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Formulation and Evaluation of Glimepiride Microparticles

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ABSTRACT

Aim: The objective of the present investigation was to formulate and evaluate microencapsulated Glimepiride produced by the emulsion - solvent evaporation method.

Methods: Microparticles were prepared using Eudragit RLPO by emulsion solvent evaporation method and characterized for their micromeritic properties, encapsulation efficiency, particle size, drug loading, FTIR, DSC, SEM analysis. In vitro release studies were performed in phosphate buffer (pH 7.4). Stability studies were conducted as per ICH guidelines.

Results: The resulting microparticles obtained by solvent evaporation method were free flowing in nature. The mean particle size of microparticles ranges from 140.49 - 189.71µm and encapsulation efficiency ranges from 91.60 - 96.24%. The infrared spectra and differential scanning calorimetry thermographs confirmed the stable character Glimepiride in the drug-loaded microparticles. Scanning electron microscopy revealed that the microparticles were spherical in nature. In vitro release studies revealed that the drug release was sustained up to 12 hrs. The release kinetics of Glimepiride from optimized formulation followed zero-order and peppas mechanism. The mechanism of drug release from the microparticles was found to be non-Fickian type.

Conclusion: Eudragit RLPO microparticles containing Glimepiride could be prepared successfully by using an emulsion solvent evaporation technique, which will not only sustain the release of drug but also manage complicity of the diabetes in a better manner.

Keywords: Glimepiride, Eudragit RLPO, Microparticles, Emulsion Solvent Evaporation, Controlled/Sustained Release.

INTRODUCTION:

Glimepiride [1-4] is the only third generation sulphonyl urea, which lowers the blood glucose level in the healthy subjects as well as in patients with type II diabetes. After oral administration, Glimepiride is completely (100%) absorbed from the GI tract. Studies with single oral doses in normal subjects and with multiple oral doses in patients with type II diabetes have shown significant absorption of Glimepiride within 1 hour after administration and peak drug levels (C_{max}) at 2 to 3 hours. Due to its low biological half life, it requires frequent administration to maintain plasma concentration. This leads fluctuations in plasma

concentration and also causes inconvenience to the patient. Therefore, development of controlled release dosage forms would clearly be beneficial in terms of decreased dosage requirements, thus increase patient compliance. Microencapsulation is a well known method for the preparation of microparticles for controlled release. Among the various methods developed for formulation of microparticles, solvent evaporation method is one of the mostly widely used one to formulate Microparticles because of its ease of fabrication without compromising the activity of drug [5]. In the present investigation Eudragit RLPO is used as a rate retardant polymer. Eudragit RLPO is a water

insoluble polymer which is widely used as a wall material for controlled release microparticles. This is due to its biocompatibility, good stability, easy fabrication and low cost [6]. In the present investigation solvent evaporation method was employed with an objective of developing microparticles for oral controlled release and subjected for evaluation in terms of drug content, encapsulation efficiency, size analysis, compatibility studies and *In-vitro* release studies.

MATERIALS AND METHODS:

Materials

Glimepiride was obtained as gift sample from Medley Pharmaceuticals Ltd., Daman Unit, Andheri East, Mumbai, India. Eudragit RLPO (Natco Pharma; Hyderabad, India), Acetone, liquid paraffin, tween 80, span 80 (Loba chemie Pvt. Ltd. Mumbai, India) and the chemical reagents used were of analytical grade.

Preparation of Microparticles:

The microparticles were prepared by emulsion solvent evaporation technique [7]. Glimepiride microparticles were formulated by varying the drug and polymer ratios and by varying the surfactants. Weighed amount of drug and polymer were dissolved in 10ml of acetone. The organic solution was then slowly added to 100ml of liquid paraffin containing 1% surfactant with constant stirring for 1hr. The resulting microparticles were separated by filtration and washed with petroleum ether. The microparticles finally air dried over a period of 12 hrs and stored in a dessicator.

Characterization of Microparticles:

Yield of Microparticles [8]:

Microparticles recovered at the end of preparation were weighed and the yield was calculated as a percentage of the total amounts of polymer and drug added during the preparation of microparticles.

Flow properties [9]:

Angle of Repose

Angle of repose is defined as the maximum angle possible between the surface of the pile of the powder and the horizontal plane. The flow characteristics of different microcapsules were studied by measuring the angle of repose employing fixed funnel method. The angle of repose was calculated by using the following formula.

$$\tan \theta = \frac{\text{Height of the pile}}{\text{radius of the base of the pile}}$$

where $\theta = \tan^{-1} (h / r)$ θ = angle of repose.

Bulk Density & Tapped Density

Bulk density and tapped density were measured by using 10 ml of graduated cylinder. The pre weighed sample was placed in a cylinder; its initial volume was recorded (bulk volume) and subjected to tapings for 100 times. Then the final volume (tapped volume) was noted down. Bulk density and tapped density were calculated from the following formula.

$$\text{Bulk Density} = \frac{\text{mass of microparticles}}{\text{bulk volume}}$$

$$\text{Tapped Density} = \frac{\text{mass of microparticles}}{\text{tapped volume}}$$

Carr's Index

Compressibility index (CI) or Carr's index value of microparticles was computed according to the following equation:

$$\text{Carr's Index (\%)} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

Hausner's Ratio

Hausner ratio of microspheres was determined by comparing the tapped density to the bulk density using the equation:

$$\text{Hausner's Ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Size Distribution and Size Analysis:

For size distribution analysis, 250 mg of the microparticles of different sizes in a batch were separated by sieving, using a range of standard sieves. The amounts retained on different sieves were weighed. The mean particle size of the microparticles was calculated by the formula [10]

$$\text{Mean Particle Size} = \frac{\sum (\text{Mean Particle Size of the Fraction} \times \text{Weight Fraction})}{\sum (\text{Weight Fraction})}$$

Estimation of drug content:

An accurately weighed portion of microparticles equivalent to 5 mg of Glimepiride were weighed and transferred in to a mortar. Powdered and dissolved in 100 ml of pH 7.4 phosphate buffer, suitably diluted the absorbance of the resulting solution was measured at 236 nm [11].

Entrapment Efficiency [12]:

Entrapment efficiency was calculated using the formula

$$\text{Entrapment efficiency} = \frac{\text{Estimated percent drug content}}{\text{Theoretical percent drug content}} \times 100$$

Estimated percent drug content was determined from the analysis of microparticles and the theoretical percent drug content was calculated from the employed core: coat ratio in the formulation of microparticles.

Morphological Characterization by SEM:

Morphology and surface characteristics were studied by Scanning Electron Microscopy. The samples for the SEM analysis were prepared by sprinkling the microparticles on one side of the double adhesive stub. The stub was then coated with fine gold dust. The microparticles were then observed with the scanning electron microscope (Leica Electron Optics, Cambridge, USA) at 10 kv).

Fourier Transform Infrared Spectroscopy (FTIR) studies

The pure drug and optimized formulations were subjected for FTIR analysis. The samples were scanned over a range of 4000-400 cm^{-1} using Fourier transformer infrared spectrophotometer. Spectra's were analyzed for drug polymer interactions.

Differential Scanning Calorimetry (DSC) studies

The pure drug and optimized formulation were subjected to differential scanning calorimeter equipped with an intra cooler (NETZSCH, Japan.). Indium/zinc standards were used to calibrate the DSC temperature and enthalpy scale. The sample were sealed in aluminum pans and heated at a constant rate 20°C/min over a temperature range of 20-250°C. An inert atmosphere was maintained by purging nitrogen gas at a flow rate of 50 ml/min.

Drug Release Studies:

Release of Glimepiride from the microparticles, was studied in phosphate buffer of pH 7.4 (900 ml) using Eight Station Dissolution Rate Test Apparatus (M/s. Electrolab) with a paddle stirrer at 100 rpm and at 37 $^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. A sample of microparticles equivalent to 5 mg of Glimepiride was used in each test. Samples were withdrawn through a filter (0.45) at different time intervals and were assayed at 228 nm for Glimepiride using Shimadzu double beam UV spectrophotometer. The drug release experiments were conducted in triplicate [13].

Dissolution kinetics:

The rate and the mechanism of release of Glimepiride

from the prepared microparticles were analyzed by fitting the dissolution data into [14], zero-order equation, $Q = Q_0 - k_0 t$ (1), where Q is the amount of drug released at time t, and k_0 is the release rate.

First order equation, $\ln Q = \ln Q_0 - k_1 t$ (2), where k_1 is the release rate constant and Higuchi's equation,

$$Q = k_2 t^{1/2} \quad (3) \text{ where } Q \text{ is the amount of the drug}$$

released at time t and k_2 is the diffusion rate constant. The dissolution data was further analyzed to define the mechanism of release by applying the dissolution data following the empirical equation,

$$\frac{M_t}{M_{\infty}} \times K t^n \quad (4)$$

Where M_t/M_{∞} is the fraction of drug released at time t. K is a constant and n characterizes the mechanism of drug release from the formulations during dissolution process.

Stability study:

The formulation was subjected to accelerated stability studies as per ICH (The International Conference of Harmonization) guidelines. The optimized formulation was sealed in an aluminum foil and stored at $25 \pm 2^{\circ}\text{C}$, $60 \pm 5\%$ RH and at $40 \pm 2^{\circ}\text{C}$, $75 \pm 5\%$ RH for 3 months (Kamlesh Dashora *et al.*, 2007). Microparticles were periodically removed and evaluated for physical characteristics and in-vitro drug release.

RESULTS AND DISCUSSION:

Glimepiride loaded Eudragit RLPO microparticles were successfully formulated by emulsion solvent evaporation method. In these formulations, span 80 and tween 80 are used as surfactants and the optimum concentration of each is 1% w/v. A total number of eight batches were formulated by varying the process variables like change in polymer concentration and type of surfactant. The detailed composition of microparticles was shown in Table 1.

These microparticles were evaluated for their percentage yield, flow properties, size analysis, percent drug content, percent encapsulation efficiency and morphological characterization, FTIR studies, DSC analysis, In vitro release studies and stability studies.

The angle of repose values of all the formulations were found to be in the range of 21.16 – 27.64, i.e. less than 30, which shows their free flowing nature of the prepared microparticles. Bulk density and tapped density showed good packability of the microparticles.

Table 1: Composition of Glimepiride Microparticles

Ingredients	Formulations							
	F 1	F 2	F 3	F 4	F 5	F 6	F 7	F 8
Glimepiride (gm)	1	1	1	1	1	1	1	1
Eudragit RLPO (gm)	1	1.5	2	3	1	1.5	2	3
Acetone (ml)	10	10	10	10	10	10	10	10
Span 80 %	1	1	1	1	--	--	--	--
Tween 80 %	--	--	--	--	1	1	1	1
Liquid Paraffin(ml)	100	100	100	100	100	100	100	100

Table 2: Micromeritic Properties of Glimepiride microparticles

Formulation code	Angle of repose (θ)	Bulk Density (g/cm^3)	Tapped Density (g/cm^3)	Carr's Index	Hausner's Ratio	Average Particle Size (μm)
F 1	27.17	0.905	1.074	15.7	1.17	140.49
F 2	25.12	0.912	1.070	14.8	1.16	156.11
F 3	22.82	0.928	1.074	13.6	1.15	167.89
F 4	21.16	0.941	1.079	12.8	1.14	179.42
F 5	27.64	0.770	0.915	15.81	1.20	147.8
F 6	25.08	0.784	0.918	14.61	1.18	163.12
F 7	23.53	0.796	0.925	13.91	1.16	172.89
F8	22.14	0.812	0.932	12.86	1.14	189.71

Table 3: Percentage yield, % drug content and % encapsulation efficiency of Glimepiride microparticles

Formulation code	% Yield	% Drug Content	% Encapsulation Efficiency
F 1	81.5	46.38	92.76
F 2	89.2	37.45	93.62
F 3	91.33	31.35	94.05
F 4	92.75	24.06	96.24
F 5	85.5	45.80	91.60
F 6	93.6	36.93	92.32
F 7	94.33	31.21	93.63
F8	96.75	23.77	95.08

Table 4: Release Kinetics of Glimepiride microparticles

Formulation Code	Correlation Coefficient Values (R^2)				Release Rate Constant (mg/hr) Ko	$t_{50\%}$	$t_{90\%}$	Wall Thickness (μ)	N value
	Zero Order	First Order	Higuchi Model	Peppas Model					
F 1	0.9932	0.8964	0.9058	0.9941	1.04	3.84	6.92	27.48	1.0157
F 2	0.9973	0.8474	0.9043	0.9978	0.92	4.34	7.82	38.29	1.0508
F 3	0.9961	0.8238	0.8979	0.9983	0.84	4.76	8.57	45.42	1.0817
F 4	0.9946	0.7977	0.8909	0.9993	0.78	5.12	9.23	55.69	1.1864
F 5	0.9996	0.8771	0.9312	0.9977	1.10	3.63	6.54	26.81	0.9402
F 6	0.9996	0.8608	0.9267	0.9992	1.04	3.84	6.92	37.49	0.9919
F 7	0.9997	0.8425	0.9228	0.9995	0.98	4.08	7.34	44.64	1.0163
F 8	0.9998	0.7833	0.9221	0.9996	0.82	4.87	8.78	51.19	1.0151

Carr's index ranges from 12.8 % to 15.81%, indicating excellent compressibility. Hausner's ratio ranges from 1.14 to 1.20, i.e. all the formulations showed that they had good flow properties. The results were tabulated in Table No. 2. The particle size analysis reveals that, with the considerable increase in the concentration of Eudragit RLPO, the mean particle size of microparticles also increased. The results were shown in Table No. 2.

It was identified that, as the polymer ratio and the product yield directly proportional to each other. The % yield varies from 81.5 to 91.65%. The % drug content in the microparticles was found to be 23.77 to 46.38. The % drug content decreases with increase in polymer concentration. The % of encapsulation efficiency ranges from 91.60 to 96.24. drug content and encapsulation efficiency data were shown in Table 3.

The SEM studies clearly showed that the obtained microparticles exhibit good spherical nature. Scanning

The encapsulation efficiency increases with the increase in polymer concentration. The yield of microparticles, % electron microscopic photographs of microparticles are shown in Fig. 1.

The Glimepiride microparticles were subjected to In-vitro release studies by employing 7.4 pH phosphate buffer and the drug release profiles were shown in Fig. 4. When the amount of drug release values are plotted against time straight lines were obtained in all the cases indicating that the rate of drug release from these microparticles followed zero order kinetics and the graphs are shown in Fig. 5. To ascertain the mechanism of drug release from various microparticles plot of $\log$ % released vs $\log$ time (peppas plots) were drawn. The plots were found to be linear with all formulations. The peppas plots are shown in Fig. 6.

Figure 1: SEM photographs of Glimepiride Microparticles

The Glimepiride pure drug showed characteristic peaks at wave numbers were 3323.07 cm^{-1} (3300-3500) (N-H) stretching, 2941.73 cm^{-1} (2850-3000) (C-H) stretching, 2853.82 cm^{-1} (3300-2500) (O-H) stretching, 1441.17 and 1524.46 cm^{-1} (1350-1550) (N=O) stretching, 1156.37 cm^{-1} (1020 -1220) (C-N) stretching 1031.83 cm^{-1} (1000 -1300) (C-O) bending. The FTIR spectrum of Glimepiride mixed with Eudragit RLPO formulation showed characteristic peaks at wave numbers were 3311.65 cm^{-1} (3300- 3500) (N-H), 2942.87 cm^{-1} (2850 - 3000) (C-H), 2974.45 cm^{-1} (3300 - 2500) (O-H), 1453.23 and 1526.72 cm^{-1} (1350 -1550) (N=O), 1203.01 cm^{-1} (1020 -1220) (C-N), 1092.65 cm^{-1} (1000 -1300) (C-O). This indicates that there were no chemical incompatibility between Glimepiride and the polymer (Eudragit RLPO) used. The FTIR Spectrums were shown in Fig. 2.

Figure 2: FTIR Spectrums of (a). Glimepiride, (b).Eudragit RLPO and (c).Glimepiride Microparticles

Pure Glimepiride exhibited an endothermic peak at 206.93 °C, which started to melt at 204.26 °C, the range of which corresponded to its melting point (205-207 °C). The optimized formulation showed an endothermic peak at 205.36 °C, nearly same temperature indicating that no interactions between the drug and excipients. The DSC thermograms were shown in Fig. 3.

Figure 3. DSC thermographs of (a). Glimepiride, (b).Eudragit RLPO and (c).Glimepiride Microparticles

Figure 4: Release Profiles of Glimepiride microparticulates (F1 to F4)

Figure 5: Zero Order Plots of Glimepiride microparticulates

Figure 6: Peppas Plots of Glimepiride microparticulates

Release Kinetic studies of Glibenclamide microparticles were shown in Table 4. The exponential coefficient (n) values were found to be in between 0.9402 to 1.1864 indicating non fickian mechanism. These results indicated that the release rate was found to decrease with increase in concentration of coating material applied. The wall thickness of microparticles was found to be increased with the increase in concentration of coating material applied. A good correlation ship sustained in between wall thickness and release rate constant and the graphs were shown in Fig. 7.

Figure 7: Correlation ship between Wall Thickness and Release Rate Constant of Glimepiride microparticles Prepared with Eudragit RLPO (*A-Tween 80 as surfactant and *B-Span 80 as surfactant).

The stability studies were carried out for the prepared microparticles. After 3 months storage of formulations at $30 \pm 2^\circ\text{C}$, $65 \pm 5\%$ RH and $40 \pm 2^\circ\text{C}$, $75 \pm 5\%$ RH, values of all parameters like percentage of drug content and encapsulation efficiency were evaluated and found to be almost similar to the initial values. The drug dissolution profile was similar to the initial profile. There was no significant change in any value and also no changes in the physical appearance. So it could be concluded that Glimepiride microparticles prepared with Eudragit RLPO is stable.

CONCLUSION:

Eudragit RLPO microparticles containing Glimepiride was prepared successfully by using an emulsion solvent evaporation method. By varying the drug: polymer ratios, is found to influence the size, entrapment efficiency and release characteristics of the microparticles. The assessment of the release kinetics revealed that drug release from microparticles was found to be non-Fickian type. Controlled release without initial peak level achieved with these formulations may reduce frequency and improves patient compliance.

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Conflict of Interest

We declare that we have no conflict of interest

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