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Research Article

Optimization of Formulation Parameters for Development of Depot Injection Containing Hormone

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ABSTRACT
Leuprolide acetate is used in treatment of choice for advanced prostate cancer and Due to shortcomings associated with subcutaneous injection of Leuprolide acetate an attempt was made to develop nanoparticle based depot injection for the same. Processing parameters i.e. concentration of surfactant, volume of internal aqueous phase of primary emulsion and volume of external aqueous phase of secondary emulsion, sonication amplitude and sonication time and polyethylene glycol -200 concentrations in internal aqueous phase were optimized. 4% w/v span-83, 0.35 % w/v polyvinyl alcohol to external aqueous phase, 250 µl as the volume of internal aqueous phase of primary emulsion and 40 ml as volume of external aqueous phase of secondary emulsion, 80W amplitude as sonication amplitude and 45 second as sonication time and 40 % w/v polyethylene glycol -200 were found as optimum parameters and selected for further studies.
Keywords: Prostate cancer, Leuprolide acetate, Nanoparticle, Depot Injection, Processing Parameters

INTRODUCTION

With rapid advances in genomic research and biotechnology, drug companies are developing new protein and peptide-based compounds such as interleukins, cytokines enzymes and many hormone analogues for a variety of diseases [1]. Currently, there are at least 90 protein or peptide-based products approved for marketing in the US alone that are used in the treatment of cancer, diabetes, multiple sclerosis, and growth deficiencies [2]. However, most of these macromolecules possess short in vivo half-lives due to physical and chemical instability or enzymatic degradation [3, 4]. Therefore, significant opportunities exist for technology solutions that are alternatives to conventional injection, reduce dosing frequency, improve safety and efficacy or improve the stability of the macromolecule[5]. Encapsulation of proteins in biodegradable polymeric devices from which the drug can be delivered locally or systemically for a prolonged period of time has been a promising solution to these problems [6].

Depot injections of peptides are mostly available as freeze dried biodegradable polymeric carriers either nanoparticles or microparticles encapsulating drug to be re-constituted in dispersing media at the time of administration. It is given usually intramuscular or subcutaneous containing pharmacological agent which releases its active compound in a consistent way over a long period of time [7, 8]. Depot injections are either solid or oil based. Depot injections may be available as certain forms of a drug or drug encapsulated in biodegradable polymeric carrier [9, 10].

Advances in polymer science have opened up possibilities for using a wide variety of polymeric materials as drug delivery systems [11]. Biodegradable polymers, by virtue of their ability to degrade in the body naturally, offer enormous advantages over conventional drug delivery systems. It eliminated the need for surgery and also does not elicit any adverse reactions from the body. Polymeric drug delivery systems are mainly intended to deliver the drug over a period of time. Some of the materials that are currently being used/studied for controlled drug delivery include poly (methyl methacrylate), poly (vinyl alcohol), polyacrylamide, polyethylene glycol, polylactic acid, poly-glycolic acid, Poly (D, L-lactide-co-glycolide), and polyanhydrides. Most biodegradable polymers are designed to degrade as a result of hydrolysis of the polymer chains into biologically acceptable and progressively smaller compounds. For example in the case of Poly (D, L-lactide-co-glycolide), the polymer would eventually break down into lactic and glycolic acid, enter the Krebs cycle and further broken down into carbon dioxide and water [12, 13]. Drugs formulated in polymeric devices are released either by diffusion through the polymeric barrier, or by erosion of the polymer material, or by a combination of both diffusion and erosion mechanisms [14, 15]. A wide variety of natural and synthetic biodegradable polymers have been investigated for drug targeting or prolonged drug release [16]. Amongst them, the thermoplastic aliphatic poly-esters like PLA, PGA, and especially PLGA have generated tremendous interest due to their excellent biocompatibility and biodegradability.

Leuprolide acetate is used in treatment of choice for advanced prostate cancer and endometriosis [17]. It is usually given subcutaneously with daily dose of 1mg. Depot injection of Leuprolide acetate may offer advantages over conventional daily subcutaneous injection such as improvement in bioavailability and patient compliance, reduction in dose required for treatment and thereby probably reduce cost of therapy.

Aim of present investigation is to optimize formulation parameters for development of depot Injection containing Hormone.Major objective of the investigation is to incorporate a water soluble protein/peptide, in Polymeric nanoparticles and assess in vivo for controlled and prolonged release of drug after IM depot injection.

MATERIALS AND METHODS

Materials

The drug sample was gifted by sun pharmaceutical, materials such as polyvinyl alcohol and polymer were purchased from BASF germany and Boehringer Inglheim

Germany respectively, other materials such as dichloromethane, polyethylene glycol -200, glycerin, potassium dihydrogen phosphate, sodium hydroxide, sodium Chloride and disodium hydrogen phosphate were purchased from s. d. fine chem India whereas, trehalose, methanol and span 83 were purchased from Loba Chemie India, S.D. Fine-Chem India, and National chemicals India respectively.

Selection of surfactant and its concentration:

SPAN-83 was selected as emulsifier for primary emulsion and PVA as stabilizer for secondary emulsion based on their HLB [18] values and biocompatibility.

SPAN-83:

w/o primary emulsions were prepared with different concentration of Span-83 and stability was checked for 3 hours.

Fixed component of emulsion includes,

Volume of aqueous phase (Distilled water-300µl)

Volume of organic phase (DCM-4ml).

PVA:

Blank nanoparticles were prepared using modified w/o/w double emulsification solvent evaporation technique with different concentration of PVA. Stability (Aggregation behavior) of colloidal dispersion of Nanoparticles after complete evaporation of organic phase was checked for 1 day.

Fixed parameters includes,

Volume of aqueous phase (Distilled water): 300µl
Volume of organic phase (DCM): 4ml
Concentration of PLGA: 20 mg/ml of DCM
Volume of outer aqueous phase: 40 ml

Optimization of volume of internal aqueous phase of primary emulsion and volume of external aqueous phase of secondary emulsion

The various batches of nanoparticles were prepared using with different combinations of the vol. of internal aqueous phase and vol. of external aqueous phase while keeping PLGA Conc.(20mg/ml in DCM), drug conc. in internal aqueous phase (4mg/100µl), sonication amplitude (75W), sonication time (45sec.) constant. The study design is illustrated in table 1. The formulations were checked for the % Entrapment Efficiency (% EE), Practical drug loading (PDL), Particle size (PS) and poly dispersivity index (PDI) as response variables.

Table1 Study design for optimization of volume of internal aqueous phase and external aqueous phase

Batch no.	Vol. of internal Aqueous phase (µl)	Vol. of external Aqueous phase (ml)	Target Drug Load
A1	150	30	9.375
A2	200	30	12.5
A3	250	30	15.625
A4	300	30	18.75
AA1	250	30	15.625
AA2	250	40	15.625
AA3	250	50	15.625

Effect of sonication amplitude and sonication time

Sonication of primary w/o emulsion in preparation of PLGA nanoparticles by double emulsification solvent evaporation technique is reported. [19] However, stability of protein and peptide on Sonication is matter of consideration. Since Leuprolide acetate is Nanopeptide, effect of Sonication on stability of peptide was ascertained by sonicating aqueous drug solution of known concentration at 90W amplitude for 1min. and analyzed for drug content up to 3 days. Sonicated drug solution show no significant change in drug content up to 3 days.

Sonication parameters viz. Amplitude and Time of Sonication are important parameters for formation of stable primary emulsion which in turn affect the size, PDI and % EE. Sonication Time and Sonication Amplitude were optimized by preparing different batches with varying combination of these two factors. Study design for optimization Sonication Amplitude and Sonication Time is illustrated in Table 2.

Table 2 Study design for optimization Sonication Amplitude and Sonication Time

Batch no	Sonication Amplitude (W)	Sonication Time (sec.)
B1	60	30
B2	70	30
BB1	80	30
BB2	80	45

Fixed parameters includes

PLGA concentration: 20mg/ml.
Drug conc. in internal aqueous phase: 4mg/100µl
Volume of internal aqueous phase (Distilled water): 300µl
Volume of organic phase (DCM): 4ml
Volume of outer aqueous phase: 40 ml

Optimization of PEG-200 as stabilizer in internal aqueous phase

Leuprolide acetate was found to be stable over period of two months at 37°C in non-aqueous protic solvent PEG. Increasing the viscosity of internal aqueous phase was found to increase the % of entrapped drug in PLGA nanoparticles prepared by double emulsification solvent evaporation technique. Therefore, PEG-200 act as stabilizer for drug and will increase % EE by increasing viscosity of internal aqueous phase. Optimum concentration of PEG-200 was determined by preparing four batches with different concentration of PEG-200 while keeping other parameters constant. Three different concentration of PEG-200 used were 20%w/v, 30%w/v, 40%w/v and 50%w/v to internal aqueous phase.

Fixed parameters includes

PLGA concentration: 20mg/ml of DCM
Drug conc. in internal aqueous phase: 6mg/0.1ml
Volume of internal aqueous phase (Distilled water):- 250µl
Volume of organic phase (DCM): 4ml
Volume of outer aqueous phase: 40ml
Sonication time and amplitude: 45 sec. and 80W respectively

RESULT AND DISCUSSION

Surfactant and its concentration

From the results shown in Table 3, 4% w/v concentration of span-83 was found to give stable emulsion and was selected as optimum concentration for this study. Results shown in Table 4 reveal that 0.35 % w/v PVA to external aqueous phase inhibit the aggregation of nanoparticles [20], so it was selected as optimum concentration for this study.

Table 3 Effect of concentration of Span-83 on stability of primary emulsion

Conc. of SPAN-83 % w/v to organic phase	Stability of primary w/o emulsion after 3 hrs.
2%	Unstable
3%	Unstable
4%	Stable

Table 4 Effect of concentration of PVA on stability of Secondary emulsion

Conc. of PVA % w/v to external aqueous phase	Stability of w/o/w emulsion after 2 day.
0.15%	Aggregation
0.25%	Moderate Aggregation
0.35%	Stable

Volume of internal aqueous phase of primary emulsion and volume of external aqueous phase of secondary emulsion

Results obtained from optimization study of Vol. of internal aqueous phase of primary emulsion and vol. of External aqueous phase of secondary emulsion are shown in Table 5. Result of batch A1 to A3 shows negligible decrease in % EE but marked increase in PDL as the vol. of internal aqueous phase of primary emulsion increases from 150 µl to 250 µl. Further increase in the vol. of internal aqueous phase from 250 µl (Batch A3) to 300 µl (Batch A4) results in significant decrease in % EE but negligible decrease in PDL. Size and PDI of Batch no A3 were also satisfactory. Therefore, 250 µl as the vol. of internal aqueous phase of primary emulsion was optimized for further study. Result of Batches AA1 to AA3 shows negligible decrease in % EE and PDL as the vol. of external aqueous phase of secondary emulsion increases from 30 ml to 50 ml. 40 ml was selected as optimum vol. of external aqueous phase of secondary emulsion based on size and PDI.

Sonication amplitude and sonication time

Since stability is matter of concern, minimum time and lowest amplitude of Sonication at which nanoparticles gives good size, PDI and % EE was selected for this study. Effect of Sonication Amplitude and Sonication Time is illustrated in Table 6. From the results tabulated in Table 6, 80W amplitude and 45 second time were selected as Sonication parameters for this study.

Effect of Polyethylene glycol-200 as stabilizer in internal aqueous phase

Polyethylene glycol-200 decrease diffusion of drug into external aqueous phase by increasing the viscosity of internal aqueous phase thus act as stabilizer for drug. Therefore use of PEG-200 in internal aqueous phase as stabilizer will increase PDL and % EE. Effect of PEG-200 as stabilizer in internal aqueous phase in preparation of nanoparticles by modified double emulsification solvent evaporation technique is shown in Table 7.

Table 5 Effect of volume of internal aqueous phase of primary emulsion and volume of External aqueous phase of secondary emulsion

Batch no.	Vol. of internal Aqueous phase (µl)	Vol. of external Aqueous phase (ml)	Target Drug Load	Practical Drug Load	% Entrapment Efficiency	Size (nm)	PDI
A1	150	30	9.375	3.89	41.46	192.53	0.213
A2	200	30	12.5	5.09	40.70	234.61	0.216
A3	250	30	15.625	6.17	39.52	267.30	0.264
A4	300	30	18.75	6.07	32.4	304.84	0.321
AA1	250	30	15.625	6.17	39.52	267.30	0.264
AA2	250	40	15.625	6.10	39.30	257.62	0.163
AA3	250	50	15.625	5.96	38.15	259.31	0.159

Table 6 Effect of Sonication Amplitude and Sonication Time

Batch no	Sonication Amplitude (W)	Sonication Time (sec.)	% Entrapment Efficiency	Size (nm)	PDI
B1	60	30	36.02	312.26	0.667
B2	70	30	38.67	296.17	0.521
BB1	80	30	39.06	276.90	0.386
BB2	80	45	38.94	261.76	0.153

Table 7 Effect of PEG-200 as stabilizer in internal aqueous phase

Batch no.	Conc. of PEG-200 in internal aqueous phase (% w/v)	PDL	% EE	Size (nm)	PDI
D0	0	7.08	37.80	263.7	0.169
D1	20	7.41	39.26	286.77	0.176
D2	30	7.56	40.37	322.15	0.241
D3	40	8.39	44.76	365.3	0.296
D4	50	8.45	45.09	406.90	0.331

Results shows that % EE, PDL and size of nanoparticles increases significantly as conc. of PEG-200 in internal aqueous phase increases from 20 % v/w to 40 %v/w. Further increase in conc. of PEG-200 in internal aqueous phase shows nonsignificant difference in % EE and PDL. Therefore batch D3 with 40 % w/v PEG-200 in internal aqueous phase was selected as optimum as concentration.

CONCLUSION

In present investigations an attempt was made to curtail the problems associated with the therapy of leuprolide acetate in management of advanced prostate cancer by developing novel formulation of depot injection containing leuprolide acetate hormone. The results of the investigation conclusively demonstrated optimization of formulation parameters of depot injection containing leuprolide acetate hormone.

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