Design and development of intraocular polymeric implant systems for long-term controlled-release of clindamycin phosphate for toxoplasmic retinochoroiditis

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Abstract

Background: The release of the anti-toxoplasmosis drug, clindamycin phosphate, from intraocular implants of the biodegradable polymers poly (D, L-lactic acid) (PLA) and poly (D, L-lactide-co-glycolide) (PLGA) has been studied *in vitro*.

Materials and Methods: The preparation of the implants was performed by a melt-extrusion method. The developed extrudates were characterized and compared in *in-vitro* release profiles for elucidating the drug release mechanism. The formulations containing up to 40% w/w of drug were prepared. Release data in phosphate buffer (pH 7.4) were analyzed by high performance liquid chromatography. The release kinetics were fitted to the zero-order, Higuchi's square-root, first order and the Korsmeyer-Peppas empirical equations for the estimation of various parameters of the drug release curves. Degradation of implants was also investigated morphologically with time (Scanning Electron Microscopy).

Results: It was observed that, the release profiles for the formulations exhibit a typical biphasic profile for bulk-eroding systems, characterized by a first phase of burst release (in first 24 hrs), followed by a phase of slower release. The duration of the secondary phase was found to be proportional to the molecular weight and monomer ratio of copolymers and also polymer-to-drug ratios. It was confirmed that Higuchi and first-order kinetics were the predominant release mechanisms than zero order kinetic. The Korsmeyer-Peppas exponent *(n)* ranged between 0.10 and 0.96. This value, confirmed fickian as the dominant mechanism for PLA formulations ($n \leq 0.45$) and the anomalous mechanism, for PLGAs ($0.45 < n < 0.90$).

Conclusion: The implant of PLA (I.V. 0.2) containing 20% w/w of clindamycin, was identified as the optimum formulation in providing continuous efficient *in-vitro* release of clindamycin for about 5 weeks.

Key Words: Clindamycin phosphate, intraocular implant, PLA, PLGA

INTRODUCTION

Conventional formulations of many drugs are known to have a short elimination half-life with significant fluctuations in therapeutic drug concentrations. Interest in polymeric matrices for long-term drug release is driven by the need for the elimination of these drawbacks of immediate release systems. Using these advanced systems, treatment outcomes and patient compliance can be enhanced.

In recent years, special investigations have focused to the development of newer systems of ocular drug delivery to attain medications with prolonged retention time and improved therapeutic efficacy.[1-5]

Toxoplasma gondii is an intracellular parasite that has been considered the leading cause of posterior uveitis in the world (one-third to half of the cases of posterior uveitis). The classic treatment of toxoplasmosis retinochoroiditis consists of oral administration of some antibiotics such as clindamycin, sulfadiazine or pyrimethamine. However, some patients are intolerant of or have infections resistant to systemic therapy. The management of ocular toxoplasmosis in patients who are unresponsive to or intolerant of oral therapy is challenging. In these cases, intraocular drug delivery is one option for these patients.[6]

For decades, intravitreal injections of clindamycin are used for treatment of many ocular infections of the posterior segment.[1-5] In the case of toxoplasmos retionochroidit, this kind of administration, through bypassing ocular barriers, can deliver a high concentration of drug to the intraocular tissues; meanwhile, it likely reduces systemic complications. So this rout is very effective in the patients who show resistance to common oral therapy and patients who developed adverse effects of the systemic drug. In addition, during pregnancy especially in the first semester, local administration can avoid of the toxic and teratogenic effects of the drug.[6-15]

Although, intravitreal injection of clindamycin, has shown promising effects, but, repeated injections are needed to maintain drug concentrations at an effective therapeutics level over a certain period of time due to the short half-life in the vitreous.[7,9] On the other hand, usually, repeated intravitreal injections result in extreme patient discomfort and may lead to complications such as vitreous hemorrhage, infections, endophtalmitis, cataract or lens injury.[1-5] Since toxoplasmosis infects many people worldwide, especially in developing countries and this infection is the leading cause of posterior uveitis, need to finding alternative treatments is felt.[9,10]

The utilization of controlled-release delivery system can be a good alternative for repeated intravitreal injections.

There are some reports of attempts for designing sustained release dosage forms of clindamycin.[7,16-21] The only investigation for development of an intraocular system for clindamycin has been reported by V.S. Rao *et al.*, They tried to fabricating a controlled release intraocular system of clindamycin using liposome. But this system was not successful for retarding drug-release rate in rabbit eyes.[7,17] In 2011, Vukomanovic *et al.*, reported successful fabricating of nanoparticles of clindamycin phosphate. This nanoparticuate-based system, which made from PLGA polymer, can retard clindamycin release for about 1 month for treatment of bone infections.[16,18-20] Although these nanospheres were developed for non-ocular applications but the promising results suggested this class of polymers as a suitable material for designing other kinds of clindamycin phosphate-controlled release devices.

Implantable solid devices offer long-term pharmacotherapeutic exposure to the posterior segment. This novel method bypasses ocular barriers, avoids systemic complications and improves compliance.

These devices have been developed employing diverse approaches such as nonbioerodible and bioerodible drug-loaded pellets, discs, plugs and also polymeric matrices with various geometries.[2] The first intraocular implant which used clinically was Vitasert©. This reservoir-type device, was fabricated for controlled-release of ganciclovir in the treatment of cytomegalovirus retinitis. The implant composed of a non-biodegradable polymer. Retisert© and Medidur© were other non-biodegradable polymer implants that approved for chronic uveitis. Despite all the advantages, applications of these systems are with some drawbacks. First of all, for these implants, surgery is necessary for implantation. This operation is associated with potential complications such as vitreous hemorrhage, retinal detachment and endophthalmitis. Also, once the drug is gone, the old implant needs to be removed with another surgery.[2-5]

Biodegradable polymer implants have some advantages over the non-biodegradables.[3,5] These devices are gradually converted to a soluble form through body reactions. Therefore, they do not need to be removed once the drug is depleted. Since, these devices can be formed into various shapes, they offer the potential to be implanted through very small incisions or even injected with a simple procedure. Therefore, common complications that have been associated with the non-biodegradable implants could be minimized.[2-5]

Among the biodegradable polymers have been investigated for formulation of dosage forms for ocular purposes, application of poly (lactic-acid) (PLA) and their copolymers with glycolic acid (PLGA) are more attractive. Favorable degradation characteristics and long clinical experiences, made them as the unique biocompatible polymers for intraocular usages. These polymers which belong to aliphatic polyester of the poly (α- hydroxy) acids class, are FDA-approved polymers that have physically strong properties which make them appropriate for fabricating the solid implants. In addition, their biodegradation properties due to hydrolysis into natural metabolites, eliminate any concern about their long-term toxicity and also any needs for removing them, after finishing therapeutic duration.[22,23] Posurdex© (A biodegradable dexamethasone implant with PLGA) is currently in clinical trials for the treatment of diabetic macular edema. This system showed a significant improvement especially in patients with persistent macular edema.[1-5] In addition, other biodegradable implants of PLGA and PLA containing wide diversity of drugs for treatment of vitreoretinal diseases are under investigation.[1-5]

The present study primarily focused on the production of a controlled-release intraocular implant of clindamycin phosphate for treatment of ocular toxoplasmosis using hot melt extrusion (HME) method.

This system, can be easily injected inside the eye (just one time in the treatment period) and keep therapeutic level of the drug for long time. Since, this system is fabricated with biodegradable polymers, there is no need to additional surgery for removing it, after finishing the drug release.

The fabricated implants were thoroughly characterized by examining the effects of polymer type and Polymer ratio on the release mechanism and related kinetic parameters. The dissolution data obtained, was fitted to various mathematical models corresponding to possible release mechanisms. These results can be useful for fabricating of effective long-term drug delivery implant of clindamycin which require accurate control of the rate of drug release over the period of device activity, to ensure efficacy and eliminate toxicity.

MATERIALS AND METHODS

Materials
The polymers used in this study, were supplied from Sigma-Aldrich. Table 1 summarizes details of these polymers, along with the data for intrinsic viscosity and molecular weights.

Clindamycin phosphate (ClP) was supplied courtesy of Behdaroo Co. (Tehran, Iran). HPLC grade-acetonitrile was obtained from Merck (Darmstadt, Germany). All other chemicals were of analytical grades and obtained from Merck.

Production process
The implants were prepared using a laboratory scale vertical ram extruder. Figure 1 shows the schematic presentation of the method.

Firstly, a homogenous mixture of the drug and polymer was prepared. In this regard, clindamycin and PLA or PLGA, with different ratio, were dissolved in a mixture of acetonitrile and distilled water (1:1). The formed solution was then placed in a freezer under -70° C. Then, the frozen solution was lyophilized for about 48 hrs (Chemical-free freeze dryer, Operon, Gyenggi, Korea).

Implants were fabricated by melt extrusion method. Approximately 50 mg of the obtained powder was fed manually, into the barrel of the extruder. The extruder cylinder was heated by using digital temperature controlled heater. In order to check the process ability of various formulations depending

Table 1: Characteristics of the polymers used in our study[a]

Polymers	Composition (L/G)	Inherent viscosity (dl/g)	Mw (kDa)
PLGA 50/50-1	50/50	0.2	12
PLGA 50/50-2	50/50	0.38	31
PLGA 50/50-3	50/50	0.52	46
PLGA 75/25	75/25	0.2	15
PDLLA-1	100/0	0.2	18
PDLLA-2	100/0	0.4	25

a.Reported by manufacturer. PLGA: Copolymers with glycolic acid , PDLLA: Poly-DL-Lactic acid
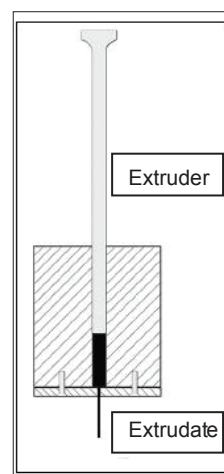
Figure 1: Schematic representation of the implant manufacturing process

on the temperature, preliminary experiments were carried out using different temperatures. On the basis of these preliminary trials, the temperature of 85°C, which allowing to successfully producing an extrudate with uniform shape and satisfactory smooth surface, was selected. The obtained semi-solid mass was extruded through a die plate with 23-G of 0.4-mm diameter, with applying appropriate pressure (which was set at 139 bars). Once the samples had cooled to ambient temperature, the rod-shaped solid extrudates were sliced up in cylinders of approximately 0.5-cm length using a hot cutter.

Different prepared formulations are shown in Table 2.

Characterization of the extrudates

Content uniformity test
For the evaluation of content uniformity of clindamycin in the implants, the procedure stated in the general chapter uniformity of dosage units of the United States Pharmacopeia 35[24] was used.

Ten implants of each batches were selected and weighed. Each implant was dissolved in a mixture of acetonitrile and distilled water as a mobile phase. After filtration and appropriate dilution, the amount of the drug was determined by high-performance liquid chromatography, according to the procedure described in the analysis method section (2.3.3).

Table 2: Composition of different formulations

Formulation code	Polymer type	Drug: Polymer ratio
F 1	PLGA5050-1	10:90
F 2	PLGA5050-1	20:80
F 3	PLGA5050-1	30:70
F 4	PLGA5050-1	40:60
F 5	PLGA5050-2	10:90
F 6	PLGA5050-2	20:80
F 7	PLGA5050-2	30:70
F 8	PLGA5050-2	40:60
F 9	PLGA5050-3	10:90
F 10	PLGA5050-3	20:80
F 11	PLGA5050-3	30:70
F 12	PLGA5050-3	40:60
F 13	PLGA7525	10:90
F 14	PLGA7525	20:80
F 15	PLGA7525	30:70
F 16	PLGA7525	40:60
F 17	PDDLA-1	10:90
F 18	PDDLA-1	20:80
F 19	PDDLA-1	30:70
F 20	PDDLA-1	40:60
F 21	PDDLA-2	10:90
F 22	PDDLA-2	20:80
F 23	PDDLA-2	30:70
F 24	PDDLA-2	40:60

PLGA: Copolymers with glycolic acid , PDLLA: Poly-DL-Lactic acid

The amounts of clindamycin in each implant (µg) were estimated and the results were expressed. The relative standard deviation (RSD) was also calculated.

In-vitro dissolution studies
The *in-vitro* release studies were performed under sink conditions over 2 months. Briefly, three implants, were immersed inside three different tubes containing 1 ml of phosphate buffer (pH 7.4). The tubes were placed inside a batch shaker which set at $37°C \pm 0.5°C$ and 50 rpm. At predetermined intervals, 1.0 ml of the medium was collected and 1.0 ml of fresh buffer was immediately replaced. Collected samples were centrifuged at 5000 rpm for 5 min, filtered and stored in the refrigerator. The amount of clindamycin released was measured by high-performance liquid chromatography (HPLC) using the method described in the section 2.3.3.

The release profile was evaluated as the cumulative percentage of clindamycin released in the medium.

High performance liquid chromatography
The reverse-phase HPLC method was developed and validated by our group for determination of clindamycin in *in-vitro* release medium and *in vivo* samples. Briefly, the chromatograph instrument was a waters system composed Waters 515 pump and Waters 2487 dual absorbance detector (Waters, USA). The stationary phase was CN-RP column (250 × 4.6 mm, 5-µm particle size) from Macherey-Nagel (Germany), made of stainless steel and the mixture of acetonitrile and water (40:60) containing 100-mM tetra methyl ammonium chloride (pH 4.2) was used as a mobile phase. Propranolol was used as an internal standard and injection volume was 100 µl. All the chromatograms were recorded at 204 nm with mobile flow rate of 1 ml/min. Before injection into the system, the implants containing drug, were dissolved in mobile phase with sonication and then filtered through 0.45-µm membrane filter. As a control, samples containing only the pure drug were diluted in the mobile phase and injected.

Dissolution data analysis
The *in-vitro* release data was fitted to various kinetic models corresponding to possible release mechanisms using Microsoft Excel 14.0. Approximately first 80% of the total drug released was fitted into the equations 1-4.

The equations 1-4, are commonly used in the drug release kinetic studies, because of their simplicity and applicability.[25,26] These models best describe the process of drug release from pharmaceutical dosage forms when either it results from a simple

phenomenon, or there is a rate-limiting step, governing the drug-release process.[27] Equation 1, the zero-order model equation; Equation 2, Higuchi's square-root equation; Equation 3 the first order and the 4 is the Korsmeyer-Peppas empirical equation.

$$M_t/M_\infty = K_0 t \tag{1}$$

$$M_t/M_\infty = K_H t_{1/2} \tag{2}$$

$$Ln\ (M_t/M_\infty) = -K_1 t \tag{3}$$

$$M_t/M_\infty = Kt^n \tag{4}$$

Where, M_t/M_∞ is the fraction of drug released at any time t; and K_0, K_H, K_1 and K are release rate constants for Equations 1,2,3 and 4, respectively. In Equation 4, n is the diffusional exponent indicative of mechanism of drug release.

For consideration of the drug release, drug-release profile was fitted in Korsmeyer-Peppas power model. The n value is used to characterize different release mechanisms as given in Table 3 for cylindrical-shaped matrices.[28]

It must be noticed that, for all the calculations, the results obtained until 24 hrs. for the *in vitro* drug release study were not considered in the kinetic analysis because the burst effect that do not correspond to the real mechanism of the drug release from implants.

Release profiles comparison and statistical analysis
The drug-release profiles were compared using a model-independent method by determining the mean dissolution time (MDT) of the formulations.[27] The MDT values were subjected to 1-way analysis of variance (ANOVA) using SPSS software version 16, to examine the statistical difference. Post-hoc analysis was carried out according to Tukey multiple comparison tests. A confidence limit of 95% was fixed and used for the interpretation of results.

$$MDT = \frac{\sum_{j=1}^{n} tj\Delta Mj}{\sum_{j=1}^{n} \Delta Mj}$$

Where, j is the sample number, n is the number of dissolution sample times, tj is the time at midpoint

between t_j and t_{j-1} (easily calculated with the expression $(t_j + t_{j-1})/2$, and ΔMj is the additional amount of drug released between t_j and t_{j-1}.

Scanning electron microscopy
The optimum formulation in the drug-release characteristics was investigated morphologically using scanning electron microscopy (SEM, Seron Technology, model AIS-2300). The implants were removed from the dissolution medium at predetermined time intervals. The samples were fixed with an adhesive sheet on a rigid support and covered with gold for their better visualization. Surface changes due to degradation in release medium were considered.

RESULTS

Preparation and evaluation of implants
Implants were prepared using a HME method without any problems and do not require for adding any additional excipients for improving shape or compressibility.

The appearance of the implants was evaluated immediately after manufacturing. Macroscopically, the rods were smooth and have the color uniformity (due to clindamycin phosphate which had a white color). Breadth was found fix as punch size and thickens was controlled as well to an average of 0.4 mm (for ease of intravitreal injection). Content uniformity, mean weight of implants and diameters are presented in Table 4.

As shown in Table 4, mean drug content values were found satisfactorily within limits. In addition, the content uniformity results, showed a uniform distribution of clindamycin phosphate in the implants. None of them were outside the acceptance range of USP 35 (85.0-115.0%)[24]of pre-indicated amount of clindamycin.
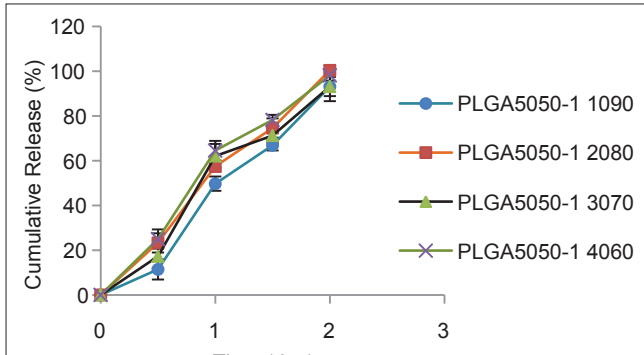
In-vitro dissolution studies
Fabricated formulations (shown in Table 2), were evaluated and compared in the release kinetic pattern for demonstration the effects of ratio of GA to LA, polymer molecular weight and drug-to-polymer ratio.
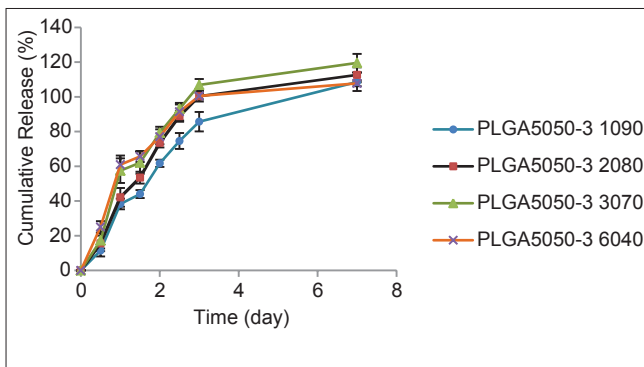
A plot of cumulative percentage released vs. time for matrix-embedded controlled release micro-cylindrs of ClP presented in Figures 2-7. As shown in the diagrams, the typical biphasic release pattern was observed for the formulations: An initial burst phase (days 0-1) and more controlled secondary phase (days 2.5 - 56). The drug released during the burst-release phase which observed for all the formulations in the first day of the experiments varied between 14% in the case of formulation (F21)

Table 3: Diffusion exponent and solute release mechanism for cylindrical shape diffusion

Exponent (*n*)	Overal solute diffusion mechanism
≤0.45	Fickian diffusion
0.45< *n* <0.89	Anomalous (non-Fickian) diffusion
0.89	0.89 case-II transport
n >0.89	Super case-II transport

and 64.5% in the case of formulation (F4). For the all formulations, as the drug loading increased from 10 to 40%, the release rate increased.

For determining the release kinetic models, the drug release data were kinetically evaluated

and fitted to the four different kinetic models (Equations 1-4).

The kinetics parameters and (r^2) values for the various models are given in Table 5. The selection of the model for each formulation was based on the selection of higher r^2. Although, there was no single kinetic model that could explain the entire release

Figure 2: Comparative release profile of clindamycin from controlled-release matrix implants prepared using different proportions of PLGA 50/50-1. Each data point represents the average of 3 samples with SD
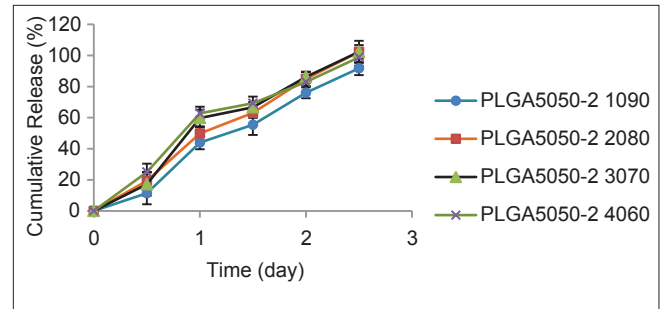
Figure 3: Comparative release profile of clindamycin from controlled-release matrix implants prepared using different proportions of PLGA 50/50-2. Each data point represents the average of three samples with SD

Figure 4: Comparative release profile of clindamycin from controlled-release matrix implants prepared using different proportions of PLGA 50/50-3. Each data point represents the average of three samples with SD
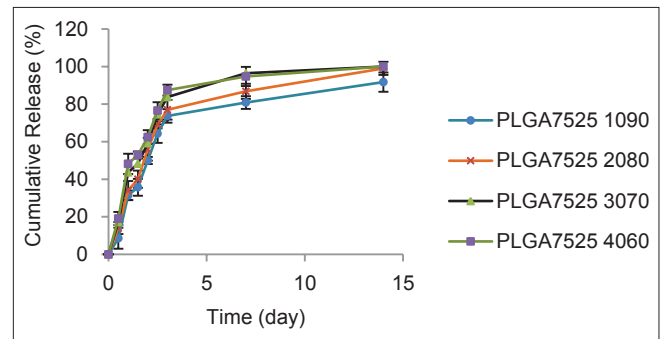
Figure 5: Comparative release profile of clindamycin from controlled-release matrix implants prepared using different proportions of PLGA 75/25. Each data point represents the average of 3 samples with SD

Table 4: Formulation components and physical characteristics of implants of clindamycin

Physical properties	Formulations codes (F1-F12)											
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Drug content (µg)	351.6	518	876	1100	352.5	526	883	1050	348.9	531	869	1150
Content variation (µg)	±0.9	±0.9	±1.8	±2.3	±2.1	±1.8	±3.1	±3.5	±1.8	±1.5	±2.5	±3.4
Weight (mg)	2.5	2.6	2.3	2.7	2.5	2.4	2.5	2.4	2.6	2.4	2.6	2.2
Weight variation (%)	±0.3	±0.5	±0.2	±0.5	±0.4	±0.2	±0.4	±0.3	±0.4	±0.1	±0.2	±0.1
Diameter (mm)	0.4	0.42	0.43	0.41	0.4	0.42	0.4	0.42	0.41	0.4	0.42	0.42
Diameter variation (mm)	±0.03	±0.02	±0.01	±0.04	±0.02	±0.02	±0.02	±0.04	±0.03	±0.02	±0.01	±0.01
	Formulations codes (F13-F24)											
	F13	F14	F15	F16	F17	F18	F19	F20	F21	F22	F23	F24
Drug content (µg)	349.0	514	865	1190	347.3	517	869	1099	348.5	535	879	1010
Content variation (µg)	±3.2	±1.7	±2.1	±5.1	±1.8	±1.6	±3.1	±2.9	±1.6	±1.4	±3.2	±4.3
Weight (mg)	2.9	2.3	2.5	2.4	2.6	2.4	2.5	2.4	2.7	2.5	2.7	2.8
Weight variation (%)	±0.5	±0.1	±0.3	±0.3	±0.3	±0.2	±0.3	±0.4	±0.4	±0.3	±0.3	±0.1
Diameter (mm)	0.41	0.39	0.38	0.38	0.4	0.41	0.4	0.4	0.39	0.38	0.39	0.39
Diameter variation (mm)	±0.02	±0.03	±0.01	±0.02	±0.02	±0.04	±0.02	±0.01	±0.01	±0.01	±0.02	±0.01

profile clindamycin from all the implants but these data indicating that, the entire release profile was best described by Higuchi model for PLA implants and first order for PLGAs.

According to this table, n values for evaluation the drug release mechanism, for different formulations ranged from 0.1 up to 0.96.

Mathematical analysis of the release kinetics indicated that, In the PLGA formulations, anomalous mechanism which is the combination of drug diffusion and polymer relaxation is responsible for drug release from matrices ($n > 0.5$).

For the PLA implants, the dominant mechanism is Higuchi and n value is more consistent with the Fickian diffusion ($n < 0.5$).

Table 6, demonstrated MDT values of the formulations. The MDTs of the drug [Table 6], can show the effects of different formulation parameters, more clearly. According to these data, MDT extended from 22 hours in the case of 40% PLGA with lowest molecular weight (F4) to more than 36 days in the case of 90% polymer-to-drug ratio of the PLA with highest molecular weight (F21). Post-hoc ANOVA, was used for statistical analysis of the MDTs.

The statistical analysis confirmed significant differences in MDTs between PLAs and PLGAs ($P < 0.05$). This difference, can show the
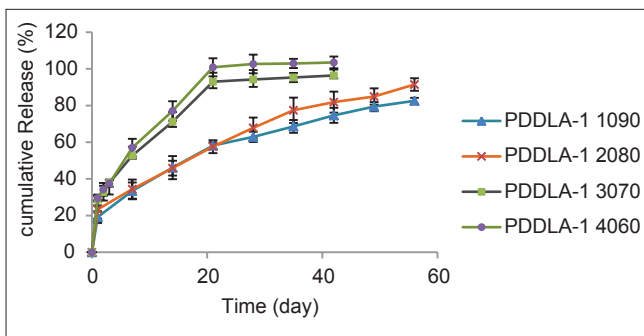
Figure 6: Comparative release profile of clindamycin from controlled-release matrix implants prepared using different proportions of PDLLA -1. Each data point represents the average of 3 samples with SD
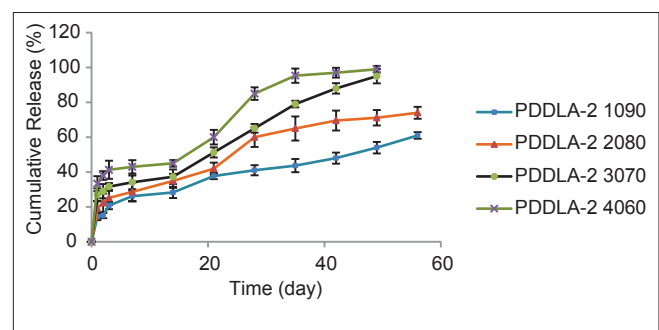
Figure 7: Comparative release profile of clindamycin from controlled-release matrix implants prepared using different proportions of PDLLA -2. Each data point represents the average of 3 samples with SD

Table 5: Kinetic values for the various formulations

Kinetic model	Parameters	Formulation codes (F9-F16)							
		F9	F10	F11	F12	F13	F14	F15	F16
Zero order	R^2	0.97	0.98	0.97	0.95	0.97	0.70	0.97	0.995
	K_0 (mg h^{-1})	27.6	35.38	29.65	25.82	22.62	3.69	24.39	23.54
Higuchi	R^2	0.95	0.97	0.999	0.98	0.98	0.74	0.97	0.989
	K_H (mg.cm^2.h$^{-1/2}$)	81.95	994	87.16	87.16	76.07	28.75	72.11	69
First order	R^2	0.99	0.99	0.973	0.99	0.99	0.99	0.98	0.96
	K_1 (h^{-1})	−0.39	−0.62	−0.74	−0.60	−0.26	−0.28	−0.34	385
KP*	R^2	0.99	0.99	0.99	0.99	0.72	0.73	0.83	0.98
	K	3.4	−2.45	−1.2	−1.3	3.41	3.69	3.2	3.66
	N	0.96	0.79	0.79	0.64	0.52	0.53	0.62	0.72
		Formulation codes (F17-F24)							
		F17	F18	F19	F20	F21	F22	F23	F24
Zero order	R^2	0.955	0.984	0.988	0.97	0.97	0.95	0.90	0.86
	K_0 (mg h^{-1})	1.24	1.39	3.14	3.6	0.75	1.03	1.46	0.988
Higuchi	R^2	0.98	0.995	0.999	0.996	0.98	0.96	0.95	0.88
	K_H (mg.cm^2.h$^{-1/2}$)	10.16	12.92	16.50	18.91	6.71	9.3	11.32	2.64
First order	R^2	0.97	0.98	0.97	0.83	0.96	0.94	0.88	0.91
	K_1 (h^{-1})	−0.01	−0.016	−0.03	−3.04	−0.005	−0.01	−0.009	0.027
KP*	R^2	0.99	0.99	0.998	0.85	0.96	0.93	0.83	0.91
	K	2.59	2.57	3.20	3.94	2.51	2.62	3.042	3.60
	N	0.47	0.48	0.39	0.32	0.41	0.42	0.32	0.084

*Korsmeyer-peppas

more important role of PLA for sustaining the drug release. On the other hand, the results did not show any significant differences between different PLGA formulations ($P > 0.05$). As shown in Table 6, in this class of implants, MDTs decreased with decreasing polymer: drug ratios or using PLGA with lower molecular weights (0.92 days in the case of lower molecular weight of PLGA 5050 with lower polymer-to-drug ratio (F4) vs. 1.55 days in the case of higher molecular weight of PLGA5050 with higher polymer-to-drug ratio (F9)) but this changes was not significant. In addition, in PLGA formulations, with an increase in LA ratio (in the case of PLGA 7527), MDT was improved, but again not significantly (MDT was 1.55 days in lower polymer-to-drug ratio to 3 days in higher ratio). So the highest MDT between PLGAs was 3 days and belonged to F13 which composed of PLGA 7527 with 10% w/w drug.

In the cases of PLAs, comparisons showed increasing in MDT from 7.06 days for F20 to 15.03 days for F17 (for PDDLA-1 polymer) and from 14.32 days for F24 to 36.38 days for F21 (for PDDLA-2 polymer). So more successful drug retardation was obtained with PLAs.

Observation of the implants surfaces

In order to verify the *in-vitro* release data and having more information about degradation characteristics of the implants in the dissolution medium, the formulation F18 (fabricated from PDDLA-1 polymer containing 20%w/w drug) which showed appropriated release pattern, was evaluated microscopically [Figure 8]. Implants surfaces were compared before and 1, 2, 3 and 10 weeks after placing in the release medium. As shown in the SEM images, negligible cracks are appearing on the implants surfaces after 2 weeks [Figure 8c]. After 3 weeks, some other little fractures, developed [Figure 8d]. After 10 weeks, we can see the highly porous surfaces of the systems which are result of drug depletion and producing more pores in the system [Figure 8e]. These images show structure of PLA implants was well maintained with evidence of little degradation during treatment.

DISCUSSION

PLA and PLGA polymers are very attractive as a matrix former for implant dosage forms because of good biocompatibility and biodegradability. These dosage forms which produced by different approaches, usually exhibit a long-term drug release for several weeks to months.[1-5,22,23,29,30] Their drug release is mainly based on diffusion through the continuous polymer matrix and on degradation and erosion of the matrix polymer.

Table 6: MDT calculated for various formulations (mean±SD)*

Formulation codes	MDT (day)
F1	1.06±0.3
F2	0.97±0.03
F3	0.94±0.04
F4	0.92±0.1
F5	1.22±0.05
F6	1.18±0.03
F7	1.11±0.07
F8	1.04±0.06
F9	1.55±0.04
F10	1.38±0.03
F11	1.0±0.1
F12	0.90±0.07
F13	3±0.4
F14	2.85±0.2
F15	1.78±0.05
F16	1.55±0.02
F17	15.03±0.9
F18	13.7±0.5
F19	10±0.3
F20	7.068±0.10
F21	36.38±1.4
F22	19.32±1.3
F23	16.34±0.7
F24	14.32±0.95

*Mean of triple samples. MDT: Mean dissolution time
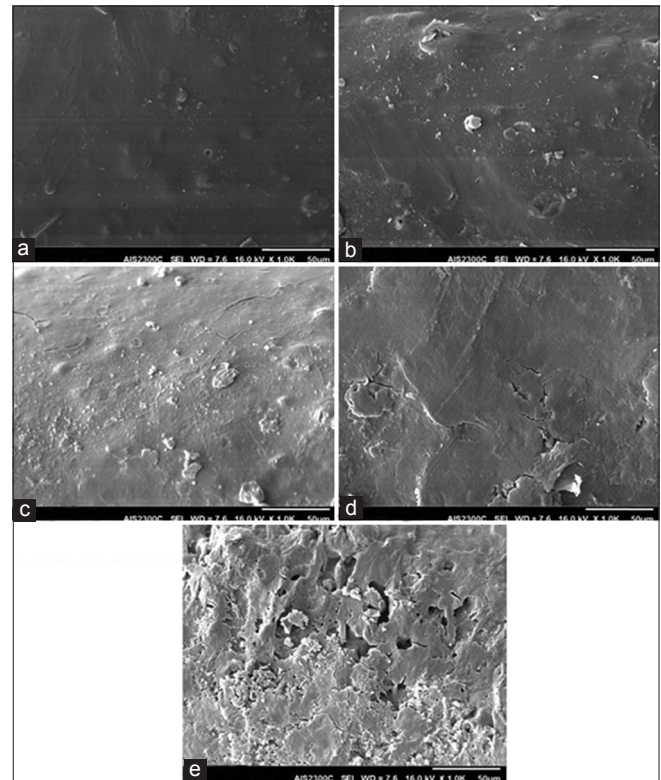
Figure 8: SEM image of the surface of PLA implants containing 20% w/w clindamycin (F18), before incubation in release medium (a), after 1 week (b), 2 weeks (c), 4 weeks (d) and 10 weeks (e) incubation

Actually, "drug release" which refers to the action in which drug solutes transfer from the primary position in the polymeric matrices to the outer surface of polymer and then to the release medium, is affected by multiple complex factors. These factors include the solutes physiochemical properties, the material system characteristics, release medium and the possible interactions between these factors.[28-31] Many publications have shown different formulation parameters which can affect drug release kinetics. These results demonstrates that the rate of drug release from polymeric carriers vary greatly with the type of drug.[31-34]

In this approach, clindamycin phosphate, a water-soluble drug (more than 150 mg/ml),[16] was used for fabricating a sustained release intraocular implant. This implant which should supply a drug for approximately 4-5 weeks, are considered to be useful for the patients who need anti-toxoplasmic treatment but cannot take medicine orally or by several intravitreal injections. This implant can be easily injected intraoculary, only once during the treatment and become a good substitute for other kinds of treatments.

Until now, there are many reports of successfully applications of intraocular implants containing wide range of drugs such as antimicrobial, antiviral and anti-inflammatory agents.[1-5,22]

In the case of clindamycin phosphate, there are some reports of attempts for designing sustained release dosage forms of this antibiotic.[7,16-21] Hascicek *et al.*, tried to design floating tablet of clindamycin. This system could produce some reductions in release rate but failed to change release mechanism.[21] In the report of V.S. Rao *et al.*, the aim was production of liposome-encapsulated clindamycin in treatment of *Staphylococcus aureus* endophthalmitis. They found, clindamycin concentration was 28 μg/ml in the rabbit eyes, after 48 hours (vs. 2.3 μg/ml after 24 hours in the case of non-encapsulated form).[7,17] Although, this study showed some kind of drug release retardation but this system could not be used as a long-term delivery system. Vukomanovic *et al.*, reported successful fabricating of controlled release formulation of clindamycin phosphate. This nanosphere-based dosage form, which made from PLGA polymer can retard clindamycin release for about 1 month and be used in treatment of bone infections.[16,18-20] This report, beside to other complimentary studies which have been done by this group, may offer the poly(α- hydroxy) acid polymers as the promising materials for fabricating of controlled-release devices of clindamycin.

Generally, controlling of hydrophilic drug release rate is more challenging, especially if the matrix is also hydrophilic and swells in the buffer solution so that the drug can rapidly diffuse through the swollen regions and "dump" into solution. Thus, long-term delivery of hydrophilic drugs like clindamycin phosphate is most likely in polymeric carriers composed of relatively hydrophobic matrices such as PLGA and PLA.

In this study, polymeric matrices were prepared with a HME method. This method is becoming a widely used method by pharmaceutical industries. Indeed, HME is a process of converting a raw material into a product of uniform density and shape by forcing it through a die. With designing the die and controlling other conditions such as temperature and pressure, the extrudates with desirable shape and size, can be obtained. This method is currently applied in the pharmaceutical industries for the manufacture of a variety of dosage forms such as tablets, pellets, granules, suppositories and implants.[35-39] HME has some advantages such as fabricating more uniform polymeric matrices, less process steps and having possible wide ranges of shapes and sizes of dosage forms.[39-43]

Different formulations containing clindamycin, using thermoplastic polymers of PLGA and PLA were fabricated with this method [Table 2]. These Implants with a rod shape and diameter of 0.4 mm were prepared with different formulations. The mixing with lyophilization method and subsequent compression with applying appropriated heat and pressure, indicating this simple compression method as a suitable technique for development of the implants with a uniform drug content.

These formulations were evaluated and compared in the release kinetic pattern for demonstration of the effects of ratio of GA to LA, polymer molecular weight and drug-to-polymer ratios.

As mentioned before, *in-vitro* release data has shown, two-phase cumulative release profile of clindamycin for all the formulations. The first phase is burst release phase, which was mainly due to drug desorption and diffusion from the surface and small pores on the surface of implants. With increasing the drug ratio, since more drug is trapped on the surface of the polymer matrix during the manufacturing process, burst effect (BE) is appeared upon activation in a release medium. In the effort to reduce the high initial burst effect and short release duration of the drug, we used polymers with higher lactide content (PLGA 75:25 and 100:0) to prepare implants. With using higher LA contents, some decreases in BE were seen. For

example, this value was reduced from 31% in the case of F13 to 19% and 14% for F17 and F21, respectively. Results showed, when LA part of copolymer increased, the initial burst effect, is decreased due to presence of more hydrophobic monomer, which depressed the initial penetration of water into the systems.

The second portion of the release curves is a sustained release phase. Duration of this part of curves was varied according to formulation types.

In order to obtain meaningful information for the release kinetic models, the drug release data were kinetically evaluated and fitted to the four different kinetic models:

* Zero-order rate Eq. (1) which describes the systems where the drug release rate is independent of its concentration
* First-order Eq. (2) which describes the release from system where release rate is concentration dependent
* Higuchi Eq. (3) which described the release of drugs as a square root of time
* Korsmeyer-Peppas Eq. (4), which with calculating parameter 'n', gives insight into the release mechanisms and can predict many kinds of drug dissolution profiles.[33,37]

Actually these four models are the major models which have best describing of drug-release phenomena.

It is possible to visualize three steps which govern the drug release from implants; penetration of the dissolution medium into the polymeric matrix, dissolution of dispersed drug particles and diffusion of the dissolved drug through the polymeric matrix. The slowest step will control the release rate. If diffusion of the drug is the rate-limiting step, the drug release follows Higuchi square root kinetics. However, if the dissolution of the drug is the rate-limiting step, the drug release follows zero-order kinetics.[27,31] Values obtained from Korsmeyer-Peppas equation, indicated that, for all the PLGA formulations n values confirm the non-Fickian mechanism which is the combination of drug diffusion and polymer relaxation as the responsible for drug release from matrices. This parameter ranged from 0.64 to 0.96 for PLGA5050 formulations and from 0.53 to 0.79 for PLGA with 75:25 LA-to-GA ratio. The lower n value in PLGA with higher LA monomer, can mean decreasing polymer relaxation role in drug release and closing the release to Fickian one.

For the PLA implants, the dominant mechanism is Higuchi and n value is more consistent with the Fickian.

According to the Higuchi model, in the present study, the release of clindamycin from the implants is mainly controlled by micropores diffusion phenomena. Indeed, because clindamycin phosphate is a water-soluble drug, it is more reasonable that, diffusion through the matrices be considered as the rate-limiting step. As other experiments also reported, Higuchi model can fit easily in the system which hydrophilic drug is entrapped in systems which matrix swelling and dissolution are negligible.[44-48] In Di Colo study, Higuchi mechanism was reported for a hydrophilic drug releasing from hydrophobic controlled release matrices of silicone elastomers and ethylene-vinyl acetate copolymers.[46] Kunou *et al.*, developed the long-term sustained release of ganciclovir from biodegradable scleral implant for the treatment of cytomegalovirus retinitis. Their implants were composed of PLA containing 20% w/w gancilovir. They reported release of the drug from homogenous matrices, by diffusion controlled mechanism which followed from Higuchi mechanism.[29] Miyajima's research group, studied release of hydrophilic substance of papaverine from biodegradable PLA biodegradable cylindrical matrix which prepared with heat compression method. They found two sequential diffusion stages in the release profile. The first stage was burst-release phase due to diffusion of papaverin through the swollen matrix. In the second release phase, the solute diffused through the water-filled micropores. With fitting in release kinetic models, Higuchi was suggested as a best model for describing the release phenomena.[47] In addition, for 5-Fluorouracil releasing from PLA polymeric nanoparticles, Higuchi was reported as a best mechanism for releasing of this hydrophilic drug by Ocal H., *et al*.[48]

In the present study, as we can see in the different release plots of the clindamycin, in formulations with PLA more controllable release pattern was seen. Among all the formulations which fabricated with PLAs, F18, which is composed of PDLLA-1 and 20% drug loading, is the best one as the clindamycin controlled-release implant. In this formulation, MDT value was calculated about 14 days. For this implant, drug release begin with the initial burst effect of 23% (equal to 125 µg of clindamycin) in the first day and continue for about 5 weeks with therapeutics drug level maintenance (ED50 reported as 1.5 µg/ml for clindamycin anti-toxoplasmosis effects [9]). So we can expect the efficient anti-toxoplasmosis effect in *in-vivo* experiments. SEM images also confirmed maintaining the structure of these devices which can guarantee more uniform drug release in duration of device activity and also preventing of secondary burst release. So F18, can be considered for more future *in-vitro* considerations and also *in-vivo* drug release in rabbit eyes.

CONCLUSIONS

In this research the cylindrical extrudates was produced using the hot-melt extrusion process for the purpose of developing a clindamycin sustained intraocular implants. The potential of this alternative device has been highlighted and it has been demonstrated that the drug release from the extrudates can be appropriately tailored through suitable selection of the dimensions of the cylinder and polymer type and ratio.

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REFERENCES

1. Bourges JL, Bloquel C, Thomas A, Froussart F, Bochot A, Azan F, *et al*. Intraocular implants for extended drug delivery: Therapeutic applications. Adv Drug Deliv Rev 2006;58:1182-202.
2. Choonara YE, Pillay V, Danckwerts MP, Carmichael TR, du Toit LC. A review of implantable intravitreal drug delivery technologies for the treatment of posterior segment eye diseases. J Pharm Sci 2010;99:2219-39.
3. Eljarrat-Binstock E, Pe'er J, Domb AJ. New techniques for drug delivery to the posterior eye segment. Pharm Res 2010;27:530-43.
4. Del Amo EM, Urtti A. Current and future ophthalmic drug delivery systems. A shift to the posterior segment. Drug Discov Today 2008;13:135-43.
5. Thrimawithana TR, Young S, Bunt CR, Green C, Alany RG. Drug delivery to the posterior segment of the eye. Drug Discov Today 2011;16:270-7.
6. Sobrin L, Kump LI, Foster CS. Intravitreal clindamycin for toxoplasmic retinochoroiditis. Retina 2007;27:952-7.
7. Peyman GA, Charles HC, Liu KR, Khoobehi B, Niesman M. Intravitreal liposome-encapsulated drugs: A preliminary human report. Int Ophthalmol 1988;12:175-82.
8. Paquejt JT, Peyman GA. Intravitreal clindamycin phosphate in the treatment of vitreous infection. Ophthalmic Surg 1974;5:34-9.
9. Soheilian M, Ramezani A, Azimzadeh A, Sadoughi MM, Dehghan MH, Shahghadami R, *et al*. Randomized trial of intravitreal clindamycin and dexamethasone versus pyrimethamine, sulfadiazine, and prednisolone in treatment of ocular toxoplasmosis. Ophthalmology 2011;118:134-41.
10. Mostafavi N, Ataei B, Nokhodian Z, Monfared LJ, Yaran M, Ataie M, *et al*. Toxoplasma gondii infection in women of childbearing age of Isfahan, Iran: A population-based study. Adv Biomed Res 2012;1:60.
11. Sallam A, Jayakumar S, Lightman S. Intraocular delivery of anti-infective drugs-bacterial, viral, fungal and parasitic. Recent Pat Antiinfect Drug Discov 2008;3:53-63.
12. Wong R, dell'Omo R, Marino M, Hussein B, Okhravi N, Pavesio CE. Toxoplasma gondii: An atypical presentation of toxoplasma as optic disc swelling and hemispherical retinal vein occlusion treated with intravitreal clindamycin. Int Ophthalmol 2009;29:195-8.
13. Lasave AF, Díaz-Llopis M, Muccioli C, Belfort R Jr, Arevalo JF. Intravitreal clindamycin and dexamethasone for zone 1 toxoplasmic retinochoroiditis at twenty-four months. Ophthalmology 2010;117:1831-8.
14. de-la-Torre A, Stanford M, Curi A, Jaffe GJ, Gomez-Marin JE. Therapy for ocular toxoplasmosis. Ocul Immunol Inflamm 2011;19:314-20.
15. Martínez Castillo S, Gallego-Pinazo R, Francés-Muñoz E, Dolz-Marco R, Vázquez Polo A, Díaz-Llopis M. Macular toxoplasmosis and intravitreal clindamycin: An alternative to oral treatment. Arch Soc Esp Oftalmol 2012;87:93-5.
16. Vukomanović M, Skapin SD, Poljanšek I, Zagar E, Kralj B, Ignjatović N, *et al*. Poly (D, L-lactide-co-glycolide)/hydroxyapatite core-shell nanosphere. Part 2: Simultaneous release of a drug and a prodrug (clindamycin and clindamycin phosphate). Colloids Surf B Biointerfaces 2011;82:414-21.
17. Rao VS, Peyman GA, Khoobehi B, Vangipuram S. Evaluation of liposome-encapsulated clindamycin in Staphylococcus aureus endophthalmitis. Int Ophthalmol 1989;13:181-5.
18. Uskoković V, Hoover C, Vukomanović M, Uskoković DP, Desai TA. Osteogenic and antimicrobial nanoparticulate calcium phosphate and poly-(D, L-lactide-co-glycolide) powders for the treatment of osteomyelitis. Mater Sci Eng C Mater Biol Appl 2013;33:3362-73.
19. Vukomanović M, Zavašnik-Bergant T, Bračko I, Skapin SD, Ignjatović N, Radmilović V, *et al*. Poly (D, L-lactide-co-glycolide)/hydroxyapatite core-shell nanospheres. Part 3: Properties of hydroxyapatite nano-rods and investigation of a distribution of the drug within the composite. Colloids Surf B Biointerfaces 2011;87:226-35.
20. Vukomanović M, Skapin SD, Jančar B, Maksin T, Ignjatović N, Uskoković V, *et al*. Poly (D, L-lactide-co-glycolide)/hydroxyapatite core-shell nanospheres. Part 1: A multifunctional system for controlled drug delivery. Colloids Surf B Biointerfaces 2011;82:404-13.
21. Hascicek C, Rossi A, Colombo P, Massimo G, Strusi OL, Colombo G. Assemblage of drug release modules: Effect of module shape and position in the assembled systems on floating behavior and release rate. Eur J Pharm Biopharm 2011;77:116-21.
22. Kimura H, Ogura Y. Biodegradable polymers for ocular drug delivery. Ophthalmologica 2001;215:143-55.
23. Athanasiou KA, Niederauer GG, Agrawal CM. Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers. Biomaterials 1996;17:93-102.
24. The United States Pharmacopeia. 35th ed. (USP 35). United States Pharmacopeial Convention Inc. Rockville: CD-ROM (Insight Publishing Productivity; 2012.
25. Li S, Shen Y, Li W, Hao X. A common profile for polymer-based controlled release and its logical interpretation to general release process. J Pharm Pharm Sci 2006;9:238-44.
26. Ritger PL, Peppas NA. A simple equation for the description of solute release, II: Fickian and anomalous release from swellable devices. J Control Release 1987;5:37-42.
27. Costa P, Sousa Lobo JM. Modeling and comparison of dissolution profiles. Eur J Pharm Sci 2001;13:123-33.
28. Korsmeyer RW, Gury R, Doelker E, Buri P, Peppas NA. Mechanisms of solute release from porous hydrophilic polymers. Int J Pharm 1983;15:25-35.
29. Kunou N, Ogura Y, Yasukawa T, Kimura H, Miyamoto H, Honda Y, *et al*. Long-term sustained release of ganciclovir from biodegradable scleral implant for the treatment of cytomegalovirus retinitis. J Control Release 2000;68:263-71.
30. Birnbaum DT, Kosmala JD, Henthorn DB, Peppas LB. Controlled release of h-estradiol from PLAGA microparticles: The effect of organic phase solvent on encapsulation and release. J Control Release 2000;65:375-87.
31. Fu Y, Kao WJ. Drug release kinetics and transport mechanisms of non-degradable and degradable polymeric delivery systems. Expert Opin Drug Deliv 2010;7:429-44.
32. Frank A, Rath SK, Venkatraman SS. Controlled release from bioerodible polymers: Effect of drug type and polymer composition. J Control Release 2005;102:333-44.
33. Rosenberg RT, Siegel SJ, Dan N. Release of highly hydrophilic drugs from poly (epsilon-caprolactone) matrices. J Appl Polym Sci J 2008;107:3149-56.
34. Siegel SJ, Kahn JB, Metzger K, Winey KI, Werner K, Dan N. Effect of drug type on the degradation rate of PLGA matrices. Eur J Pharm Biopharm 2006;64:287-93.
35. McGinity JW, Koleng JJ, Repka MA, Zhang F. Hot-melt extrusion technology. In: Swarbrick J, Boylan JC, editors. Encyclopedia of pharmaceutical technology. New York: Marcel Dekker; 2001. p. 203-26.
36. Breitenbach J. Melt extrusion: From process to drug delivery technology. Eur J Pharm Biopharm 2002;54:107-17.
37. Albers J, Alles R, Matthée K, Knop K, Nahrup JS, Kleinebudde P. Mechanism of drug release from polymethacrylate-based extrudates and milled strands

prepared by hot-melt extrusion. Eur J Pharm Biopharm 2009;71:387-94.

38. Young CR, Koleng JJ, McGinity JW. Production of spherical pellets by a hot-melt extrusion and spheronization process. Int J Pharm 2002;242:87-92.

39. Brabander C, Vervaet C, Remon JP. Development and evaluation of sustained release mini-matrices prepared via hot-melt extrusion. J Control Release 2003;89:235-47.

40. Cheboyina S, Wyandt CM. Wax-based sustained release matrix pellets prepared by a novel freeze pelletization technique II. *In vitro* drug release studies and release mechanisms. Int J Pharm 2008;359:167-73.

41. Verhoeven E, Siepmann F, De Beer TR, Van Loo D, Van den Mooter G, Remon JP, *et al.* Modeling drug release from hot-melt extruded mini-matrices with constant and non-constant diffusivities. Eur J Pharm Biopharm 2009;73:292-301.

42. Fukuda M, Peppas NA, McGinity JW. Properties of sustained release hot-melt extruded tablets containing chitosan and xanthan gum. Int J Pharm 2006;310:90-100.

43. Schilling SU, Bruce CD, Shah NH, Malick AW, McGinity JW. Citric acid monohydrate as a release-modifying agent in melt extruded matrix tablets. Int J Pharm 2008;361:158-68.

44. Gurny R, Doelker E, Peppas NA. Modelling of sustained release of water-soluble drugs from porous, hydrophobic polymers. Biomaterials 1982;3:27-32.

45. Higuchi T. Mechanism of sustained action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J Pharm Sci 1963;52:1145-9.

46. Di Colo G. Controlled drug release from implantable matrices based on hydrophobic polymers. Biomaterials 1992;13:850-6.

47. Miyajima M, Koshika A, Okada J, Kusai A, Ikeda M. Factors influencing the diffusion-controlled release of papaverine from poly (L-lactic acid) matrix. J Control Release 1998;56:85-94.

48. Ocal H, Arıca-Yegin B, Vural I, Goracinova K, Calış S. 5-Fluorouracil-loaded PLA/PLGA PEG-PPG-PEG polymeric nanoparticles: Formulation, *in vitro* characterization and cell culture studies. Drug Dev Ind Pharm. Epub 2013 Apr 18.