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In-Vitro Drug Release Studies Of Insulin Loaded Eudrajit L Microspheres.

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Research Article

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ABSTRACT

The speculation of this research was to observe whether Eudrajit L microspheres have the potential to serve as an oral carrier for peptide drugs like insulin. Eudragit L-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 production yield was found to be between 61-79% for PS1-PS4. In the first hour drug release of different Microsphere formulations SP1- SP4 was noted to be 19-29%. This may be attributed to the drug present in the pores of the Microspheres. The overall cumulative percent release for different Microsphere formulations PS1-PS4 at the end of eight hours was found to be 56-86 %.

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]. 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 [12,13,14,15,16,17,18]. 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 [19,20,21,22,13]. 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 [24,25,26].

MATERIALS AND METHODS

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

Microspheres preparation using Eudragit L 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

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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 compositions of various microspheres formulations are given in Table 1.

Table 1: Composition of Eudragit L-100 based microspheres 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

In-vitro release studies were carried out in USP basket apparatus with stirring rate 50 rpm at 37±0.5 ^{0}C. Initial drug release was carried out in 900 ml of 0.1N hydrochloric acid for 2 hours followed by phosphate buffer pH 6.8 for next 6 hour. Samples were withdrawn at regular intervals and analyzed spectrophotometrically at 249 nm [29]. All the readings were taken in triplicate. The same procedure was followed for *in-vitro* release studies of Insulin loaded Microspheres. The samples were analyzed at 420 nm. The *in-vitro* release data of Insulin loaded Microspheres are given in Table 2 - Table 5.

RESULTS AND DISCUSSION

The different Microsphere formulations of Insulin were subjected to *in-vitro* release studies using USP XX1V dissolution assembly. It was observed that for each formulation the drug release decreased with increase in the amount of polymer. This may be due to the fact that the release of drug from the polymer matrix takes place after complete swelling of the polymer and as the amount of polymer in the formulation increases the time required to swell also increases. The release showed a bi-phasic pattern with initial burst effect. In the first hour drug release of different Microsphere formulations PS1- PS4 was noted to be 19-29%. This may be attributed to the drug present in the pores of the Microspheres. The overall cumulative percent release for different Microsphere formulations PS1-PS4 at the end of eight hours was found to be 56-86 %.

Table 2: *In-vitro* drug release data for formulation PS1

Time in Hrs T	√T	Log T	Cum. % drug released	Log Cum % drug released	Cum. % drug remained	Log Cum. % drug remained
0	0	-	0	-	100	2
1	1	0	16.55±0.002	1.2188	83.45	1.9214
2	1.4142	0.3010	18.97±0.001	1.2780	81.03	1.9086
3	1.7321	0.4771	32.98±0.012	1.5183	67.02	1.8262
4	2.0000	0.6021	38.08±0.010	1.5807	61.92	1.7919
5	2.2361	0.6990	45.48±0.019	1.6578	54.52	1.7366
6	2.4494	0.7782	49.67±0.012	1.6961	50.33	1.7018
7	2.6458	0.8451	52.45±0.001	1.7198	47.55	1.6772
8	2.8284	0.9031	53.45±0.006	1.7279	46.55	1.6679

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Table 3: *In-vitro* drug release data for formulation PS2

Time in Hrs T	√T	Log T	Cum. % drug released	Log Cum % drug released	Cum. % Drug remained	Log Cum. % drug remained
0	0	-	0	-	100	2
1	1	0	19.66±0.023	1.2936	80.34	1.9049
2	1.4142	0.3010	24.56±0.034	1.3902	75.44	1.8776
3	1.7321	0.4771	40.89±0.003	1.6116	59.11	1.7717
4	2.0000	0.6021	50.98±0.056	1.7074	49.02	1.6903
5	2.2361	0.6990	60.54±0.078	1.7820	39.46	1.5966
6	2.4494	0.7782	64.16±0.011	1.8073	35.84	1.5544
7	2.6458	0.8451	66.57±0.012	1.8233	33.43	1.5241
8	2.8284	0.9031	66.67±0.005	1.8239	33.33	1.5229

Table 4: *In-vitro* drug release data for formulation PS3

Time in Hrs T	√T	Log T	Cum. % Drug released	Log Cum % drug released	Cum. % Drug remained	Log Cum. % drug remained
0	0	-	0	-	100	2
1	1	0	27.14±0.009	1.4336	72.86	1.8625
2	1.4142	0.3010	30.42±0.007	1.4832	69.58	1.8425
3	1.7321	0.4771	41.44±0.001	1.6174	58.56	1.7676
4	2.0000	0.6021	51.67±0.010	1.7132	48.33	1.6842
5	2.2361	0.6990	59.59±0.005	1.7752	40.41	1.6065
6	2.4494	0.7782	63.60±0.000	1.8034	36.4	1.5611
7	2.6458	0.8451	68.14±0.001	1.8334	31.86	1.5032
8	2.8284	0.9031	71.69±0.020	1.8555	28.31	1.4519

Table 5 *In-vitro* drug release data for formulation PS4

Time in Hrs T	√T	Log T	Cum. % drug released	Log Cum % drug released	Cum. % Drug remained	Log Cum. % drug remained
0	0	-	0	-	100	2
1	1	0	30.43±0.001	1.4833	69.57	1.8424
2	1.4142	0.3010	36.75±0.009	1.5653	63.25	1.8011
3	1.7321	0.4771	41.49±0.098	1.6179	58.51	1.7672
4	2.0000	0.6021	53.58±0.086	1.7290	46.42	1.6667
5	2.2361	0.6990	61.40±0.077	1.7881	38.60	1.5866
6	2.4494	0.7782	69.45±0.045	1.8417	30.55	1.4850
7	2.6458	0.8451	77.06±0.034	1.8869	22.94	1.3606
8	2.8284	0.9031	82.62±0.023	1.9171	17.38	1.2400

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CONCLUSION

Insulin loaded microspheres Eudrajit L and conclude that proper concentration of polymer and emulsification agents give us better formulation and production yield. This may be due to the fact that the release of drug from the polymer matrix takes place after complete swelling of the polymer and as the amount of polymer in the formulation increases the time required to swell also increases.

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