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OPTIMIZATION OF FORMULATION PARAMETERS FOR LEUPROLIDE ACETATE LOADED DRUG DELIVERY SYSTEM

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Abstract:

Leuprolide acetate is peptide indicated for many cancers especially prostate cancer. It has disadvantages like higher solubility, instability and low drug loading during formulation. The objective of this study was to evaluate the effect of process (homogenization speed and evaporation type and time, temperature) and formulation (surfactant type and concentrations) variables on the preparation of leuprolide acetate loaded PLA microparticles using double emulsion solvent evaporation technique. The best formulation was selected on the basis of particle size and drug loading. The final formulation was also characterized for its morphology and interaction pattern through FT-IR and DSC. It was observed that the formulation consists of 0.15% of Poloxamer 188(minimal surfactant concentration), cooled below 15°C with high external phase volume, high stirring speed with extended time of stirring with overhead evaporation was effective. The lyoprotectant mannitol with 0.1 and 0.15% concentration had good dispersability with no aggregates. Thus, it was concluded that the particle size of the microparticles could be reduced further and the leuprolide loaded microspheres could be adjusted to nanosize using above optimized conditions.

Keywords: *Leuprolide acetate, microparticles, poloxamer 188, lyoprotectant.*

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INTRODUCTION:

Despite of several advantages, there are few limitations associated with nanodrug delivery systems. Due to intrinsic and extrinsic factors, there may be some variations in rate of controlled release dosage form. For control release parental, low drug loading is limiting the usage. The difference in the release is observed from one dosage form to another and moreover it carries the risk of interacting with blood components and forming complexes on parental drug delivery. Once the carrier is administered, there is difficulty in removing the carrier from the body in case of adverse and toxic event and dose dumping can be also the reason for failure of therapy. For oral delivery, there are heterogeneous factors like food; mucin turnover and the transition rate through gut that varies the rate of drug release. For formulation design, selection of polymer (Castro-aguirre, 2016), surfactant, and process parameters are challenging and reproducibility, scale-up issues are also present.

Leuprolide acetate is a peptide-based drug used in prostate cancer, mammary cancer, endometriosis, and precocious puberty. It is a luteinizing hormone-releasing hormone agonist analog which suppress circulating luteinizing and sex hormones. Leuprolide acetate stimulates gonadotropin secretion in acute doses and produces antagonist action on chronic use at high dose. Peptide-based drugs are found to be better because these drugs don't exhibit any serious side effects. It is a highly water-soluble drug with molecular weight of 1269.47. It is less stable in body fluids and excreted rapidly due to its short biological half-life and low bioavailability through gastrointestinal tract (by oral administration). To overcome this disadvantages, peptide-based drugs are given by intravenous route (IV) and subcutaneous route is preferred for very low half-life molecules to extended duration of action of the peptide therapeutics. Daily injection of therapeutics in case of chronic diseases like cancer is the drawback with patient compliance and there is a need of hospitalization for supervision in case of treatment. To overcome these disadvantages, implants are been introduced for which also care to be taken to avoid infection (Wischke and Schwendeman 2008; Wade et al. 2013; US006036976A).

To overcome all these disadvantages, different strategies were studied to provide patient compliance by self-administrable dosage form by different route of administration like nasal, oral, rectal, and vaginal delivery. Due to the lower bioavailability of delivery system with different strategies, they are not found to be suitable for delivery of potent therapeutics. Therefore, sustained release nano- or micro-formulations have become suitable strategy for long-term delivery of peptide-based drugs. These matrix type drug carriers have extended drug release for longer duration of time and they can be easily administered via oral route after protecting it within suitable carriers viz. biodegradable and non-biodegradable polymers (Okada 1997; Woo et al. 2001). Synthetic polymers are more preferred in comparison to natural polymers due to their additional advantages. Synthetic polymers exhibit highly controlled consistent degradation with limited immunogenicity and infections where natural polymers lack these features. The physical and chemical properties like tensile strength, elastic modulus, and degradation rate are reproducible in case of synthetic polymers. Polymers like poly[α-hydroxy esters], poly[lactic acid] (PLA), poly[glycolic acid] (PGA) and copolymers poly[lactic-co-glycolide] (PLGA), poly acrylic polymers, poly caprolactone are widely used for such preparations (Gentile et al.,2004). These polymers convert into monomers (hydrolytic degradation) by de-esterification and these monomers are excreted from body by natural pathways without causing any toxicity. These polymers are approved by food and drug administration (FDA) (Conn et al. 1995). The formulation of leuprolide acetate also challenging, since it is peptide, the harsh environment during formulation alter its conformation leading to structural instability. Therefore, this study aims in formulating oral nano-drug delivery system after optimizing formulation parameters and characterizing the formulation for peptide stability through which conforming the retention of leuprolide biological activity (Eroğlu et al., 2001)

MATERIALS AND METHODS:

2.1 Pure leuprolide acetate was purchased from PolyPeptide Pvt. Ltd., Mumbai. The polymers PLA, gelatine, poloxamer, and PVA, PMMA, Eudrogit, polycaprolactone are obtained sigma Aldrich. All other chemicals used were of analytical grade and purchased from CDH, Mumbai.

Optimization of Formulation Parameters

The formulation parameters were optimized using poly lactic acid (PLA) polymer which already had proven record. The effect of stirring condition, speed and time, choice of surfactant type/concentration, type of evaporation method, temperature, lyophilizing agents on particle size and drug entrapment which will be useful for developing leuprolide formulation were analyzed (Table 1).

Formulation Method

Leuprolide acetate microspheres were prepared by multiple emulsion solvent evaporation technique (W/O/W) which is one of the most favoured method for commercially production of nano/microsphere. Leuprolide is dissolved in aqueous phase and polymer (PLA- DCM) was dissolved in respective organic phase. Both drug solution and organic phase are mixed together to form primary emulsion (W/O emulsion). The primary emulsion was then emulsified in external aqueous phase in which stabilizer is dissolved to form microparticles (W/O/W emulsion). The multiple emulsion was stirred continuously to evaporate the organic phase so polymer get solidified and drug get encapsulated in polymer matrix. Finally, freeze-dried with cryoprotectant.

Characterization

Particle Size

Dynamic light scattering method (Malvern ZetasizerNano ZS, etc.) was used for measuring of both size and surface charge of prepared formulations.

Morphology

5 mg of lyophilized formulation is dispersed milli-q water and placed on slide which is covered by cover slip before taking images under microscope using 10× magnification (Nikon polarizing microscope).

Percentage Encapsulation efficiency

The drug entrapment efficiency was calculated by centrifugation method (indirect method). After formulation, the preparations were centrifuged and supernatant were collected to determine free drug content using UV-Vis spectrophotometry and calibration curve method.

The drug entrapment efficiency is calculated using the formula as follows:

$$\% \text{ Encapsulation efficiency} = \frac{\text{Initial drug amount} - \text{free drug amount in supernatant}}{\text{Initial drug amount}} \times 100 \quad (1)$$

Percentage Drug loading

The drug loading also carried out by above said indirect method but using following formula:

$$\% \text{ Drug loading} = \frac{\text{Amount of drug entrapped in theformulation}}{\text{Total amount of formulation obtained}} \times 100 \quad (2)$$

FT-IR Analysis

Leuprolide acetate drug is triturated with potassium bromide and Fourier transformer infrared (FTIR) spectroscopy (IR prestige-21 FTIR shimadzu).

Differential Scanning Calorimetry

Two grams of drug and formulation were crimped in aluminum pan. Analysis was performed using DSC-60 plus shimadzu, the set temperature rate was 10°C/min, and the measurement was performed from 30°C to 300°C.

RESULTS AND DISCUSSION:

Optimization of Formulation Parameters (Table 1)

In the microsphere preparation utilizing (homogenization of primary emulsion at 5000 rpm for 5 min) 0.1% and 0.25% PVA (poly vinyl alcohol) solution and organic solvent evaporation by Rota-evaporator, aggregation of polymer was observed with 97% of unentrapped drug in external phase. Therefore, change in surfactant from PVA to poloxamer was preferred which might stabilize the microparticles and reduce aggregation of polymer. There was no aggregation of micron size particles but the unentrapped drug was 92% in external phase when preparation utilized 0.25% poloxamer solution (organic solvent evaporation by Rota-evaporator). Microsphere preparation utilizing 0.1% poloxamer (reduced surfactant concentration) and 0.2% there was slight aggregation and no aggregation of the particles respectively and unentrapped drug was determined to be 64% for both. Particle size of centrifuged particles was 5.84 µm. Trial when primary emulsion was cooled to 15°C to increase viscosity (to enhance entrapment efficiency), there was no aggregation and unentrapped drug was determined to be 3 mg (50%). This strongly suggest that the peptide drug formulation will be effective only when the preparation is carried out at low temperature preferably below 15°C particularly at 4–8°C which is the favorable temperature of most of protein and peptide. In another trial of utilizing 0.2% poloxamer solution, the homogenization was avoided completely in case of primary emulsion formation. It was not effective and thus proves essentiality of homogenization for primary emulsion. In one more trial gelatin was added to aqueous phase of primary emulsion to observe the improvement in the entrapment of drug but entrapment was very poor (4.2 mg).

Table 1: Various trials with formulation factors

Trials	Excipients concentration	Process condition	Results	Inference
001	PVA (0.1%)	Homogenization:5000 rpm/5 min; Rota evaporation: 383 Mbar pressure, 1 h 30 min	Aggregation	Increase in surfactant may stabilize, decrease aggregation
002	PVA (0.25%)		97% drug unentrapped	Preferred change in surfactant
003	Poloxamer (0.25%)		No aggregation but 92% unentrapment	Change in surfactant had effect in reduction of aggregation, for higher entrapment cooling of primary emulsion preferred
004	Poloxamer (0.25%)	Primary emulsion formed and cooled to 15°C	3 mg untrapped drug with particle size of 7 µm	Cooling increased entrapment, but surfactant concentration have to be altered to reduce particle size
005	Poloxamer (0.1%)		Slight aggregation untrapped drug 3.85 mg with particle size of 5.8 µm	Change in surfactant concentration preferred
006	Poloxamer (0.2%)		No aggregation with 50% of untrapped drug	Change in surfactant concentration effect entrapment Homogenization effect was checked
007	Poloxamer (0.2%)	Homogenization of primary emulsion in Step I was avoided. Just stirring 800 rpm for 10 min	Aggregation, no particle formation. Direct addition & stirring for 10 min at 800 rpm was not effective	Homogenization is essential
008	Poloxamer (0.2%) + gelatin	Homogenization	No aggregation but larger particle size with 4.2 mg of free drug	External phase volume and temperature modification
009	0.25% PVA in 50 ml of external phase + gelatin	Overhead stirring for 2 h at 1000 rpm instead Rota evaporation	No aggregation with 5.3 µm particle size with 1.8 mg of free drug	Higher external phase volume has good effect which allows more space for particle formation
010	0.25% PVA	Without homogenization, over head stirring	Aggregation and no particle formation	Essentiality of homogenization was proved
011	0.25% PVA	With homogenization and below 15°C cooling, and over head stirring	No aggregation with particle size 11.5 µm, unentrapped drug was 48% and foam formed	Might be due to higher stabilizer concentration
012	0.15% PVA		No aggregation of 11 µm particle formation with 50% unentrapped drug. No foam formation	Polaxamer may be the choice for reducing particle size

013	0.25% Polaxamer/ 50 ml external phase		Slight aggregation with 60% unentrapment	Reduction in concentration might effect
014	0.15% Polaxamer		Slight aggregation of 11.6 µm with 55% unentrapment	Decrease of internal phase volume was trial out
015	0.15% Polaxamer 20µl instead of 60µl		Slight aggregation with 755 free drug with 10.2 µm particle size was observed	Decrease in internal phase volume not effective, increase in speed of multiple emulsion might effect
016	0.15% Polaxamer	Increase in speed with over head stirrer to 1200 rpm	No aggregation with reduced particle size and 35% of free drug	Cooling below 15°C, high external phase volume, high speed with extended time of stirring with minimal surfactant concentration is effective
017	Trial with optimized formula	Trial with optimized condition-	No aggregation with nanoparticle formation and 50% drug loading	pre formulation stirring of polymer alone in solvent for 2 h, formulation with higher speed 1200 rpm, extended time to 4 h stirring, maintenance of temperature at 4–8°C until completion of formulation was effective

To study the effect of external phase volume, temperature on microparticles formation and entrapment in primary emulsion and evaporation of organic phase with overhead stirrer instead of Rota evaporator trial with 0.25% PVA (poly vinyl alcohol) solution was carried out. The external phase was kept five times increment in volume than used in previous trials (external phase volume was increased from 10 ml to 50 ml) and gelatin was added to primary emulsion to check improvement in entrapment efficiency. External phase was cooled to 15°C before addition of primary emulsion and organic solvent was evaporated by continuously stirring multiple emulsion under over head stirrer for 2 h. There was no aggregation, the particles formed are 5.3 µm and unentrapped drug was found to be 1.8 mg. Thus cooling and increase in external phase volume has impact on microspheres formation. To check the feasibility of process without gelatin alone utilizing high external phase volume and overhead stirrer, trial with microsphere preparation utilizing reduced concentration of PVA (poly vinyl alcohol) solution as external phase with five times increment in volume (external phase volume was increased from 10 ml to 50 ml) and without gelatin was carried out. There was no aggregation with size of 11 µm, unentrapped drug was 50%, but there was foam formation. To check the feasibility of process with poloxamer as stabilizer instead of PVA utilizing high external phase volume and overhead stirrer, trial with microsphere preparation utilizing reduced poloxamer solution 0.15% (PVA is replaced with increased external phase volume (50 ml) was carried out. There was slight aggregation with particle size of 11.6 µm and unentrapped drug was 55%. Trials also with reduced quantity of internal aqueous phase utilizing high external phase volume and overhead stirrer was trial out which had no improved quality of microparticles.

Table 2: Effect of PLA concentration on particle size and encapsulation efficiency

S. No	Excipient	Wt in mg	Wt in mg	Wt in mg
1	PLA	25 mg	20 mg	30 mg
2	Leuprolide drug solution in milli-Q	30 µl (=3 mg)	30 µl (=3 mg)	30 µl (=3 mg)
3	DCM	1 ml	1 ml	1 ml
4	Poloxamer solution 0.15%	25 ml	25 ml	25 ml
	Parameters	Results		
5	Particle size	6.5	6.1	6.9
6	Entrapment	38.3	37.8	38.8

Change in the concentration of polymer in ±20% have no significant effect on the particle size and entrapment efficiency of the drug. Alteration of polymer in minute concentration during process may not effect the formulation to large extent.

There was no aggregation with size of 8.2 µm with unentrapped drug 35% was observed when following condition was utilized. Therefore, trial with microsphere preparation utilizing 0.15% poloxamer solution (reduced stabilizer/surfactant concentration), with homogenization speed of 5000 rpm for 5 min, increased external phase volume (50 ml) along with cooling below 15°C and organic solvent evaporation by continuous stirring of multiple emulsion under overhead stirrer for 2 h with increased speed (1000 rpm) was optimized as formulation parameters. Trial with stirring of polymer alone for 2 h for 1000 rpm before addition of drug solution, and stirring of multiple emulsion for 2 h at 1000 rpm results in size reduction to nanosize and 50% of drug loading which is utilized for further processing.

Table 3: Effect of poloxamer solution on particle size and encapsulation efficiency

S. No	Excipient	Wt in mg	Wt in mg	Wt in mg
1	PLA	25 mg	25 mg	25 mg
2	Leuprolide drug solution in milli-Q	30 µl (=3 mg)	30 µl (=3 mg)	30 µl (=3 mg)
3	DCM	1 ml	1 ml	1 ml
4	Poloxamer solution 0.15%	25 ml	20 ml	30 ml
	Parameters	Results		
6	Particle size	6.5	7.3	6.3
7	Entrapment	38.3	38.9	37.9

Alteration in concentration of poloxamer has high impact on particle size and entrapment. Effect of 0.15% poloxamer solution (external phase) quantity on size and entrapment was studied by altering ±20% volume. Based on results there was no significant effect of volume on size and entrapment efficiency.

The change in the concentration of polymer in ±20% have no significant effect on the particle size and entrapment efficiency of the drug. Alteration of polymer in minute concentration during process may not affect the formulation to large extent. The change in the concentration of DCM has no significant effect on the particle size and entrapment efficiency of the drug. Decrease in DCM concentration is not appropriate as it is difficult to homogenize hence it is not performed. However, increase in DCM concentration in small range do not have any significant effect on formulation. Alteration in concentration of poloxamer solution has high impact on particle size and entrapment. Effect of 0.15% poloxamer solution (external phase) quantity on size and entrapment was studied by altering ±20% volume. Based on results, there was no significant effect of volume on size and entrapment efficiency.

Table 2 Effect of DCM concentration on particle size and encapsulation efficiency

S. No	Excipient	Wt in mg	Wt in mg	Wt in mg
1	PLA	25 mg	25 mg	25 mg
2	Leuprolide drug solution in milli-Q	30 µL (=3 mg)	30 µL (=3 mg)	30 µL (=3 mg)
3	DCM	1 mL	2 mL	3 mL
4	Poloxamer Solution 0.15%	25 mL	25 mL	25 mL
	Parameters	Results		
6	Particle size	6.5	7.3	7.9
7	Entrapment	38.3	37.8	37.0

Change in the concentration of DCM have no significant effect on the particle size and entrapment efficiency of the drug. Decrease in DCM concentration is not appropriate as it is difficult to homogenize hence it is not performed. However increase in DCM concentration in small range do not have any significant effect on formulation.

The effect of process parameters on drug formulation were evaluated using optimized formulation composition. The effect of homogenization speed on optimized formulation was evaluated with ±1000 rpm. There was significant change in particle size and entrapment efficiency by change in homogenization speed. Stirring time on optimized formulation was evaluated with ±1 h. There was alteration in particle size and entrapment efficiency by increasing stirring time. ±200 rpm increase in speed has effect on particle size which was reduced emphasizing the importance of increase in stirring speed and time in formation of micro- to nano-particle size and adequate drug loading. In case of lyoprotectant optimization among mannitol and sucrose with various concentration, mannitol with 0.15% found to be effective as it was free flowing, easily redispersible on shaking and there were no aggregates.

Table 4: Effect of process parameters on drug formulation using optimized formulation composition

Table 4A: Optimized formulation composition

S. No	Excipient	Wt in mg	Wt in mg	Wt in mg
1	PLA	25 mg	25 mg	25 mg
2	Leuprolide drug solution in milli-Q	30 µl (=3 mg)	30 µl (=3 mg)	30 µl (=3 mg)
3	DCM	1 ml	1 ml	1 ml
4	Poloxamer Solution 0.15%	25 ml	25 ml	25 ml

Table 4B Homogenization speed

Homogenizer RPM	10000	9000	11000
Particle size	6.5	6.8	6.2
Entrapment efficiency	38.3	38.5	37.9

Effect of homogenization speed on optimized formulation was evaluated with ±1000 rpm. There is significant effect in particle size and entrapment efficiency change in homogenization speed.

Table 4C Stirring time

Stirring time	2 h	1 h	4 h
Particle size	5.5	6.7	1.0
Entrapment	38.3	38.4	45.0

Effect of stirring time on optimized formulation was evaluated. There was significant affect in particle size and entrapment efficiency.

Table 4D Stirring speed

Stirring speed	800	1000	1200
Particle size	6.5	4.8	3.0
Entrapment	38.3	38.5	43.5

Effect of stirring speed on optimized formulation was evaluated with ±200 RPM. There was no significant effect in particle size and entrapment efficiency by small change in stirring speed. Foam formation was observed slightly high in 1000 rpm and decreased particle size to small extent.

Table 4E Lyoprotectant optimization

S. No	Excipient	Concentration	Observation
1	Mannitol	0.05%	Free flowing, easily redispersible on shaking. There were few agglomerates (doublets and triplets) of particles
2	Mannitol	0.1%	Free flowing, easily redispersible on shaking. There were no aggregates
3	Mannitol	0.15%	Free flowing, easily redispersible on shaking. There were no aggregates
4	Sucrose	0.05%	Free flowing, aggregates were observed on re-dispersion
5	Sucrose	0.1%	Free flowing, aggregates were observed on re-dispersion
6	Sucrose	0.15%	Free flowing, aggregates were observed on re-dispersion

Interaction Studies Proving Drug Encapsulation

The major functional groups present in FTIR of leuprolide acetate (Fig.1) are 3300 cm^{-1} due to NH_2 and NH stretching, stretching at 1670 cm^{-1} due to stretching of carbonyl group (C=O), stretching at 1570 is due to phenyl ring, bending at 1400 is cm^{-1} due to CH_3, CH_2 bending, amide bending is observed at 1500 cm^{-1}. Polylactic acid (PLA) was used to encapsulate leuprolide acetate microsphere formulation the encapsulated drug is masked by PLA in microspheres (Anderson and Shive, 2012). The lactic acid consists of acid group and hydroxyl group as mentioned below. The FTIR of microsphere formulation has stretching at 3300 cm^{-1} which is indicative for hydroxyl group the broadness in peak differentiates the peak of amide group of leuprolide acetate. The carboxyl group of lactic acid has stretching at 1770 cm^{-1} and CH_3 stretching at 2900 to 3000 cm^{-1}. This indicates encapsulation of drug in PLA.

In crystalline materials when heat is supplied at a particular temperature phase transition take place due to breakage in bonds the energy required to change solid state to liquid state is called as heat of fusion and temperature at which change take place is called as melting point. Amorphous substances are solids without any fixed arrangements in particles hence amorphous materials melt at large range of temperature.

Leuprolide acetate is amorphous substance which has low change in melting point where as the PLA which exhibit melting at 150°C to 170°C. Hence there is a variation in peak shape in DSC thermogram of leuprolide acetate formulation as the drug is masked by PLA (Fig 2).

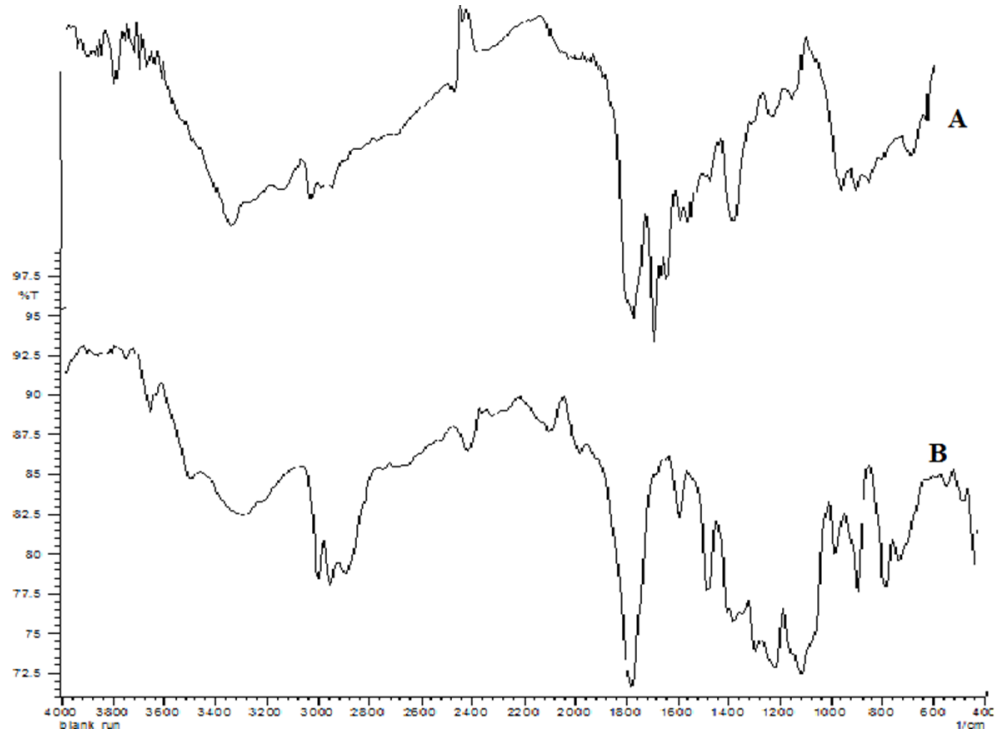

Figure 1: Comparative FT-IR Spectra of leuprolide acetate (A) and optimized formulation (B).

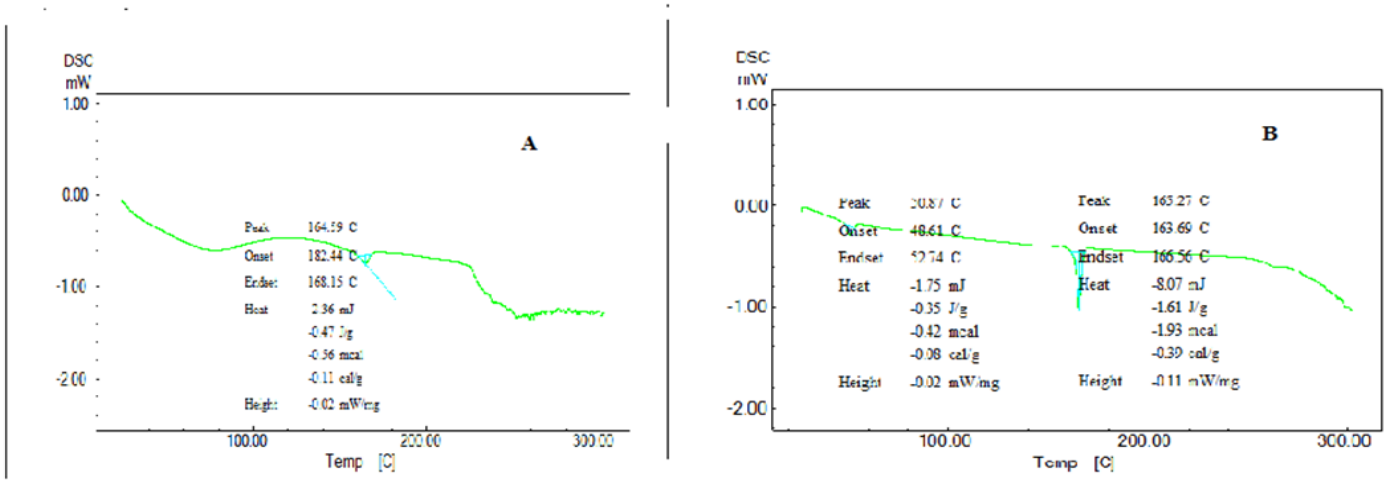

Figure 2: Comparative DSC spectra of leuprolide acetate (A) and optimized formulation (B).

DISCUSSION:

Effect of surfactants and its Concentrations on Particle Size/EE

The formation of an emulsion is the most important step in micro/nanoparticle preparation because the size of the emulsion droplets is directly related to the final nanoparticle size. This emulsion, which is formed by mixing an organic phase consisting of polymer with an aqueous phase containing a surfactant or stabilizer, is broken down into droplets by applying external energy, and these microdroplets lead to microparticle formation upon evaporation of the organic solvent. First, we determined the optimal concentration of surfactant, i.e., polyvinyl alcohol or Poloxamer 188, in the aqueous phase. The concentration of these surfactants was varied to identify the effect on particle size, keeping the other parameters constant as in the standard procedure. The surfactant concentration in the aqueous phase was varied from 0.1% to 0.25% for polyvinyl alcohol or 0.1% to 0.25% for Poloxamer 188; all other processing parameters, including homogenization time and drug concentration, were kept constant.

Poloxamer has advantages over PVA which are used as stabilizing agents. The particle size decreased from 11 µ to 8 µ when concentrations of poloxamer was increased from 0.1% to 0.15%. But at the concentration of 0.25% the particle size was not appreciable, increase in concentration resulted in increase in particle size. It has been known that increasing the viscosity of water exerts the stabilizing effect of Poloxamer 188. The sufficiently high viscosity prevents movement of emulsified multiple microparticles. Poloxamer 188, as a non-ionic emulsifier, has been reported to act as a co-emulsifier during the emulsification process, resulting in smaller particle size and narrower size distribution [Madhavan et al.,2010]. An interaction between Poloxamer 188 and polyester linkage of PLA in methylene chloride solution has been emphasized by an associative thickening effect [Jyothi et al.,2010, Makadia et al., 2010]. The hydrophobic propylene chain of Poloxamer 188 may have aligned on the oil/water interface thus reducing the interfacial tension between the oil and water phase and resulting in lower particles size. But, as the concentration of Poloxamer 188 was increased, the hydrophilic chains of one particle may have interacted with hydrophilic chains of the other particle and this inter-particle interaction of chains may have resulted in agglomeration at higher concentrations.

Poloxamer 188 had essentially had only slight effect on the encapsulation efficiency. The hydrophobic propylene chain of Poloxamer 188 may have aligned on the oil/water interface to cover the oil nanodroplets more efficiently, thus sterically stabilizing the oil nanodroplets. As all the nanodroplets were stabilized by the presence of surfactant and co-surfactant on the interface, drug may not partition out to the aqueous phase and hence less change in the encapsulation efficiency was seen. Along with other formulation parameters only the varied concentrations of Poloxamer 188 have effect on encapsulation efficiency which represents the importance of other formulation parameters also.

The monomers of poloxamer comprising the copolymer blocks are chemically dissimilar (e.g., polar and non-polar), rendering the block copolymers amphiphilic and leading to surface active properties. The block segregation gives rise to interesting and useful structures, which are spontaneously formed in solution (self-assembly). Poloxamer exhibit an amphiphilic character in aqueous solution on the basis of the PEO solubility in water and the PPO insolubility. The PEO blocks are thus hydrophilic, while the PPO block is hydrophobic. Poloxamer with long PPO blocks require lower block copolymer concentrations or temperatures for micellization to occur [ref]. In this study, different concentration of PVA (0.1%–0.15%–0.25%) were investigated. It was shown that the concentration of PVA has significantly changed the size and encapsulation efficiency and its optimum utilization ratio was found to be 0.25% but along with gelatin. The effect of PVA alone not enough to trap the drug inside the matrix. The gelation properties of gelatin give crowding effect which enhances the property of formulation along with the PVA. But the poloxamer itself has the power to give good properties to the formulation,

Effect of Temperature on Particle Size/ Encapsulation Stability/Stability

The temperature-induced emulsification methodology utilizing poloxamers allows for lower processing temperatures. In this procedure, a given drug is mixed with a biocompatible solubilizing agent and then heated under mixing (or other method of homogenization) along with the poloxamer until the entire mixture is a homogeneous melt. The drug and solubilizing agent form an oil phase, which is encapsulated by the poloxamer. The solution is then rapidly cooled below to 15°C kinetically “trap” the emulsion. Upon rehydration, dispersed structures can be formed, the properties of which vary depending on which additives are used. Drug dissolved in water was successfully encapsulated in poloxamer by melting the ingredients together up to 60°C and then rapidly cooling the homogenized mixture. The maximum stable drug load (ratio of the weight of drug solubilized in delivery vehicles to the total weight of the delivery vehicles) was good, with an encapsulation efficiency of around 50%. The cooling to lower temperature not only effect in particle size and encapsulation efficiency, also renter stability of peptide drug leuprolide acetate.

When temperature in high the secondary, tertiary structures will be disturbed and denaturation of peptide results which lead to decrease in activity of proposed. Therefore, maintain the temperature as lower as possible not only after formulation and also during formulation would results in effective particle formation with higher stability.

Effect of Processing Parameters

Effect of Homogenization

The smallest particles were obtained with increasing homogenization speed from 5000 to 10000 rpm for 5 min (Table 4B). Thus, the homogenization power was likely to decrease the nanoparticle size, probably because this energy also increased the energy released by emulsification and decreased the mean

particle diameter.

Effect of Evaporation Method and Time

The method used to remove the organic solvent had a significant influence on nanoparticle size (Table 4C and 4D). We obtained a submicron particle size with a Rotavapor and overhead stirring, but it was least possible to obtain a submicron-sized particle of required size with Rota evaporator. The results show that over head stirring for 2 h had the fastest evaporation rate for organic phase removal, but also resulted in a less particle size than with Rota evaporation. It could be suggested that the evaporation rate for removing organic solvent is also an important parameter. Less time may lead to inefficient evaporation by which residual solvent interfere with further processing and evaluation with two, four, and six hours of stirring showed a spherical shape. Therefore, we concluded that a minimum of six hours of evaporation with magnetic stirring is needed to remove the organic solvent.

CONCLUSION:

The outcomes of this study exposes that the process and formulation variables have influences on effective formulation and could be improved to accomplish the preferred features, such as stability, particle size, entrapment efficiency, and in vitro drug release kinetics. The use of 0.15% of poloxamer, pre-formulation stirring of polymer alone in solvent for 2 h, formulation with higher speed 1200 rpm, extended time to 4 h over head stirring, maintenance of temperature below 15°C preferably at 4–8°C until completion of formulation was found to be effective.

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