Turk J. Pharm. Sci. 7 (1), 49-56, 2010

Original Article

EFFECTS OF SOLVENT COMBINATIONS ON DRUG RELEASE FROM INJECTABLE PHASE SENSITIVE LIQUID IMPLANTS

Evren ALĞIN YAPAR, Tamer BAYKARA*

University of Ankara, Faculty of Pharmacy, Department of Pharmaceutical Technology, 06100 Tandoğan, Ankara-TURKEY

Abstract

In this study, effects of solvent combinations on granisetron HCl release from injectable in situ forming implants (ISFIs) were investigated. Solvents having decreasing hydrophilicities in the rank of dimethylsulphoxide (DMSO), N-methyl-2-pyrrolidone (NMP) and prophylene carbonate (PC) were used in 1:1 combination with hydrophobic benzyl benzoate (BB). Glycerin (GL), polyethylene glycol 400 (PEG 400) and benzyl alcohol (BA) were used as additives in 1:5 combination with BB. Investigated ISFIs contain 64% solvent, 32% poly(DL-lactide-co-glycolide) (Resomer RG 502) and 4% drug. In vitro dissolution test was carried out in a shaker bath (30 rpm and 37°C) and samples were analyzed by UV spectrophotometer. Drug release and initial burst increased by using solvent systems in the rank of BB:DMSO>BB:NMP>BB:PC and also increased by using solvent-additive systems in the rank of BB:GL>BB:PEG 400>BB:BA. Though solvent systems gave lower initial burst of drug, they caused irregular release profiles compared to solvent-additive systems while BB alone gave a sigmoid like release profile with lowest initial burst. Between all formulations better release profile (initial burst 19.15% in the first day) was obtained with BB:BA system. According to kinetic evaluation, formulations containing solvent systems best fitted to Korsmeyer-Peppas while formulations containing solvent-additive systems fitted to Higuchi kinetic model best. As a conclusion it was observed that hydrophobic solvent combinations could be useful to control the release of drug from in situ forming implant systems.

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

Enjekte Edilebilen Faz Duyarlı Sıvı Implanttan Etkin Madde Salımına Çözücü Bileşimlerinin Etkilerinin Değerlendirilmesi

Bu çalışmada, enjekte edilebilen in situ oluşan implantlardan granisetron HCl salımına çözücü bileşimlerinin etkileri incelenmiştir. Azalan hidrofillik sıralamasında dimetilsülfoksit (DMSO), N-metil-2-pirolidon (NMP) ve propilen karbonat (PC) hidrofobik benzil benzoat (BB) ile 1:1 bileşimde kullanılmıştır. Gliserin (GL), polietilen glikol 400 (PEG 400) ve benzil alkol (BA) katkı maddesi olarak 1:5 oranında BB ile kullanılmıştır. ISFI`lar %64 çözücü, %32 poli(DL-laktid-ko-glikolid) (Resomer RG 502) ve %4 etkin madde içermektedir. Çözünme hızı testi çalkalayıcı su banyosunda yürütülmüş ve numuneler UV spektrofotometre ile analiz edilmiştir. Etkin madde salımı ve başlangıç doz boşalması, çözücü sistemlerinin kullanılmasıyla BB:DMSO>BB:NMP>BB:PC sıralamasında ve aynı zamanda çözücü-katkı sistemlerinin kullanılmasıyla BB:GL>BB:PEG 400>BB:BA sıralamasında artmıştır. Yalnız BB en düşük başlangıç doz boşalması ile sigmoid benzeri salım profili verirken çözücü sistemi, çözücü-katkı sistemine göre düşük başlangıç doz boşalmasına rağmen düzensiz etkin madde salımına neden olmuştur. Tüm formülasyonlar arasında BB:BA sistemi ile elde edilen salım profili (birinci günde %19.15 doz boşalması) en iyi bulunmuştur. Kinetik değerlendirmeye göre çözücü sistemleri içeren formülasyonlar en iyi Korsmeyer-Peppas`a uyarken, çözücü-katkı sistemleri içeren formülasyonlar en iyi Higuchi kinetik modeline uymuştur. Sonuç olarak hidrofobik çözücü bileşimlerinin in situ oluşan implant sistemlerden etkin madde salımını kontrol etmede yararlı olabileceği görülmüştür.

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

***Correspondence:** E-mail: Tamer.Baykara@pharmacy.ankara.edu.tr
Tel: +90-312- 212 71 28, Fax: +90-312-212 71 28

INTRODUCTION

Injectable drug delivery based on polymer solution platforms has gained attention in recent years, particularly for protein-based therapies (1-3). Such systems consist of a biodegradable polymer dissolved in a biocompatible solvent along with the desired bioactive agent. On injection into the water based physiologic environment, the polymeric solution undergoes phase inversion, forming a membrane structure consisting of polymer-rich and solvent–nonsolvent-rich domains. The thermodynamics and mass-transfer characteristics associated with the *in vivo* liquid de-mixing process play important roles in establishing the membrane morphology and associated drug release characteristics (4). Two major classes of membrane-forming solutions are the so-called fast phase inversion (FPI) systems and the delayed or slow phase inversion (SPI) systems (4,5). The former are characteristic of solutions based on strong, water soluble solvents and the latter are characteristic of solutions based on relatively weak solvents that are either sparingly water soluble or completely insoluble (4). Phase inversion in SPI systems generally takes place on the order of days to weeks, leading to a dense polymer-rich phase, relatively free of observable pores. These systems tend to show limited or no burst and exhibit relatively uniform release kinetics over time scales less than the erosion times of the depots. However they exhibit high viscosity of solution which makes the injection hardener (4). With FPI systems, phase inversion and membrane formation take place on the order of minutes or even seconds, leading to highly porous networks of interconnected solvent–nonsolvent-rich domains surrounded by a polymer-rich matrix (4,6). FPI solutions exhibit lower viscosities, making their injection easier and provide a more hydrophilic environment that increases biocompatibility. The release characteristics of phase sensitive systems can be controlled by the use of additives limiting or eliminating undesirable burst effects of drug (3,7).

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 (8).

The purpose of this study was to evaluate the effects of solvent combinations on granisetron HCl release from injectable biodegradable *in situ* forming implants for 21 days.

EXPERIMENTAL

Materials

The following chemicals were obtained from commercial suppliers and used: Granisetron hydrochloride (Cipla Limited, India), poly(D,L-lactide-co-glycolide) (PLGA 50:50, Resomer RG 502, M_w 18 kDa) (Boehringer Ingelheim GmbH, Ingelheim, Germany), Dimethylsulphoxide (Merck), N-methyl-2-pyrrolidone (Merck), Propylene carbonate (Sigma-Aldrich), Benzyl benzoate (Sigma), Glycerin (Sigma), Polyethylene glycol 400 (Sigma), Benzyl alcohol (Merck), Disodium hydrogenphosphate (Merck), Potassium dihydrogenphosphate (Merck), Sodium chloride (Merk).

Preparation of in situ forming drug delivery systems

In situ implants (polymer solutions) were prepared by mixing PLGA with solvent or mixture of two solvents (BB or 1:1 for DMSO:BB, NMP:BB, PC:BB and 1:5 for GL:BB, PEG 400:BB, BA:BB) in glass vials until the formation of a clear solution. For the *in situ* implants polymer, solvent and drug concentration was kept constant at 32%, 64% and 4% by weight respectively and GRN HCl was dissolved/suspended (Bandelin Sanoplus HD 2070, Germany) in the polymer solution. Implant solutions were then sealed and heated to 65 °C to remove trapped air bubbles. The code and content of formulations is given in Table 1.

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

Content/Code	F0	F1	F2	F3	F4	F5	F6
DMSO	-	150	-	-	-	-	-
NMP	-	-	150	-	-	-	-
PC	-	-	-	150	-	-	-
BB	300	150	150	150	250	250	250
GL	-	-	-	-	50	-	-
PEG 400	-	-	-	-	-	50	-
BA	-	-	-	-	-	-	50
Resomer RG 502	150	150	150	150	150	150	150
GRN HCl	20	20	20	20	20	20	20
Injectability (20G needle)	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Dimethylsulphoxide: DMSO
N-methyl-2 pyrrolidone: NMP
Prophylene carbonate: PC
Benzyl benzoate: BB
Glycerin: GL
Polyethylene glycol 400: PEG 400
Benzyl alcohol: BA
Resomer RG 502: PLGA 50:50
GRN HCl: Granisetron HCl

Drug release studies

After injectabilities of all formulations from 20G needle were determined (Table 1), formulations were injected into 10 ml phosphate buffer saline pH 7.4 containing vials 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 through 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. Drug release kinetics was evaluated by Zero order, First order, Higuchi and Korsmeyer-Peppas kinetic models which were calculated by GraphPad Instat 3.0.

RESULTS AND DISCUSSION

In this study the effects of solvent combinations on drug release were compared and evaluated by means of their solubility parameter LogP (1-oktanol/water partition coefficient) which were calculated by ALOGPS 2.1 on-line software program (9). Important properties of solvents were given in Table 2 (10).

Table 2. Properties of solvents used in *in situ* forming injectable implant systems (10).

Solvent	LogP	Melting point (°C)	Boiling point (°C)	LD_{50} (mg/kg)
GL	-1,76	+18	+290	Oral, rat: 12600 IP, mice: 63 SCU, rat: 100 IV, rat: 5566
DMSO	-1,09	+18,5	+189	IV, rat: 5360 IP, rat: 8200 SCU, rat: 12000 Oral, rat: 14500 Dermal, rat: 40000
NMP	-0,71	-24	+202	IV, mice: 155 IP, mice: 3050 Oral, rat: 3914 Dermal, rabbit: 8000
PEG 400	-0,44	+4	+250	IV, rat: 7313 IP, rat: 9708
PC	0,14	-50	+243	Oral, rat: 29100 Dermal,rabbit: 20001
BA	1,10	-15	+205	IV, mice: 480 Oral, rabbit: 1040 Dermal, rabbit: 2000
BB	3,43	+18	+323	Oral, rabbit: 1680 Dermal, rabbit: 4000

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

As seen in Table 2, all of the solvents were liquid at temperatures of injection (24°C) and dissolution (37°C). Their amounts in formulations were under the toxicity limits for human that was predicted from LD_{50} values as seen in Table 2. Highly hydrophobic BB was used in all solvent systems to reduce the initial drug burst from the formulations (11). As seen in Figure 1, lonely BB as solvent prevented the initial burst and caused a slow release of drug for 144h from F0 formulation which resulted as a sigmoid like release profile with a lowest initial burst. To increase the release of drug in the first week and obtain a uniform release profile BB decided to combine with hydrophilic DMSO to form F1 formulation. Relatively hydrophilic solvent system consists of BB and DMSO showed a fast drug release following a high initial burst from F1 formulation as shown in Figure 1. To obtain a release profile between F0 and F1 hydrophobic character of solvent system increased by using NMP and PC at second and third stages to form F2 and F3 respectively. Relatively hydrophobic solvent systems consist of BB and PC decreased the initial burst best from F3 formulation compared to F1 and F2, however it caused an irregular release profile of drug. Solvent system consists of BB and NMP having a hydrophilicity between the others solvent systems gave a release profile of F2 similar with the above mentioned two formulations and an initial burst similar with BB:DMSO system. Irregular release profiles having stages from such systems were also obtained by other researchers (12,13). However combination of NMP with BB gave lower initial burst and more regular release profile of drug compared to the study of Refienia et al. (13) which investigated the release of bethametazone acetate from an *in situ* implant formulation containing NMP and high M_w PLGA.

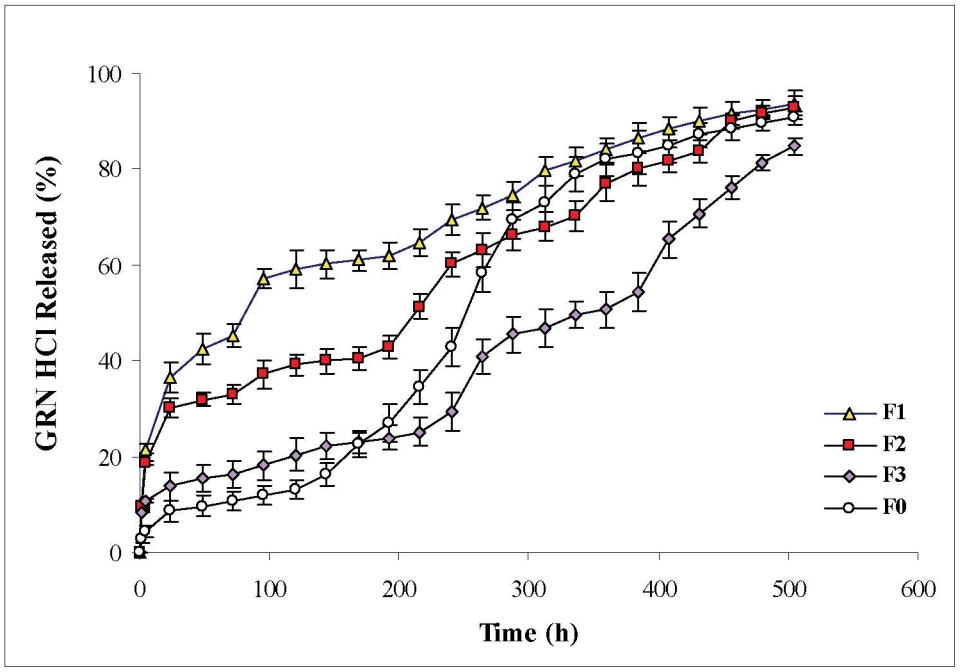

Figure 1. Dissolution profiles of F0, F1, F2 and F3 formulations.

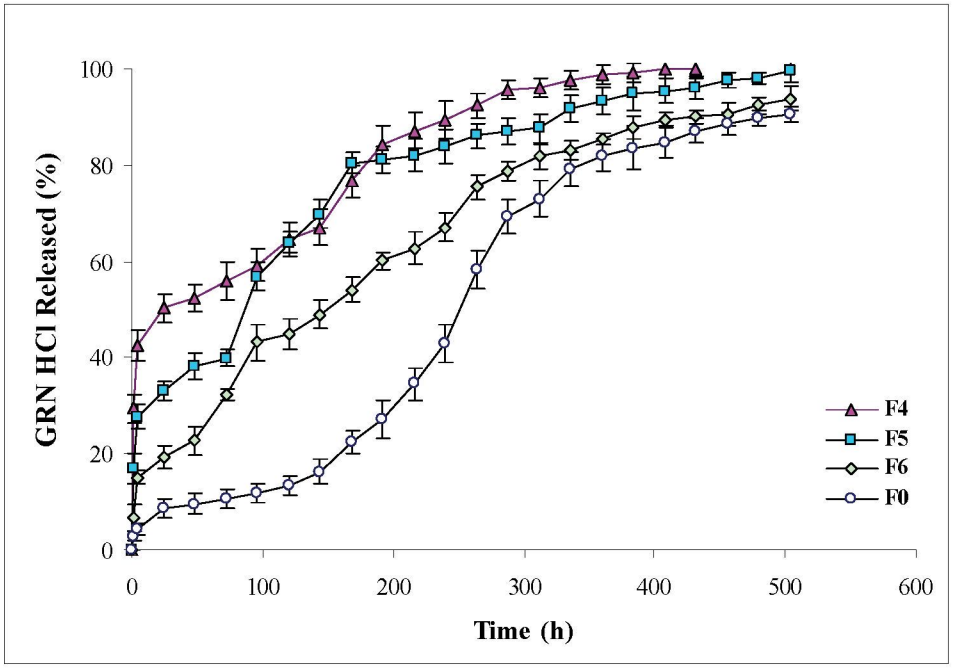

Figure 2. Dissolution profiles of F0, F4, F5 and F6 formulations.

Effects of solvent-additive systems on drug release are presented in Figure 2. Drug release profiles showed that the initial drug burst from F4, F5 and F6 formulations increase by using additive solvents in the rank of GL>PEG 400>BA related with their increasing hydrophilicity.

F4 containing BB:GL solvent-additive system showed highest initial burst with a release of 50.2% of drug in the first day and following release was also fast. F5 containing BB:PEG 400 solvent-additive system showed a decrease in initial burst and drug release was 33.1% in the first day. Following release was bimodal from F5 formulation which was fast for 168 h (the first week) and was slow between 168-504 hours. F6 containing BB:BA solvent system gave the lowest initial burst with a value of 19.15% and following release of drug was slower than F5 and F6. In this group increasing water solubility of additive solvents caused an increase in water entering to the inside of formulations and resulted as increase in release of water soluble low M_w drug. However water entering also provided fast phase inversion and depot formation which controlled the release of drug after initial burst. This phenomenon was better adjusted in F6 and between the all formulations F6 gave lower initial release of drug (19.15%) and relatively regular release profile as seen in Figure 3. The local anesthetic efficacy of BA also adds an advantage to F6 by reason of being an injectable system. Our results are supported by the study of Chen and Singh (14). Kinetic evaluation of investigated formulations according to higher determination coefficient (r^2) and lower residual mean square (RMS) values (15) showed that F1, F2 and F3 containing solvent systems best fitted to Korsmeyer-Peppas while F4, F5 and F6 containing solvent-additive systems fitted to Higuchi kinetic model best as seen in Table 3. Although highest r^2 and lowest RMS values were obtained for Zero order kinetic model from F0 containing highly hydrophobic BB alone, the release profile of F0 was not support this result as seen in Fig 2.

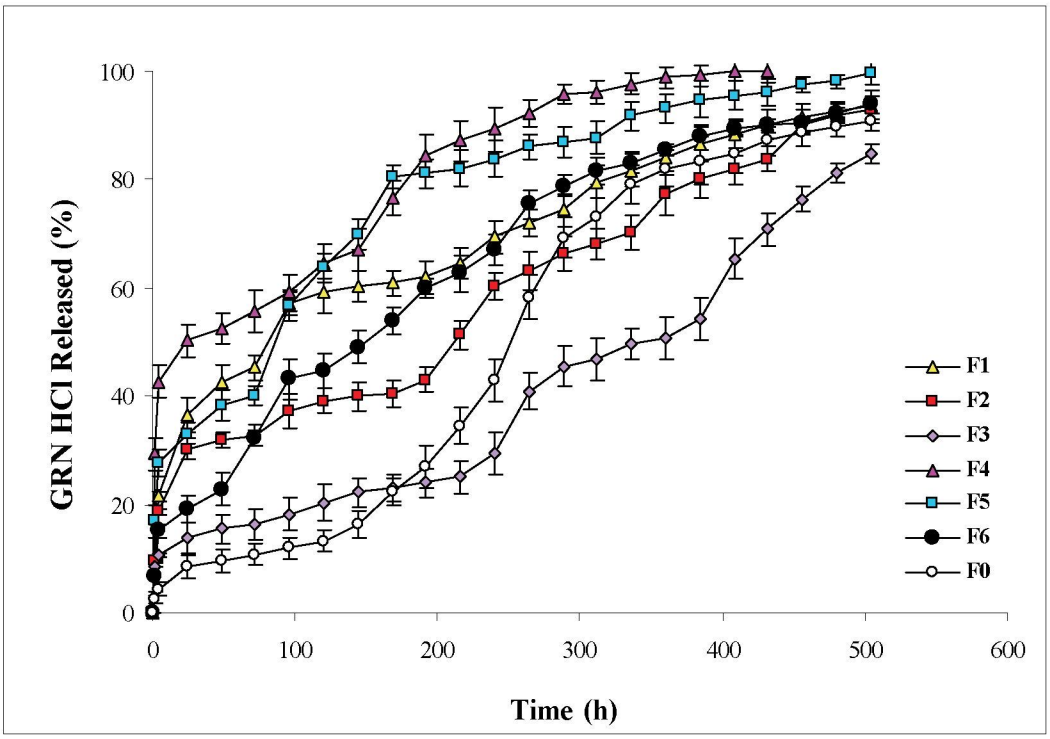

Figure 3. Dissolution profiles of F0, F1, F2, F3, F4, F5 and F6 formulations.

Table 3. Kinetic evaluation of F0, F1, F2, F3, F4, F5 and F6 formulations.

Model	Parameter	F0	F1	F2	F3	F4	F5	F6
Zero Order	k_o	0,0416	0,0271	0,0304	0,0293	0,0306	0,0294	0,0337
	SE	0,0412	0,0527	0,0542	0,0651	0,0731	0,0616	0,0778
	r^2	***0,9458***	0,9000	0,9762	0,9483	0,9113	0,8432	0,9352
	RMS	***74,76***	1287	445,3	45,22	2347	1734	552,7
First Order	k_1	0,0005	0,0003	0,0004	0,0003	0,0004	0,0003	0,0004
	SE	0,0020	0,0030	0,0040	0,0050	0,0004	0,0030	0,0040
	r^2	0,9334	0,9731	0,9190	0,8381	0,8773	0,8836	0,9906
	RMS	310,9	23,90	94,00	139,4	617,2	66,35	19,65
Higuchi	k	1,010	0,7189	0,7583	0,6895	0,7343	0,7924	0,8755
	SE	0,3130	0,2120	0,2450	0,3730	0,5110	0,4150	0,5760
	r^2	0,8665	0,9912	0,9448	0,8185	***0,9676***	***0,9543***	***0,9836***
	RMS	165,6	9,72	35,40	110,9	***17,09***	***31,68***	***13,45***
Korsmeyer -Peppas	n	0,5126	0,3259	0,2947	0,3217	0,1456	0,2508	0,3976
	SE	0,0690	0,0386	0,0582	0,1290	0,0147	0,0341	0,0290
	r^2	0,8214	***0,9783***	***0,9368***	***0,9629***	0,9538	0,9001	0,9494
	RMS	0,0330	***0,0015***	***0,0034***	***0,0166***	0,0007	0,0049	0,0052

CONCLUSION

As a conclusion hydrophobic solvent combinations could be useful to control the release of water soluble low M_w drug from phase sensitive *in situ* forming implant systems up to one month.

REFERENCES

1. **Dunn, R.L., Tipton, A.J.,** "Polymeric compositions useful as controlled release implants", US Patent 5.702.716, **1997**.
2. **Brodbeck, K.J., Gaynor-Duarte, A.T., Shen, T.T.I.,** "Gel composition and methods", US Patent 6.130.200, **2000**.
3. **DesNoyer, J.R., McHugh, A.J.,** "The effect of Pluronic on the protein release kinetics of an injectable drug delivery system", *J. Control. Release*, 86, 24– 55, **2003**.
4. **Brodbeck, K.J., DesNoyer, J.R., McHugh, A.J.,** "Phase inversion dynamics of PLGA solutions related to drug delivery: Part II. The role of solution thermodynamics and bath side mass transfer", *J. Control. Release,* 62, 333–344, **1999**.
5. **Tsay, C.S., McHugh, A.J.,** "Mass transfer modeling of asymmetric membrane formation by phase inversion", *J. Polym. Sci., B, Polym. Phys.*, 28, 1327–1365, **1990**.
6. **Graham, P.D., Brodbeck, K.J., McHugh, A.J.,** "Phase inversion dynamics of PLGA solutions related to drug delivery", *J. Control. Release*, 58, 233–245, **1999**.
7. **Raman, C., McHugh A.J.,** "A model for drug release from fast phase inverting injectable solutions", *J. Control. Release,* 102, 145–157, **2005**.
8. **Aapro, M.,** "Granisetron: an update on its clinical use in the management of nausea and vomiting", *The Oncologist*, 9, 673, **2004**.

9. ALOGPS 2.1 Virtual Computational Chemistry Laboratory, **2001**. http://www.vcclab.org/lab/alogps/
10. **Alğın Yapar, E.,** "Development of Sustained Drug Delivery Systems as *In situ* Forming Injectable Implants", *PhD Thesis.,* **2009**.
11. **Mchugh, A.J.** "The role of polymer membrane formation in sustained release drug delivery systems", *J. Control. Release,* **109**, 211-221, **2005**.
12. **Astaneh, R., Erfan, M., Moghimi, H.R., Mobedi, H.** "Changes in morphology of in situ forming plga implant prepared by different polymer molecular weight and its effect on release behavior", *J. of Pharm. Sci.,* DOI 10.1002/jps.21415, **2008**.
13. **Rafienia, M., Mirzadeh, H., Mobedi., H., Jamshidi, A.** "In vitro evaluation of drug solubility and gamma irradiation on the release of betamethasone under similated in vivo conditions", *J. Bioactive and Compatible Polymers,* **22**, 443-459, **2007**.
14. **Chen, S., Singh, J.,** "Controlled delivery of testosterone from smart polimer solution based systems: in vitro evaluation", *Int. J.Pharm.,* **295**, 183-190, **2005**.
15. **Alpar R., Öner L.,** "Regresyon çözümlemesi", *Fabad J. Pharm. Sci.,* **13**: 556-565, **1988**.

Received: 9.07.2009
Accepted: 30.7.2009