44	8.16
3	32.22	14.17	12.80	11.78
6	37.49	18.85	14.67	15.50
12	42.17	23.01	18.63	21.14
24	47.30	27.45	23.04	29.74
72	53.87	33.84	31.73	34.97
120	57.93	41.11	35.82	37.35
240	63.07	44.29	44.38	41.74
360	69.61	58.19	53.80	55.97
480	86.19	70.16	61.16	66.55
550	93.38	77.5	64.68	73.38
600	98.91	86.29	67.22	84.89
720	-	94.54	69.77	95.72

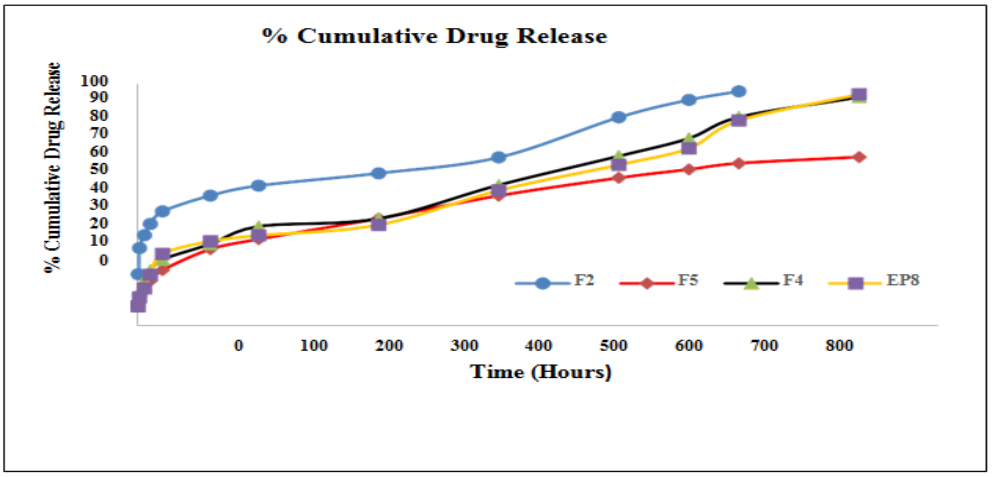

Figure 12. *In-vitro* drug release study of ISFI and ISM

Post Injection Tissue Irritation Test

The given study conducted with approval of institutional ethical committee approval number SDPC/AFC12017/12 and OECD, 1991 guidelines. To examine for tissue irritation at the injection site, a male albino Wister rat was given an intramuscular injection of formulation F4 (test). At the same time, the control group got saline via the i.m. route. After 48 hours, the rats were euthanized, and tissue irritation was measured using a dissection of the leg muscle as described in the literature. The injection site (on the skin) and adjacent muscles showed no symptoms of irritation [23-25]. It was confirmed when the results were compared to those of the control group. The irritation score of treated group was found to be 0.1 and when compared to control group [26]. As demonstrated in (Figure 13), the solid implant formed during dissection was also evident.

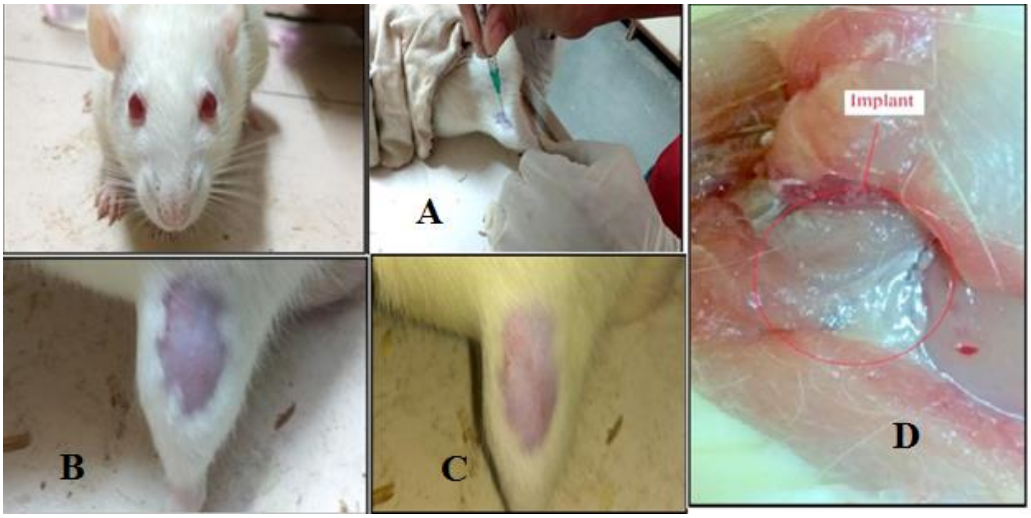

Figure 13. Local Tissue irritation test (A) Injection Site, (B) Group A: With normal Saline (post Injection) (c) Group B: ISFI formulation (post Injection), (D&E) Section of injection Site which shows no irritation.

Drug Release Kinetics

The method of releasing drugs from PLGA-based drug delivery systems is complicated. It occurs primarily through four drug release mechanisms: diffusion through a water-filled polymer, (ii) osmotic pumping, and (iv) polymer erosion (i.e. no drug transport). The release data was submitted to multiple kinetic models to determine the exact release mechanism of the medication from creating formulations. Various mathematical models have been utilized to describe drug release mechanisms from PLGA-based DDSs, including zero order, Higuchi and Korsmeyer, and Peppas models. According to the results, the regression coefficient value (R^2=0.9773) for the Korsmeyer Peppas model was higher than the other models in every occurrence of ISFI (Table 7). It appears that the drug's release follows the Korsmeyer Peppas paradigm as shown in Figure 14. The regression coefficient (R^2) value indicates the model's decent correlation fit. Table 8 shows the n value obtained from the graph, whereas in the case of ISM, drug release follows zero-order kinetics with an R^2 value of 0.9824 as shown in Figure 14.

Table 7. R^2 and n value of Korsmeyer-peppas model for ISFI formulations

Model/Formulation code	**R^2 Value**			
	F2	**F4**	**F5**	**EP8**
Korsmeyer peppas model	0.9584	**0.9773**	0.9947	0.9797
n Value	0.2	0.3	0.3	0.2
Drug transport mechanism	Fickian diffusion	Fickian diffusion	Fickian diffusion	Fickian diffusion

Table 8. R^2 and n value of Zero order model for ISM formulations

Model/Formulation code	**R^2 Value**			
	F2	**F4**	**F5**	**EP8**
Zero order model	0.918	0.9715	0.9411	**0.9824**
n Value	0.2	0.3	0.3	0.2
Drug transport mechanism	Fickian diffusion	Fickian diffusion	Fickian diffusion	Fickian diffusion

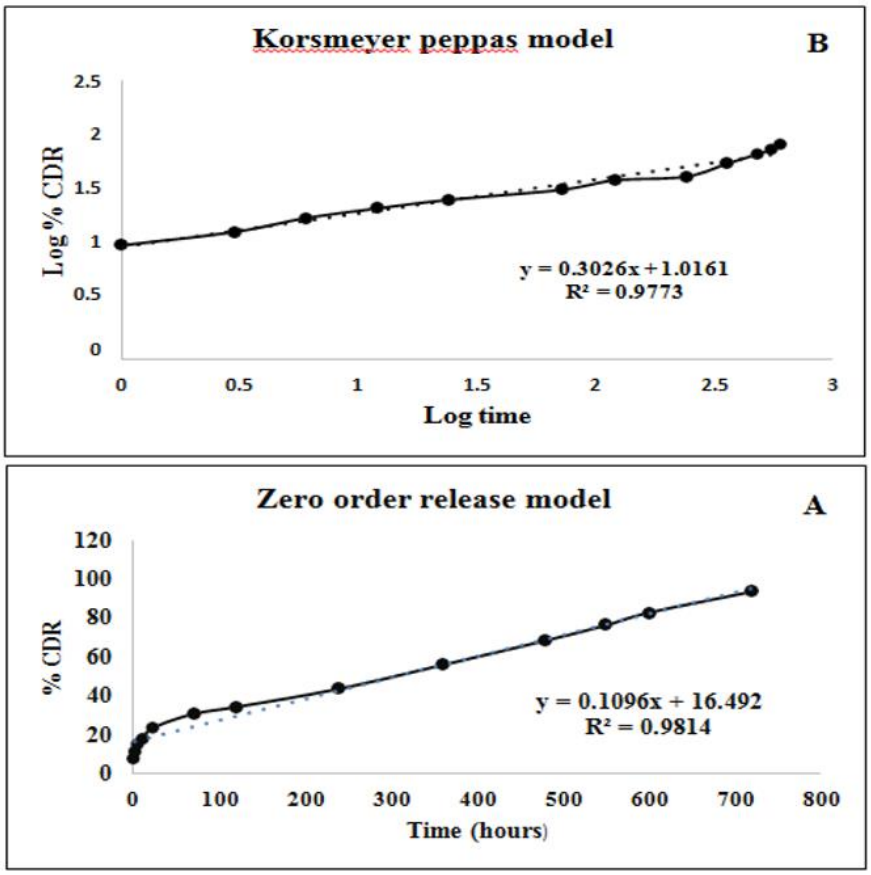

Figure 14. Release kinetics of formulation Zero order release model for ISM (EP8) Korsmeyer peppas model for ISFI (F4)

Stability Study

Final formulations were tested for stability according to ICH guideline Q1C, as shown in table 9. The ISFI (F4) and ISM (EP8) formulations were held at 40±2°C and 75%±5%RH. The pH and medication content were measured for up to one month. The analysis discovered that raising the temperature greatly reduced the drug concentration in the formulations. Physical features of both formulations were similar; they were both stable, did not change colour, and solidified in the buffer after injection [27].

Table 9: Stability of formulations

Temperature of storage	Time	pH		% Drug Content	
		ISFI	ISM	ISFI	ISM
40±2°C&75%±5%RH	Day1	7.95 ± 0.054	8.11 ± 0.077	100	100
	Day15	7.71 ± 0.89	7.89 ± 0.054	98.12	97.74
	Day30	7.52 ± 0.81	7.81 ± 0.062	96.68	96.45

The first burst release of drug within the first 24 hours of therapy is one of the key disadvantages of sustained-release formulations. Levothyroxine depot formulations' high initial drug release boosts the drug serum levels, which can cause serious adverse effects include tachycardia and fatigue. Because the hydrogen bonding between the NMP molecules and the polymer chains in the ISFI formulation hindered NMP from rapidly diffusing into the release medium, resulting in a decreased initial drug release, PLGA polymer was employed in this work to alleviate this problem (controlling the early drug release).

According to our findings, the copolymer and solvent NMP are biodegradable, biocompatible, and capable of delivering the medicine for a long time (30 days *in-vitro* drug release).

ISFI and ISM are biodegradable and biocompatible, requiring no surgical removal after use. As a result, long-acting Levothyroxine sodium formulations (ISFI & ISM) would be a better therapeutic option, allowing dose frequency to be reduced from "per day" to "per week" & "per month." This aids in maintaining a more consistent hormone plasma concentration in the body and lowers patient non-compliance, such as irregular administration, drug-food combinations, missing doses, and so on. This innovative drug delivery method has the potential to safely and effectively distribute different therapeutic compounds, particularly those employed in various chronic illness situations.

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AUTHOR CONTRIBUTIONS

Concept: M.G., M.S., R.J.; Design: M.G., R.J.; Control: M.S.; Sources: M.G., R.J Materials: R.J.; Data Collection and/or Processing: M.G., M.S., R.J.; Analysis and/or Interpretation: M.S., R.J.; Literature Review: R.J.; Manuscript Writing: M.S.; Critical Review: M.S.; Other: M.S.

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is required for this study (Proposal Number: SDPC/AFC/2017/12). The given study was conducted at Shree Dhanvantary pharmacy college, Kim.

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