ORIGINAL ARTICLE

Design, preparation and characterization of novel poly-lactic-co-glycolic acid-hyaluronic acid implants containing triptorelin acetate

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Hormones and their derivatives are widely used to treat different types of diseases such as prostate cancer which is treated by agonists of gonadotropin-releasing hormone. Triptoreline salts are the first therapeutics of this group launched into the market in the form of microparticles (microspheres). Implants, as one of attractive injectable dosage forms, have many advantages over multi-particulate systems. Some of these advantages are dose adjustability, drug absorption improvement, constant release profile, etc. In this research, a new composite of poly-lactic-co-glycolic acid and hyaluronic acid was designed and prepared in the form of implants containing triptorelin acetate for administration as an injection under the skin (subcutaneously) in arm or thigh area. The manufactured implants characterized by Fourier transform infrared spectroscopy, thermas gravimetric analysis, X-ray diffraction and scanning electron microscopy to assess different aspects of structure and morphology. The drug release profile was assessed by high performance liquid chromatography. These characterizations confirmed that the newly designed drug delivery has a good stability during manufacturing process. The release pattern of the implant was also studied and revealed that the release of the model drug follows a zero-order and erosion mechanism. The compatibility between the components of the newly designed implants and the release profile of the delivery system make it a promising device for drug delivery.

Key words: *Drug delivery system, gonadotropin-releasing hormone, hyaluronic acid, implants, prostate cancer*

INTRODUCTION

The treatment of a wide range of illnesses and disorders is accomplished using hormones or their derivatives. Hormones are used to treat a variety of problems and disorders such as cancers (prostate, ovaries and breast),[1-3] aging,[4,5] sex reassignment,[6] intersex conditions such as Klinefelter's and Turner syndrome,[7,8] and hormonal deficiencies, for example in growth deficiencies and hypothyroidism.[9,10] The mechanisms of therapeutic effects of hormones are both direct and indirect, in that hormones can either affect directly on their target organs as an exogenous source or regulate the secretion of other endogenous hormones based on positive or negative feedbacks.

Prostate cancer in men is the second most prevalent cancer and the cause of death in one sixth of the male population.[11] It is therefore crucial to control the level of testosterone in blood, mostly in the form of its metabolite as 5α-dyhydrotestosterone (DHT), in men who suffer from benign or malignant prostate cancer. Hence, adjustment of the blood level of this hormone is the main target in treatment of this disease. Two types of medications are used for the treatment of prostatic cancer. One type of medication is using inhibitors of 5α-reductase isoenzymes (mostly type II) responsible for conversion of testosterone to DHT, such as finasteride and dutasteride.[12] The second class of drugs is agonists of gonadotropin-releasing hormone (GnRH) such as leuprolide, buserelin, nafarelin, histrelin, goserelin, deslorelin and triptorelin which are used in the treatment of prostatic cancer.[13] These are synthetic analogs of GnRH decapeptide and work by

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suppressing the hypothalamus-hypophisis axis in long-term treatment through down-regulation of GnRH receptors.

Triptorelin salts, including pamoate and acetate, are widely used in the palliative treatment of advanced prostate cancer. Triptorelin salts are also indicated in other hormone-responsive cancers such as breast cancer and other hormonal disorders like precocious puberty, estrogen-dependent conditions such as endometriosis or uterine fibroids and in assisted reproduction. These salts are usually formulated in a sustained-release formulation for long-term therapy. Poly-lactic-co-glycolic acid (PLGA) is the main constituent of most of these formulations which is synthetic polyester with attractive properties such as biocompatibility, biodegradability, availability in both crystalline and amorphous forms and flexibility related to processing.[14,15] These properties enable the formulator to have the best choice among the different grades of this polymer to achieve a desirable product with an appropriate release profile. In industrial scale, the triptorelin salt is encapsulated in the polymer with other ingredients as microparticles usually by preparing water in oil in water double emulsion and solvent evaporation. The resulted microspheres are then mixed with other excipients and lyophilized as a finished product. The product is reconstituted with a suitable diluent before use and is injected intramuscularly.

During the past decades, implants have been used as novel drug delivery systems for their ability to result in controlled release profile and protecting the active molecules.[16] They have many advantages over microparticles such as direct administration to the side of action, lower dose frequency, longer duration of action, better patient compliance and better pharmaceutical therapeutic profile.[17-21] A major drawback in using implants is that their insertion as well as their expelling at the termination stage of the treatment may need surgery which is considered to be an invasive method.[20,21] Local immunological reactions with implants as a foreign object and fibrous formation around the implant are also problems which can affect the pattern of drug release from the implant and may work as barriers against drug release to the capillaries and hence prevent systemic absorption.[22,23] Use of biodegradable materials makes implants overcome many of these problems.

Hyaluronic acid (HA) is a natural anionic polymer on which copious research has been done on its ability to be used as a drug delivery system with controlled release property.[24-26] It consists of non-sulfated glycosaminoglycan units, is found in skin, connective and neural tissues and has different functions in the body such as lubrication of joints, moisturizing of skin, healing of wounds, cell activity regulation, etc.[27,28] HA is used as a hydrophilic part of the formulation for incorporation of triptorelin acetate in the implants and it could probably reduce the immunologic reactions at the site of administration and fibrous formation around the implant.

As mentioned earlier, the benefits of PLGA and HA polymers, have been considered in this study. It has been postulated that designing a composite which contains both polymers in an optimized ratio and preparing an implant carrying a GnRH agonist such as triptorelin acetate for subcutaneous application in arm or thigh could create a drug release pattern similar to or even better than the existing products in the market. This could represent a promising and potential vehicle for other protein and peptide molecules.

MATERIALS AND METHODS

Materials

HA (with two different MWs of 100 and 2170 kDa) was purchased from Cadila, India, and PLGA (with PL/PG ratio of 50/50MW of 5000 Da) was purchased from Puracs, The Netherlands; Triptorelin acetate was obtained from Bachem, Switzerland; pharmaceutical grade propylene glycol (PG) and polyethylene glycol (PEG) 100 and reagent grade dichloromethane were supplied from Merck, Germany. For analysis purposes high performance liquid chromatography (HPLC) grade acetonitrile and analytical grade potassium dihydrogen phosphate were obtained from Merck, Germany. All other reagents were of an analytical grade.

Methods

Preparation of implants

Ten different formulations containing different grades and amounts of HA were prepared employing polymer dispersion using the solvent removal method [Table 1]. To give a brief description, an accurately weighed amount of HA was dispersed in 2 ml of dichloromethane and an exact amount of triptorelin acetate, equivalent to 3.75 mg per each centimeter of final implants, was added. The mixture was then homogenized by an ultrasonicator (Hielscher, Germany) which was set at 50 watt for 30 seconds. Separately, an accurately weighed amount of PLGA was dissolved in dichloromethane to obtain a clear solution in a final concentration of 0.75 mg/ml. This solution was added drop-wise to the HA and triptorelin dispersion while stirring and then depending on the formulation 3.7-6.3% propylene glycol and 1.8-3.2% polyethylene glycol 100 was added to the mixture drop wise. These two ingredients were used to decrease the *Tg* temperature and to stabilize the formulation for better uniformity. Afterwards, the mixture was homogenized by an ultrasonicator which was set at 50 watt for 3 minutes. A large portion of the solvent evaporated during sonication resulting in a consolidated paste. This paste was then injected into a stainless steel mold [Figure 1] using a syringe and discharged from the die employing a punch made from stainless steel. The resulting extrudates were cut in 1 cm pieces by a cutter and were dried at room temperature under vacuum.

Characterization of implants

Fourier transform infra red (IR) spectrophotometry

FT-IR spectrum of PLGA, HA, triptorelin acetate, and implant having both desirable structural strength and proper release

Table 1: Proportion of PLGA and HA in different formulations

Ingredients	% During preparation	% After drying under vacuum
Formula 1		
PLGA	4.83	29.77
LMW HA	0.80	4.96
API	0.95	5.84
PEG 100	3.17	19.55
PG	6.34	39.11
Solvent	83.91	0.78
Formula 2		
PLGA	4.83	29.71
LMW HA	1.61	9.90
API	0.95	5.83
PEG 100	2.90	17.86
PG	5.80	35.73
Solvent	83.91	0.97
Formula 3		
PLGA	4.83	29.71
LMW HA	2.41	14.85
API	0.95	5.83
PEG 100	2.63	16.21
PG	5.27	32.43
Solvent	83.91	0.97
Formula 4		
PLGA	4.83	29.59
LMW HA	3.22	19.73
API	0.95	5.80
PEG 100	2.37	14.51
PG	4.73	29.01
Solvent	83.91	1.35
Formula 5		
PLGA	4.83	29.54
LMW HA	4.83	29.54
API	0.95	5.79
PEG 100	1.83	11.20
PG	3.66	22.39
Solvent	83.91	1.54
Formula 6		
PLGA	4.83	29.65
HMW HA	0.80	4.94
API	0.95	5.81
PEG 100	3.17	19.48
PG	6.34	38.95
Solvent	83.91	1.16
Formula 7		
PLGA	4.83	29.77
HMW HA	1.61	9.92
API	0.95	5.84
PEG 100	2.90	17.90
PG	5.80	35.80
Solvent	83.91	0.78
Formula 8		
PLGA	4.83	29.59
HMW HA	2.41	14.80
API	0.95	5.80
PEG 100	2.63	16.15
PG	5.27	32.30
Solvent	83.91	1.35
Formula 9		
PLGA	4.83	29.71
HMW HA	3.22	19.81
API	0.95	5.83
PEG 100	2.37	14.56
PG	4.73	29.13
Solvent	83.91	0.97
Formula 10		
PLGA	4.83	29.65
HMW HA	4.83	29.65
API	0.95	5.81
PEG 100	1.83	11.24
PG	3.66	22.48
Solvent	83.91	1.16

PLGA: Poly-lactic-co-glycolic acid, PEG: Polyethylene glycol, PG: Propylene glycol, LMW: Low molecular weight, API: Active pharmaceutical ingredient, HA: Hyaluronic acid

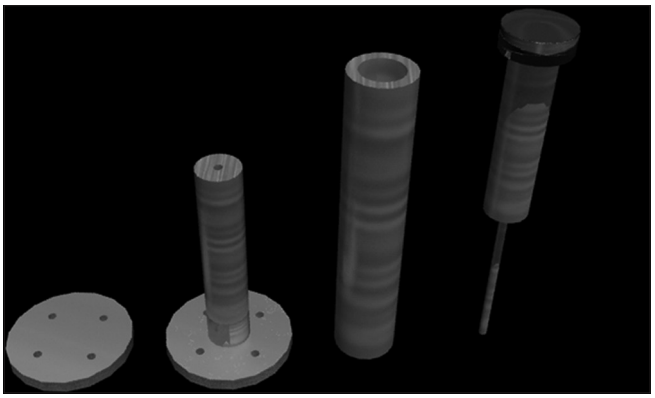

Figure 1: A schematic view of mold used to prepare the implants

patterns (implant of formulation No. 8) were obtained in solid state (KBr as filler), at room temperature in the range of 4000-400 cm^{-1} using the Nicolet FT-IR Magna 550 spectrophotometer (Thermo Electron Corporation, USA).

Thermal gravimetric analysis

Thermal gravimetric analysis (TGA) using the Perkin Elmer-Pyris Diamond TG/DTA analyzer (PerkinElmer Life and Analytical Sciences, USA) was performed to assess the physicochemical changes of the polymer system of implant and thus to ensure that the stability of drug within delivery system remained intact during the manufacturing process. The temperature range was set from room temperature to 700°C with increments of 20°C per minute under N_2 atmosphere. For a better interpretation of the results the analysis was also done on PLGA and HA alone and the results were compared.

X-ray diffraction crystallography

Crystalline structure of pure PLGA, HA and implant of formulation No. 8 were studied by X-ray diffraction (XRD) method, using an X-ray diffractometer (Siemens, X'Pert MPD, The Netherlands) equipped with a Cu tube set at 40 kV and 30 mA and a horizontal goniometer. Samples were placed in a sample holder and scanned at a rate of 0.02° sec^{-1} from 0° to 90°.

Scanning electron microscopy

The morphology of the implant of formulation No. 8 was assessed by scanning electron microscopy (SEM) (FESEM, MIRA II LMU, TESCAN, Czech Republic). The implant was cut in very thin slices which were initially coated by gold. This operation in DC-magnetron sputtering was carried out for 10 minutes in DC plasma condition using Argon gas with DC voltage of 6 kV and DC current of 6 mA. The accelerator voltage for scanning was 15 kV (Vacc = 15 kV). Size diameter was determined using the CLEMEX® particles image analysis software package.

Release study

The release pattern of implants obtained from 10 formulations was assessed using phosphate buffer pH = 7.5 as a release medium. To explain briefly, 6.706 gram potassium dihydrogen phosphate was dissolved in about 950 ml of demineralized water and the pH was adjusted at 7.5 using NaOH 1 M. Implants of different formulations in 1 cm pieces were placed in 10 ml Falcon tubes and 10 ml of medium was added to each tube and placed in a bain-marie shaker (Memmert-Germany) which was set at 37°C with 60 strokes per minute and shaken for 31 days. Five hundred microliter samples were withdrawn on days 1, 2, 3, 4, 6, 8, 10, 13, 17, 21, 25 and 31 with substitution of the equivalent amount of fresh medium. The samples were filtered by 0.45 µm filters and analyzed in triplicate using Agilent 1260 series HPLC equipped with a Perfectsil Target (Germany) 150 × 4.6 mm column containing ODS 3-5 µm, quaternary pump, and photodiode array detectorset on 220 nm. A mixture of 70% phosphate buffer pH = 7.5 and 30% acetonitrile was used as mobile phase. The release profile of sufficient amount of Diphereline microspheres equivalent to 3.75 mg triptorelin acetate was also measured in the same manner to compare it with the release pattern of the implants.

Assessing release kinetics and mechanisms using mathematical models

Based on the results of the release study, three different mathematical models were used to describe the mechanism of release kinetic and to predict the mechanism of drug release from different formulations. Zero-order describes a uniform release from a matrix independent of drug concentration in matrix and is presented by the following equation:[29]

$$\frac{M_t}{M_\infty} = K_0 t$$

where M_t/M_∞ is the fractional drug release, K_0 is the zero-order kinetic constant and t is the time.

The Higuchi model is the first example of a mathematical model to explain drug release from a matrix system by diffusion according to Fick's law and relates the drug release to square root of time.[30] It is presented by the following equation:[31]

$$\frac{M_t}{M_\infty} = K_H t^{1/2}$$

in which M_t/M_∞ is the fractional drug release, K_H is the Higuchi constant and t is the time.

The Korsmeyer-Peppas model is usually used in case of an exponential relationship between release and time.[32] It is a powerful tool to distinguish between different release mechanisms, including Fickian diffusion, Non-Fickian transport, case II (relaxation or swelling controlled) transport, and supper case II (erosion controlled) transport. The model is presented by the following equation:

$$\frac{M_t}{M_\infty} = K_p t^n$$

in which M_t/M_∞ is the fractional drug release, K_p is the model constant, t is the time, and n is the release exponent which its value characterizes the release mechanism.

The mean of all experimental data was fitted by Sigma Plot 10 software to assess the mathematical models of drug release.

Statistical analysis

All data is presented as mean ± standard deviation from three independent experiments. SigmaPlot 10.0 software was used to analyze the drug release data and measuring the fitness of release kinetics with different mathematical models and probability values <0.05 were assumed significant. Statistical significant differences were assessed employing SPSS 11.5 software, using one-way ANOVA and probability values <0.05 assumed significant.

RESULTS

Drug-Polymer interaction and drug stability in polymer matrix

FT-IR spectrophotometer and thermal gravimetric analysis are the most useful tools for the study of the interaction between components of a polymeric drug delivery system as well as the stability of drug substance in such a device.

Figure 2 represents the IR spectrums of PLGA, HA, triptorelin acetate, and implant of formulation No. 8. The characteristic O-H stretching vibrations of carboxylic acid can be seen in both PLGA (b) and HA (a) spectrums in 3475-3400 cm^{-1} which has remained in implant (d) N-H amid stretching vibration

of triptorelin acetate (c) at 3329 cm^{-1} has overlapped with other stretching vibrations in this region. In the region of 2959-2878 cm^{-1}, well-known cyclic aliphatic C-H stretching vibrations can be seen in HA and triptorelin acetate spectrums as well as the implant. Ester C = O stretching in both PLGA and implant spectrums have appeared in the same region 1751 and 1750 cm^{-1}, respectively, and amid C = O stretching of HA and implant are almost in the same wave numbers 1619 and 1625 cm^{-1}, respectively. There is a strong and sharp band at 1667 cm^{-1} in triptorelin acetate which could be attributed to N-H bending vibration which has overlapped in the implant with other vibrations in this region. Common alcoholic O-H in-plane bending vibrations can be seen at 1405-1400 cm^{-1}, and, alcoholic C-O and ether C-O-C stretching vibrations at 1250-1000 cm^{-1} region in all four spectrums. Weak bands in region 900-650 cm^{-1} are unique for aromatic C-H bending vibrations which can only be seen in triptorelin acetate and implants spectrums. It could imply the presence of the drug molecule in the implant and reveals a rough estimation of drug stability in the polymer matrix.

TGA of PLGA, HA and implant of choice are illustrated in Figure 3. Significant weight changes for HA starts at about 230°C which is related to polymer decomposition [Figure 3a]. PLGA shows a good stability up to about 300°C with only a small drop in weight which could be attributed to evaporation of volatile materials, but a significant change happens over 300°C which is due to polymer decomposition [Figure 3b]. Biphasic weight changes can be seen for the implant of formulation No. 8, one between 100 and 200°C which could mainly be due to evaporation of plasticizers and, to a lesser extent, due to the solvent system used for manufacturing of the implant and the residual water probably trapped in plasticizers [Figure 3c]. This is confirmed by viewing the thermogram of PG [Figure 3d] which shows a weight loss between 100 and 200°C due to evaporation, though PEG 100 [Figure 3e] remain unchanged in this temperature range. The second weight change starts from 200°C which is due to polymer system decomposition.

Morphology and structural change of the implant

XRD and SEM, as the most common technique used for characterization of drug delivery systems, were employed to study the morphology and structural changes of intact implant as well as during release study in microscopic levels to better understand the mechanism of drug release.

Based on XRD spectrum [Figure 4], PLGA is amorphous [Figure 4b] as it has no crystalline arrangement. HA, on the other hand, shows percentages of crystalline order in its structure which has been preserved in implant [Figure 4a and c].

Figure 5 illustrates the cross-section of the implant of formulation No. 8 by SEM in three stages: Just before starting the release study (a), 15 days after the release study (b), and at the termination of the study in day 31 (c). Primarily, the implant has possessed a uniform texture without any cracks, cavities, and pores. Interestingly, it has almost maintained its uniform

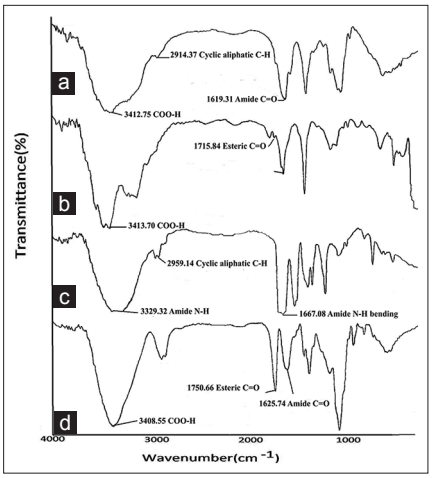

Figure 2: FT-IR spectrums of PLGA (a), HA (b), triptorelin acetate (c), and implant of formulation 8 (d) in solid state

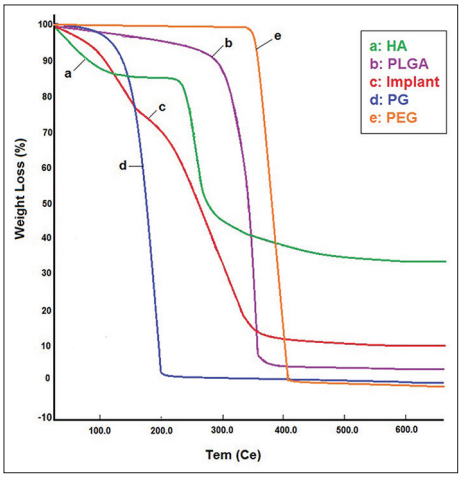

Figure 3: TGA of HA (a), PLGA (b), and implant of formulation 8 (c)

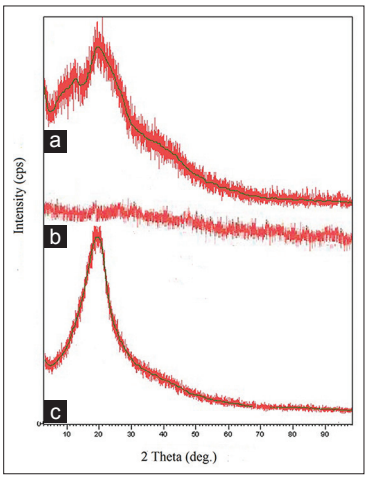

Figure 4: XRD of HA (a), PLGA (b), and implant of formulation 8 (c)

texture even 15 days after it had been in contact with the release medium. At the end of the release study, degradation of polymer system of the drug delivery device started to happen and cavities and pores hardly smaller than 5 µm appeared.

Release study

Table 2 illustrates the percentage release of triptorelin acetate from 10 formulations at the last time point (day 31). As it can be seen from the data, all implants containing HA with low MW have released higher amounts of their contents in comparison with implants containing HA with high MW at the end of release study. The percentage release of triptorelin acetate at the last time point among all implants was enhanced by an increase in the amount of HA polymer in the formulation and reached a maximum level in formulation No. 3 and 8 and then decreased by further increasing the amount of HA.

Drug releases from implants of different formulations are presented in Figure 6. The release data was examined by three mathematical models and extend of fitness in these models are tabulated in Table 3. As it can be seen from Table 3, a comparison of the R^2 values reveals that all formulations are fitted with an exponential release kinetic except for formulations No. 5 and 10 which are mostly fitted on zero-order kinetic. Formulations No. 1, 6 and 7 are best fitted with the Korsmeyer-Peppas drug release model with a Non-Fickian drug release mechanism from the implant based on release exponent of these formulations with quantities between 0.45 and 0.89, whereas all other formulations follow an erosion mechanism with exponent values of more than 0.89. It can be concluded that the ratio of HA in the formulation of implants could be a detrimental parameter which would govern the drug release mechanism. It could also be the explanation for why the release of triptorelin increases with an increase in the amount of HA ratio in formulations, reach an optimum level and then decrease with further increase in the ratio of this component. It is probable that further increases in the amount of HA could reduce the rate of

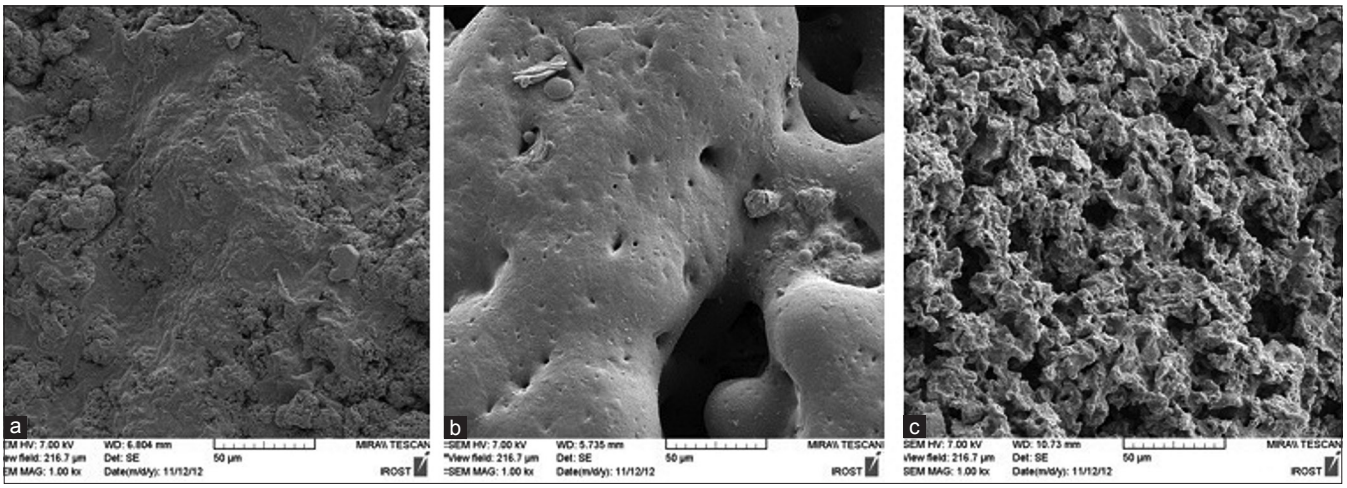

Figure 5: SEM of implant of formulation 8 before starting the release study (a), 15 days after release study (b), and at the termination of the study in day 31 (c)

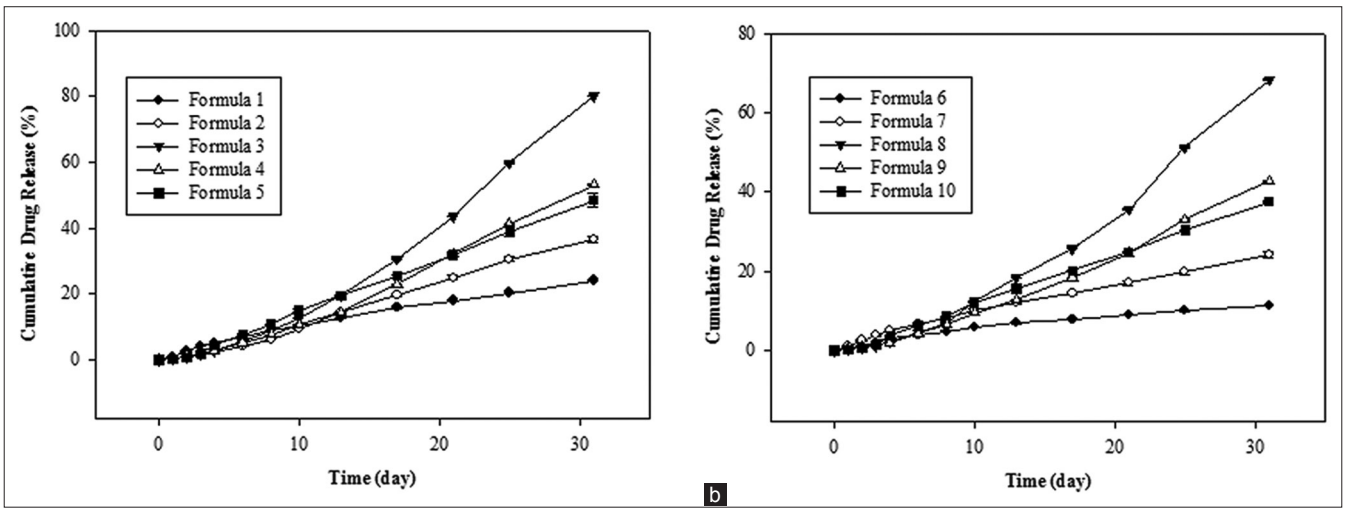

Figure 6: Release profile of triptorelin acetate from formulations No. 1-5 (a) and 6-10 (b) (mean of 3 experiments ± SD)

erosion and the number of water-filled pores. A comparison of the release rates of different formulations shown in Figure 6 confirms this hypothesis. Except for formulations No. 2 and 9 which have a similar release profile ($P > 0.05$), release profile of all other formulations are significantly different from each other ($P < 0.05$).

As mentioned earlier, one of the advantages of implants is their ability to deliver their loaded drug steadily and in a smooth manner within a specific time period, which is in contrast to multiparticulate systems such as microshperes. As the implant showing both robust structure and sufficient amount of drug release at the end of release study, the implant obtained from formulation No. 8 was selected and its release rate compared with an existing commercial product, Diphereline 3.75 mg [Figure 7]. A fast release of the drug molecule can be seen within first 8 days for Diphereline reaching a maximum on day 8. Then the release decreases a little and maintains a plateau up to day 25 where the second increase in release starts. The drug release from the implant, on the other hand, starts with almost a constant rate and continues to the end of release study.

Table 2: Percent release of triptorelin acetate from ten implants with different formulations on day 31

Formulation	Percent of drug released
1	24.1±0.18
2	36.6±0.9
3	80.1±0.06
4	53.12±0.12
5	48.4±2.1
6	11.3±0.1
7	24.2±0.9
8	68.3±0.3
9	42.7±0.2
10	37.6±0.2

Table 3: The parameters of fitting of different implants formulations in three mathematical models of drug release kinetics

Formulation no.	Mathematical model of drug release kinetic		
	Zero-order	Higuchi	Korsmeyer-peppas
1	K=0.77 R^2 = 0.982	k=1.71 R^2 = 0.960	*n*=0.76 R^2 = 0.998
2	K=1.26 R^2 = 0.992	k=2.30 R^2 = 0.980	*n*=1.21 R^2 = 0.994
3	k=2.55 R^2 = 0.962	k=4.36 R^2 = 0.925	*n*=1.61 R^2 = 0.999
4	k=1.73 R^2 = 0.978	k=3.05 R^2 = 0.954	*n*=1.43 R^2 = 0.994
5	k=1.61 R^2 = 0.998	k=3.05 R^2 = 0.992	*n*=1.11 R^2 = 0.997
6	K=0.36 R^2 = 0.954	k=0.85 R^2 = 0.908	*n*=0.67 R^2 = 0.992
7	K=0.75 R^2 = 0.988	k=1.66 R^2 = 0.969	*n*=0.78 R^2 = 0.998
8	k=2.17 R^2 = 0.964	k=3.72 R^2 = 0.928	*n*=1.59 R^2 = 0.998
9	k=1.38 R^2 = 0.982	k=2.45 R^2 = 0.961	*n*=1.39 R^2 = 0.999
10	k=1.25 R^2 = 0.998	k=2.39 R^2 = 0.994	*n*=1.08 R^2 = 0.997

DISCUSSION

Drug-Polymer interaction and drug stability in polymer matrix

A comparison between IR spectrum of the implant of formulation No. 8 and its components reveals that there are no significant interactions between the drug and the drug delivery system. Data obtained from TGA also shows that the polymers are stable enough in working temperature (room temperature) in which the implant has been prepared and there is no particular interaction between the components of the implant for at least up to 100°C.

Morphology and structural change of the implant

A morphology study of the implant of formulation No. 8 and its components by XRD spectrometry reveals a rough estimation of the drug stability. It could be expected that the process which was used to manufacture the implants did not do any harm to the structure of the formulation components which is crucial for triptoreline acetate as it is a peptide drug which can be sensitive to harsh conditions during processing in a dosage form.

Monitoring the structure of the implant of choice during release study by SEM reveals that at the beginning of the release process swelling or polymer relaxation could have been the dominant process. Erosion occurred later during the release study.

Release profile

Release of drugs from PLGA matrices is more complex due to different events that may happen sequentially.[33] Water

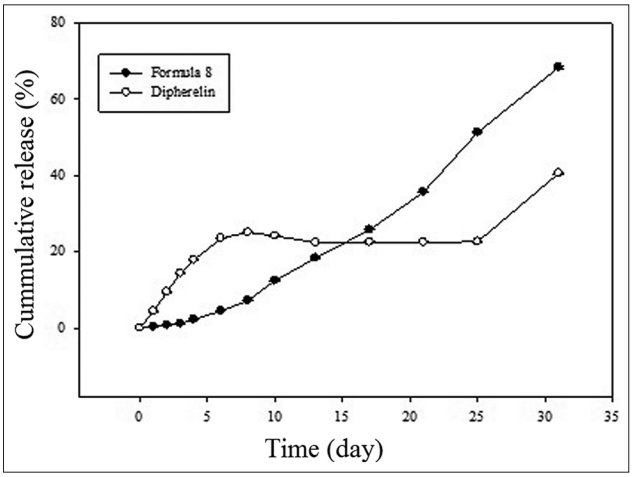

Figure 7: Comparison between profile of release of implant obtained from formulation No.8 and a commercial sample of Diphereline 3.75 mg (mean of 3 experiments ± SD)

absorbs into the drug delivery system rapidly followed by swelling of the polymer. Then hydrolysis would start which would result in subsequent erosion. These events lead to four main release mechanisms: (i) diffusion through water-filled pores, (ii) diffusion through the polymer, (iii) osmotic pumping, and, (iv) erosion (with no drug transport). Triptorelin acetate is a hydrophilic and medium size molecule so it cannot move through the polymer phase, whereas it can diffuse through water-filled pores. Moreover, observation of all implants at the end of the release course showed that they had been swollen and therefore release by osmotic pressure could not have been the main release mechanism. The weight of implants was measured at the completion of the drug release process following which a mass reduction was observed in all the implants. As a result, erosion could be assumed to be one of the involved release mechanisms. This is confirmed by SEM pictures of the implant of formulation No. 8. Therefore, it is postulated that diffusion through water-filled pores during swelling of the polymer system and later erosion are the only ways for the drug molecules to be transported into the release medium. Diffusion through the pores is usually increased by water absorption but at the same time the pores may close up through swelling and rearrangement of polymer chains (polymer relaxation), especially in low MW PLGA.[33] HA, as the second component of the implants of this study, could form a hydrogel upon contact with water. The higher the amount of the HA, the more robust a hydrogel is formed and the longer it can remain in implants. Interestingly, the amount of drug released in termination of release study increased as the amount of HA increased in the formulation and reached a maximum in formulations No. 3 and 8 but decreased as the amount of HA further increased more than 15% [Table 2]. This could be due to the swelling of the implants when HA percentage was increased in formulation leading to formation of more water-filled pores which resulted in easier drug release, hence the total amount of drug released rises and reaches to a maximum value. When HA percentage passes an optimum value, a pore closure phenomenon starts since the microenvironment pH changes to acidic condition due to hydrolysis of PLGA polymer chain that may cause the polymer to become more hydrophobic in nature. Bearing in mind that HA also contains carboxylic groups in its structure, acidic pH may render it more hydrophobic as well. The overall effect is a cessation in the number of water-filled pores and trapping of the drug molecules in the drug delivery system.

The amount of the HA in the formulation could govern not only the amount of drug released from the implants but also the mechanism of drug release. Based on the fitting studies in different mathematical models of drug release [Table 3] it can be seen that by increasing the amount of HA in the formulation, the release mechanism shows a tendency toward the zero-order kinetic. The kinetic parameters of the implants of formulations 5 and 10 confirm this hypothesis.

CONCLUSION

Cancers and other diseases linked to sex hormone disorders are prevalent illnesses which the treatment is remarkably costly. In addition, due to vast progress in pharmaceutical technologies and growing of biopharmaceutical products, traditional therapeutic chemical molecules are going to be substituted with their biological peers. Because of their nature, these new therapeutics require special delivery systems and techniques to reach their target in human body. Designing new delivery devices can improve the efficiency of the existing therapeutics as well as the novel active pharmaceutical substances.

The outcome of different characteristic techniques revealed that a composite consisting of PLGA and HA and the technique employed to make an implant show a good compatibility between the components and a reasonable stability, at least during the release, of the loaded drug. The drug release from the newly designed drug delivery system has a promising pattern in comparison to its counterpart products in the market and further attempts for its development and optimization seems to be worthwhile.

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REFERENCES

1. Shaw G, Oliver RT. Intermittent hormone therapy and its place in the contemporary endocrine treatment of prostate cancer. Surg Oncol 2009;18:275-82.
2. Zhou B, Sun Q, Cong R, Gu H, Tang N, Yang L, *et al*. Hormone replacement therapy and ovarian cancer risk: A meta-analysis. Gynecol Oncol 2008;108:641-51.
3. Martin LJ, Minkin S, Boyd NF. Hormone therapy, mammographic density, and breast cancer risk. Maturitas 2009;64:20-6.
4. Staren ED, Omer S. Hormone replacement therapy in postmenopausal women. Am J Surg 2004;188:136-49.
5. Cutler WB, Genovese-Stone E. Wellness in women after 40 years of age: The role of sex hormones and pheromones. Dis Mon 1998;44:421-546.
6. Murad MH, Elamin MB, Garcia MZ, Mullan RJ, Murad A, Erwin PJ, *et al*. Hormonal therapy and sex reassignment: A systematic review and meta-analysis of quality of life and psychosocial outcomes. Clin Endocrinol 2010;72:214-31.
7. Handelsman DJ, Zajac JD. 11: Androgen deficiency and replacement therapy in men. Med J Aust 2004;180:529-35.
8. Bolar K, Hoffman AR, Maneatis T, Lippe B. Long-term safety of recombinant human growth hormone in turner syndrome. J Clin Endocrinol Metab 2008;93:344-51.
9. Akehurst RL, Barnett D, Berry SC, Bird S, Buxton M, Claxton K, *et al*. Guidance on the use of human growth hormone (somatropin) in children with growth failure. Technology Appraisal Guidance No. 42 National Institute for Clinical Excellence. 2002.

10. Bunevicius R, Kazanavicius G, Zalinkevicius R, Prange AJ Jr. Effects of thyroxine as compared with thyroxine plus triiodothyronine in patients with hypothyroidism. N Engl J Med 1999;340:424-9.
11. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61:69-90.
12. Aggarwal S, Thareja S, Verma A, Bhardwaj TR, Kumar M. An overview on 5alpha-reductase inhibitors. Steroids 2010;75:109-53.
13. Kraus S, Naor Z, Seger R. Gonadotropin-releasing hormone in apoptosis of prostate cancer cells. Cancer Lett 2006;234:109-23.
14. Giteau A, Venier-Julienne MC, Aubert-Pouessel A, Benoit JP. How to achieve sustained and complete protein release from PLGA-based microparticles? Int J Pharm 2008;350:14-26.
15. Jiang W, Gupta RK, Deshpande MC, Schwendeman SP. Biodegradable poly (lactic-co-glycolic acid) microparticles for injectable delivery of vaccine antigens. Adv Drug Deliv Rev 2005;57:391-410.
16. Powell EM, Sobarzo MR, Saltzman WM. Controlled release of nerve growth factor from a polymeric implant. Brain Res 1990;515:309-11.
17. Yasukawa T, Kimura H, Tabata Y, Ogura Y. Biodegradable scleral plugs for vitreoretinal drug delivery. Adv Drug Deliv Rev 2001;52:25-36.
18. Fournier E, Passirani C, Montero-Menei CN, Benoit JP. Biocompatibility of implantable synthetic polymeric drug carriers: Focus on brain biocompatibility. Biomaterials 2003;24:3311-31.
19. Nitsch MJ, Banakar UV. Implantable drug delivery. J Biomater Appl 1994;8:247-84.
20. Shi Y, Li LC. Current advances in sustained-release systems for parenteral drug delivery. Expert Opin Drug Deliv 2005;2:1039-58.
21. Iyer SS, Barr WH, Karnes HT. Profiling *in vitro* drug release from subcutaneous implants: A review of current status and potential implications on drug product development. Biopharm Drug Dispos 2006;27:157-70.
22. Hetrick EM, Prichard HL, Klitzman B, Schoenfisch MH. Reduced foreign body response at nitric oxide-releasing subcutaneous implants. Biomaterials 2007;28:4571-80.
23. Ratner BD. Reducing capsular thickness and enhancing angiogenesis around implant drug release systems. J Control Release 2002;78:211-8.
24. McTaggart LE, Halbert GW. Assessment of polysaccharide gels as drug delivery vehicles. Int J Pharm 1993;100:199-206.
25. Coviello T, Matricardi P, Marianecci C, Alhaique F. Polysaccharide hydrogels for modified release formulations. J Control Release 2007;119:5-24.
26. Xu X, Jha AK, Duncan RL, Jia X. Heparin-decorated, hyaluronic acid-based hydrogel particles for the controlled release of bone morphogenetic protein-2. Acta Biomater 2011;7:3050-9.
27. Tammi R, Ågren UM, Tuhkanen AL, Tammi M. Hyaluronan metabolism in skin. Prog Histochem Cytochem 1994;29:1-81.
28. Trommer H, Wartewig S, Böttcher R, Pöppl A, Hoentsch J, Ozegowski JH, *et al*. The effects of hyaluronan and its fragments on lipid models exposed to UV irradiation. Int J Pharm 2003;254:223-34.
29. Sadighi A, Ostad SN, Rezayat SM, Foroutan M, Faramarzi MA, Dorkoosh FA. Mathematical modelling of the transport of hydroxypropyl-beta-cyclodextrin inclusion complexes of ranitidine hydrochloride and furosemide loaded chitosan nanoparticles across a Caco-2 cell monolayer. Int J Pharm 2012;422:479-88.
30. Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic modeling on drug release from controlled drug delivery systems. Acta Pol Pharm 2010;67:217-23.
31. Higuchi T, Connors KA, Phase-solubility techniques. Adv Anal Chem Instrum 1965;4:117-22.
32. Korsmeyer RW, Gumy R, Doelker E, Buri P, Peppas NA. Mechanisms of solute release from porous hydrophilic polymers. Int J Pharm 1983;15:25-35.
33. Fredenberg S, Wahlgren M, Reslow M, Axelsson A. The mechanisms of drug release in poly (lactic-co-glycolic acid)-based drug delivery systems--a review. Int J Pharm 2011;415:34-52.

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