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Research Article

OPTIMIZATION OF FORMULATION OF INSULIN MICROSPHERES FOR ORAL DELIVERY

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ABSTRACT

The speculation of this research was to observe whether Eudrajit L or Eudrajit RL microspheres have the potential to serve as an oral carrier for peptide drugs like insulin. Eudragit RL-100 based Insulin loaded Microspheres were prepared by quasi-emulsion solvent diffusion method with polysorbate 20 as dispersing agent in the internal aqueous phase (IAP) and PVA/PVP as stabilizer in the external aqueous phase. The morphology of the Microspheres was studied by scanning electron microscopy (SEM).The mean particle size of formulations SP1-SP4 and PS1-PS4 in the ratios of 3:1, 6:1, 9:1 and 12:1 were found to be between 60-44µm and 62-41 µm respectively. An increase in amount of polyvinyl alcohol (emulsifying agent) from 0.5 % to 1.0 % w/v resulted in decreased production yield and increased mean particle size. An increased amount of emulsifying agent increased the mean particle size from 60 µm to 71 µm and 53 µm to 64 µm for the formulations SP1, PS1 respectively. The production yield was found to be between 70-79% for SP1-SP4, and 68-77% for PS1-PS4. The actual drug content was found to be between 62-81% for SP1-SP4, and 67-83% for PS1-PS4.

KEYWORDS: *Insulin, Oral, Eudrajit L, Eudrajit RL, Microspheres, Hypoglycemic.*

INTRODUCTION

Peptides show the widest structural and functional variation and involve to the regulation and maintenance of all biological processes. Application of formulated therapeutic proteins is very challenging and difficult task. The key to achievement of proteins as pharmaceuticals is to have in place an efficient drug delivery system that allows the protein drugs to gain access to their target sites at the right time and for proper duration. Four factors that must be considered in order to fulfill this goal are pattern of drug release, route of administration, fabrication of formulation and method of delivery.[1]

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The delivery of insulin by non-parenteral routes has gained significant attention over last two decades. The alternate routes explored are ocular [2, 3], nasal [4], buccal [5, 6], rectal [7], pulmonary [8, 9] and oral [10, 11]. Among all alternative routes of administration of insulin, the oral route offers maximum advantage in terms of patient compliance. However, there are several limitations of oral route. These include low oral bioavailability due to degradation in the stomach, inactivation and digestion by proteolytic enzymes in the luminal cavity, poor permeability across intestinal epithelium because of its high molecular weight and lack of lipophilicity.[12-18]

Eudrajit L dissolves at pH above 6, thus it would liberate insulin in small intestine but it will be chances to destroy by trypsin and chymotrypsin. [19-23] Insulin loaded Eudrajit

L microspheres made by quasi-emulsion solvent diffusion method, given orally with a permeation enhancer. Thus a polymer that would liberate the drug at above pH 6 appears to be suitable for oral insulin delivery. Eudrajit L is such type of a polymer. It is an anionic polymer synthesized from methacrylic acid and methyl methacrylate and it has a pH dependent solubility. It is slowly soluble in the region of the digestive tract When used to entrap insulin in microspheres, it is expected to protect insulin from degradation by gastric juice and allow it to be released in the region of the GIT of pH > 6 i.e. large intestine or colon where proteolytic enzymes are low in concentration. [24-26]

MATERIALS

Human insulin, Porcine insulin injection, Eudrajit L 100 & Eudrajit RL 100, Polysorbate 20 Poly vinyl alcohol, Poly vinyl pyrrolidone, Potassium dihydrogen phosphate, Ethanol, Dichloromethane, Isopropyl alcohol, Hydrochloric acid

METHODS

Microspheres preparation using Eudragit RL 100

Eudragit RL-100 based Insulin loaded Microspheres were prepared by quasi-emulsion solvent diffusion method. The internal phase consisted of Eudragit RL-100 (200mg) and triethylcitrate (1% v/v, as plasticizer) dissolved in 5 ml dichloromethane. The drug was added to this with gradual stirring (500 rpm). The internal phase was then poured into 0.5% w/v polyvinyl alcohol (PVA, molecular weight 30,000-70,000) solution in water, the external phase. After 8 hour of stirring the Microspheres were formed due to removal of Dichloromethane from the system. The Microspheres were filtered and dried at 40°C for 12 hours. [27-28] The same method was used for the preparation of Microspheres with Eudragit L-100 except the stirring rate which was kept at 1000 rpm. The compositions of various microspheres formulations are given in Table 1 & 2.

Table 1 Composition of Eudragit RL-100 based microspheres formulations

Name of ingredients	*Formulation code/amount*			
	SP1	***SP2***	***SP3***	***SP4***
Insulin (mg)	40	50	60	70
Eudragit RL-100 (mg)	200	200	200	200
Triethylcitrate (%v/v)	1	1	1	1
Dichloromethane (ml)	5	5	5	5
PVA (% w/v)	0.5	0.5	0.5	0.5

Table 2 Composition of Eudragit L-100 based microsphere formulations

Name of ingredients	*Formulation code/amount*			
	PS1	***PS2***	***PS3***	***PS4***
Insulin (mg)	40	50	60	70
Eudragit L-100 (mg)	200	200	200	200
Triethylcitrate (%v/v)	1	1	1	1
Dichloromethane (ml)	5	5	5	5
PVA (% w/v)	0.5	0.5	0.5	0.5

Effect of drug to polymer ratio on the size of Microspheres

The drug and polymer in the ratios 3:1, 6:1, 9:1, 12:1 were taken to prepare different Microsphere formulations. In each formulation, the amounts of polymer (200 mg), dichloromethane (5 ml), PVA (0.5% w/v) were kept constant. The Microsphere formulations were prepared using mechanical stirrer (Remi RQ1217-D) at a stirring rate of 500 rpm for Eudragit RL-100 based Microspheres and 1000 rpm for Eudragit L-100 based Microspheres for 8 hours.

Effect of the amount of emulsifying agent on the production yield and size of Microsphere

Two different concentrations viz. 0.5 % and 1.0 % w/v were taken to study the effect of amount of emulsifying agent (PVA) on the Microsphere formulations (SP1 and PS1). The effect of emulsifying agent on Microsphere formulations is presented in Table 3.

Table 3 The effect of emulsifying agent on Microsphere formulations

Formulation Code	*PVA (% w/v)*	*Yield (%)*	*Mean Diameter (µm ± S.D.)*
PS1	0.5	73.06±0.21	52.54±5.24
PS1	1.0	64.82±0.82	63.59±5.64
SP1	0.5	79.01±0.57	60.25±5.67
SP1	1.0	61.34±3.67	71.02±4.28

RESULTS AND DISCUSSION

Quasi-emulsion solvent diffusion method was used for preparation of Microspheres because of its simplicity and reproducibility. Moreover, it has advantage of avoiding solvent toxicity. The drug and polymer in the ratios 3:1, 6:1, 9:1, 12:1 were taken to prepare different Microsphere formulations. In each formulation, the amounts of polymer (200 mg), dichloromethane (5 ml), PVA (0.5% w/v) were kept constant. The Microsphere formulations were prepared using mechanical stirrer (Remi RQ1217-D) at a stirring rate of 500 rpm for Eudragit RL-100 based Microsphere and 1000 rpm for Eudragit L- 100 based Microsphere for 8 hours. The various Microsphere formulations namely SP1, SP2, SP3, SP4 containing Drug:Eudragit RL-100 in the ratios 3:1, 6:1, 9:1, 12:1, respectively and PS1, PS2, PS3, PS4 containing Eudragit L100:drug in the ratios 3:1, 6:1, 9:1, 12:1, respectively were prepared.

The effect of various variables like drug to polymer ratio, amount of emulsifying agent on the nature of Microspheres was studied.

Effect of drug-polymer ratio on the size of Microspheres

The morphology of the Microspheres was studied by scanning electron microscopy (SEM). The Microspheres were observed to be spherical and uniform with no drug crystals on the surface. It was noted that drug-polymer ratio has considerable effect on the morphology and size of Microspheres. It was

observed that as the ratio of drug to polymer was increased, the particle size decreased. The mean particle size of formulations SP1-SP4 and PS1-PS4 in the ratios of 3:1, 6:1, 9:1 and 12:1 were found to be between 60-44µm and 62-41 µm respectively. This could probably be due to the fact that in high drug to polymer ratios, the amount of polymer available per Microsphere was comparatively less. Hence fewer polymers surrounded the drug resulting in smaller Microspheres.

Effect of amount of emulsifying agent on the production yield and size of Microspheres

An increase in amount of polyvinyl alcohol (emulsifying agent) from 0.5 % to 1.0 % w/v resulted in decreased production yield and increased mean particle size.

The amount of emulsifying agent significantly effected the production yield and mean particle size. Due to non-ionic nature of the emulsifier some hydrophobic region might have formed which dissolved some of the drug and polymer resulting in lower production yield. An increased amount of emulsifying agent decreased the production yield from 79% to 61%, 73% to 65% for the formulations SP1, PS1, respectively The increase in the amount of emulsifying agent resulted in larger Microspheres, probably due to increased viscosity, wherein larger emulsion droplets formed resulting in larger Microspheres. An increased amount of emulsifying agent increased the mean particle size from 60 µm to 71 µm and 53 µm to 64 µm for the formulations SP1, PS1 respectively.

The production yield was found to be between 70-79% for SP1-SP4, and 68-77% for PS1-PS4. The actual drug content was found to be between 62-81% for SP1-SP4, and 67-83% for PS1-PS4. The encapsulation efficiency ranged from 82-98%. The mean particle size was found to be between 60-44 µm for SP1-SP4, and 53-34 µm for PS1-PS4. The data obtained for various formulations in respect to production yield, actual drug content, and encapsulation efficiency were subjected to t-test at 95% level of significance. No significant difference in relation to these parameters was observed amongst various formulations at $p < 0.05$.

CONCLUSION

Effect of drug-polymer ratio on the size of Microspheres and Effect of amount of emulsifying agent on the production yield and size of Microspheres Optimization of Insulin loaded Eudrajit L microspheres Eudrajit RL and conclude that proper concentration of polymer and emulsification agents give us better formulation and production yield.

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