Original Article

Thermoanalytical characterization of clindamycin-loaded intravitreal implants prepared by hot melt extrusion

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Abstract

Background: The aim of the present study was to evaluate a non-destructive fabrication method in for the development of sustained-release poly (L, D-lactic acid)-based biodegradable clindamycin phosphate implants for the treatment of ocular toxoplasmosis.

Materials and Methods: The rod-shaped intravitreal implants with an average length of 5 mm and a diameter of 0.4 mm were evaluated for their physicochemical parameters. Scanning electron microscopy (SEM), differential scanning calorimetry (DSC), Fourier-transform infrared (FTIR), and nuclear magnetic resonance (1H NMR) studies were employed in order to study the characteristics of these formulations.

Results: Drug content uniformity test confirmed the uniformity in different implant batches. Furthermore, the DSC, FTIR, and 1H NMR studies proved that the fabrication process did not have any destructive effects either on the drug or on the polymer structures.

Conclusion: These studies showed that the developed sustained-release implants could be of interest for long-term sustained intraocular delivery of clindamycin, which can provide better patient compliance and also have good potential in terms of industrial feasibility.

Key Words: Clindamycin phosphate, intravitreal implant, melt extrusion, poly (L, D-lactic acid)

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INTRODUCTION

A wide range of posterior segment ophthalmic diseases may benefit from the development of novel drug delivery systems.[1] Implantable sustained-release intraocular device technology has being given much impetus due to the perceptible benefits afforded over the use of topical drop, systemic administration, and repeated intravitreal injections as modes of drug delivery to the posterior segment of the eye. Solid biocompatible implants for sustained or controlled intravitreal drug delivery to the ocular posterior segment have been developed using different kinds of materials.[1,2]

Among the wide ranges of materials which are applied for this purpose, biodegradable polymers may offer some advantages over the non-biodegradable ones.

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The biodegradable implants which can be formed into various shapes are implanted by a very simple procedure or even injected as an office procedure. These devices are converted to a soluble form through either enzymatic or nonenzymatic reactions in the body. Therefore, they do not need to be removed once the drug is delivered.[1-5]

Although a number of different polymers have been investigated for formulation of biodegradable products, polymers of poly (L, D-lactic acid) (PLA) and their copolymers with glycolic acid (PLGA) are found to be more promising. These polymers are attractive, especially in the fabrication of ocular dosage forms, due to the favorable biocompatibility and bioresorability characteristics. These aliphatic polymers of poly (α-hydroxy) acids are hydrolyzed into natural metabolites (lactic and glycolic acids), which are removed from the body through the citric acid cycle. These polymers provide wide ranges of degradation rates depending on the molecular weights and composition of the monomers.[6-8]

For a long time, intraocular injections of clindamycin (CLP) have been used for the treatment of ocular infections of the posterior segment.[9-11] In the case of toxoplasmosis retinochoroiditis, this kind of administration is very effective in the patients who show resistance to common oral therapy and in those who develop adverse effects of the systemic drug. In addition, during pregnancy, especially in the first semester, local administration can avoid toxic and teratogenic effects of the drug.[9-11]

In spite of its promising effects, repeated injections are needed to maintain the drug concentration at an effective therapeutic level over a certain period of time due to clindamycin's short half-life in the vitreous.[9-11] On the other hand, usually repeated intravitreal injections result in extreme patient discomfort and may lead to serious complications. Since toxoplasmosis is the leading cause of posterior uveitis, especially in the developing countries, the need to find alternative treatments is felt.[9-11]

Controlled-release intraocular implants of clindamycin can be a good substitute for repeated intravitreal injections. Due to miniaturized size, this system can be easily injected inside the eye (just one time in the treatment period) and maintains the therapeutic level of the drug for a long time. Since this system is fabricated with biodegradable polymers, there is no need for additional surgery for removing it after the drug release is performed.

Hot melt extrusion (HME) has been developed as a new preparation technique in recent years. In this method, applying appropriate heat and pressure, the raw materials are passed through a die and converted into products. HME is mainly used to process controlled-release formulations, and producing a homogeneous mixture. Some advantages, including simple fabrication process, lack of residual solvents, decreased environmental hazards, low cost, and also continuous and efficient process, make this method as an interesting method in pharmaceutical industries.[12-14] PLA and PLGA, due to their thermoplastic properties, can be softened and melted on heating and shaped in a variety of implants using several methods such as melt extrusion.[15-17]

In spite of all the advantages, since the dosage forms are fabricated at high temperatures (usually between 80°C and 170°C) and under high shearing forces in HME, there are some concerns about stability. Under these conditions, either the polymer or drug may be degraded or may lose its crystallinity. This condition can also affect the degradation rate and the subsequent drug release. So, in the case of using such preparation techniques, selection of appropriate methods of characterization such as quality control is an important step to assure a reproducible and effective product.[16]

A number of experimental techniques [i.e. differential scanning calorimetry (DSC), Fourier transform infrared (FTIR), nuclear magnetic resonance (1H NMR), scanning electron microscopy (SEM), etc.] have been used to investigate the stability of final dosage forms and the interaction between drug and polymer.[18,19] Most of these methods are used directly on pharmaceutical preparations. So, by applying them, valuable information on physicochemical properties of final dosage formulations can be obtained. This information can facilitate development of new dosage forms.[20]

The goal of this study was to apply some analytical experiments for evaluating the biodegradable implants for the prolonged release of CLP in the treatment of toxoplasmosis retinochoroiditis. The physicochemical characterizations of the implants were evaluated by SEM for morphological evaluations; DSC was employed for studying the crystallinity changes of PLA and CLP; and FTIR and 1H NMR were used for studying the polymer and drug chemical structures and the possible interactions.

MATERIALS AND METHODS

Materials

The polymer studied was PLA R203 (molecular weight 18 kDa) (Sigma Aldrich Inc., Munich, Germany). The

organic solvent used was methylene chloride (Labsynth Ltd, Diadema, Brazil). Clindamycin phosphate was the model drug used (Behdaroo Co., Tehran, Iran). High-performance liquid chromatography (HPLC) grade acetonitrile was supplied by Merck (Darmstadt, Germany). All other chemicals were of analytical grade and obtained from Merck.

Implant fabrication

Clindamycin phosphate-loaded PLA implants were prepared in a two-step process by melt extrusion method. In the first step, the drug was co-lyophilized with PLA to obtain a homogenous mixture. Briefly, clindamycin and PLA (20:80) were dissolved in a mixture of acetonitrile and distilled water (1:1). The obtained solution was placed in the freezer (–70°C). Afterward, the frozen solution was lyophilized (Chemical-free Freeze Dryer; Operon, Gyenggi, Korea) for 48 h. Then, the powder was removed from the tube and ground using an agate mortar. Evaluation of the content uniformity of the final implants was done to find if the mixing process was successful.

In the second step, for compressing the mixture, approximately 50 mg of the lyophilized powder was introduced into the barrel of extruder, represents the image of the lab-scale extruder. The cylinder of extruder was heated to 85°C by using digital temperature controlled heater. This temperature is appropriate for obtaining a plastic, semi-solid mass which could be extruded through a die plate with 0.4 mm diameter. Once the polymer started plasticizing, on applying appropriate pressure (set at 139 bars), rod-shaped solid extrudates were formed, and after cooling to the room temperature, they were cut into 5 mm length.

Content uniformity test

Content uniformity test of clindamycin-containing implants was done according to the procedure stated in the general chapter "Uniformity of Dosage Units" in the United States Pharmacopeia 35 (USP 35).[21]

As described in the guideline, ten implants were selected and weighed. Each implant was dissolved in the mobile phase (mixture of acetonitrile and distilled water). After filtration and suitable dilution, the drug content was determined by HPLC. HPLC was performed under the following conditions: Column, CN-RP column (250 × 4.6 mm, 5 μm particle size) (Macherey-Nagel, Duren, Germany); mobile phase, mixture of acetonitrile and water (40:60) containing 100 mM tetramethyl ammonium chloride (pH 4.2); flow rate, 1 ml/min; and detection, ultraviolet at 204 nm.

In vitro release test

In vitro release studies were performed under sink conditions over 40 days. Briefly, three implants were placed inside three different vials containing 1 ml of phosphate buffer as the dissolution medium (pH 7.4). The tubes were placed inside a batch shaker (37°C ± 0.5°C and 50 rpm). At desirable time intervals, the whole volume of the medium (1 ml) was collected and replaced with 1.0 ml of fresh buffer. After filtration and dilution, the amount of drug release was measured by HPLC method (described in the previous section).

Scanning electron microscopy

Clindamycin phosphate implant degradation was studied by SEM (model AIS-2300; Seron Technology, Gyeonggi-do, Korea). The implant's surface changes were compared before and 4 and 10 weeks after immersing in the dissolution medium. The samples were coated by gold and observed at magnification of ×1000.

Differential scanning calorimetry

The physical state of clindamycin phosphate loaded in the implants was characterized by DSC thermogram analysis (Mettler ToleDo; Star system, Columbus, USA). The samples measuring up to 10 mg were weighed and sealed in aluminum pans using the press in DSC instrument. An empty pan was used as the reference. They were heated from 20°C to 250°C at a heating rate of 5°C/min. DSC curves covering a range of 20°C-250°C were recorded in order to evaluate CLP and polymer stability and CLP-polymer interaction.

As control, the pure polymer as well as the physical and lyophilized mixtures of CLP and the polymer were analyzed. All DSC data analysis was performed using STAR SW version 9.10.

Fourier transform infrared studies

FTIR spectra were recorded on the apparatus (WFQ-510, Rayleigh, Beijing, China) for pure PLA, pure CLP, physical and lyophilized mixture of the polymer and drug, and clindamycin-loaded implants. The samples were mixed with KBr and the obtained disks were analyzed in the range of 4000 cm^{-1} to 400 cm^{-1} at a resolution of 4 cm^{-1}. The spectra were the mean of five consecutive scans of the same sample.

Nuclear magnetic resonance

1H NMR spectra were recorded at room temperature on a spectrometer instrument (AC300; Brucker, Karlsruhe, Germany) equipped with a Broadband inverse BBI probe. All samples were dissolved in deuterated dimethyl sulfoxide (DMSO-d6) as the solvent, which contained teramethylsilane (TMS) as the internal standard. The 1H NMR spectra were obtained at 300 MHz. The chemical shifts of the

implant containing clindamycin, as well as of the pure polymer and drug were analyzed using this method.

RESULTS

Implants' morphology

Figure 1 shows the clindamycin phosphate implants. Smooth and uniform color of the implants (due to clindamycin phosphate which has a white color) is clearly seen in the image. Macroscopically, the implants exhibited a rigid structure with 0.41 ± 0.04 mm diameter. The mean weight of the developed rods was 2.4 mg ± 0.20 mg. The extrudates were cut in 5 mm length and used for more studies.

The surfaces changes of implants with time were evaluated by SEM. As shown in Figure 2a, before immersing in the release medium, the implants exhibited smooth and dense surfaces. Figure 2b shows the well-maintained structure of PLA implants after 4 weeks. In this image, some pores appearing on the surface as a result of drug depletion from the system is observed [Figure 2b]. After 10 weeks of incubation, the implants were more eroded and significant cracks appearing in them are noticeable [Figure 2c].

Content uniformity test

The content uniformity test 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%)[21] for the pre-indicated amount of clindamycin (20% w/w, corresponding to approximately 517 μg ± 1.6 μg of the drug per implant).

In vitro release studies of clindamycin from the implants

Figure 3 presents the *in vitro* cumulative release of clindamycin from the implants during the period of study (about 2 months).

During the first day, fast release of clindamycin in the burst stage was observed. In this phase, 23% of the drug, which is equal to 125 μg, was released. After this phase, the more controlled release phase was observed. During the first week, about 30% of the drug was released. This process continued for the following several weeks, but in different rates. During the second and third weeks, the release happened in approximately the same rate (about 60 μg in each week). During this period, the largest quantity of the total clindamycin was released. Between the fourth and fifth weeks, the release of clindamycin phosphate was decreased, and the same trend continued during the following weeks. Although the release continued over 2 months, 50% inhibitory concentration of clindamycin against *Toxoplasma gondii* (the parasite responsible for ocular toxoplasmosis) was achieved by our implant during the first 5 weeks.[9,10]

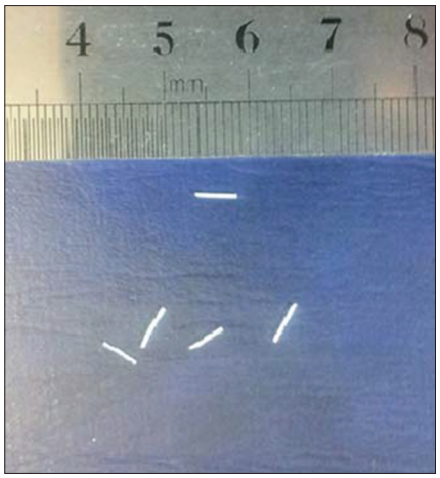

Figure 1: Macroscopic view of the fabricated implants

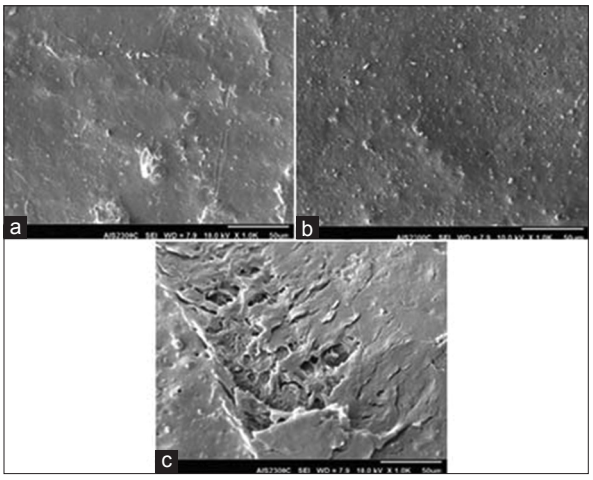

Figure 2: Microscopic images of implants: (a) before immersing in the release medium (b) 4 weeks and (c) 10 weeks after incubation

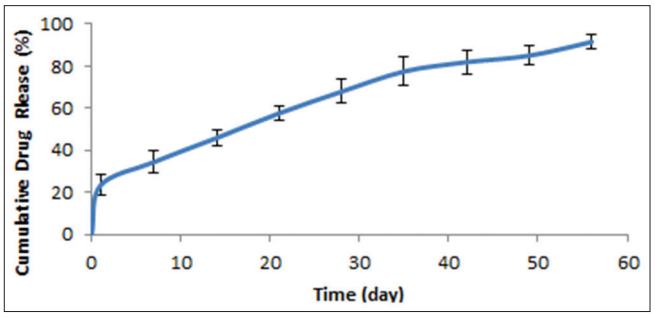

Figure 3: The *in vitro* release diagram of clindamycin from PLA implants (the values are shown as mean ± SD). Each data point represents the average of three samples

Differential scanning calorimetry

In Figure 4, the DSC curves of clindamycin phosphate, PLA, clindamycin mixed with PLA, and the final implant are shown.

DSC curves indicate the expected peaks reported in the literature and by the suppliers.[22,23] In Figure 4a and b, the endothermic event related to the melting of pure crystalline clindamycin phosphate (Tm) at 170.5°C and glass transition temperature (Tg) of PLA as an endothermic peak at 52.1°C are demonstrated, respectively.[22,23]

For the drug and polymer physical and lyophilized mixture, there are the two peaks which show the drug melting at about 172.4°C and the polymer glass transition at 55.3°C. These two peaks can be a simple superposition of the peaks of the pure polymer and drug alone, although in the case of the mixture, a peak related to melting point of the drug appears a little smaller than that of the drug alone. This decrease can be the result of dilution of the drug in the presence of higher portion of the polymer in the mixture that can affect the thermogram curves of the drug.

In addition, the glass transition temperature can have a significant role in defining the propensity of amorphous material to crystallize at determined temperatures ranges.[24] According to our results, it is obvious that no changes were observed in the amorphous state of PLA during the freeze-drying process. Moreover, the presence of clindamycin phosphate could not make significant alteration in the Tg value of the polymer.

The parameters determined by DSC for the final clindamycin-loaded PLA implants showed thermal transition of the polymer at 51.3°C. The glass transition temperature of PLA in the final implants did not show any significant changes in compared to the unprocessed polymer. This indicates that presence of the drug and also the fabrication process did not affect thermal characteristic of the polymer. In the case of the drug, we could not see the melting point of the drug. This disappearance of the drug peak in the thermogram can be the result of transformation of the drug from the unstable crystalline form to the its stable amorphous form. This can also be as a result of homogenous drug mixing and dissolving throughout the polymer matrix.[25] In addition, this phenomenon can be the result of the interaction between the drug and polymer, which can be confirmed with additional information obtained from the FITR and 1H NMR analytical methods.

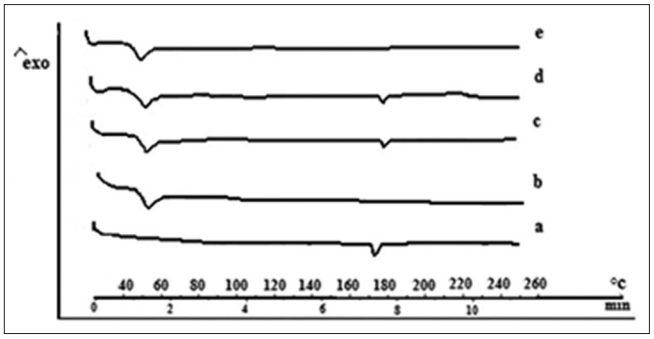

Figure 4: DSC thermograms of samples: (a) pure CLP, (b) pure PLA, (c) physical mixture of CLP/PLA, (d) lyophilized mixture of CLP/PLA, and (e) clindamycin-containing implants

Fourier transform infrared studies

In the present work, pure PLA and clindamycin alone, physical mixture of CLP/PLA, and also the prepared implants were subjected to FTIR studies. Figure 5a and b shows the chemical structures of PLA and clindamycin, respectively.

The spectra obtained are shown in Figure 6. The pure clindamycin sample [Figure 6a] shows the main peaks, indicated by the appearance of skeletal vibrations in the fingerprint region, between 1600 cm^{-1} and 600 cm^{-1}. These skeletal vibrations originate from the vibrations of pyrrole and saccharide rings, which are the main components of the structure of clindamycin molecules.The group of bands that are indicated in this region are mainly attributed to the stretching vibrations of the C double bond; length as C–C of aromatic groups at 1570 cm^{-1} as well as the stretching vibrations of the amide group in the plane of the ring, which are observed in the interval between 750 cm^{-1} and 680 cm^{-1}. Within the intervals between 1480 cm^{-1} and 1370 cm^{-1}, both the spectra consist of bands at 1466 cm^{-1} and 1381 cm^{-1}, which correspond to the C–H deformation vibrations in combination with the vibrations of the aromatic saccharide ring. The stretching vibration of the C–O groups bonded to the saccharide ring is observed at 1157 cm^{-1}, while the bands at 1319 cm^{-1} and 526 cm^{-1} correspond to the in-plane and out-of-plane vibrations of the O–H groups attached to the aromatic ring, respectively. In

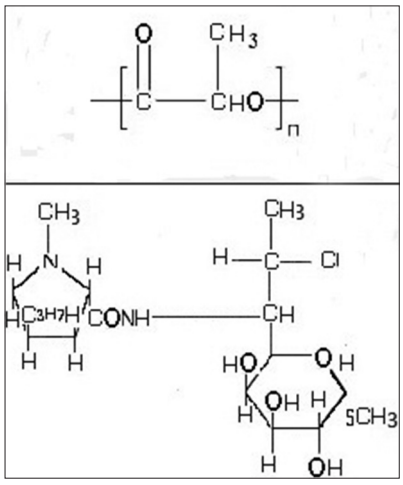

Figure 5: General chemical structures of (a) PLA and (b) clindamycin phosphate. Reprinted with permission from references[26,27]

the region of higher wavenumbers, which corresponds to the region of vibrations of double bonds, the stretching vibrations of the carbonyl group bonded to the amide (NH–CO) can be observed (at 1685 cm^{-1}). We can also see the broad band with a maximum at 3346 cm^{-1}, which corresponds to the vibrations of the O–H groups of aromatic alcohols.[28,29]

In the bulk PLA sample [Figure 6b], we can see the main peaks such as –CH, –CH2, –CH3 stretching (2850-3000 cm^{-1}), carbonyl –C = O stretching (1700-1800 cm^{-1}), C–O stretching (1050-1250 cm^{-1}), and –OH stretching (3200–3500 cm^{-1}), which were broad. These absorbance peaks almost matched those in previous reports.[30-32]

FTIR spectra of the physical mixture of clindamycin and PLA and also clindamycin-containing PLA implants are shown in Figure 6b, c. The spectra are the result of summation of the PLA and clindamycin individual spectra, suggesting that no interactions occur between the drug and the polymer in their physical mixtures. This analysis indicated that the specific functional groups of polymeric material in the implant surface have almost the same chemical characteristics as those of the pure polymer and the drug entrapped. The study suggested that no molecular interactions have occurred that could alter the chemical structure of the drug at the time of study. For example, we can find a sharp peak band at 1757 cm^{-1} for PLA and CLP. This value lies within the range of 1700-1800 cm^{-1}, so this peak is attributed to the C = O group. At 3200-3500 cm^{-1}, we can see the peak of –OH stretching of clindamycin and PLA. In 2999, 2951, and 2787 cm^{-1}, we can see the low-intensity peaks of clindamycin and PLA of sp3 hybridized CH stretch.

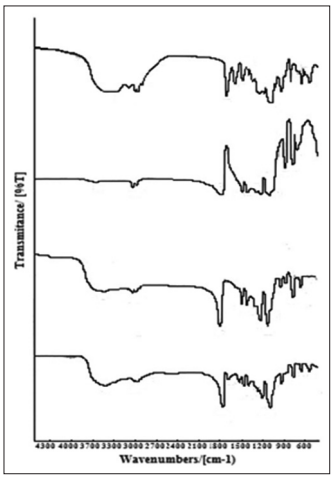

Figure 6: Infrared spectra of (a) pure CLP, (b) pure PLA, (c) physical mixture PLA/CLP, and (d) CLP-containing implant

Nuclear magnetic resonance studies

Figure 7 demonstrates 1H NMR spectroscopy of the samples. According to the literature reports, in 1H NMR spectra of the pure PLA, the peaks around 5.25 ppm are due to the methine protons in the lactic acid repeat units and the peaks at 4.35 ppm are related to the same protons in the end groups of the polymer. In addition, the sharp peaks at 1.6 ppm are a characteristic signal of the methyl protons in the repeated units in the main chain.[33,34]

The 1H NMR spectrum of clindamycin displays cluster of methine resonances between 3.5 and 5.3 ppm which are expected for a sugar moiety. Resonance of the amide group is observed at 8.64 ppm. Chemical shifts of the protons of the pyrrole group are seen in 0.9 ppm until 2.6 ppm.[35,36]

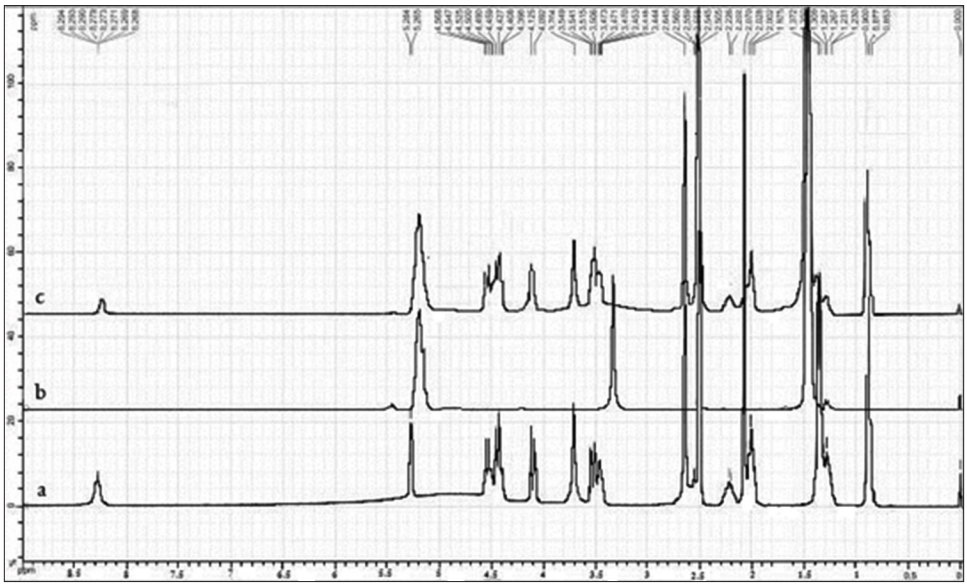

Figure 7: 1H NMR of (a) pure clindamycin, (b) pure PLA, (c) and drug-loaded PLA implant

For the drug-containing implant, we can see the main peaks of the CLP and PLA alone with the same intensity and without significant shift (which can occur due to some kind of chemical interaction) or appearance of additional peaks (as a result of degradation of either polymer or drug), which suggest occurrence of no drug-polymer interaction or degradation of either of them.[33]

DISCUSSION

Development of implantable drug delivery systems is perhaps the most interesting application of biodegradable polymers. Owing to their transient nature, such kinds of polymers do not require additional surgical procedures for removing the devices after their intended application period. Therefore, they can overcome some problems related to the long-term safety of non-degradable counterparts.[1,2,37,38]

To our knowledge, this is the first report of fabricating of clindamycin-sustained release device for implantation into the ocular posterior segment. Different formulation parameters such as polymer grades and drug/polymer ratio were investigated in our previous studies. Eventually, according to the *in vitro* release data, the best formulation for more characterization and for the *in vivo* studies was selected.[39]

PLA and PLGA polymers have generated immense popularity due to their well-established biodegradability and biocompatibility. These characteristics make them as one of the few US Food and Drug Administration (FDA)-approved polymers for drug delivery. In addition, their unique physicochemical properties make them as easy materials to be formulated into various drug carrier devices. In recent years, PLA has attracted much interest as a base biomaterial. Since PLA is characterized by a slower degradation rate, it is used more than PLGA, for the delivery of drugs for which more prolonged release profile is desirable. In addition to favorable degradation rates and drug release characteristics, PLA seems to be better tolerated than the other kind of polymers in intraocular applications.[15,37,38]

In this study, PLA (with average molecular weight of 18 kDa) was used as a matrix former in the fabrication of the implants. These implants could achieve the desirable clindamycin release rate. The release diagrams show that the drug concentrations are maintained at an effective therapeutic level for about 5 weeks. Since the therapeutic duration with intravitreal injections of clindamycin is reported to be between 4 and 6 weeks,[40-44] using this system, any concerns related to repeated intravitreal injections (due to short half-life of clindamycin in the vitreous) can be eliminated. On the other hand, the miniature size allows for easy injection of these implants through a needle inside the vitreous cavity and without any serious injuries.

In the present study, HME was used for the development of the implants. In this process, by applying heat and pressure, the drug was embedded in the polymer carrier.

Evaluation of formulations produced via HME is done using several methods. Along with the good quality control tools, these evaluating methods can provide useful information about the physical and chemical characteristics of the dosage form.

DSC examines the system as a function of temperature and is used for the evaluation of the raw materials, their possible drug-polymer incompatibility, and possible alterations of the components that are induced by the fabrication process.[22,43,45] It can also differentiate between solid solutions (molecularly dispersed drug), solid dispersions in which drug is only partly molecularly dispersed, and physical mixtures of drug and carrier. This information is useful for determining the state of the drug in the carrier. In the current study, thermal behavior of PLA was unchanged during lyophilization and extrusion. In the case of clindamycin, the only difference in comparison to the pure drug was seen in the final product. In the clindamycin-loaded implant, the disappearance of the drug peak which can be attributed to changes in the molecular state of the drug in the carrier, possible polymer-drug interactions, or any degradation of clindamycin during fabrication. So, using complementary methods such as FTIR and 1H NMR can provide more clear information about the changes in the physicochemical characteristics of clindamycin and PLA.

Infrared analysis is a powerful tool to characterize the organic and inorganic materials. Since in this spectroscopic method there is selective and unique light absorption corresponding to the vibration mode of specific chemical bonds, IR spectrum was used as a fingerprint pattern for each substance. So, infrared spectroscopy can be utilized to qualitatively identify different compounds. In the development and characterization of drug delivery systems, FTIR is also used as a valuable tool for investigation of the possible interactions that can occur between the drug and excipients or any degradation that occurs in the formulation, with consideration of the alterations in the intensity and frequency of the involved atoms in such interactions.[46-48] In the present study, the main characteristic peaks of both drug and polymer

were seen in the lyophilized mixture and implant. So, any hypothesis related to degradation can be rejected with FTIR.

1H NMR is another accurate method that is used frequently in studies for determining any instability or interactions in the formulations.[48,49] In the current study, in 1H NMR peaks, no proton deshielding or additional peaks due to drug-polymer interactions or degradation were observed.

CONCLUSIONS

In the present study, a sustained release implant system was fabricated to avoid the clinical inconvenience and the dangerous side effects of repeated intravitreal injections of clindamycin phosphate used as an effective anti-toxoplasmosis drug. The thermoanalytical studies of clindamycin-containing implants suggest that CLP is in an amorphous state or in a disordered crystalline phase of molecular dispersion in the PLA polymeric matrix. The thermal stability evaluations show that since all materials analyzed did not suffer from appreciable decomposition below 200°C in the analytical conditions, this thermal treatment can be suggested as a safe production process. The infrared results and the results obtained from 1H NMR showed that in the length of time studied, strong chemical interaction between PLA and CLP did not occur. This suggests that the implant fabrication process did not interfere with the chemical structure of the polymer and preserved the structural drug integrity. Thus, the methods employed to produce the dosage form and the analytical method to characterize samples are proven to be appropriate.

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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. Mainardes RM, Evangelista RC. Praziquantel-loaded PLGA nanoparticles: Preparation and characterization. J Microencapsulation 2005;22:13-24.
7. Uhrich KE, Cannizzaro SM, Langer RS, Shakesheff KM. Polymeric systems for controlled drug release. Chem Rev 1999;99:3181-98.
8. Shive MS, Anderson JM. Biodegradation and biocompatibility of PLA and PLGA microspheres. Adv Drug Deliv Rev 1997;28:5-24.
9. Rao VS, Peyman GA, Khoobehi B, Vangipuram S. Evaluation of liposome-encapsulated clindamycin in *Staphylococcus aureus* endophthalmitis. Int Ophthalmol 1989;-13 ;181-5.
10. Peyman GA, Charles HC, Liu KR, Khoobehi, Niesman M, Paquejt JT, *et al*. Intravitreal liposome-encapsulated drugs: A preliminary human report. Int Ophthalmol 1988;12 :175-82.
11. Sobrin L, Kump LI, Foster CS. Intravitreal clindamycin for toxoplasmic retinochoroiditis. Retina 2007;27:952-7.
12. Forster A, Hempenstall J, Tucker I, Rades T. Selection of excipients for melt extrusion with two poorly water-soluble drugs by solubility parameter calculation and thermal analysis. Int J Pharm 2001;226:147-61.
13. Crowley MM, Schroeder B, Fredersdorf A, Obara S, Talarico M, Kucera S, *et al*. Physicochemical properties and mechanism of drug release from ethyl cellulose matrix tablets prepared by direct compression and hot-melt extrusion. Int J Pharm 2004;269:509-22.
14. Bruce LD, Shah NH, Malick AW, Infeld MH, McGinity JW. Properties of hot-melt extruded tablet formulations for the colonic delivery of 5-aminosalicylic acid. Eur J Pharm Biopharm 2005;59:85-97.
15. Fialho SL, da Silva Cunha A. Manufacturing techniques of biodegradable implants intended for intraocular application. Drug Deliv 2005;12:109-16.
16. Rothen-Weinhold A, Besseghir K, Vuaridel E, Sublet E, Oudry N, Kubel F, *et al*. Injection-molding versus extrusion as manufacturing technique for the preparation of biodegradable implants. Eur J Pharm Biopharm 1999;48:113-21.
17. Gosau M, Müller BW. Release of gentamicin sulphate from biodegradable PLGA-implants produced by hot melt extrusion. Pharmazie 2010;65:487-92.
18. Bruni G, Amici L, Berbenni V, Marini A, Orlandi A. Drug-excipient compatibility studies: Search of interaction indicators. J Therm Anal Calorim 2002;68:561-73.
19. Wyttenbach N, Birringer C, Alsenz J, Kuentz M. Drug-excipient compatibility testing using a high-throughput approach and statistical design. Pharm Dev Technol 2005;10:499-505.
20. Verma RK, Garg S. Compatibility studies between isosorbide mononitrate and selected excipients used in the development of extended release formulations. J Pharm Biomed Anal 2004;35:449-58.
21. "<905>Uniformity of Dosage Units" The United States Pharmacopeia. 35th ed. (USP 35) United States Pharmacopeial Convention Inc., Rockville: CD-ROM (Insight Publishing Productivity); 2012.
22. Bragagni M, Beneitez C, Martín C, Hernán Pérez de la Ossa D, Mura PA, Gil-Alegre ME. Selection of PLA polymers for the development of injectable prilocaine controlled release microparticles: Usefulness of thermal analysis. Int J Pharm 2013;441:468-75.
23. Available from: http://www.sigmaaldrich.com/materials-science/polymer-science/resomer.html. [Last accessed on4/16/2013].
24. Hatakeyama T, Quinn FX, editors. Thermal Analysis-Fundamentals and Applications to Polymer Science. London: John Wiley and Sons company; 1994. p. 65-105.
25. Hariharan M, Price JC. Solvent, emulsifier and drug concentration factors in poly (D, L-lactic acid) microspheres containing hexamethylmelamine. J Microencapsul 2002;19:95-109.
26. Deng H, Lei Zh. Preparation and characterization of hollow Fe_3O_4/SiO_2@PEG–PLA nanoparticles for drug delivery. Compos Part B: Eng 2013;54:194-9.
27. Joshi Sh. HPLC separation of antibiotics present in formulated and unformulated samples. J Pharm Biom Anal 2002;28:795-809.
28. Bharati CH, Jayaram P, Sunder Raj J, Saravana Kumar M, Bhargavi V, Handa VK, *et al*. Identification, isolation and characterization of impurities of clindamycin palmitate hydrochloride. J Pharm Biomed Anal 2008;48:1211-8.
29. 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.

30. Moona SI, Leeb CW, Taniguchia I, Miyamotoa M, Kimuraa Y. Melt/solid polycondensation of l-lactic acid: An alternative route to poly (l-lactic acid) with high molecular weight. Polymer 2011;42:5059-62.
31. Li Y, Sun XS. Preparation and characterization of polymer-inorganic nanocomposites by *in situ* melt polycondensation of L-lactic acid and surface-hydroxylated MgO. Biomacromolecules 2010;11:1847-55.
32. Auras R, Harte B, Selke S. An overview of polylactides as packaging materials. Macromol Biosci 2004;4:835-64.
33. Nagarwal RC, Kumar R, Dhanawat M, Pandit JK. Modified PLA nano *in situ* gel: A potential ophthalmic drug delivery system. Colloids Surf B. Biointerfaces 2011;86:28-34.
34. Essa S, Rabanel JM, Hildgen P. Effect of aqueous solubility of grafted moiety on the physicochemical properties of poly (d, l-lactide) (PLA) based nanoparticles. Int J Pharm 2010;388:263-73.
35. Verdier L, Bertho G, Gharbi-Benarous J, Girault JP. Lincomycin and clindamycin conformations. A fragment shared by macrolides, ketolides and lincosamides determined from TRNOE ribosome-bound conformations. Bioorg Med Chem 2000;8:1225-43.
36. Available from: http://www.chem.agilent.com/Library/applications. [Last accessed on 02/23/2011].
37. Merkli A, Tabatabay C, Gurny R, Heller J. Biodegradable polymers for the controlled release of ocular drugs. Prog Polym Sci 1998;23:563-80.
38. Athanasiou KA, Niederauer GG, Agrawal CM. Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers. Biomaterials 1996;17:93-102.
39. Tamaddon L, Mostafavi A, Dorkoosh F, Karkhane R, Riazi-Esfahani M, Rafiee-Tehrani M. Design and development of intraocular polymeric implant systems for long-term controlled-release of clindamycin phosphate for toxoplasmic retinochroiditis. Adv Biomed Res In press 2013.
40. 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.
41. 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.
42. Kishore K, Conway MD, Peyman GA. Intravitreal clindamycin and dexamethasone for toxoplasmic retinochoroiditis. Ophthalmic Surg Lasers 2001;32:183-92.
43. Wei G, Jin J, Xu L, Liu Y, Lu W. Preparation, characterization and *in vivo* pharmacodynamic evaluation of thymopentin loaded poly (lactide acid)/poly (lactide-co-glycolide acid) implants. Int J Pharm 2010;398:123-9.
44. Tsuji H, Ikarashi K. *In vitro* hydrolysis of poly (L-lactide) crystalline residues as extended-chain crystallites. Part I: Long-term hydrolysis in phosphate-buffered solution at 37 degrees C. Biomaterials 2004;25:5449-55.
45. Hsiue GH, Liao CM, Lin SY. Effect of drug-polymer interaction on the release characteristics of methacrylic acid copolymer microcapsules containing theophylline. Artif Organs 1998;22:651-6.
46. Sherman Hsu CP, editor. Infrared spectroscopy. Handbook of Instrumental Techniques for Analytical Chemistry. New Jersey?: Prentice Hall; 1997. p. 247-83.
47. Stuart BH, editor. Infrared Spectroscopy: Fundamentals and Applications. Chichester: John Wiley and Sons Company; 2004. p. 113-36.
48. Hanafy AF, El-Egaky AM, Mortada SA, Molokhia AM. Development of implants for sustained release of 5-fluorouracil using low molecular weight biodegradable polymers. Drug Discov Ther 2009;3:287-295.
49. Nagarwal RC, Kumar R, Dhanawat M, Pandit JK. Modified PLA nano *in situ* gel: A potential ophthalmic drug delivery system. Colloids. Surf B Biointerfaces 2011;86:28-34.

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