Formulation and *in vitro / in vivo* Evaluation of Novel Biodegradable Microspheres for Treatment of Hormone Responsive Cancers

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ABSTRACT

Objectives: The present work relates with developing long acting controlled release poly(DL-lactide-co-glycolic acid) (PLGA) microspheres of Goserelin acetate for treatment of breast and prostate cancer. **Methods:** Microspheres were prepared by using biodegradable polymers PLGA 50:50 and PLGA 75:25 employing modified emulsification method by using static mixer. Evaluate the effect of process and formulation variables on the characteristics of microspheres were studied. Particle size distribution, surface morphology, encapsulation efficiency, *in vitro* drug release and *in vivo* drug release profile in Wistar rats were investigated. **Results:** Preparative variables such as concentrations of stabilizer, drug-polymer ratio, stirring rate and ratio of internal to external phases were found to be important factors for the preparation of Goserelin acetate loaded PLGA microspheres. These changes were also reflect *in vitro* and *in vivo* drug release profiles. The result indicated that the morphology of Goserelin acetate PLGA microspheres presented as spherical shape with smooth surface, homogenous drug distribution and the particle size distributed from 88.11 to 116.78 μm. *In vitro* and *in vivo* release profile showed controlled initial burst release followed by one month sustained release profile of the Goserelin acetate loaded PLGA microspheres. **Conclusion:** The process and formulation variables could be effectively modified to achieve the desired characteristics and one month sustained release of Goserelin acetate loaded biodegradable polymeric microspheres showed potential for treatment of hormone responsive cancers.

Key words: Breast cancer, Prostate cancer, Goserelin acetate, Microspheres, poly(D, L-lactide-*co*-glycolide).

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INTRODUCTION

The basic principle for controlled drug delivery system is to change the biopharmaceutical, pharmacokinetics and pharmacodynamics properties of pharmacologically active moieties by using novel drug delivery systems or by modifying the molecular structure and physiological parameter inherent in preferred route of administration.[1] The primary goals of controlled drug delivery are to ensure safety and to improve efficacy of drugs as well as patients compliance.[2] This is achieved by higher management of plasma drug levels and fewer frequent dosing. In general, the dosing interval is also magnified either by modifying the drug molecule to decrease the rate of elimination (K_{cl}) or by modifying the release rate of a dosage form to decrease the rate of absorption (K_a). Both the approaches look for decrease fluctuations in plasma level throughout multiple dosing, permitting the dosing interval to extend while not either over dosing or beneath dosing.[3]

The controlled drug delivery can be designed according to various routes of administration.[4] Although oral administration of drugs could be wide accepted route of drug delivery, bioavailability of drugs usually varies as a result of gastrointestinal absorption, degradation by first-pass effect and hostile surrounding of gastrointestinal tract. Transdermal administration for percutaneous absorption of drug is limited by the impermeable nature of the stratum corneum.[5] Therefore, in such situation, the parenteral route is the most viable approach, of the various ways in which biodegradable polymeric microspheres are one of the better means of controlled drug release over a longer period of time to achieve long-term parenteral drug delivery.[6]

Microspheres formulations are particularly well suited for parenteral route of administration and consist of polymeric materials in which active agents are encapsulated at high efficiency.[7] Biodegradable microspheres are made of biodegradable products, which can be injected with as syringe into the body and once injected, solidify to form a semi-solid deposit.[8] Drug is gradually release on erosion or by diffusion from the particles. These systems offer certain unique advantages, which has sparked people's interest. In addition to ease of use, these include targeted delivery for a site-specific action, longer delivery times, reduced medication dose with corresponding reduction in potential undesirable side effects common to most types of systemic delivery and enhanced patient compliance and comfort.[9] Poly (glycolic acid) (PGA), poly (lactic acid) (PLA) and its copolymers poly (DL-lactide-co-glycolic acid) (PLGA), have attracted attention due to their remarkable features such as biodegradability, biocompatibility andsafety.[10]

Biodegradable Microspheres are usually prepared by various methods such as Emulsification, Spray drying, Phase separation (Conservation), Solvent extraction, Evaporation base processes and Supercritical technology.[11] Emulsification extraction method is a common technique for preparation of controlled release biodegradable microparticles. Emulsification is critical step in microencapsulation process and has

strong impact on the microspheres properties such as particle size distribution, morphology, encapsulation efficiency, drug loading, burst release etc.[12]

The major challenges in biodegradable microspheres are reduced initial burst release and achieve constant release rate. The initial burst release occurs due to the heterogeneous drug distribution and surface morphology of the microspheres appears pores and cracks.[13,14]

Therefore, it is important to understand the effect of the process parameters on the emulsion quality and to control the process accordingly. In present studies, developed the processes to improve understanding of the emulsion production with static mixers. Static mixer consist of motionless mixer elements with cross-bars fitted in a cylindrical tube. Flow pattern in such mixer may be laminar or turbulent. Mixing in these systems is achieved by dividing and re-uniting fluid streams. Unit operations carried out by static mixers are the blending of fluids, heat transfer and solids blending. Static mixers are usually multiphase applications with the blending of a continuous liquid phase and a dispersed phase or immiscible liquid phase.[15] The numbers of advantages of using static mixer are easily controlled particle size distribution, homogenous drug distribution, higher efficiency, easily scaled up and suited for aseptic manufacturing.

Certain cancers, notably those of the reproductive tract-prostate and testis cancer in men and breast, ovarian and endometrial cancer in women, are described as hormone-dependent cancers. The sex steroid hormones such as androgens, estrogens and progestins play crucial role in both normal development and maintenance of these organs, as well as premalignant and malignant growths.[16,17]

Goserelin acetate is the most widely used drug for the chemotherapy of the prostate and breast cancer and approved by the United States of Food and Drug Administration (USFDA). It acts as a potent inhibitor of pituitary gonadotropin secretion and suppression of luteinizing hormone (LH) and serum testosterone levels.[18,19]

Controlled release drug delivery system based biodegradable Goserelin acetate microspheres for subcutaneous administration to treat hormone responsive cancers such as prostate and breast cancer. It decreases male sex hormone testosterone and female sex hormones estrogen and progesterone, which may stimulate the growth of cancer cell. However in order to effectively improve patient compliance with fewer side effects.

The present work was to design the controlled release biodegradable microspheres of Goserelin acetate by modified emulsification technique using static mixer and evaluate the effects of various process as well as formulation variables were carried out their *in vitro* and *in vivo* characterization. The pharmacokinetics studies are essential to determine the exposures of these formulations and to correlate their *in vivo* exposures with *in vitro* drug release pattern. Wistar rats were used to perform the *in vivo* studied. These species are considered to be more appropriate to perform such studies as their disposition profile is close to human.

MATERIALS AND METHODS

Materials

Goserelin acetate was purchased from Hemmo Pharmaceutical Private Limited, India. poly(D, L-lactide-*co*-glycolide) (PLGA)lactide: glycolide ratio, 50:50 (Resomer RG505, Resomer RG504, Resomer RG504H) and lactide: glycolide ratio, 75:25 (Resomer RG750) were purchased from Evonik Roehm Gmbh Germany. Polyvinyl alcohol4-88 (PVA) and Ethyl acetate were supplied by Merck Ltd. All chemicals and reagents used were of injectable grade.

Preparation of microspheres

Goserelin acetate loaded PLGA microspheres were prepared by emulsification method using static mixer.[20] (Table 1 and Figure 1).
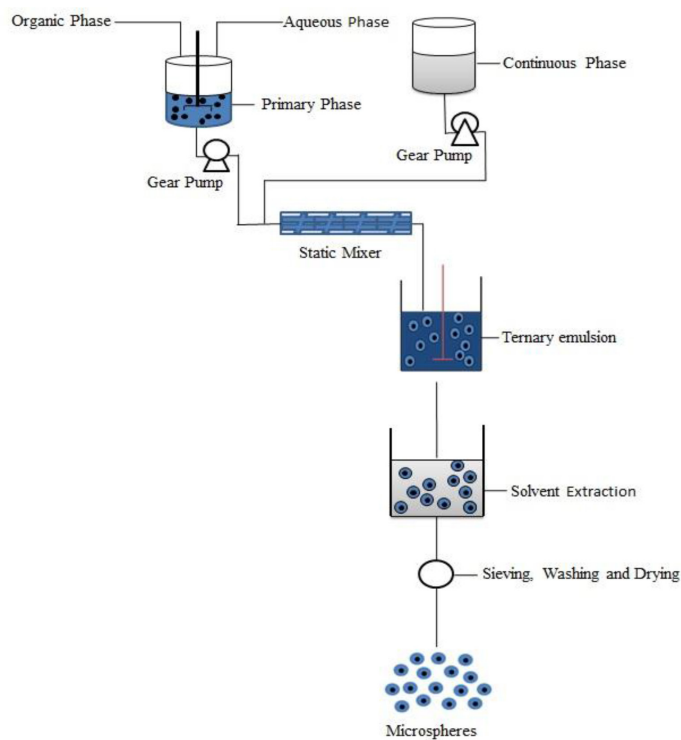

Figure 1: Preparation of microspheres.

Table 1: Composition of Goserelin acetate PLGA microspheres.

Formulation code	Goserelin acetate (mg)	Manufacturing variables						
		PLGA 50:50 (mg)			PLGA 75:25 (mg)	Water for Injection (mL)	Ethyl acetate (mL)	Primary phase: continuous phase ratio
		RG505	RG504	RG504H	RG750S			
M1	10	90	-	-	-	0.5	5	1:1
M2	10	90	-	-	-	0.5	5	1:3
M3	10	90	-	-	-	0.5	5	1:5
M4	10	-	90	-	-	0.5	5	1:5
M5	10	-	-	90	-	0.5	5	1:5
M6	10	-	-	-	90	0.5	5	1:5

Preparation of the Primary phase: poly-(DL-Lactide-co-glycolide) was dissolved in ethyl acetate to obtain an organic phase and Goserelin acetate was dissolved in water for injection to obtain an aqueous phase. This aqueous phase emulsified with organic phase using silver son homogenizer at 1000 rpm for 2 min.

Preparation of Continuous phase: Polyvinyl alcohol (1w/v %) and ethyl acetate was dissolved in water for injection and filter through a 0.22μ filter.

Preparation of the ternary emulsion (water/oil/water): Primary phase was pumped simultaneously with a continuous phase through a ¼ inch static mixer at varying flow ratio (1:1, 1:3 and1:5).

Solvent Extraction: The emulsion was transferred into tank containing water for injection(2°C-8°C) and mixed for 240 min for extraction (extraction Ratio 3%) of the solvent from the emulsion and hardening of microspheres.

Sieving and drying of the microspheres: Slurry contains microspheres from tank was passed through a 28μm sieve and subsequently a 150μm sieve, washed with water for injection.

Microspheres were collected into drying device and dried at 28°C for 16 hrs. Dried microspheres were collected in glass vials and stored at 2-8°C.

Evaluation of microspheres

Microsphere morphology
The shape and surface morphology of the Goserelin acetate loaded PLGA microspheres were

Particle size analysis
The volume average particle diameter (Mean size) and size distribution of all Goserelin acetate loaded PLGA microspheres were analysed by a laser diffraction particle size analyser (Mastersizer 2000, Malvern, UK).

Encapsulation efficiency
The encapsulation efficiency of Goserelin acetate loaded PLGA microspheres was determined by dissolving 55mg of microspheres in 15mL of acetonitrile and make up volume upto 50mL with Phosphate buffer pH 7.4. The solution was filtered through 0.45μm Millex-HV filters and suitable dilution with phosphate buffer pH 7.4. Goserelin acetate concentration in these samples were measured by reverse phase high performance liquid chromatography (HPLC) using a column C_{18} column [octadecylsilane (ODS), 150×4.6mm, pore size 5μm, Phenomenex Inc., Japan] and a mobile phase of phosphate buffer pH7.4:acetonitrile in ratio of 70:30 v/v. The flow rate was set at 1mL/min. The absorbance values at 220nm corresponding to concentration were then evaluated.

To calculate the % Encapsulation efficiency following equation used.

$$Drug\ Loading\ (\%) = \frac{Weight\ of\ drug\ in\ microspheres}{Weight\ of\ microspheres} \times 100$$

$$Encapsulation\ efficiency\ (\%) = \frac{Actual\ drug\ loading\ (\%)}{Thoretical\ drug\ loading\ (\%)} \times 100$$

Inherent Viscosity
Inherent viscosity of Goserelin acetate loaded PLGA microspheres was measuring by using Microostwald viscometer.[21] Inherent viscosity is calculated as ratio of the natural logarithm of the relative viscosity and the mass concentration of the polymer (C). Approximately 55mg Microspheres were dissolved in 10mL of chloroform. Microostwald viscometer having capillary constant 0.01mm²/s was used. The viscometer was kept in auto rinse mode and allowed 1min pretampering wash and

then sample analysis was repeated three times by which Kinematic and Inherent viscosities were obtained as

Calculation formula:

$$Rleative\ Viscosity\ \eta r = \eta S/\eta 0 = t_s/t_0$$
$$\eta inh\ (dl/g) = (ln\ \eta r)/C$$

Differential scanning colorimeter (DSC)
The glass transition temperature (Tg) of Goserelin acetate loaded PLGA microspheres, physical mixture, polymer carrier and pure drug were analyzed using a DSC-821 differential scanning colorimeter (Mettletr Toledo, Germany). Samples of about 5mg were placed in 50μl perforated aluminum pans and sealed using 10°C/min heating rate. The analysis was performed at heating rate 10°C/min from 20°C to 280°C using dry nitrogen as the effluent gas.

X-ray diffraction (XRD)
X-ray diffraction pattern of the Goserelin acetate loaded PLGA microspheres, polymer and pure drug were analysed using a Equinox 3000 XRD system equipped with Cu-Ka radiation source (40 kV, 30 mA). The scans were recorded in the 2Ө angle range from 0°C to 40°C.

In vitro drug release
The *in-vitro* drug release was performed in release media under sink conditions on shaker incubator at constant temperature (37ºC). The 40mg Goserelin acetate loaded PLGA microspheres were dispersed screw capped bottles containing 50mL release media phosphate buffer pH7.4 were fixed onto the platform in water bath for shaking at 50rpm in shaker incubator. At the set points, 2mL sample was removed by syringe for analysis through 0.45μm mdi nylon filters. Any microspheres drawn into the syringe filter were returned back by washing with 2mL replacement fresh media (pH7.4) and supernatants was analysed by HPLC at 220nm.

Drug Release kinetics
The drug release profile of optimized formulation was fitted to various kinetics models. Zero-order, First-order and Higuchi's model to analyze the mechanism of the drug release from microspheres.[22]

In vivo drug release
The experimental protocol for *in vivo* studies were reviewed and approved by the Institutional Animal Ethics Committee. Healthy male Wistar rats weighing between 250g and 280g (Average age 8-10 weeks) were used. These rats were divided into two groups (*n*=4). One group for injection of Goserelin acetate loaded RG505 microspheres (Formulation M3) and the other group for injection of Goserelin acetate loaded RG504H microspheres (Formulation M5) at a dose 1mg. Microspheres were suspended in diluent containing Sodium carboxymethyl cellulose and Polysorbate 20. The formulation was injected into the subcutaneously at buttock (back side the thigh region) using a 21-gauge needle. Blood samples were taken at 1 day before injection and then at 1h, 6h, 1d, 3d, 7d, 10d, 14d, 17d, 21d, 24d, 28d, 31d and 35 days after injection. 0.5mL of blood samples were withdrawn from the Retro-orbital plexus at predetermined time interval. All blood samples were collected in EDTA tubes and were immediately centrifuged. Plasma samples were frozen and stored at -20°C until analysis. The drug content was determined by LC-MS/MS.[23-26]All the animals' procedures approved by Animal Welfare Division, Government of India (ref no.: CPCSEA/IAEC/ P'Ceutics-34/2017-18/133. For ethical reasons all the animals were euthanized using carbondioxide gas chamber at the end of the study.

In vitro and in vivo correlation (IVIVC)
IVIVC is a predictive mathematical models used to describe the relationship between *in vitro* drug release behaviour of a formulation and relevant *in vivo* response. There are different levels of IVIVC that

have been described in the regulatory agency guidance.[27-29] Level A correlation is most desirable and most informative is point to point correlation. Fraction of absorption is calculated by deconvolution techniques using Wanger Nelson method.

RESULTS

Microsphere morphology

The morphology of the Goserelin acetate loaded PLGA microspheres using high resolution digital optical microscope and scanning electron microscopy, analysed the surface structure and cross section of the microspheres. The microparticles showed smooth nonporous surface with spherical shape as shown in Figure 2 and Figure 3 and homogenous drug distribution in microspheres as shown in cross section of microspheres (Figure 4).

Particle size analysis

Mean particle size (d 0.5) of Goserelin acetate microspheres was found to be from 116.78 ± 2.05 to 81.96± 1.55 μm and span value from 1.20± 0.02 to 1.08± 0.05 of microspheres prepared with PLGA 50:50 and 88.11± 2.33 μm and span value 1.15± 0.022 of microspheres prepared with PLGA75:25 (Table 2).

Encapsulation efficiency

Encapsulation efficiency of Goserelin acetate in microspheres was found to be between 90.85± 1.11% to 95.50± 1.73% as shown in Table 2. The encapsulation efficiency for microspheres formulated with PLGA 50:50 and PLGA75:25 were almost similar, despite difference in their lactide content.Primary phase and continuous phase ratio 1:5 to produce the optimum particle size and to achieve better encapsulation efficiency.

Inherent Viscosity of microspheres

Inherent viscosity of PLGA 50:50 was found to be between 0.58± 0.04 to 0.71± 0.04 dL/g and PLGA 75:25 was found to be 0.96 ± 0.03 dL/g
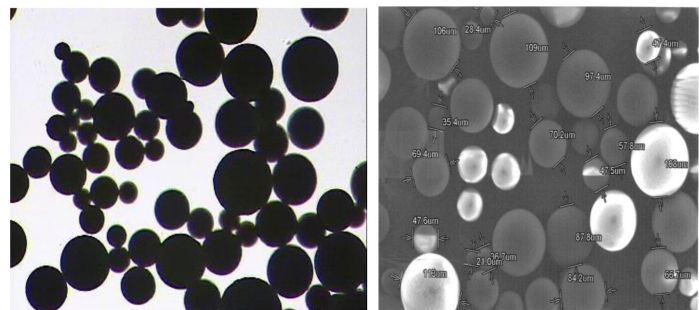

Figure 2: Optical microscopy photograph of microspheres.
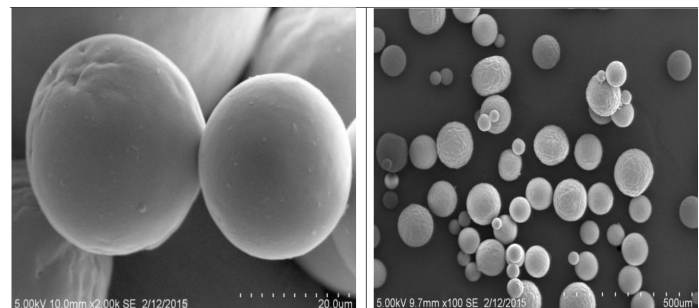

Figure 3: Scanning electron microscopy (SEM) of surface morphology microspheres.
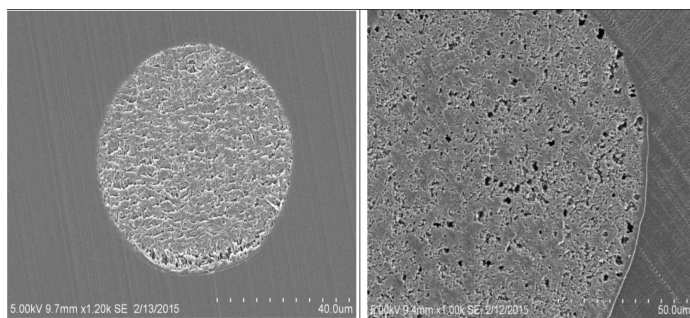

Figure 4: Scanning electron microscopy (SEM):Cross section/ Drug distribution of microspheres.

Table 2: Characterization of Polymer and Microspheres.

Formulation Code	Encapsulation Efficiency (%) (± SD)	Inherent Viscosity (dl/gm) (± SD)	Mean Particle Size (μm) (± SD)	Span Value (± SD)
PLGA 50:50				
Resomer RG505	-	0.71 ± 0.04	-	-
M1	90.85 ± 1.11	0.59 ± 0.01	116.78 ± 2.05	1.20 ± 0.02
M2	92.45 ± 1.32	0.55 ± 0.05	102.77 ± 2.11	1.12 ± 0.04
M3	94.13 ± 0.59	0.60 ± 0.02	89.31 ± 0.85	1.11 ± 0.02
Resomer RG504	-	0.58 ± 0.04	-	-
M4	92.11 ± 0.63	0.48 ± 0.02	81.96 ± 1.55	1.08 ± 0.05
Resomer RG504H	-	0.60 ± 0.04	-	-
M5	95.50 ± 1.73	0.53 ± 0.03	86.01 ± 1.65	1.13 ± 0.03
PLGA 75:25				
Resomer RG750	-	0.96 ± 0.03	-	-
M6	93.69 ± 0.91	0.79 ± 0.01	88.11 ± 2.33	1.15 ± 0.02

were suitable for the microsphere formulations capable of degradation in 1 month and 3 month. Inherent viscosity of Goserelin acetate loaded PLGA 50:50 microspheres was found to be between 0.48 ± 0.02 dL/g to 0.60 ± 0.02 dL/g and Goserelin acetate loaded PLGA 75:25 was found to be 0.79 ± 0.01 dL/gas shown in Table 2.

Differential scanning colorimeter (DSC)

DSC studies of PLGA, Goserelin acetate and Goserelin acetate loaded PLGA microspheres were performed. Thermogram of pure Goserelin acetate, PLGA 50:50 (Resomer RG505) and PLGA 75:25 (Resomer RG750) has sharp endothermic peak at171.20°C, 40.56°C and 47.06°C, which corresponds to its melting point (Figure 5). Thermogram of microspheres, the decrease in intensity of Goserelin acetate endothermic peak in drug loaded microspheres may be due partial conversion of crystalline to amorphous form. The DSC thermograms indicated that Goserelin acetate molecularly dispersed in microspheres.

X-ray diffraction (XRD)

X-ray diffraction (XRD) patterns of PLGA50:50 (Resomer RG505) and PLGA 75:25 (Resomer RG750), Goserelin acetate and Goserelin acetate loaded PLGA microspheres are shown in Figure 6 and Figure 7. Goserelin
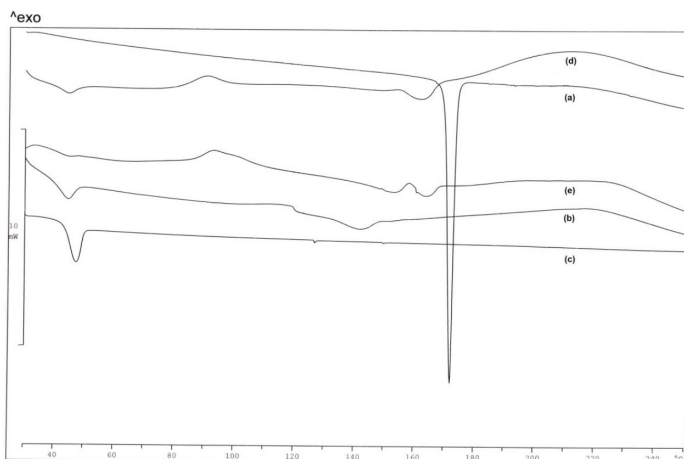
Figure 5: DSC Thermogram of (a) Goserelin acetate (b) PLGA 50:50 (c) PLGA 75:25 (d) Goserelin acetate PLGA 50:50 microspheres (e) Goserelin acetate PLGA 75:25 microspheres.
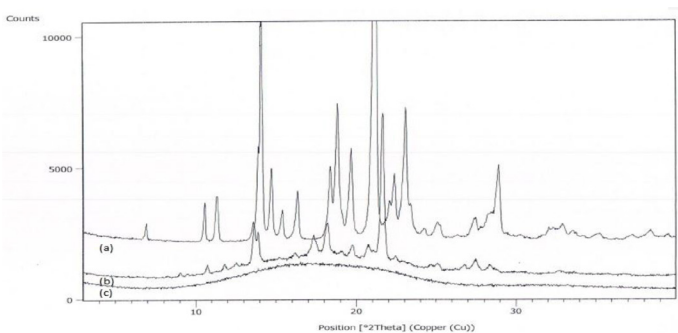

Figure 6: XRD of (a) Goserelin acetate (b) PLGA 50:50 (c) Goserelin acetate PLGA 50:50 microspheres.
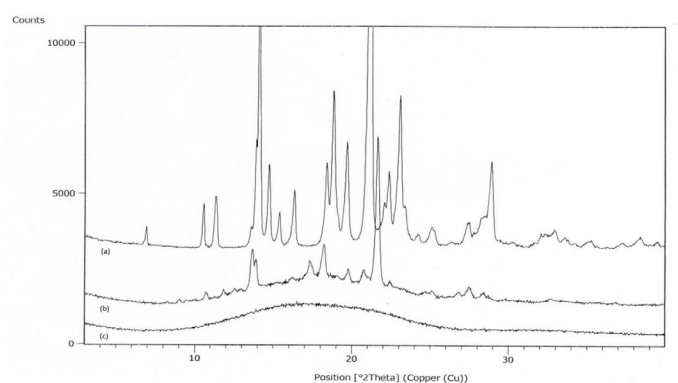

Figure 7: XRD of (a) Goserelin acetate (b) PLGA 75:25 (c) Goserelin acetate PLGA 75:25 microspheres.

acetate presence of 2Ө peaks values at 14.15°, 18.47°, 21.23°, 23.11°, 28.92° and 39.52° indicated that crystalline form. The absence of these peaks in XRD patterns of Goserelin acetate loaded PLGA microspheres indicated that Goserelin acetate is amorphous form in the matrix of PLGA50:50 and PLGA 75:25.

In vitro drug release

Real time *in vitro* release profile of Goserelin acetate loaded PLGA microspheres were depicted in Figure 8. Drug released through M1, M2,

M3, M4 and M5 formulation containing PLGA 50:50, a burst release was observed within 1day which was 25.63 ± 3.91 %, 21.23 ± 1.39 %, 15.22 ± 1.46%, 33.12 ± 1.09 % and 23.29 ± 1.39 % followed by a longer lag phase up to 14 days and erosion controlled release (plateau) 67.21 ± 1.65 %, 83.39 ± 2.31 %, 94.45 ± 1.22%, 88.23 ± 1.16 %, 90.56 ± 1.39 % at the end of 30 days. Drug released through M6 formulation containing PLGA 75:25, where only 44.49 ± 1.59 % drug was released ate the end of 30 days.

Drug Release Kinetics

The above drug release data of optimized formulation M3 and M5 were fitted kinetics models Zero-order, First-order and Higuchi's model. The correlation coefficient of determination values as shown in Table 3. This result may indicate that the drug release mechanism was mainly due to the combination of diffusion of drug through the polymer and polymer erosion of the PLGA microspheres.

In vivo drug release

The optimized formulation Goserelin acetate loaded PLGA 50:50 microspheres, formulation M3 (Resomer RG505) and formulation M5 (Resomer RG 504H)microspheres were selected for *in vivo* study. The mean plasma concentration time profile of Goserelin acetate following subcutaneous administration of the Goserelin acetate loaded PLGA microspheres in rat model are shown in Figure 9 and Table 4. The plasma concentration of Goserelin acetate raised to the maximum concentration (C_{max}) of formulation M3 and M5 were found to be 25.28± 6.11 ng/mL and 37.69 ± 6.51 ng/mL at 6h (T_{max}); then the decreased level steadily and maintained at value about 7.22 ± 2.06ng/mL to 8.59 ± 1.22 ng/mL and 11.64 ± 2.43 ng/mL to 13.17 ± 1.62 ng/mL from day 3 to day 7; after that the plasma concentration of Goserelin acetate rise gradually, reaching a second maximum plasma level of 53.03± 6.96 ng/mL and 61.69 ± 2.86 ng/mL at the Day 14, followed by a slowly decrease to below 0.50 ± 0.22 ng/mL and 0.36 ± 0.15 ng/mL at 35 Day. Thus, the duration of Goserelin acetate release from the PLGA 50:50 (Resomer RG505 and Resomer RG 504 H) microspheres was about one month. In addition, the area under the curve (AUC) of formulation M3 and M5 were found to be 13857 ± 567.48 ng.h/mL and 14515 ± 708.1ng.h/mL.
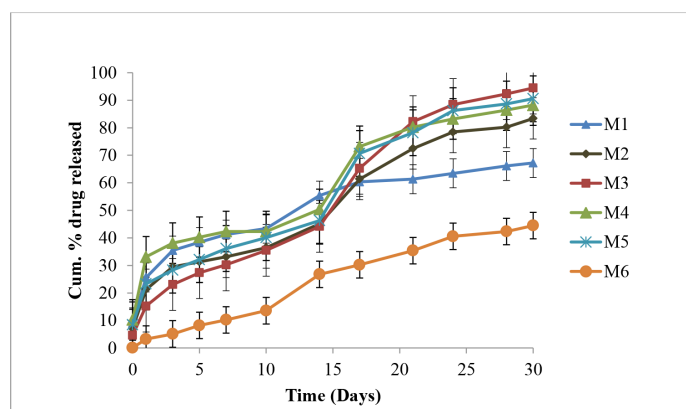

Figure 8: *In vitro* release behaviour of Goserelin acetate microspheres.

Table 3: Release kinetics studies of Goserelin acetate PLGA microspheres.

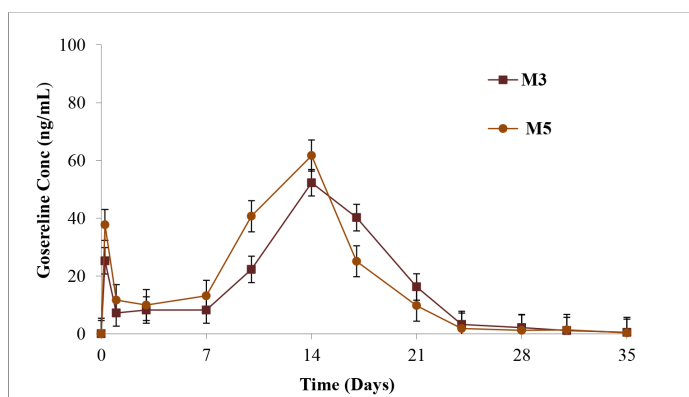| Formulation code	Zero order (R²)	First order (R²)	Higuchi model (R²)	Korsemeyer-Peppas (R²)
M3	0.956	0.961	0.981	0.923
M5	0.968	0.978	0.927	0.911

Figure 9: *In vivo* release behaviour of Goserelin acetate microspheres.

Table 4: Pharmacokinetic parameters of Goserelin acetate PLGA microspheres.

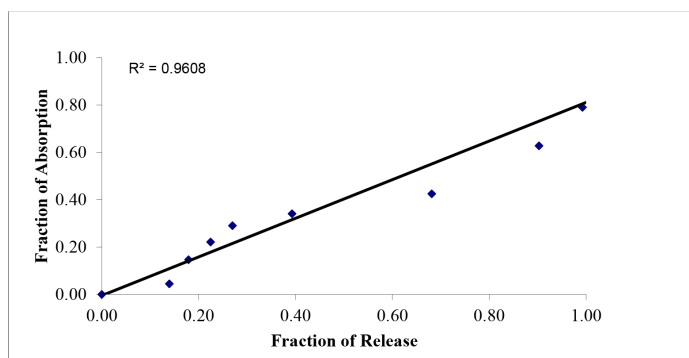| Formulation code	T_{max} (Days)	C_{max} (ng/mL) (± SD)	AUC_{last} (ng.h/mL) (± SD)
M3	14	53.03 ± 6.96	13857 ± 567.48
M5	14	61.69 ± 3.73	14515 ± 708.1

Figure 10: Correlation of Formulation M3 fraction of absorption *in vivo* Vs fraction of release *in vitro*.
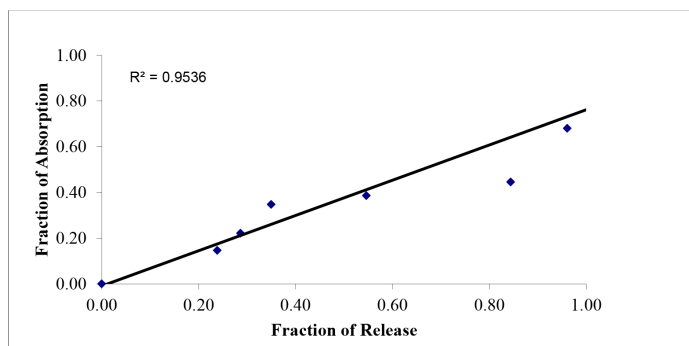

Figure 11: Correlation of Formulation M5 fraction of absorption *in vivo* Vs fraction of release *in vitro*.

In vitro and in vivo correlation (IVIVC)

The amount of the drug absorbed was determined using Wanger-Nelson. The cumulative percentage of the drug dissolved and cumulative fraction of the drug absorbed was plotted (Figure 10 and Figure 11). The correlation coefficient (r^2) of M3 and M5 formulation were found to be 0.9608 and 0.9536. Thus it can be concluded that good IVIVC was observed and

in vitro drug release test seemed to be rational anticipate *in vivo* release performance of Goserelin acetate loaded PLGA microspheres.

DISCUSSION

Process variables

The most suitable method for encapsulation of hydrophilic drug into PLGA microspheres is the double emulsion solvent extraction techniques. The drug Goserelin acetate, being water soluble, the solvent emulsification using static mixer method was selected. Multiple parameters exhibit that PLGA can easily be formulated into drug carrying devices as microspheres.

Morphology and particle size are important characteristics of microspheres, because of its effect on degradation rate, drug loading and initial burst release of microspheres.[30] The preparation of emulsion using static mixer, the particle size of the resulting microspheres can easily be controlled by the flow rates and ratio of primary phase and continues phase to be mixed.[31] It was found that an increase the in the phases flow rates resulted in a decrease in microspheres mean size. These results findings may be explained on the basis of change in the viscosity of the primary and ternary emulsion formed during emulsification processing. Increase viscosity of the emulsion by alternation of primary emulsion and continuous phase ratio resulted in high viscous resistance against the shear force during the forming of microspheres.[32] The external energy in the form of emulsification and the concentration of emulsifier in continuous phase causing droplet breakdown were kept constant; thus on increasing continuous phase volume, the same amount of energy must now be distributed in the large volume, resulting to less droplet breakdown and hence bigger microspheres were obtained.[33]

The porosity of the microspheres decreases with increasing continuous phase ratio and thus lower continuous phase ratio could have resulted in more porous microspheres leading to burst release, can probably be explained by the difference in the distribution of drug in microspheres due to different evaporation rates of the organic solvent. At lower continuous phase ratio, ethyl acetate will distribute into the continuous phase slowly, which is controlled by ethyl acetate evaporation rate; therefore, the drug will tend to migrate onto the microspheres surface together with ethyl acetate because of more solubility in the organic solvent, resulting in drug-rich microspheres surface. When the continuous phase ratio is high, ethyl acetate distributes in water quickly and the drug solidifies in the polymer matrix rapidly. The drug near the surface will diffuse out of the microspheres first, causing burst release. These findings are in agreement with the published research, where the effect of process and formulation variables on Goserelin acetate loaded PLGA microspheres 'internal morphology' was evaluated.

The encapsulation efficiency increase with increase in the polymer concentration, from the result it can be inferred that there is proper distribution of Goserelin acetate in microspheres. The encapsulation efficiency based on the fast polymer precipitation on the surface of the primary phase or dispersed phase can prevent loss into the continuous phase. Solidification of the primary phase or dispersed phase is delayed; the encapsulation efficiency becomes low because more drug are diffuse into the continuous phase.[34]

The cumulative amount of drug release up to 30 days increased with increasing concentration of continuous phase used in the preparation of microspheres. The difference in the release profiles due to difference in the surface morphology and particle size of microspheres. It has been reported that large amount of continuous phase results in faster precipitation of the polymer with a smoother and less porous surface of the microspheres.[35]

Formulation variables

The influence of PLGA polymer properties such as molecular weight, inherent viscosity, hydrophilicity and lactide/glycolide ratio on particle size, morphology, encapsulation efficiency, *in vitro* release study and *in vivo* release study of Goserelin acetate loaded PLGA microspheres were studied. PLGA50:50 Resomer RG505, Resomer RG504, Resomer RG504H and PLGA75:25 Resomer RG750 were used for this study. The particle size of Goserelin acetate loaded PLGA microspheres turned into specifically affected by the molecular weight of polymers. At consistent solvent volume in organic phase, the viscosity of the polymeric solution becomes proportional to the molecular weight of the polymer. Low viscosity polymer solution might be dispersed in the external aqueous phase to a more quantity than high viscosity polymer solution and consequently reduced the microspheres to smaller size. The Goserelin acetate loaded PLGA microspheres showed smooth nonporous surface with spherical shape, there were no significant differences in surface morphology of the microspheres between Resomer RG505, Resomer RG504, Resomer RG504H and Resomer RG750.

The encapsulation efficiency of Goserelin acetate was highly dependent on the molecular weight and hydrophilicity of PLGA polymers. Goserelin acetate encapsulation efficiency higher for Resomer RG505 and Resomer RG750 compared to Resomer RG504 and Resomer RG504H. This might be due to the higher viscosity of polymeric solution as result of high molecular weight of the polymer which could prevent the partitioning of Goserelin acetate into internal and external phase. Despite the difference in their lactide content, the encapsulation efficiency of Goserelin acetate microspheres formulated with Resomer RG505 and Resomer RG750 were almost similar may be this ratio does not affect the encapsulation efficiency of Goserelin acetate microspheres. The Resomer RG504H also exerted positive influence on encapsulation efficiency compared to Resomer RG504. This might be due to the strong interaction of carboxylic group present in polymer chain with the amine group of the drug molecules.

Higher inherent viscosity of PLGA (Resomer RG505 and Resomer RG750) resulted in longer lag times and less initial drug release because of the higher molecular weight of PLGA and thus slower PLGA degradation.[36] The rapid release phase due to PLGA degradation was delayed as result of the longer lag time. PLGA polymers with low inherent viscosities have higher hydration and swelling properties, therefore leading to faster diffusion of surface-associated drug into the release medium.

DSC and XRD techniques were carried out as the useful tool in investigation of physicochemical properties of Goserelin acetate in microspheres formulation. The crystallinity changes of drug during microspheres formulation process clearly influence the drug release rate and bioavailability.[37] The obtain DSC thermograms and their respective endothermic values indicated that the drug and manufacturing process had slight impact on the thermal properties of the polymer. These findings are highly consistent with the XRD results, that the dispersion of drug in the core of Goserelin acetate loaded PLGA microspheres leads to the crystalline Goserelin acetate molecules melting peak disappearance. Moreover, in view of the objective of the current formulation to provide a constant drug release, the molecular dispersion of Goserelin acetate in microspheres seems to give sustained release profiles for the intended drug delivery applications.

The *in vitro* release profile of microspheres are intended to help in predicting the final behavior of a given microspheres formulation. The release of Goserelin acetate from PLGA50:50 and PLGA75:25 showed different release properties. This might be due to difference in their lactide/glycolide ratio and higher content glycolide leads to faster degradation rate. The Resomer RG504 showed faster degradation rate and higher initial burst release compared to Resomer RG504H. This was attributed to the polymer terminal carboxylic group because hydrophilicity of polymer which cause favourable interaction between free carboxylic end group within the polymer chains and Goserelin acetate. It was noticeable that the polymer composition was very important factor to controlled release profile of Goserelin acetate from microspheres.

The mechanism of Goserelin acetate release from PLGA microspheres was investigated by correlation coefficient values and drug release was found to be controlled by diffusion of drug through polymer matrix. *In vivo* study the difference in the release profile of two formulation are easily discernible. Different plasma concentration of formulation with Resomer RG505 and Resomer RG504H were detected depending on the system which Goserelin acetate was released because different hydrophobic behavior of polymer. It indicates that sustainable release properties of Goserelin acetate loaded PLGA microspheres for treatment of hormone responsive cancers.

The IVIVC predictive can allow *in vitro* dissolution study as a substitute for *in vivo* study from controlled release dosage form. Result of the *in vitro* release showed that optimized formulation resulted in one month and complete release of the drug from the microspheres. Simple *in vitro* study may not however reflect the same *in vivo* effects. Hence, we also performed studies to determine drug release profile *in vivo*. Interestingly, the optimal formulation resulted in one month and complete release *in vivo*. In addition, also established IVIVC. This correlation was 100 percent suggesting that result from *in vitro* studies are valid.

CONCLUSION

In present study, Goserelin acetate was successfully encapsulated into PLGA microspheres by modified emulsification technique using static mixer. The results of this investigation explicate that the process and formulation variables could be effectively modified to achieve the desired characteristics, such as homogenous drug distribution, particle size distribution, surface morphology, encapsulation efficiency, controlled initial burst release, *in vitro* and *in vivo* drug release profile of Goserelin acetate loaded biodegradable polymeric microspheres.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ABBREVIATIONS

PLGA: Poly(DL-lactide-co-glycolic acid); **PGA:** Poly(glycolic acid); **PLA:** Poly(lactic acid); **K_{el}:** Rate of elimination; **K_a:** Rate of absorption; **USFDA:** United States of Food and Drug Administration; **LH:** Luteinizing hormone; **PVA:** Polyvinyl alcohol; **DSC:** Differential scanning colorimeter; **Tg:** Glass transition temperature; **XRD:** X-ray diffraction; **HPLC:** High performance liquid chromatography; **EDTA:** Ethylene diamine tetraacetic acid; **LC-MS/MS:** Liquid chromatography with tandem mass spectrometry; **IVIVC:** *In vitro* and *in vivo* correlation.

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