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Research Article

PREPARATION AND CHARACTERIZATION OF GOSERELIN ACETATE LOADED MICROSPHERES

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ABSTRACT

The objective of the present work was to formulate and evaluate Goserelin acetate microspheres for treating prostate and breast cancer effectively in order to improve patient compliance with fewer side effects. Goserelin acetate is used as an antineoplastic agent and it is considered as one of the several first-line options for hormonal therapy. The marketed preparation of Goserelin acetate is available in the form of implants which has less patient compliance because of its side effects like hot flushes and pain. Goserelin acetate was prepared in the form of microspheres by solvent evaporation method for subcutaneous administration for controlled release. The influence of various parameters, such as the polymer concentration, organic solvent, and homogenization speed on encapsulation efficiency and particle size were investigated. Different formulations using synthetic biodegradable polymer Poly (Lactide-co-Glycolide) acid were developed and evaluated for percentage yield, entrapment efficiency, surface morphology (SEM), particle size analysis, In-vitro drug release and Stability studies. The SEM picture showed that the shape of the microspheres was spherical. FTIR studies showed that there was no disappearance or significant shift in the peak position of the drug with polymer. In-vitro drug release for optimized formulation was found to be 95%. From the experimental results it is evident that the controlled release of Goserelin acetate microspheres can be a suitable alternative in the treatment of prostate and breast cancer for improving patience compliance with fewer side effects.

Keywords: Goserelin acetate, Controlled release, PLGA, Microspheres, Implants

INTRODUCTION

Goserelin acetate is a synthetic gonadotropin- releasing hormone analog (GnRHa) like naturally occurring luteinizing hormone releasing hormone(LHRH) that is produced by the hypothalamus, initial or intermittent administration of goserelin stimulates release of gonadotropins, luteinizing hormone(LH) and follicle stimulating hormone (FSH), from the anterior pituitary. Long –term sustained use of goserelin is associated with an early phase of increased LH and FSH levels, followed by their suppression.

The marketed preparartion of goserelin acetate is available under the brand name Zoladex implants manufactured by Astra Zeneca Pharma.

The objective of the present work was to formulate and evaluate controlled release goserelin acetate microspheres for subcutaneous administration for treating prostate and breast cancer effectively in order to improve patient compliance with fewer side effects. Different formulations were prepared by following solvent evaporation technique (double emulsion) using biodegradable poly (Lactide-co-Glycolide) acid and evaluated for percentage yield, entrapment efficiency, surface morphology (SEM), particle size analysis, in-vitro drug release and stability studies.The optimized formulations of goserelin acetate microspheres with controlled release were attempted for a release upto atleast one month.

MATERIALS AND METHODS

Materials

PLGA (50:50 mole ratio of lactide to glycolide) was purchased from Evonik Roehm Gmbh, (Germany). Goserelin acetate was purchased from Hemmo Pharmaceutical Private Limited, (Mumbai). Polyvinyl alcohol 1% (PVA) and Dichloromethane (DCM) was obtained from SD fine chemicals. PVA was used as an emulsifying agent. Ethyl acetate was obtained from Qualigens.

Microsphere preparation

The preparation method is adopted from the work of Rassoul Dinarvand et al.[13, 14] 500mg of Polymer PLGA Poly (Lactide-co-glycolide) acid 50-50 was dissolved in 2ml of organic phase DCM (Dichloro methane).To this organic phase, 0.2ml of aqueous drug solution was added and the two phases were emulsified using high speed homogenizer (IKA) operating around 10000 rpm for about 1 minute to prepare water[1] /oil (w^1/o) primary emulsion. This primary emulsion was added to 50ml of external aqueous phase containing surfactant (1% poly vinyl alcohol was used to prepare w/o/w emulsion) at homogenizer speed around 8000 rpm for 3 minutes. The contents were then stirred at 1000 rpm for 1 hour at 2-8°C and at room temperature for the next 2 hours to permit evaporation of DCM. The microspheres obtained were collected by centrifugation followed by filtration and dried.

As the polymer is commonly used in the preparation of microspheres, only three concentrations were attempted based on the literature survey. The composition of various formulations of Goserelin acetate prepared using different concentrations of PLGA and Ethyl acetate were shown in Table 1.

The composition of various formulations of Goserelin acetate microspheres were prepared by using different concentrations of DCM are shown in Table 2.The effect of different primary and secondary homogenization speeds on the formulation was also investigated in Formulations F8 to F12 as shown in Table 2.

Table 1: The composition of various formulations of Goserelin acetate microspheres.

Composition		Formulations				
		F1	F2	F3	F4	F5
Drug(mg)		100	100	100	100	100
WFI(ml)		0.2	0.2	0.4	0.2	0.2
PLGA 50-50(mg)		**1000**	**800**	**500**	500	500
DCM(ml)		2	2	2	-	-
PVA 1% (ml)		50	50	50	50	50
Ethyl acetate		-	-	-	**4**	**2**
Temperature (°c)		2-8	2-8	2-8	2-8	2-8
Homogenization speed (rpm)	Primary(1min)	10,000	10,000	10,000	10,000	10,000
	Secondary(3min)	8000	8000	8000	8000	8000

Table 2: Composition of Goserelin acetate microspheres

Composition		Formulations						
		F6	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**
Drug(mg)		100	100	100	100	100	100	100
WFI(ml)		0.2	0.2	0.2	0.2	0.2	0.2	0.2
PLGA 50-50(mg)		500	500	500	500	500	500	500
DCM(ml)		**2**	**4**	2	2	2	2	2
PVA 1% (ml)		50	50	50	50	50	50	50
Ethyl acetate		-	-	-	-	-	-	-
Temperature (^{0}C)		2-8	2-8	2-8	2-8	2-8	2-8	2-8
Homogenization speed (rpm)	Primary(1min)	10,000	10,000	10,000	10,000	10,000	**12,000**	**14,000**
	Secondary(3min)	8000	8000	**4000**	**6000**	**8000**	8000	8000

Evaluation of Microspheres

FTIR studies

FTIR studies were carried out for goserelin acetate plain drug, PLGA, goserelin acetate microspheres and scanned from 4000 cm-1 to 400 cm-1 in Bruker IR spectrophotometer and checked for any shifts in functional peaks.

Determination of percentage yield

Microspheres were weighed and the yield of microspheres was calculated using the formula:

Percentage yield = Practical yield (gm) / Theoretical yield × 100

Determination of drug entrapment efficiency

The amount of drug entrapped was estimated by dissolving 100mg of microspheres in DCM and water in 3:1 ratio, under vigorous shaking for 1hr, the resultant solution was centrifuged. Both layers were separated. As the goserelin acetate was soluble in water but not in DCM, the drug content in aqueous solution was analyzed by using HPLC at 220 nm with further dilutions against appropriate blank.

The amount of the drug entrapped in the microspheres was calculated using the formula:

Encapsulation efficiency = Actual weight of drug in sample x 100

Theoretical weight of drug in sample

Scanning electron microscopy

Microspheres were observed and photographed with scanning electron microscopy (SEM) (Using Hitachi-S-3700N). Scanning electron microscopy was carried out to study the morphological characteristics of Goserelin acetate PLGA microspheres. The samples for the SEM analysis were prepared by sprinkling the microspheres on one side of adhesive stub. Then the microspheres were coated with gold (100A°) before microscopy. Finally the morphology of the microspheres was observed with the scanning electron microscopy.

Particle size analysis

The average particle size of the optimized formulations was measured using Malvern instruments**.**

***In-vitro* drug release**

The in-vitro drug release from the microspheres was carried out by using a regenerated cellulose membrane dialysis apparatus Float-A-lyzer.2ml of microspheres suspension containing known amount of drug was placed in the Float-A-lyzer and this was placed in 50ml of PBS (pH 7.4), maintained at 37^{o}C and stirred with the help of a magnetic stirrer. Aliquots (2ml) of release medium were withdrawn at different time intervals and the sample was replaced with fresh PBS (pH 7.4) to maintain constant volume. The samples were analyzed for drug content by HPLC at 220nm. Upon completion of one week, the complete medium was withdrawn and replaced by fresh medium to avoid saturation of the medium.

***In-vitro* drug release kinetics**

The obtained data was fitted into various kinetic models like zero order, first order, Highuchi model and korsemeyer equation/ peppas model in order to describe the kinetics and mechanism of drug release from the microsphere formulations.

Stability studies

To assess the physical and chemical stability of the microspheres, stability studies were conducted for 3 months under various storage conditions mentioned in ICH guidelines. The optimized formulation was placed in vials and stored at 25±2^{0}c/ 60±5% RH. After 90 days the formulations were checked for physical appearance and drug content

RESULTS AND DISCUSSIONS

Effect of polymer concentration

As the amount of polymer increased, the particle size of Goserelin acetate was found to increase and encapsulation efficiency was found to decrease (Table1 and Table 2). The polymer matrix might have saturated with Goserelin acetate after a certain optimized concentration. Upon further increase in polymer, the free drug tends to escape from the polymer and therefore the encapsulation efficiency is found to decrease.

Effect of Organic Solvent

Two organic solvents namely Ethyl acetate and DCM were studied. When the polymer was dissolved in ethyl acetate, encapsulation efficiency was found to decrease because of its high boiling point (55^{o}c) which must have resulted in slow solvent removal rate (Table1). When the polymer was dissolved in DCM (BP- 40^{o}c), high encapsulation efficiency was obtained indicating faster rate of solvent removal. DCM is also more soluble in water and its solubility allowed relatively fast mass-transfer between the dispersed and the continuous phase and led to fast precipitation of the polymer.

Effect of stirring speed

When the secondary homogenization speed (rpm) was decreased as seen in Table 2 in case of F8, F9 and F10 formulations, the particle size was also found to decrease. When homogenization speed was increased to 8000 rpm, particle size was found to be 81 micro meters which was optimized. In F11 and F12 formulations, as the primary homogenization speed was increased from 10,000 rpm to 14,000 rpm, further reduction in the particle size of microspheres was observed.

Formulation optimization

The microspheres were prepared by using double emulsion technique using homogenizer. Formulations were optimized basing on particle size, entrapment efficiency and *in vitro* release profiles. As shown in Table 2, the optimized formulation was F10 with Goserelin acetate 100mg, 500 mg of polymer, 50ml of 1%PVA, 2ml of DCM and 0.2ml of aqueous phase volume.

Evaluation of Microspheres

FTIR studies

The FTIR spectra of the plain drug,polymer and the formulation are shown in Fig 1,2 and 3.The position of peak in FT-IR spectra of pure

drug was compared with those in FT-IR spectra of drug with the polymer. It was observed that there was no disappearance or significant shift in the peak position of drug in any spectra of drug with polymer which proved that the drug and polymers used for the study are compatible.

Percentage Yield, Entrapment Efficiency & mean particle size

The percentage yield, encapsulation efficiency and mean particle size were determined for all the formulations from F1 to F12. The percentage yield for optimized formulation (F10) was found to be 73.3%, encapsulation efficiency was found to be 85% and rounded mean particle size was found to be 81µm.

Mean particle size distribution

The obtained mean particle size distribution values for all the formulations were rounded to the nearest whole number as seen in Table 3. As the polymer concentration decreased the particle size also was found to decrease.

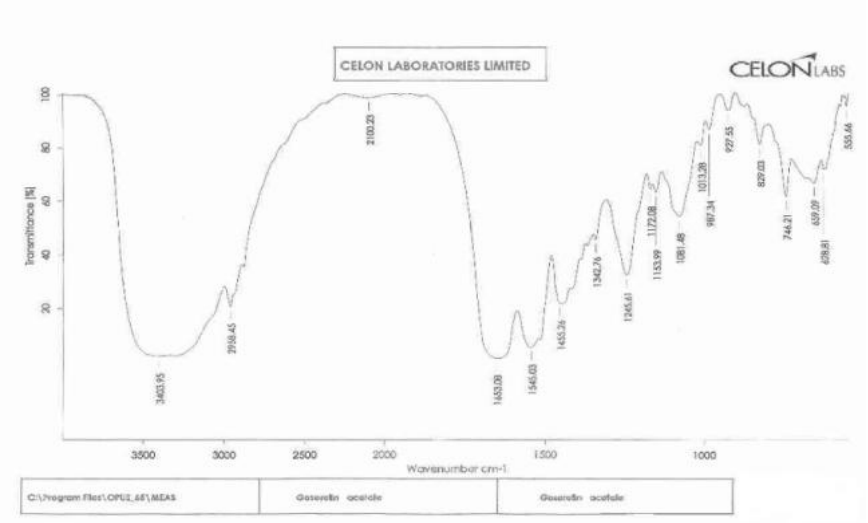

Fig. 1: FTIR Spectrum of Goserelin acetate plain drug

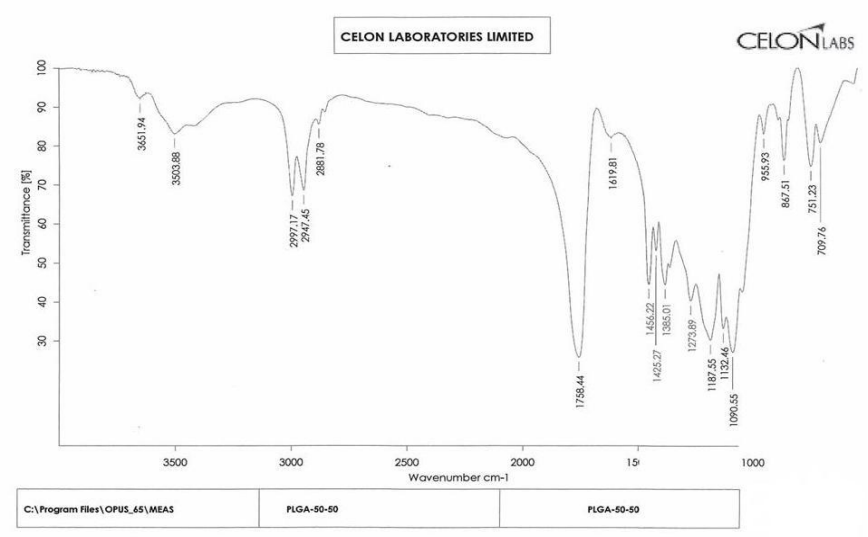

Fig. 2: FTIR spectrum of Poly (Lactide-co- Glycolide) acid 50-50

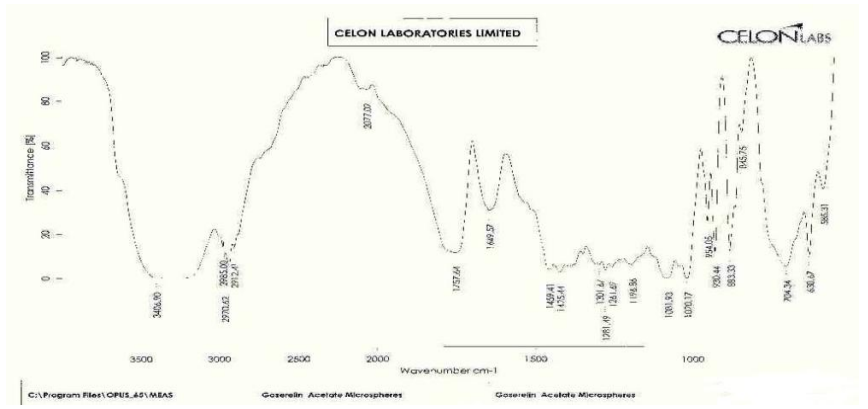

Fig. 3: FTIR Spectrum of Goserelin acetate microspheres

Table 3: Percentage yield, entrapment efficiency and rounded mean particle size of various formulations.

Code	Percentage yield	Entrapment Efficiency%	Rounded mean particle size
F1	72.7%	80	85
F2	68.8%	75	81
F3	63.3%	64	45
F4	48.3%	55	55
F5	51.6%	60	65
F6	70.8%	80	90
F7	71.6%	81	100
F8	70.2%	80	72
F9	70.8%	82	70
F10	**73.3%**	**85**	**81**
F11	69.1%	72	60
F12	58.3%	65	50

Although lower particle size was observed with ethyl acetate, DCM was selected based on the %Entrapment Efficiency. The primary homogenization speed did not seem to have any effect on the particle size and entrapment but increase in the secondary speed was found to increase the particle size although in the micrometer range. However F10 was optimized based on the particle size of 81µm as shown in Fig 5 and highest entrapment efficiency of 85%.

Scanning electron microscopy

SEM was performed on optimized goserelin acetate microspheres at 120X magnification. The SEM picture showed that the shape of the microspheres was spherical and the coated surface was clearly visible as seen in Fig 4.

In vitro release studies

The release profiles showed a characteristic initial burst release followed by a lag period and further initiation of controlled release. After the initial lag, a nearly linear and continuous release was observed over 12- 18 days, around 95% at day 30. Comparison of *in vitro* drug release profiles for some formulations F7, F9 and F10 showing better entrapment efficiency are shown in Fig 6 and the data is shown in Table 4.

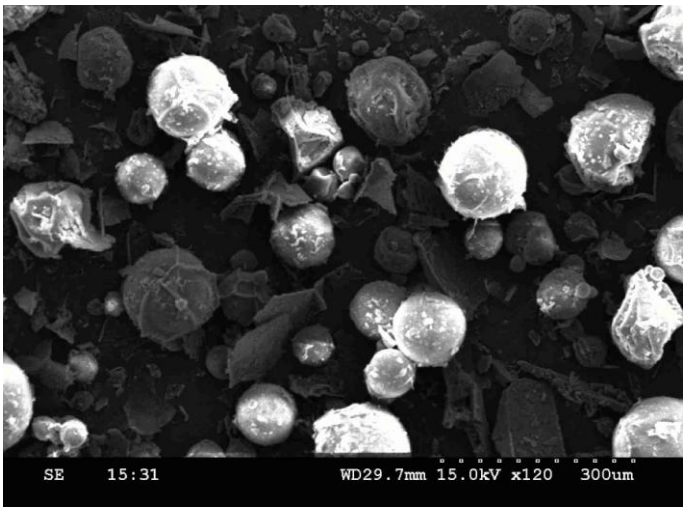

Fig. 4: SEM picture of optimized formulation of Goserelin acetate microspheres.

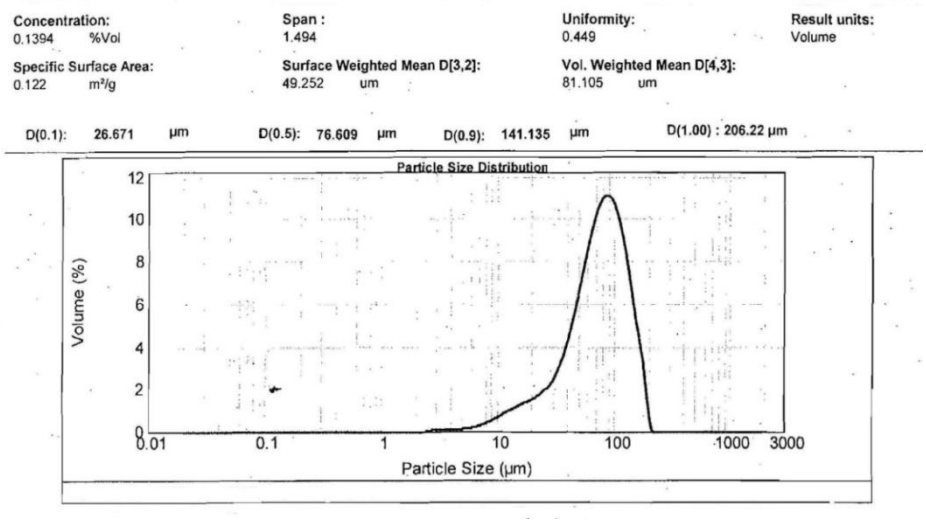

Fig. 5: Mean Particle Size for optimized formulation of Goserelin acetate microspheres

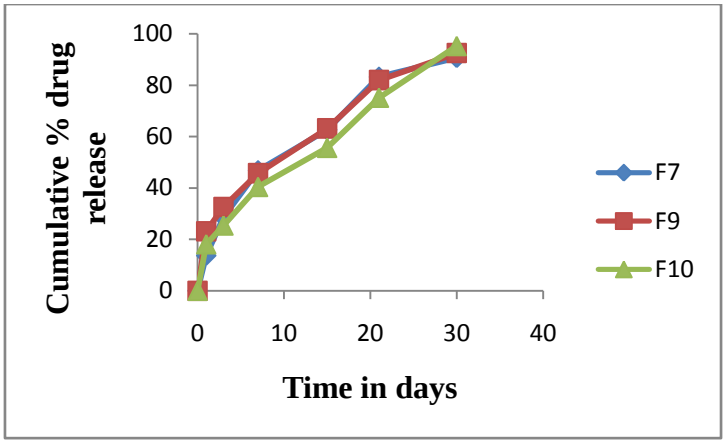

Fig. 6: Comparison of in-vitro drug release profiles of goserelin acetate from formulations F7, F9 & F10

Table 4: In-vitro drug release profiles of goserelin acetate from the formulations F7, F9 &F10

Time (Days)	Cumulative% drug release F7	F9	F10
0	0	0	0
1	13.7	23.1	18.1
3	28.6	32.6	25.4
7	46.8	45.7	40.4
15	62.6	63.1	55.6
21	83.2	81.9	75.1
30	90.6	92.4	**95.2**

Table 5: Curve Fitting Data of Drug Release Profiles for Optimized Formulations F8, F9 & F10

Formulation Code	Zero order (r^2)	First order (r^2)	Higuchi (r^2)	Korsmeyer Peppas (n)
F7	0.936	0.982	0.987	0.990
F9	0.973	0.973	0.990	0.987
F10	0.992	0.894	0.977	0.979

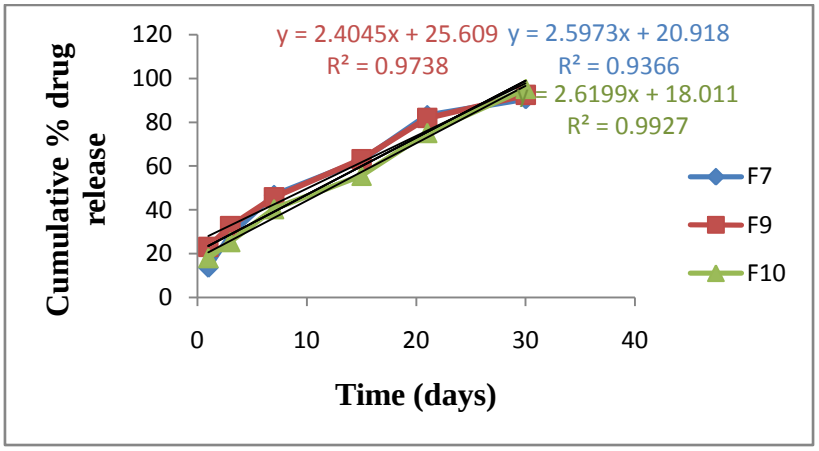

Fig. 7: Comparison of Zero order drug Release profiles of optimized formulations F7,F9&F10

Table 6: Accelerated stability data of Goserelin acetate microspheres at 25±2⁰C/ 60± 5%RH

Test	0 days	15days	30 days	45 days	60 days	75 days	90 days
Description	White to off-white	White to off-white	White to off-white	White to off-white	White to off-white	White to off-white	White to off-white
Assay of F7 formulation	81.3%	80.5%	79.2%	78.3%	76.4%	75.1%	74.3%
Assay of F9 formulation	82.4%	82.2%	81.4%	80.8%	79.5%	78.4%	77.3%
Assay of F10 formulation	85.3%	85.1%	84.5%	83.6%	83.2%	82.3%	81.2%

In vitro release kinetics

The release kinetics of F7, F9, F10 formulations was studied and fitted into various models such as Zero Order, First Order, Higuchi and Korsmeyer Peppas as shown in Table 5.The zero order release is shown in Fig 7. From the data it can be said that F10 formulation followed zero order kinetics based on the R^2 value.

Stability studies

Accelerated stability studies of Goserelin acetate microspheres at temperature 25±2^{0}C/60±5% RH as per ICH guidelines were studied for 90 days. The assays and appearance of samples were determined as a function of the storage time. There was no color change in the physical appearance and assay was found to be 81.2% after 90 days. From the data, it is observed that there was negligible change in the drug content indicating chemical stability.

CONCLUSIONS

In the present study, attempts were made to prepare Goserelin acetate microspheres for controlled release by solvent evaporation technique using PLGA 50-50 polymer. The selection of organic solvent and the stirring rate were found to have played a predominant role in the preparation. The formed microspheres were found to be uniform and spherical in shape. The optimized formulations exhibited 95% in vitro controlled release for one month. From the experimental results it is evident that the controlled release microspheres of Goserelin acetate can be successfully formulated for subcutaneous administration in the treatment of prostate and breast cancer with fewer side effects.

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