

Effects of Formulation Parameters on the Characteristics of Biodegradable Microspheres of Goserelin Acetate

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Abstract

Aim: The purpose of this study was to develop biodegradable microspheres controlling drug delivery for treatment of prostate and breast cancer. The effects of formulation parameters on the characteristics of microspheres were studied. **Material and Methods:** Emulsification/extraction method was used to prepare goserelin acetate controlled-release poly(D, L-lactide-co-glycolide) (PLGA)-based biodegradable microspheres. Microspheres were formulated and characterized in terms of encapsulation efficiency, particle size distribution, surface morphology, and drug release profile. **Results and Discussion:** Preparative variables such as concentrations of stabilizer, drug-polymer ratio, stirring rate, and the 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 reflected in drug release profile. **Conclusion:** The *in vitro* goserelin acetate release study from PLGA microspheres proved that the present microspheres had the properties of an ideal controlled release formulation for anticancer therapy.

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

INTRODUCTION

Controlled release drug delivery systems are being developed to address many of the difficulties associated with traditional methods of administration. The development of novel technologies in the area of drug discovery such as genetic engineering, combinatorial chemistry, and high-throughput screening leads to numbers of drug candidates with high therapeutic potentials. However, majority of them have poor oral absorption or a short biological half-life. The emerging of these complex active ingredients has drawn considerable attention on development of novel techniques to deliver them in an effective and efficient way. Parenteral controlled release of drugs represents one of such approach. Single-dose administration of these systems can maintain the drug in the desired therapeutic range for days, weeks, months, and for some products, even years.^[1]

One of the technological resources used to improve the performance of drugs at the site of action is the use of therapeutic systems prepared using biodegradable polymers. Biodegradable polymers show increasing importance in the

development of sustained release drug delivery system. Biodegradable polyanhydrides and polyesters are useful materials for controlled drug delivery. They have hydrophobic backbones with hydrolytically labile anhydrides and/or esters that may hydrolyze to dicarboxylic acids and hydroxy acid monomers when placed in an aqueous medium. Fatty acids are suitable candidates for the preparation of biodegradable polymers because they are natural body components and are hydrophobic and thus may retain an encapsulated drug for longer periods when used as drug carriers.^[2]

Biodegradable polymers are useful material for controlled release drug delivery system such as disks, rods, pellets, or microparticles that encapsulate drug and control release rates for extended period. Such systems offer several potential

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advantages over traditional methods of administration. The advantage is that the drug release can be controlled; microparticles have a long duration of action and dosage frequency and adverse effects can, therefore, be reduced. A poly (lactic-co-glycolic acid) (PLGA) microsphere formulation was developed which incorporates carboxylic acid groups into the microsphere surface. These functional groups are suitable for coupling to a variety of ligands and form linkages that remain stable in aqueous environments for extended period. The ligand binding capacity of these microspheres compares favorably to that of comparably sized carboxylated microspheres, which are commonly used as model particles for targeted microsphere delivery studies.^[3]

Microspheres made of biodegradable products, can be injected with a syringe into the body and, once injected, solidify to form a semi-solid deposit. Drug is gradually released on erosion or by diffusion from the particles.^[4] These systems offer certain unique advantages, which has sparked people's interest. Apart from ease of application, these provide localized delivery for a site-specific action, prolonged delivery periods, decreased drug dosage with concurrent reduction in possible undesirable side effects common to most forms of systemic delivery and improved patient compliance and comfort.

Since depot injections are frequently used for those who find taking oral medication on a regular basis difficult or unacceptable. The fact that the patient must attend for injection, the delivery of a known quantity of medication is guaranteed. Receiving an injection fortnightly or monthly is more straightforward than having to take tablets once or twice daily. Moreover, these agents enable the treatment team to rapidly detect non-compliance.

Hormone-dependent cancers of the prostate and testis cancer in men and breast, ovarian, and endometrial cancer in women are most common cancer. The sex steroid hormones, androgens, estrogens, and progestins play an essential role in both normal development and maintenance of these organs, as well as premalignant and malignant growths.^[5]

Breast cancer is one of the leading causes of cancer deaths among women. Nearly 1 million new cases are diagnosed each year.^[6] One of the major problems facing cancer chemotherapy is the achievement of the required therapeutic concentration of the drug at the tumor site for a desired period of time without causing undesirable effects, on the other organs, while it circulates in the body.^[7]

Goserelin acetate acts as a potent inhibitor of pituitary gonadotropin secretion and widely used in the chemotherapy of the breast cancer and prostate cancer.^[8] Goserelin acetate was then approved by the United States of Food and Drug Administration for use as an agent for advanced breast cancer

palliative treatment in premenopausal and perimenopausal women and prostate cancer.

The objective of the present work was to design the controlled release microspheres of goserelin acetate using biodegradable polymer-based drug delivery system for subcutaneous administration to treat prostate and breast cancer. Goserelin acetate decreases male sex hormone testosterone and female sex hormones estrogen and progesterone. However in order to improve patient compliance and minimize the side effect.

Several microencapsulation techniques have been developed for this purpose; however, the appropriateness of such techniques depends on the nature of the drug/polymer. The most suitable microencapsulation techniques are emulsion solvent evaporation, phase separation, interfacial polymerization, and spray drying of these methods, emulsion-solvent evaporation is the method of choice for microencapsulation of water-insoluble drugs using a water-insoluble polymer.

The effects of a series of formulation parameters of emulsion on the microencapsulation and release behavior of goserelin acetate PLGA microspheres also investigated.

MATERIALS AND METHODS

Chemicals

Goserelin acetate was purchased from Hemmo Pharmaceutical Private Limited, Mumbai. PLGA Resomer RG505 (lactide: glycolide ratio, 50:50) was purchased from Evonik Roehm GmbH, Germany. Poly(vinyl alcohol) (PVA) and dichloromethane (DCM) were supplied by Merck Ltd. All chemicals and reagents used were of analytical grade.

Preparation of goserelin acetate-loaded PLGA microspheres

Goserelin acetate-loaded PLGA microspheres were prepared by an emulsification/extraction method.^[9-10] [Table 1 and Figure 1].

Preparation of the primary emulsion (water/oil): PLGA was dissolved in DCM 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 Silverson homogenizer at 1000 rpm for 2 min.

Preparation of continuous phase: PVA was dissolved in water for injection and filter through a 0.22 μ filter.

Preparation of the ternary emulsion (water/oil/water): Primary emulsion was pumped simultaneously with a continuous phase into a Silverson L4RT mixer to obtain an emulsion at 900–1200 rpm for 5 min.

Table 1: Composition or manufacturing variables of goserelin acetate PLGA microspheres

Formulation Code	Manufacturing variables						
	Goserelin acetate (mg)	PLGA 50:50 (mg)	Water for Injection (mL)	DCM (mL)	PVA concentration (%)	Homogenization speed (rpm)	Extraction ratio (%)
M1	5	95	0.5	5	0.50	900	3
M2	10	90	0.5	5	0.50	900	3
M3	15	85	0.5	5	0.50	900	3
M4	10	90	0.5	5	0.75	900	3
M5	10	90	0.5	5	1.00	900	3
M6	10	90	0.5	5	1.00	1000	3
M7	10	90	0.5	5	1.00	1200	3

PLGA: Poly (D, L-lactide-co-glycolide), DCM: Dichloromethane, PVA: Poly (vinyl alcohol)

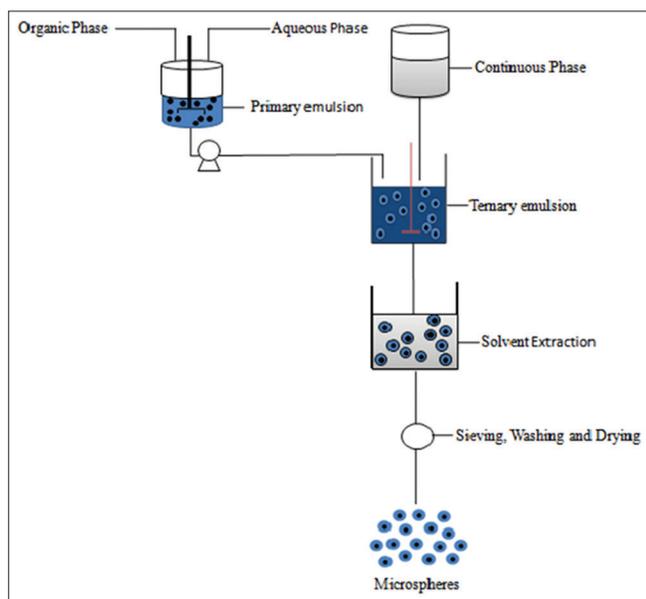


Figure 1: Production of microspheres by the solvent emulsification

Solvent extraction: The emulsion was transferred into a tank containing water for injection (2°C–8°C) and mixed for 120 min for extraction of the solvent from the emulsion and hardening of microspheres.

Sieving and drying of the microspheres: Slurry of microspheres from tank was passed through a 28 µm sieve and subsequently a 150 µm sieve, washed with water for injection. Microspheres were collected and vacuum dried at 28°C for 180 min.

Microsphere morphology and size analysis

The shape and surface morphology characteristics of microspheres were examined by digital optical microscope (Motic B3 professional series) and scanning electron microscopy HITACHI SU 1500, Japan, JEOL JFC-1100E Ion sputter, before analyzing the samples, the goserelin acetate PLGA microspheres were coated with platinum under

the vacuum condition.^[11] Mean diameters and particle size distribution of microspheres were measured by particle size analyzer (Mastersizer, Malvern, UK).

Residual solvent determination

Residual solvent DCM into the microsphere was determined by the headspace gas chromatography method using gas chromatograph (GC 8610) using BP5 Capillary Column (0.25–1.5-mm film thickness) equipped with the headspace system.^[12] Ultra high-purity nitrogen (≥99.99%) was used as the carrier gas. Detection was carried out with a flame ionization detector. Accurately weighed microsphere formulation (100 mg) was taken in a 4 mL glass vial equipped with a Teflon septum closure and sealed. The vial was incubated at 60°C in oven for 10 min. and the headspace was subsequently onto to the GC column. The column temperature was initially equilibrated to 40°C for 1 min and ramped from 40°C to 75°C at 10°C/min. The peak area of DCM was determined. The amount of residual DCM in the microspheres was determined by the one-point analysis method using DCM (1000 ppm) as standard.

Determination of residual PVA

Residual PVA present in the microspheres was determined by colorimetric method based on the formation of a colored complex between two adjacent hydroxyl groups of PVA and iodine molecules.^[13] Briefly, microspheres sample was treated with 0.5M sodium hydroxide for 15 min at 60°C. Sample was neutralized with 900 µl of 1N hydrochloric acid and the volume was adjusted to 5 mL with distilled water. To the sample, 3 mL of 0.65 M boric acid followed by 0.5 mL of I₂/KI (0.05 M/0.15 M) was added and incubated for 15 min. the absorbance of the sample was measured at 690 nm.

Determination of encapsulation efficiency

Encapsulation efficiency of goserelin acetate PLGA microspheres was determined by measuring the total

amount of goserelin acetate present in each 55 mg sample (i.e., actual core loading) and comparing this value with the expected amount of goserelin acetate in each of the samples based on the drug loading during the preparation (i.e., theoretical core loading). Microspheres containing goserelin acetate were dissolved in 15mL of acetonitrile and make up volume up to 50 mL by Phosphate buffer pH7.4. The solution was filtered using 0.45 μm membrane filters and after suitable dilution with phosphate buffer pH 7.4. The absorbance values at 220 nm corresponding to concentration were then evaluated.

To calculate the % Encapsulation efficiency following equation used.^[14]

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

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

Inherent viscosity of microspheres

Inherent viscosity is calculated as ratio of the natural logarithm of the relative viscosity and the mass concentration of the polymer (C).^[15] Approximately 46 mg microspheres were dissolved in 10 mL of chloroform. Micro-Ostwald viscometer having capillary constant 0.01 mm²/s was used. The viscometer was kept in auto rinse mode and allowed 1 min pre-tampering wash and then sample analysis was repeated three times by which kinematic and inherent viscosities were obtained as

Calculation formula:

$$\text{Relative Viscosity } \eta_r = \eta_s / \eta_0 = t_s / t_0$$

$$\eta_{inh} (\text{dl/g}) = (\ln \eta_r) / C$$

Determination of *in vitro* release of goserelin acetate from microspheres

The *in vitro* drug release was performed in release media under sink conditions on shaker incubator at constant temperature (37°C). The 40 mg microspheres were dispersed screw capped bottles containing 50 mL release media phosphate buffer pH 7.4 were fixed onto the platform in water bath for shaking at 50 rpm in shaker incubator. At the set points, 2 mL sample was removed by syringe for analysis through 0.45 μm mdi nylon filters. Any Microspheres drawn into the syringe filter were returned back by washing with 2 mL replacement fresh media (pH 7.4) and supernatants was analyzed by HPLC at 220 nm.

RESULTS AND DISCUSSION

Morphological studies, particle size and residual DCM analysis in the Goserelin acetate PLGA microspheres

The morphology of the goserelin acetate PLGA microspheres prepared with PLGA Rsomer RG505(50:50) showed smooth nonporous surface with spherical shape as visualized in SEM images shown in Figures 2 and 3.

Particle size is one of the important characteristics of microspheres because of its effect on degradation rate, drug loading, and initial burst release of microspheres. The average particle diameters of goserelin acetate PLGA microspheres were from 153.72 to 91.57 μm with a good dispersibility in Table 2.

It is well known, that DCM has a high acute toxicity, for example, central nervous system affects, heart rhythm

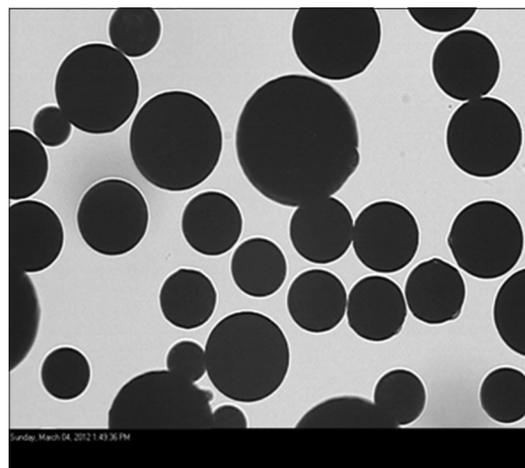


Figure 2: Optical microscopy photograph of behavior of goserelin acetate poly(D, L-lactide-co-glycolide) microspheres

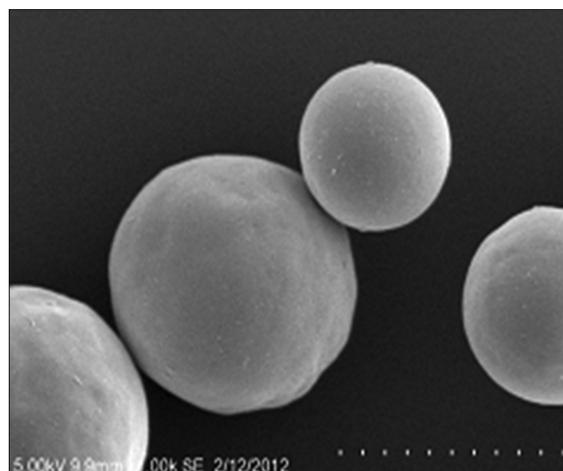


Figure 3: Scanning electron microscopy of goserelin acetate poly(D, L-lactide-co-glycolide) microspheres M7

disturbance, and respiratory paralysis. As a result, attention has been paid to find possibilities, which avoid DCM during microencapsulation in PLGA. The residual DCM contents in all goserelin acetate PLGA microspheres were below 500ppm, which was in accord with the requirements of the ICH standard.

Effects of drug:polymer ratio

The effect of different concentrations of drug:polymer ratio from 0.5:9.5%, 1:9%, and 1.5:8.5% mentioned in Table 1. The results indicate that the variation in the concentration of drug:polymer ratio effects on the microspheres and *in vitro* release of goserelin acetate from microspheres are shown in Figure 4. Found that increasing the PLGA polymer concentration in the organic phase resulting in increases the particle size and increasing the concentration of goserelin acetate in aqueous phase resulting in increases the particle size of microspheres, similarly. The increasing the drug concentration leads to the more drug molecules overlaid the surface of the microspheres (loosely trapped in the particles). The higher concentration of polymer in oil phase increases the drug encapsulation of PLGA microspheres because an increase in the viscosity of the oil phase prevents drug from diffusion Table 2. While the low concentration of drug and higher concentration of polymer initial burst release shown very higher or slow and increase the concentration of drug the initial burst release of the microsphere in the first 5 days and the higher concentration of drug the initial burst release of the drug was slightly fast starting at 3 days. Batch containing 1:9% drug:polymer concentration in aqueous solution having better encapsulation efficiency, smaller particle size, and drug loading with better syringeability.

Effect of stabilizer or PVA concentrations

The influence of different PVA concentrations in the continuous phase from 0.5 to 1% was studied. The mechanism of PVA binding has been proposed to be due to the interpenetration of PVA and PLGA molecules during microspheres formulation. The hydrophobic segments of PVA penetrate into the organic phase and remain entrapped into the polymeric matrix of the microspheres.^[16] The results indicate that the variation in the concentration of PVA in the dispersion medium affects the

size of the microspheres. It was observed that the minimum concentration of PVA required for the stabilization of organic droplets in the aqueous medium was 0.5%. Below this value, the droplets were not stabilized and finally adhered on the stirrer. The results in Table 2 indicate that as the percentage of PVA increases, the size of the microspheres decreases. At 0.5% and 0.75% PVA, most of the microspheres were in the range 133.60–153.72 μm and 125.03 μm . However, at 1 % PVA, microspheres in the range 91.57–116.90 μm were obtained.

The effect of PVA concentration in the aqueous phase of the emulsion on the encapsulation efficiency in microspheres is

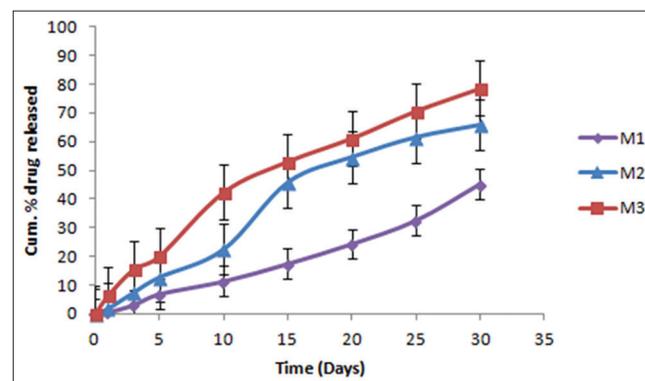


Figure 4: Effects of drug:polymer ratio on *in vitro* release behavior of goserelin acetate poly(D, L-lactide-co-glycolide) microspheres

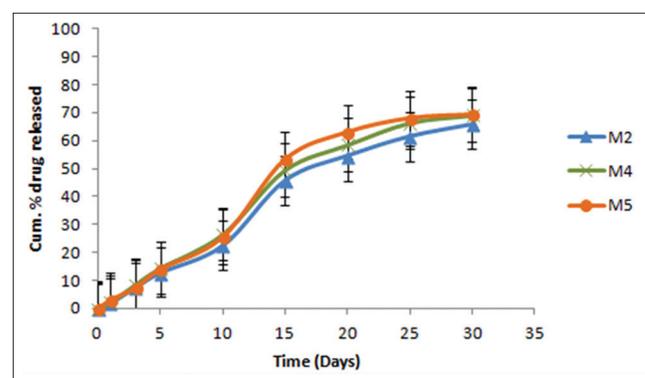


Figure 5: Effect of stabilizer or poly(vinyl alcohol) concentrations on *in vitro* release behavior of goserelin acetate poly(D, L-lactide-co-glycolide) microspheres

Table 2: Characterization of microspheres

Formulation Code	Particle size d (0.9) μm	Inherent viscosity (dl/gm)	Theoretical drug loading (%)	Actual drug loading (%)	Encapsulation efficiency (%)
M1	137.12	0.81	5	4.34	86.85
M2	133.60	0.79	10	9.44	94.45
M3	153.72	0.72	15	13.44	89.63
M4	125.03	0.76	10	9.45	94.53
M5	116.90	0.74	10	9.55	95.50
M6	99.26	0.78	10	9.75	97.53
M7	91.57	0.77	10	9.88	98.84

shown in Table 2. The results indicate that the encapsulation efficiency increased with increasing PVA concentration in the external aqueous phase. This can be related to the increasing viscosity of the PVA solution with increasing concentration of PVA leading to the resistance to the outward diffusion of goserelin acetate from the internal aqueous phase and to the better stabilization of the emulsion at higher PVA concentrations. Furthermore, higher amount of PVA at the interface of organic phase and the external aqueous phase could have contributed to the higher resistance to goserelin acetate diffusion out of the polymeric phase leading to higher encapsulation efficiency in microspheres prepared with higher amount of PVA.

The effect of PVA concentration in the aqueous phase of the emulsion on the *in vitro* release of goserelin acetate from microspheres is shown in Figure 5. The release profiles were biphasic for both the formulations, with an initial burst release attributed to surface associated drug followed by a slower release phase as the entrapped drug slowly diffuses out into the release medium. While, the microspheres with low concentration of PVA 0.5% has shown very high initial burst release and increase in the concentration of PVA to 0.75% has shown the initial burst release of the microspheres in the first 5 days and the higher concentration of PVA at 1% initial burst release of the drug was slightly fast starting after 5 days. Increasing the PVA concentration in the continuous medium reduced the particle size and loading efficiency of the Goserelin acetate microspheres. It was found that goserelin acetate-loaded PLGA microspheres prepared with high PVA concentration was more narrowly and evenly distributed than the lower PVA.

Effect of stirring speed

It was observed that an increase in stirring speed from 900 rpm to 1200 rpm decreases the microsphere size significantly from 153.72 μm to 91.57 μm shown in Table 2, found that with increase in stirring speed, size of the PLGA-loaded goserelin acetate microspheres was decreased. It was observed that batch M7 having particle size of 91.57 μm which shows no change in release profile as compared to batch M5 and M6 having comparatively larger particle size of 99.26 μm and 116.90 μm . Thus, an optimum batch M7 having stirring speed 1200 rpm was chosen to produce optimum particle size so as to achieve a better encapsulation, drug loading, and percent release. The batch M7 shows better syringeability, narrow size distribution, and shows uniform shape. The results summarized in Figure 6 and Table 2 shows that the increase in the stirring speed causes a decrease in the size of the microspheres. This is because of the breaking down of the larger droplets of the dispersed phase into smaller droplets with increase in stirring speed.

In vitro release of goserelin acetate from microspheres

As reported in an earlier research, the drug release of PLGA microspheres is impacted by numerous parameters, such as the types of polymer, the physicochemical properties of drug, particle

size of microspheres, and preparation process.^[17] To investigate the effects of formulation parameters on *in vitro* release of goserelin acetate from microspheres, a series of formulation of goserelin acetate PLGA microspheres were designed and prepared by emulsification/extraction method [Table 1]. Results showed that the parameters during the preparation of the microspheres had significant influence on the *in vitro* release behavior of goserelin acetate PLGA microspheres.

The cumulative *in vitro* release of goserelin acetate from microspheres is shown in Figures 4-6. The release profiles were biphasic for both the formulations, with an initial burst release attributed to surface associated drug, followed by a slower release phase as the entrapped protein slowly diffuses out into the release medium.^[18] To investigate the effects of formulation parameters on *in vitro* release of goserelin acetate from microspheres. Results showed that the parameters during the preparation of the microspheres had significant influence on the *in vitro* release behavior of goserelin acetate PLGA microspheres.

CONCLUSIONS

Goserelin acetate PLGA microspheres were designed and prepared successfully through the emulsification/extraction method. The results of a formulations revealed that the drug-to-polymer ratio, PVA concentration, and stirring speed are imperative to acquire sustained release and entrapment efficiency. The formulations described in this study released goserelin acetate constantly for 4 weeks. The *in vitro* goserelin acetate release study from PLGA microspheres proved that the present microspheres had the properties of an ideal sustained release formulation for anticancer therapy.

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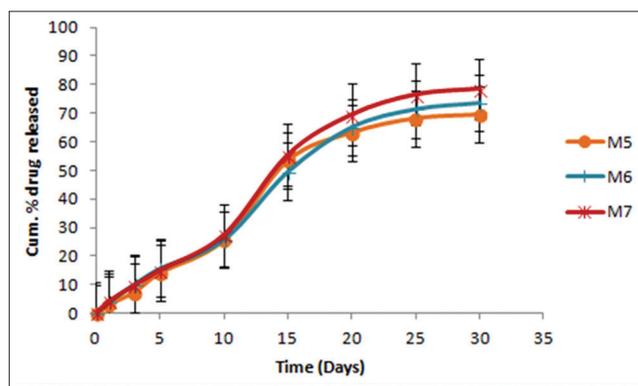


Figure 6: Effect of stirring speed on *in vitro* release behavior of goserelin acetate poly(D, L-lactide-co-glycolide) microspheres

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