RESEARCH ARTICLE

Design and Development of Insulin Loaded Microspheres for Oral Delivery

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ABSTRACT

Protease inhibitor-encapsulated human insulin microspheres were developed and evaluated in this research in order to determine the optimal formulation. Manufacturing human insulin microspheres involved coating them with eudragit S-100, stabilizing them with polyvinyl alcohol, and evaporating the solvent from w/o/w multiple emulsions. Excellent encapsulation efficiency and pH-dependent controlled release were demonstrated by human insulin-loaded eudragit L-100 microspheres with a protease inhibitor. This helped encapsulate and transfer insulin to the best absorption zone. Thus, insulin absorption and physiological response increased. Thus, insulin formulations enhance efficacy, making microsphere insulin injection more efficient as a diabetic treatment. Drug-polymer ratio affects microsphere size and form. The effect of emulsifying agent amount on microsphere size and manufacturing yield. Eudrajit RL, microspheres loaded with insulin, are more successfully formulated and manufactured at optimal concentrations of polymer and emulsifying agent.

Keywords: Design, Development, Insulin, Microspheres, Oral Delivery.

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INTRODUCTION

The investigation, advancement, and development of proteins and peptide pharmaceuticals for the treatment of an extensive array of medical conditions have garnered increasing attention over past several decades. In contrast to the production and development of ordinary treatments, these drugs need the most cutting-edge pharmaceutical technology available. Insulin stands out among therapeutic proteins and peptides created by recombinant DNA technology due to the essential role it plays in medicine as well as the considerable pharmacological analysis that has been conducted on it. In the year 1922, Frederick Banting and Charles Best were the first people to successfully extract insulin from the bovine pancreas. Insulin had previously only been successfully extracted from the human pancreatic. People who have diabetes are given exogenous insulin in an effort to make their insulin production as similar as possible to the insulin production that occurs naturally in healthy people. The production of endogenous insulin by the pancreas may be replaced by the production of exogenous insulin by subcutaneous infusion of exogenous insulin, which makes it easier to keep diabetes under control. Research on insulin administration via the subcutaneous route has been considerable, and it has been employed.[1,2]

The approaches used by parents are suitable for the great majority of circumstances in their entirety. Variables such as peripheral hyperinsulinemia, smooth muscle cell proliferation stimulation, and glucose absorption into the lipid of artery walls all possess the capacity to contribute to the progression of diabetes microangiopathy and macroangiopathy. Nevertheless, these factors have the capacity to contribute to the development of diabetic microangiopathy and macroangiopathy. Additionally, parenteral insulin administration is accompanied by a number of additional issues, some of which include the pain that is brought on by daily injections, physiological stress, irritation, high expenses, possible hazards, the risk of infection, and difficulty in efficiently monitoring insulin levels. Because localized insulin deposition at the injection sites might lead to localized fat deposition and hypertrophy, these side effects are possible. Recent developments in the field of recombinant DNA technology have made it feasible to manufacture human therapeutic recombinant proteins that meet very specific requirements. In the process of manufacturing insulin, the use of recombinant DNA technology has led to the synthesis of huge amounts of very affordable insulin crystalline granules. This has made insulin more accessible to people with diabetes.[3-5]

Insulin is a protein that has received a significant amount of attention as a topic of interest because to the vast research done on its non-parenteral delivery. Consequently, a considerable volume of research has been devoted to various facets of insulin

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delivery, culminating in the attainment of significant findings. The pharmaceutical industry has exhibited considerable interest in recent years in the evaluation and investigation of the viability of non-invasive insulin administration methods, in addition to the development of products associated with such methods. Numerous routes of administration are included among the non-invasive techniques for the delivery of drugs. These routes include the buccal, oral, nasal, pulmonary, transdermal, ocular, and rectal systems. The objective of this research endeavor was to examine drug loading capacity and *in-vitro* release properties of human insulin encapsulated with protease inhibitors within eudragit S100 microspheres. For the objective of regulated oral administration, the polymer eudragit S-100, which has a wide range of applications, was employed. Encapsulating human insulin, a therapeutic protein that is extensively utilized was accomplished by a process called w/o/w multiple emulsion solvent evaporations. Insulin is functioning as a typical model for this research project.[6,7]

MATERIALS AND METHODS

Materials

The following materials were provided by Astron Research Limited, Ahmedabad, India: human insulin (Abbott India Ltd), methacrylic acid copolymer (Eudrajit RL 100 &EudrajitL 100), and polysorbate 20. The chemicals potassium dihydrogen phosphate and polyvinyl pyrrolidone were obtained from Astron Research Limited, located near Ahmedabad. HPLC-grade solvents were used.

Methods

Preformulation studies

Before undertaking a systematic process of formulating any concept or idea, it is essential to do thorough research as a first step in the formulation process. The term "Investigation of physical and chemical properties of drug substance alone and combined with the excipients" may be regarded as a unified concept. The main aim of preformulation testing is to provide formulators with data that may be used in the development of stable dosage forms that are both bioavailable and suitable for bulk manufacturing[8-10]. The study has the following objectives,

- The primary objectives are to determine the physical features of the substance and assess its compatibility with the excipients.
- In order to ascertain the kinetic rate profile.

Formulation of microspheres

- *Microspheres preparation using eudragit RL 100*

Microspheres encapsulating insulin and comprised of eudragit RL-100 were manufactured using the quasi-emulsion solvent diffusion method. To generate the internal phase, a solution was prepared by dissolving 1% v/v triethyl citrate (a plasticizer) and 200 mg eudragit RL-100 in 5 mL of dichloromethane. After the drug was added, the mixture was subjected to agitation at a gradually increasing speed of 500 rpm. The internal phase was subsequently added to the outer phase, which consisted of a 0.5% w/v aqueous solution of polyvinyl alcohol (PVA) with a molecular weight ranging from 30,000 to 70,000. Following a period of eight hours characterized by intense agitation, the removal of dichloromethane from the solution resulted in the emergence of microspheres. The microspheres underwent filtration and subsequent drying at 37°C for a period of 12 hours.[11-13]

- *Microspheres preparation using eudragit L 100*

Equipped with insulin, microspheres made of Eudragit L-100 were produced by means of the quasi-emulsion solvent diffusion technique. In the internal phase, which was 1% v/v triethyl citrate and 200 mg of eudragit L-100 diluted in 5 mL of dichloromethane made up the plasticizer. The agitation was carried out gradually while administering the drug at a rate of 1000 rpm. The internal phase was subsequently added to outer phase, which was a 0.5% w/v solution of PVA in water with a molecular weight range of 30,000 to 70,000. Following a period of eight hours of intense stirring, the removal of dichloromethane from the solution resulted in the emergence of microspheres following filtration, microspheres were dried at 37°C for 12 hours. Methodology for the production of the eudragit L-100 microspheres was followed, with the exception of maintaining a stirring rate of 1000 rpm.[14,15]

Optimization of formulation

- *Effect of drug to polymer ratio on the size of microspheres*

Different microsphere formulations were generated by combining the medication with polymer in the ratios of 5:1, 4:1, 3.33:1, and 2.86:1. None of the formulas had altered quantities of polymer (200 mg), dichloromethane (5 mL), or polyvinyl alcohol (0.5% w/v). The microsphere mixtures were made with a Remi RQ1217-D motorized agitator that was turned on for eight hours at a speed of 500 rpm for eudragit RL-100 microspheres and 1000 rpm for eudragit L-100 microspheres[16].

- *Effect of volume of internal phase on production of microspheres*

There are 2 distinct volumes. In order to investigate the impact of the volume of dichloromethane (internal phase solvent) on Microsphere formulations SP1 and PS1, samples of 5 and 10 mL were used.[17]

- *Effect of stirring speed on size of microspheres*

Impact of varying stirring speeds on mean size of microspheres was investigated by conducting experiments at varied stirring speeds. Specifically, stirring rates of 300, 400, and 500 rpm were used for formulations SP1, whereas formulations PS1 were subjected to stirring speeds of 500 and 1000 rpm.[18]

- *Effect of amount of emulsifying agent on production yield and size of microsphere*

A study was conducted to regulate outcome of variable concentrations of emulsifying agent (PVA), specifically 0.5% and 1.0% w/v, on the formulations of microspheres (SP1 and PS1).

RESULTS AND DISCUSSION

Pre-Formulation Study

Determination of melting point

Melting point of insulin was 233°C (451°F).

Differential scanning colorimetric

The melting point of insulin was determined using the technique of differential scanning calorimetry (DSC) (Figure 1). Precisely measured samples, weighing 2 mg each, were put into aluminum pans and then sealed. The experimental procedure included subjecting all samples to a heating rate of 20°C/min during 40 to 430°C, by Shimadzu DSC-60 instrument.

Identification of Insulin

- *Sulfated ash*

Calculation

Weight of crucible + sample: 18.4010 g
Weight of crucible: 17.3956 g
Taken weight of the sample: 1.0054 g

After ignition

Weight of crucible + ash: - 17.3968 g (Before Ignition)
Weight of crucible: - 17.3956 g (After Ignition)
Weight of ash: -0.0012 g.
Sulphated ash% = 0.1193%

- *Loss on drying*

Calculation

Weight of bottle: 27.4151 g
Weight of bottle + sample: 28.4165 g
Taken weight of sample: 1.0014 g

After drying

Weight of bottle + sample: - 28.4165 g (Before Ignition)
Weight of bottle + sample: - 28.3913 g (After Ignition)
Loss in weight: -0.0252 g
Loss on drying %= 2,516%

Determination of absorption maxima (λ_{max})

The λ_{max} of the insulin is shown in Table 1.

Infra red spectroscopy

Table 2 presents the interpretation of the FTIR spectra, which are depicted in Figures 2, 3, and 4.

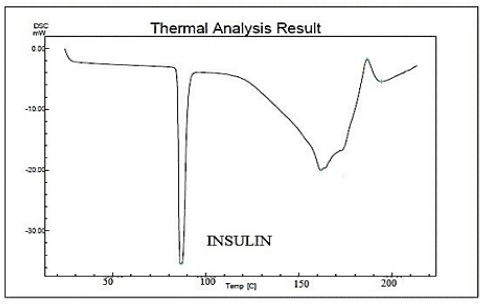

Figure 1: DSC thermogram of insulin

Inference

No displacement or removal of the distinct peaks associated with the medication and polymer was seen in FTIR spectra of both physical combination and improved formulation. This finding implies that there is a lack of interaction between the drug and the polymer[19]. Hence, it may be posited that the pharmaceutical compound stays chemically unmodified and does not suffer any kind of interaction with eudragit L 100.

Formulation of microspheres

Composition containing eudragit RL-100 is displayed in Table 3 and formulation with eudragit L 100 is detailed in Table 4.

Optimization of formulation

- *Effect of stirring speed on size of microspheres*

Study used a light microscope (RXLr-3T, Radical, India) to look at how the rate of stirring affected the size of microspheres. In order to see how the stirring rate affected the shape of the microspheres, the mixture with a lower drug to polymer ratio (3:1) was chosen. Formulations that used eudragit RL-100 had their stirring rate changed between 300 and 500 rpm. Formulations that used eudragit L-100 had their stirring rate

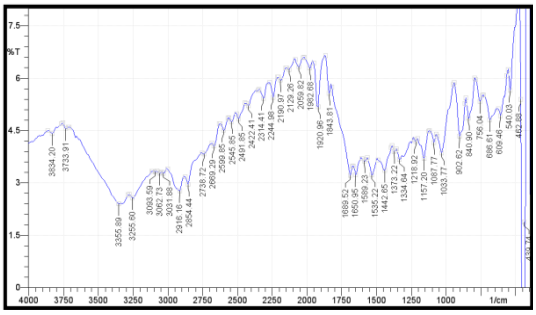

Figure 2: FTIR Spectrum of Insulin

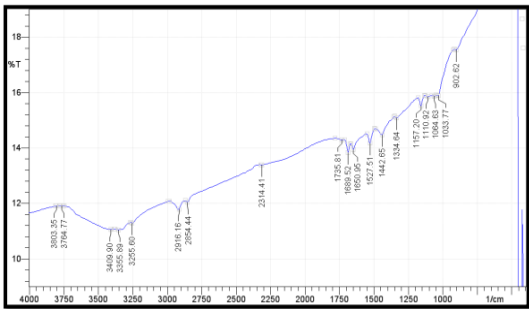

Figure 3: FTIR Spectrum of Eudragit RL 100

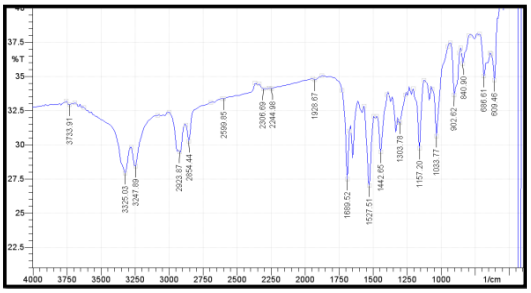

Figure 4: FTIR Spectrum of Eudragit L 100

Table 1: Proportional values of particular parameters used to detect drug

S. No.	*Drug*	λ_{max}*(nm)*
1.	Insulin	276 nm

Table 2: Explanation of FTIR

Drug	*Reported peaks (cm^{-1})*	*Reported peaks (cm^{-1})*	*Inference*
Insulin	850–750	840.10	Para-disubstituted aromatic ring
	1250–1270	1263.40	C-N-H group
	1250–1020	1138.09	C-N stretching
	1300–1000	1223.80	C-O stretching
	1570–1515	1560.38	Amide II band
	1655–1620	1679.90	C=O (amide) stretching
	1725–1700	1720.45	C=O (ester) stretching
	3000–2840	2867.89	C-H stretching
	1950–1980	1967.46	Disulphide bond
	3400–3200	3300.90	O-H stretching
	3500–3100	3413.77	N-H stretching

Table 3: Composition of eudragit RL-100-based microspheres formulations

Ingredients	*Formulation code/amount*			
	SP1	*SP2*	*SP3*	*SP4*
Insulin (mg)	40	50	60	70
Eudragit RL-100 (mg)	200	200	200	200
Triethyl citrate (% v/v)	1	1	1	1
Dichloromethane (mL)	5	5	5	5
PVA (% w/v)	0.5	0.5	0.5	0.5

changed between 500 and 1000 rpm. It was seen how the speed of the stirring affected the formation of microspheres and spread of medicine and polymer in the water phase. The increase in speed resulted in the production of smaller microspheres that exhibited consistent size and spherical shape. The number provided is 177. The microspheres produced using Eudragit RL-100 as the basis material exhibited a spherical shape, with an average particle size ranging from 72 to 60 µm for formulation SP1, upon increasing the stirring rate to 300 to 500 rpm. Spherical microspheres with a mean particle size ranging from 74 to 53 µm were produced using eudragit L-100 as the basis material. This particle size range was achieved by increasing the stirring rate to a range of 500 to 1000 rpm. Table 5 displays the influence of stirring rate on size of microspheres, whereas Figures 5 and 6 visually depicts this correlation.

- *Effect of amount of emulsifying agent on production yield and size of microsphere*

The manufacturing yield was reduced and the average particle size was increased when the concentration of the emulsifying agent polyvinyl alcohol was increased from 0.5 to 1.0% weight/volume. The manufacturing yield and mean particle size were both affected by the amount of emulsifying agent used. The presence of a hydrophobic region might potentially arise as

Table 4: Composition of eudragit L-100 based microsphere formulations

Ingredients	*Formulation code/amount*			
	PS1	*PS2*	*PS3*	*PS4*
Insulin (mg)	40	40	40	40
Eudragit L-100 (mg)	200	200	200	200
Triethyl citrate (% v/v)	1	1	1	1
Dichloromethane (mL)	5	5	5	5
PVA (%w/v)	0.5	0.5	0.5	0.5

Table 5: Result of stirring speed on size of microsphere formulations

Stirring speed (rpm)	*Size (um)*	
	SP1	*PS1*
300	71.73 ± 7.24	-
400	63.91 ± 5.21	-
500	60.25 ± 5.67	74.37 ± 6.88
1000	-	52.54 ± 5.24

(n = 3)*, Mean ± S.D.

Table 6: 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

* Mean ± S.D. (n = 3)

a consequence of the non-ionic properties of the emulsifier, resulting in the dissolution of a portion of the medicine and polymer, hence reducing the overall production yield. The manufacturing yield of formulations SP1 and PS1 saw a decrease from 79 to 61% and 73 to 65%, respectively, as a result of an increase in the amount of the emulsifying ingredient. The production of larger microspheres was achieved by increasing the amount of emulsifying agent, possibly due to the resulting rise in viscosity. This, in turn, led to the generation of larger emulsion droplets and subsequently larger microspheres. The average particle size for SP1 and PS1 formulations rose from 60 to 71 µm and 53 to 64 µm, respectively, due to an increase in the amount of emulsifying agent. The production yield for SP1–SP4 and PS1–PS4 was found to range from 70 to 79% and 68 to 77%, respectively. The drug content of SP1–SP4 was found to range from 62 to 81%, but for PS1–PS4, it ranged from 67 to 83%. The encapsulation efficiency exhibited a range of 82 to 98%. The investigation revealed that the mean particle size for SP1–SP4 varied between 60 to 44 µm, whereas for PS1–PS4 it ranged from 53 to 34 µm. For every formulation, we ran a t-test with a 95% significance level on the data related to production yield, actual drug content, and encapsulation efficiency. No statistically significant differences were seen in these parameters across the various formulations at an importance level of $p < 0.05$. Table 6 presents the impact of the emulsifying agent on the compositions of microspheres.

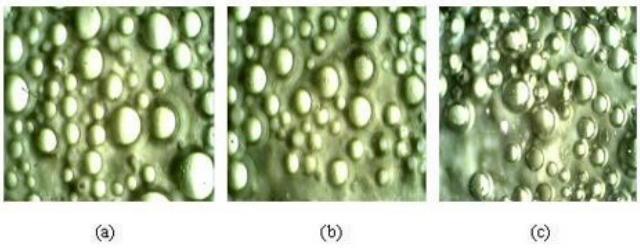

Figure 5: SEM photomicrographs of insulin loaded microspheres (SP1) prepared at different stirring rates (a) 300 rpm; (b) 400 rpm; (c) 500 rpm

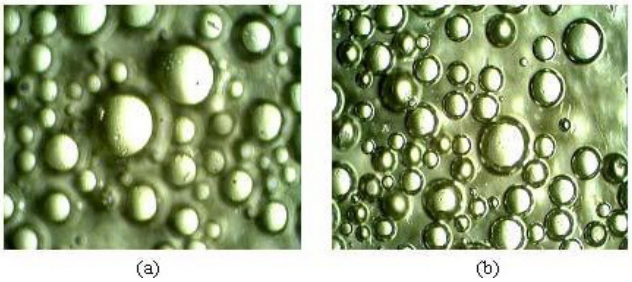

Figure 6: SEM photomicrographs of insulin-loaded microspheres (PS1) prepared at different stirring rates (a) 500 rpm; (b) 1000 rpm

- *Effect of drug-polymer ratio on size of microspheres*

SEM was used to look at the shape of microspheres. There were no drug crystals visible on the microspheres' surface, which shows that they had a uniform circular shape. The research showed that the amount of drug to polymer has a big effect on the microspheres' size and shape. The study found that particle size got smaller as the amount of medicine to polymer went up. During the study, it was found that the average particle sizes of SP1-SP4 and PS1-PS4 mixtures with ratios of 5:1, 4:1, 3.33:1, and 2.86:1 were 60 to 44 µm and 62 to 41 µm, respectively. This phenomenon may be attributed to the considerably lower polymer concentration per microsphere seen at higher drug-to-polymer ratios. The production of smaller microspheres resulted from a reduction in the amount of polymer around the medicine.

- *Effect of volume of internal phase on formation of microspheres*

The research showed that microspheres did not form when the internal phase amount was amplified from 5 to 10 mL. This could be because the inner part has less viscosity, which is a possible explanation. As the dichloromethane amount was increased, spherical drops that were almost like emulsions were seen spreading out evenly in the solvent while it was being stirred. However, upon cessation of stirring, the emulsion droplets exhibited a tendency to adhere to one another and undergo coalescence.[20] Therefore, the formation of microspheres was deemed unattainable. These results show how important it is to keep the dichloromethane level in the right range so that you can change both how the quasi-emulsion droplets form at first and how the drug and polymer solidify inside these droplets later on. A volume of three to five milliliters of dichloromethane was used in the synthesis of microspheres.[21]

CONCLUSION

The objective of the current research endeavor was to address the aforementioned circumstance. It was hypothesized that the use of microspheres in medicine delivery systems specifically designed for the colon would enhance the targeting of bioactive compounds, resulting in improved efficiency and prolonged residence durations. Microspheres refer to polymeric drug delivery systems that possess physiological inertness, non-irritating properties, non-mutagenic characteristics, non-allergenic nature, and non toxic attributes. The researchers have shown significant potential in the effective transportation of pharmaceuticals to their designated sites. Microspheres, also referred to as porous microspheres, are generated by the use of cross-linked polymers, mostly styrene-divinyl benzene or substituted acrylates. The present study included the training and assessment of colon-specific tablet formulations using microspheres. In the experimental procedure, the insulin microspheres were fabricated using a quasi-emulsion solvent diffusion technique, using eudragit RL 100 and eudragit L-100 as the primary materials.

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