International Research Journal of Pharmaceutical and Applied Sciences (IRJPAS)
Available online at **www.irjpas.com**
Int. Res J Pharm. App Sci., 2013; 3(4):166-169

Research Article

IN-VITRO DRUG RELEASE STUDIES OF INSULIN LOADED EUDRAJIT RL MICROSPHERES

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Abstract: The speculation of this research was to observe whether 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 production yield was found to be between 70-79% for SP1-SP4. In the first hour drug release of different Microsphere formulations SP1- SP4 was noted to be 15-30%. This may be attributed to the drug present in the pores of the Microspheres. The overall cumulative percent release for different Microsphere formulations SP1-SP4 at the end of eight hours was found to be 61-94 %.
Key**words:** Insulin, oral, 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].

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[2-26].

MATERIALS

Human insulin, Porcine insulin injection, 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 compositions of various microspheres formulations are given in Table 1.

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

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 SP1-SP4 was noted to be 15-30%. This may be attributed to the drug present in the pores of the Microspheres. The overall cumulative percent release for different Microsphere formulations SP1-SP4 at the end of eight hours was found to be 61-94 %.

Table 2 *In-vitro* drug release data for formulation SP1

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	16.43±0.078	1.2156	83.57	1.9221
2	1.4142	0.3010	19.68±0.034	1.2940	80.32	1.9048
3	1.7321	0.4771	37.45±0.056	1.5735	62.55	1.7962
4	2.0000	0.6021	46.56±0.004	1.6680	53.44	1.7279
5	2.2361	0.6990	51.21±0.009	1.7094	48.79	1.6883
6	2.4494	0.7782	56.56±0.004	1.7525	43.44	1.6379
7	2.6458	0.8451	57.90±0.008	1.7627	42.10	1.6243
8	2.8284	0.9031	58.91±0.002	1.7702	41.09	1.6137

Table 3 *In-vitro* drug release data for formulation SP2

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	18.24±0.008	1.2610	81.76	1.9125
2	1.4142	0.3010	22.69±0.004	1.3558	77.31	1.8882
3	1.7321	0.4771	40.01±0.043	1.6022	59.99	1.7781
4	2.0000	0.6021	53.41±0.067	1.7276	46.59	1.6683
5	2.2361	0.6990	62.14±0.098	1.7934	37.86	1.5782
6	2.4494	0.7782	63.21±0.010	1.8008	36.79	1.5657
7	2.6458	0.8451	65.43±0.056	1.8158	34.57	1.5388
8	2.8284	0.9031	67.62±0.034	1.8301	32.38	1.5103

Table 4 *In-vitro* drug release data for formulation SP3

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	24.48±0.020	1.3888	75.52	1.8787
2	1.4142	0.3010	27.88±0.002	1.4453	72.12	1.8581
3	1.7321	0.4771	47.14±0.003	1.6734	52.86	1.7231
4	2.0000	0.6021	57.59±0.004	1.7603	42.41	1.6275
5	2.2361	0.6990	66.14±0.040	1.8205	33.86	1.5297
6	2.4494	0.7782	70.26±0.007	1.8467	29.74	1.4733
7	2.6458	0.8451	73.29±0.004	1.8650	26.71	1.4267
8	2.8284	0.9031	74.01±0.020	1.8692	25.99	1.4148

Table 5 *In-vitro* drug release data for formulation SP4

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	30.41±0.045	1.4830	69.59	1.8425
2	1.4142	0.3010	34.08±0.089	1.5325	65.92	1.8190
3	1.7321	0.4771	47.50±0.043	1.6767	52.50	1.7201
4	2.0000	0.6021	56.19±0.056	1.7497	43.81	1.6416
5	2.2361	0.6990	67.59±0.067	1.8299	32.41	1.5107
6	2.4494	0.7782	79.59±0.043	1.9009	20.41	1.3099
7	2.6458	0.8451	85.71±0.012	1.9330	14.29	1.1550
8	2.8284	0.9031	86.01±0.014	1.9345	13.99	1.1458

CONCLUSION

Insulin loaded microspheres Eudrajit RL 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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