

INVESTIGATION OF *IN VITRO* AND *INVIVO* PERFORMANCE OF INJECTABLE *IN SITU* IMPLANTS

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Abstract

In this study, effect of solvent system on in vitro granisetron HCl release from injectable in situ forming implants were investigated also by means of gamma irradiation sterilization. Implant formulations contain 56% solvent, 38% polymer and 6% drug. For the preparation of the formulations, hydrophobic benzyl benzoate and moderately hydrophobic propylene carbonate were used as solvents while medium molecular weight poly(DL-lactide-co-glycolide) (Resomer RG 503H) was used as polymer and the formulation containing the solvents in 1:1 combination showed low initial burst and acceptable regular release of drug for 21 days. It was determined that drug release from this formulation was increased by application of gamma irradiation and the sterilization effect were investigated on solidified implant system by the morphological analysis, which used as descriptive for in vitro release behavior of drug. In vivo performance of the mentioned sterile formulation was investigated on rabbits and it was determined that plasma drug concentrations reached to steady state on 10 to 21 days. As a conclusion encouraging results were obtained for the investigation of in situ implant systems.

Key words: *Injectable, In situ implant, Granisetron HCl, Poly(DL-lactide-co-glycolide), Rabbit.*

Enjekte Edilebilen *In situ* İmplantların *In vitro* ve *In vivo* Performanslarının Araştırılması

Bu çalışmada, enjekte edilebilen in situ oluşan implantlardan in vitro granisetron HCl salımına çözücü sisteminin etkisi, gamma radyasyonla sterilizasyon işlemiyle birlikte incelenmiştir. İmplant formülasyonları %56 çözücü, %38 polimer ve %6 etkin madde içermektedir. Formülasyonların hazırlanmasında çözücü olarak hidrofobik benzil benzoat ve daha az hidrofobik propilen karbonat çözücü olarak kullanılırken, polimer olarak orta molekül ağırlıklı poli(DL-laktid-ko-glikolid) (Resomer RG 503H) kullanılmış ve çözücülerin 1:1 bileşimde yer aldığı formülasyondan etkin madde doz boşalmasının azaldığı ve 21 gün düzenli kabul edilebilecek etkin madde salımının sağlanabildiği görülmüştür. Bu formülasyondan etkin madde salımını gamma radyasyonla sterilizasyon işleminin arttırdığı belirlenmiş ve sterilizasyon işleminin katılmış implant sistemi üzerindeki etkileri morfolojik analiz ile incelenerek in vitro etkin madde salımını açıklayıcı olarak kullanılmıştır. Bahsedilen steril formülasyonun in vivo performansı tavşanlar üzerinde incelenmiş ve plazma etkin madde konsantrasyonlarının 10 dan 21. güne kadar sürede denge durumuna ulaştığı belirlenmiştir. Sonuç olarak in situ implant sistemlerin araştırmalarında umut verici sonuçlar elde edilmiştir.

Anahtar kelimeler: *Enjekte edilebilen, In situ implant, Granisetron HCl, Poli(DL-laktid-ko-glikolid), Tavşan.*

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INTRODUCTION

A major development of the past decade has been the fabrication of implantable delivery systems based on biocompatible or biodegradable polymers. A novel biodegradable injectable polymeric system namely *in situ* forming implant (ISFI) has been developed and looks very promising in drug delivery which has disclosed a delivery approach to prolonged zero-order release over 2 weeks to 6 months (1-3). The main parts of ISFIs are; a non-reactive synthetic biodegradable polymer preferably aliphatic polyesters such as poly(DL-lactide-co-glycolide) (PLGA), additive and drug which are dissolved in a biocompatible and pharmaceutically acceptable solvent. This system has several advantages over existing systems: it is based on pharmaceutically acceptable excipients, the fabrication process is simple, does not require toxic solvent and it is likely to have greater acceptance by patients (4). After subcutaneous injection of ISFI, organic solvent dissipates into the surrounding tissue as water penetrates in. This leads to phase separation and precipitation of the polymer forming a depot at the injection site. The way the implant solution respond to its physiological surroundings, determines their release characteristics and morphology (5-8). The lag time between injection and formation of solid implant causes an initial burst release of drug, which may lead to tissue irritation and sometimes systemic toxicity (7). To control the initial burst effect, formulation variables such as type and amount of solvent, polymer and drug have been studied by researchers and still under investigation (6-11). Because of their resorption in the body, it is necessary to sterilize the complete product before application. For PLGA based implants most preferable sterilization method is gamma irradiation, which characteristically highly penetrating with a low dose rate (kGy/hour) (12). Nevertheless, effect of gamma irradiation have been reported on the release behavior of polymeric systems are different in results like; release of drug decrease or increase interestingly (13-15). *In vivo* performance of these systems has been evaluated for protein release in dogs (16) and rats (17) as sterilized form with gamma irradiation and nonsterile form as well in rats (18).

Granisetron is a potent and selective 5-HT₃ receptor antagonist which is an effective and well-tolerated agent in the management of chemotherapy induced, radiotherapy induced and postoperative nausea and vomiting in adults and children (19). The present study was aimed to evaluate *in vitro* and *in vivo* performance of *in situ* injectable polymeric implant systems regarding to the release of granisetron HCl.

EXPERIMENTAL

Materials

The following chemicals were obtained from commercial suppliers: granisetron hydrochloride (Cipla Limited, India), poly(D,L-lactide-co-glycolide) (PLGA 50:50, Resomer RG 503H, M_w 34 kDa, acid number: minimum 3) (Boehringer Ingelheim GmbH, Ingelheim, Germany), propylene carbonate (Sigma-Aldrich), benzyl benzoate (Sigma), disodium hydrogenphosphate (Merck), potassium dihydrogenphosphate (Merck), sodium chloride (Merk), N-(1-naphthyl) ethylenediamine dihydrochloride (Merck), acetonitrile (Merck), sodium hydroxide (Merck), sodium dihydrogen phosphate (Merck), toluene (Merck), ortho phosphoric acid (Merck), formaldehyde (Merck), ketamine hydrochloride (Ketalar[®], Pfizer, Turkey) and all other chemicals were analytical grade.

Preparation of *in situ* forming drug delivery systems

ISFIs (polymer solutions) were prepared by mixing PLGA with solvent (benzyl benzoate or prophylyene carbonate) or mixture of two solvents (benzyl benzoate and prophylyene carbonate) in glass vials until the formation of a clear solution. Then granisetron HCl was homogenized (Bandelin Sanoplus HD 2070, Germany) in the polymer solution. The implant solutions were then sealed and heated to 65 °C to remove trapped air bubbles. Polymer, solvent and drug concentration was kept constant at 38%, 56% and 6% by weight respectively in the composition of *in situ* implants. Code, content and injectability of the formulations are given in Table 1.

Table 1. The code, content (given in %) and injectability of *in situ* implant formulations.

Content (%) /Code	FB	FP	FBP/RBP*
Benzyl benzoate	56	-	28
Prophylyene carbonate	-	56	28
Resomer RG 503H	38	38	38
Granisetron HCl	6	6	6
Injectability (20G needle)	Yes	Yes	Yes/Yes

*Gamma irradiated form of FBP formulation.

Sterilization process

The implant formulations in liquid form were placed in glass and aluminum sealed vials and then irradiated with a ⁶⁰Co source (Tenex Issledovatel, Russia). A 25 kGy dose was applied according to the European Pharmacopoeia recommendations for an effective sterilization (20).

In vitro drug release studies

After injectabilities of all formulations from 20G needle were determined (Table 1), the formulations (500 mg) were injected into the vials containing 10 mL phosphate buffer saline pH 7.4 and *in vitro* dissolution test was carried out in a shaker bath (GFL 1086, Germany) at 30 rpm and 37°C (n=3). Replenished, filtered and collected dissolution media at predetermined time points (1h, 4h, 24h, once a day during 2-21 days) were analyzed by UV spectrophotometer (Shimadzu 1240, Japan) at 301 nm (after accomplished calibration and method validation stages) and drug release profiles were obtained.

Scanning Electron Microscopy

Among the formulations, *in situ* forming implant system with a best *in vitro* release profile and its irradiated form were investigated by morphological analyses with a scanning electron microscope (JEOL JSM-6490, USA). Injected formulations into the dissolution media (n=3) were removed at the end of 1, 3 and 10 days. After drying in desiccators, the hardened matrices were cooled in liquid N₂ and then cut with a razor blade. Samples were sputtered under an argon atmosphere with gold to a thickness of 8 nm and were then observed with scanning electron microscope at room temperature with a magnification of 200-1000 for micrographs.

In vivo drug release studies

All animal care and studies were carried out in accordance with current guidelines for investigations and experiments in conscious animals were approved by the Animal Welfare and Ethics Committee of the University of Ankara with the approval number of 2006/29 and the

date of 28th June 2006. Adult male New Zealand white rabbits with the baseline weight range of 3-3.5 kg were chosen to evaluate the *in vivo* performance of selected sterile *in situ* forming implant formulation. One rabbit did not receive the injection was used as control. Following the anesthesia by 15 mg/kg ketamine hydrochloride (Ketalar®) administered intramuscularly, liquid implant formulations were injected to the rabbits. On the start day of the study, rabbits were weighed and given a single subcutaneous injection of the selected formulation at the hair removed back region using a 20-gauge needle and needle was not taken out for 3 seconds to prevent leak out of the formulation. Later on the injection area was signed. As the total contents of syringes were injected, syringes were weighed before and after the injections to determine the injected amount of formulation (app. 500 mg). Approximately 1 mL of blood was collected from the dorsal ear vein of rabbits, transferred into Li-heparin containing tubes. The plasma was separated by centrifugation (Nüefuge CN180) at 3000 g for 3 minutes and was frozen at -45°C for later analysis by HPLC (Shimadzu LC-10AD, Japan). The concentration of granisetron HCl in the plasma samples was analyzed by the HPLC method (after accomplished calibration and method validation stages) developed by Pinguet et al. (21).

RESULTS AND DISCUSSION

In this study, the effects of solvents on drug release were evaluated by means of their solubility parameter LogP (1-octanol/water partition coefficient) descriptive for hydrophobicity, which were calculated by ALOGPS 2.1 on-line software program (22) and important properties of solvents are given in Table 2 (23).

Table 2. Properties of solvents used for *in situ* forming injectable implant systems (23).

Solvent	LogP	Melting point (°C)	Boiling point (°C)	LD ₅₀ (mg/kg)
Propylene carbonate	0,14	-50	+243	Oral, rat: 29100 Dermal,rabbit: 20001
Benzyl benzoate	3,43	+18	+323	Oral, rabbit: 1680 Dermal, rabbit: 4000

IP: Intra peritoneal, SCU: Subcutaneous, IV: Intravenous

As seen in Table 2, solvents were liquid at the temperatures of injection (24°C) and dissolution (37°C). Their amounts in formulations were under the toxicity limits for human that was predicted from LD₅₀ values given in Table 2. Dissolution profiles of FB, FP and FBP formulations are presented in Figure 1. FB formulation containing highly hydrophobic BB showed low initial burst and a slow release for 120 h, following release was relatively fast until 360 h but irregular in general, characterized a tri-phase release profile. Moderately hydrophobic propylene carbonate providing a low initial burst was used in FP to achieve porous depot formation for regular release independent by polymer degradation. Despite its hydrophobic character, propylene carbonate caused a high initial burst probably due to the hydrophilic character of Resomer RG 503H having -COOH groups resulting in an increased water affinity in combination with propylene carbonate. Following release was fast until 216 h but again irregular and bi-phasic in general from FP formulation. To obtain a release profile between FB and FP, benzyl benzoate and propylene carbonate was decided to use together as a 1:1

combination to form FBP formulation in the light of our previous study (11). Release pattern of FBP was between the others and better when it is compared to them as seen in Figure 1. In radiotherapy, it has reported that medication with granisetron HCl could be used with high doses at the beginning (24) and considering this as an initial drug release in FBP formulation. To investigate FBP for *in vivo* conditions it was necessary to obtain drug release profile of sterile form of FBP formulation. Comparison of dissolution profiles of sterile and nonsterile forms of FBP are presented in Figure 2. As seen in the figure, sterilization with gamma irradiation caused an increase in release of drug from the sterile formulation represented as RBP. Expected increase in drug release due to polymer degradation in RBP formulation caused a slight increase in initial burst but much more increase for following release which was in accordance with our previous study (15). However release profile of RBP was more regular compared to nonsterile FBP.

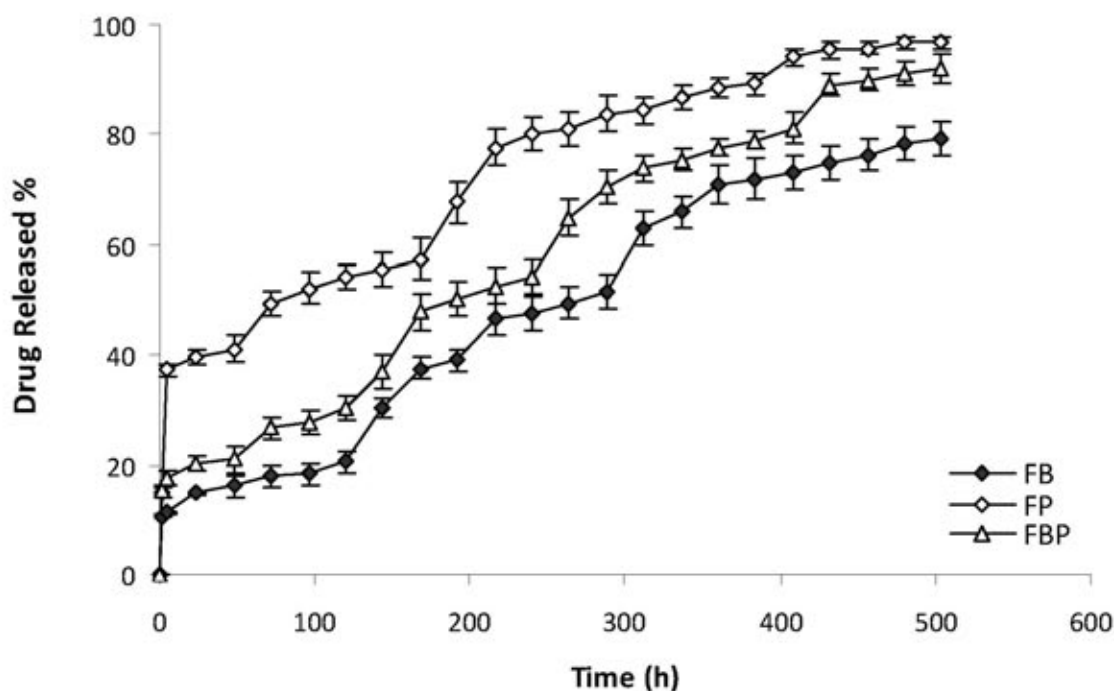


Figure 1. Dissolution profiles of FB, FBP and FP formulations.

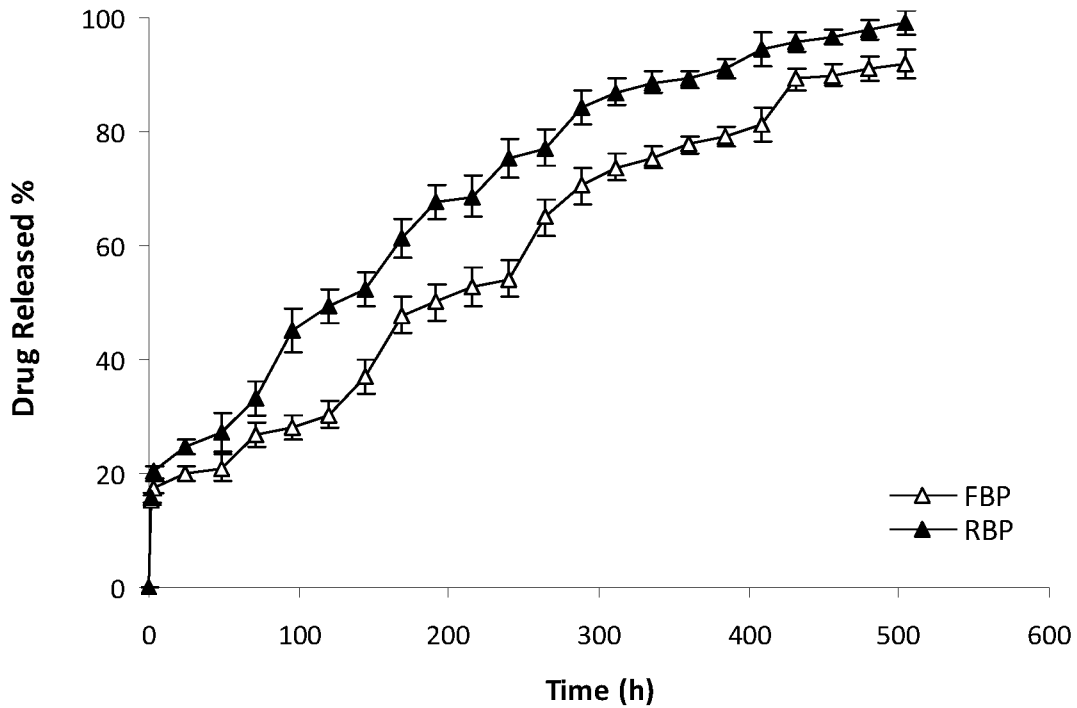


Figure 2. Dissolution profiles of FBP and RBP formulations.

Comparisons of micrographs of FBP and RBP by means of depot morphology obtained by scanning electron microscope are presented in Figure 3 – Figure 7. After solidification of the formulations following injection into dissolution medium with parallels for each sample, FBP and RBP formulations were removed from the dissolution medium and observed by micrographs in the first, third and tenth days. Surface morphologies of FBP and RBP at the end of first and third days were similar but slightly larger pores were observed on the surface of RBP as presented in Figure 3 and Figure 4 respectively, which are concordant with their dissolution profiles presented in Figure 2. Micrographs of cross sections of FBP and RBP in the third day were also similar. Surface morphologies of FBP and RBP were different in the tenth day; larger pores on the surface of RBP demonstrated the higher drug release from RBP compared to FBP as seen in Figure 2. Degradation of polymer appeared on the surface and cross section of RBP was much more than FBP regarding to the pores formed either on surface or inner parts of solidified formulations that in accordance with the dissolution profiles of mentioned forms.

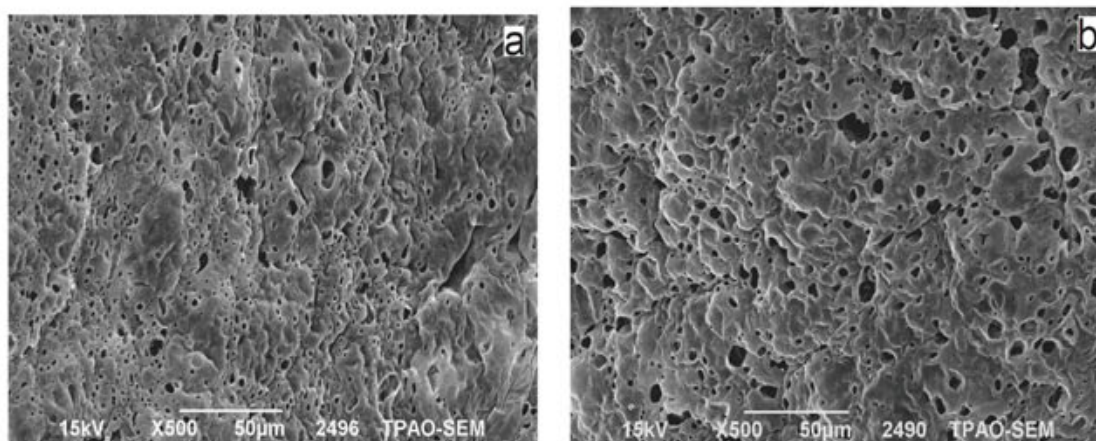


Figure 3. Surface morphologies of FBP (a) and RBP (b) formulations after solidification in the first day.

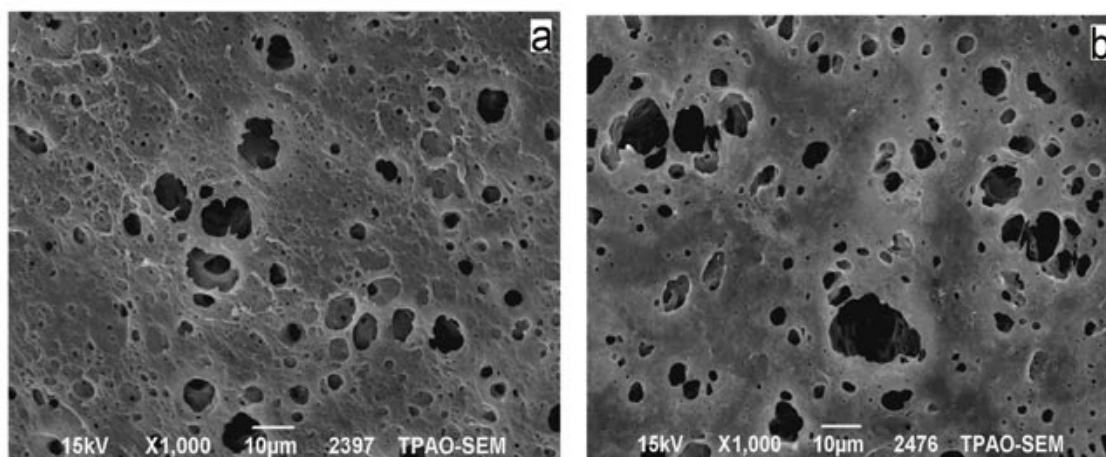


Figure 4. Surface morphologies of FBP (a) and RBP (b) formulations after solidification in the third day.

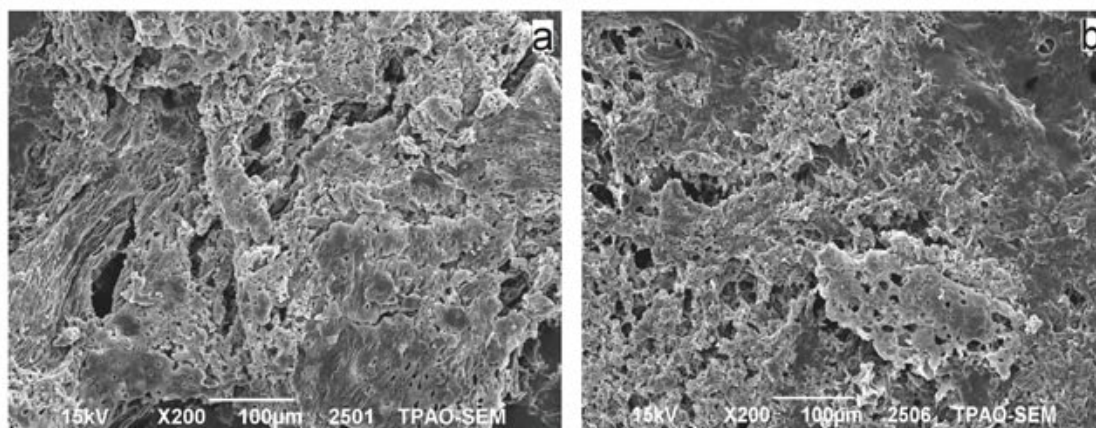


Figure 5. Cross section morphologies of FBP (a) and RBP (b) formulations after solidification in the third day.

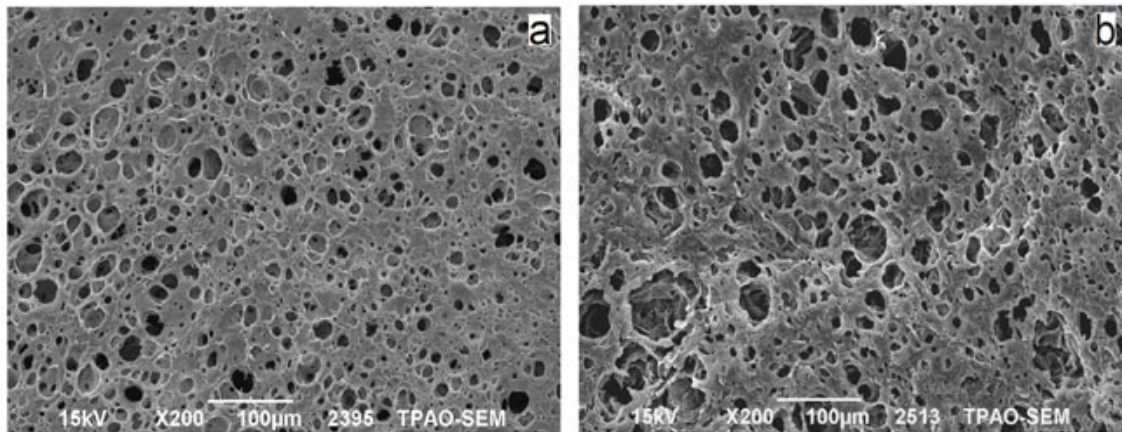


Figure 6. Surface morphologies of FBP (a) and RBP (b) formulations after solidification in the tenth day.

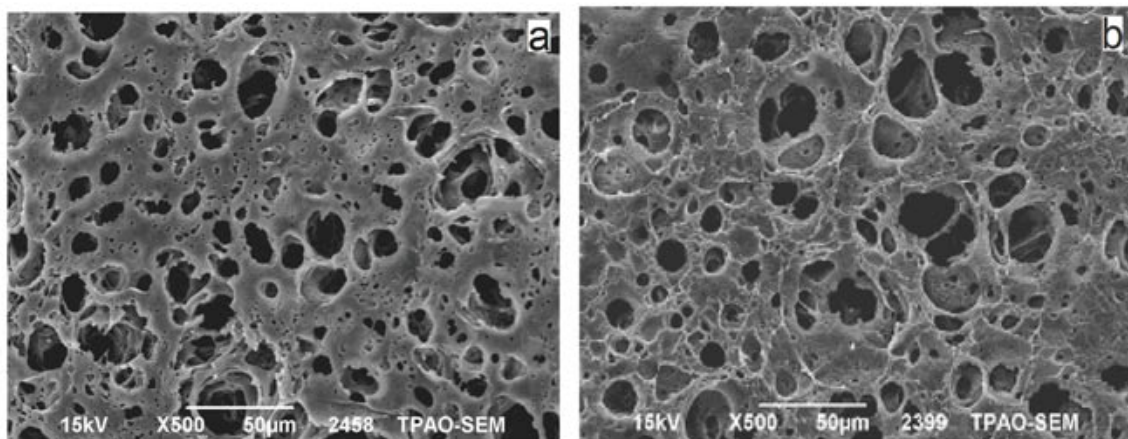


Figure 7. Cross section morphologies of FBP (a) and RBP (b) formulations after solidification in the tenth day.

In vivo performance of RBP investigated by means of plasma drug concentrations given as individual profiles of rabbits (n=4) are presented in Figure 8. Following subcutaneous injection of RBP, during solidification in physiological environment at the end of first hour, drug plasma concentrations were obtained between 18.78 – 29.04 mcg from the rabbits. From 4 to 72 hours, plasma drug concentrations decreased approximately three folds and at the end of 96 h unexpected increase in plasma drug concentration, which was discordant with *in vitro* release profile of RBP was observed. This could be explained by drug saturated tissue appearing as a lag time in drug transportation that ended a burst in plasma drug concentrations. Following profiles were similar for rabbit B and C while the others were different. Mean plasma drug concentrations obtained from rabbits are presented with standard deviations in Figure 9. As seen in the figure, around about steady state between 240-504 h from RBP in *in vivo* conditions was obtained while previous release needs to be modification for acceptable plasma profile of RBP.

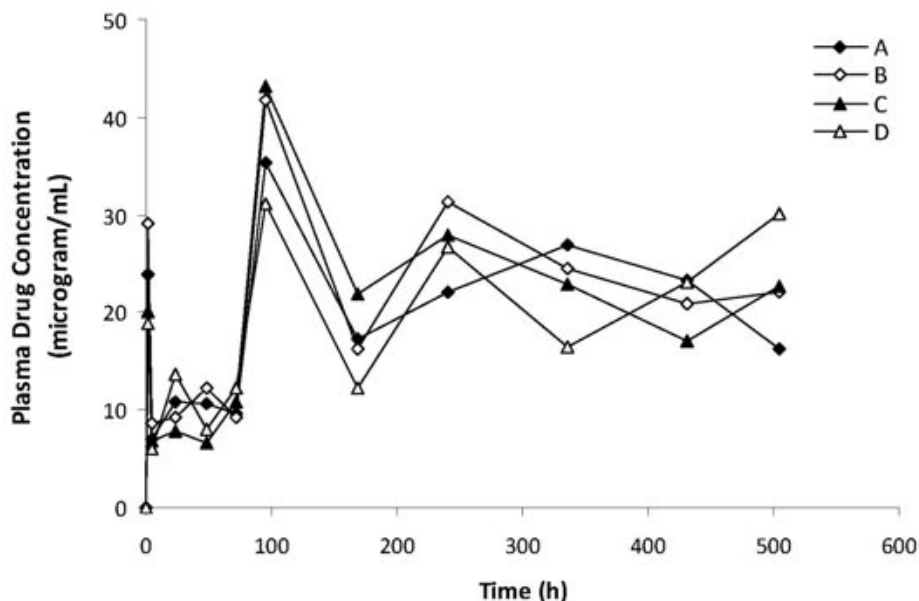


Figure 8. Plasma drug profiles of RBP formulation obtained from rabbits (n=4).

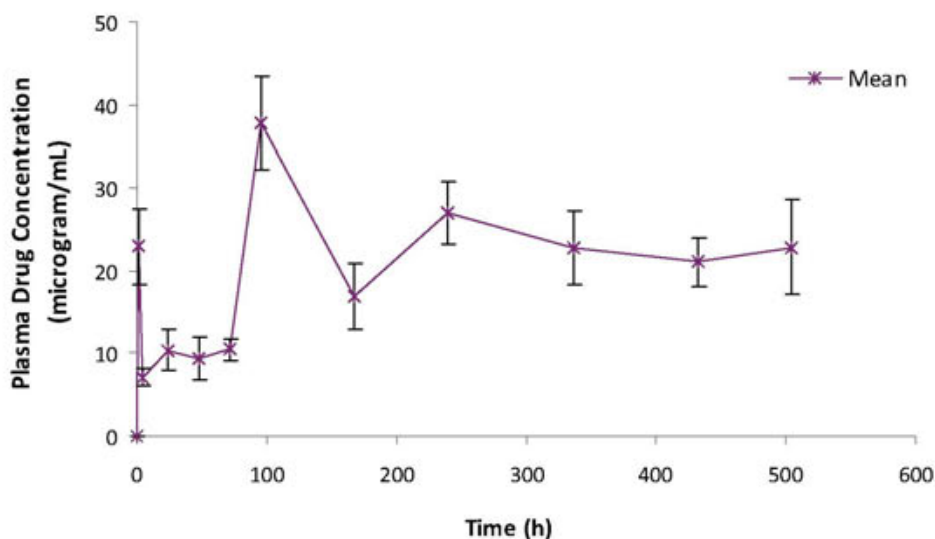


Figure 9. Mean plasma drug profile of RBP formulation obtained from rabbits (n=4).

CONCLUSION

As a conclusion, low molecular weight drugs with high water solubility caused an initial burst followed by an acceptable *in vitro* drug release from phase sensitive injectable *in situ* implant systems. It is evident from this study and our previous study (15) that *in situ* implant systems are sensitive to gamma irradiation sterilization regarding to drug release. Monitored morphological analyses of solidified implants were found descriptive on *in vitro* drug release from these systems. *In vivo* performance of these systems could be encouraging for further investigations.

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