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Original Research Article

BIODEGRADABLE MICROSPHERES FOR SUSTAINED RELEASE DELIVERY OF REPAGLINIDE.

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Abstract: The objective of the present study is to prepare repaglinide microspheres for the sustained delivery of the drug for better patient care in the management of diabetics. The biodegradable microspheres of repaglinide is prepare using poly(lactic-co-glycolic acid) (PLGA) by emulsion solvent evaporation technique. The microspheres are prepared with different drug-to-carrier ratios and considering other variables (i.e. solvent, surfactant and stirrer speed) as well. The evaluation of microspheres prepared are perform on the basis of various parameters like particle size, percentage yield, drug entrapment efficiency, surface morphology (SEM), drug-polymer interaction (FT-IR study), *in vitro* drug release kinetics and stability studies. SEM reveals that microspheres are spherical and has nearly smooth surface morphology. The percentage yield and drug entrapment efficiency is quite well for all the formulations. FT-IR spectra show that there is no chemical interaction between the drug and the polymer. The *in vitro* release study data shows that the repaglinide release from all the formulations are slow and sustained upto 7days. The various kinetic equations indicate that the *in vitro* drug release is of zero order release with initial burst from repaglinide microspheres. There is no appreciable difference is observed in the stability study observations.

Key words: Biodegradable, Microspheres, Poly(lactic-co-glycolic acid) (PLGA), Repaglinide, Surface Electron Microscopy (SEM) and Fourier's Transformation Infra Red Spectroscopy (FT-IR).

Introduction: In the recent time more importance was given on modified release dosage forms to achieve and maintain therapeutic amount of drug in the blood or tissue to improve pharmacokinetics of drug and increase patient compliance as well as reducing side effects for a prolong period of time. [1,2] Microspheres comprise matrix systems which contain drug throughout their structure and are potential candidates for oral controlled release. Microspheres can be defined as solid spherical

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particles ranging from 1 to 1000 μm in size. [3-4] These particles consists of the drug which is the core material and a polymeric coating material. The coating material can be of various types ranging from natural polymers (chitosan, albumin, gelatin,) to synthetic polymers (PVA, PLGA, PEG, poly(ε-caprolactone), blok copolymers etc).[5-6] Among the various coating materials used for the development of sustained release formulations, PLGA has been reported to be advantageous as it is biodegradable, biocompatible, and has a very low glass temperature. [7,8] Apart from single PLGA now-a-days polymeric blend or diblock copolymer with protein repellents (like Poloxamer, Ploxamine and PEG) have been used to impart a stealth characteristic to polymeric micro/nano-particles and ultimately achieved controlled release of the drug. This has led to its application in the preparation of different delivery systems in the form of microspheres, nanoparticles, and implants. [9]

Repaglinide belongs the meglitinide class of drugs is a fast- and short-acting drug with a very short plasma half-life (about 1 hr) and low bioavailability (50%).[10] Repaglinide was chosen as the model drug in the present study for formulation of microspheres to achieve the controlled drug release profile suitable for peroral administration.

Materials and Methods

Materials: Repaglinide and polymer were received as a gift sample from M/S Torrent Pharmaceuticals, Ahmadabad, India. Dichloromethane, Methanol, PVP, Polysorbate 80 were purchased from Loba Chem. Pvt. Ltd. And SD fine chemicals, Mumbai, India. All other reagents used were of analytical grade.

Preparation of Microspheres: Solvent evaporation method was used for the preparation of repaglinide microspheres.[11] Total 36 formulations were prepared by using different drug-to-carrier ratios (1:2,1:1& 2:1), different stirrer speed (500rpm, 1000rpm & 1500 rpm), different surfactant (PVP & Polysorbate 80) and different solvent (Methanol & DCM) An accurately weighed quantity (calculated) of the polymer was dissolved in 10 mL of dichloromethane/methanol and weight amount of repaglinide was dissolved in this polymer phase. This solution was emulsified in 100 mL of 0.5% PVP/Polysorbate 80 using continuous stirring for two hours at 500/1000/1500 rpm. The microspheres formed were filtered and washed three times with 50 mL of distilled water to remove surface adhered surfactants and dried at room temperature for 6 h. The dried microspheres were weighed and the % yield of the microspheres prepared was calculated using the formula:[12]

Percent Yield = Amount of Microspheres Obtained (g)/ Theoretical Amount (g) × 100

Determination of the mean particle size and surface morphology: Particle size analysis was carried out by using optical microscopy.[13] About 100 microspheres were selected from each formulation randomly and their size was determined using an optical microscope fitted with a standard micrometer scale. Surface morphology and topography of the microspheres were examined by scanning electron microscopy (S-3000N, magnification=x5.0k, WD=33.3mm) and SEM photomicrographs of suitable magnification obtained.

Determination of percentage drug entrapment: For determination of drug content a weighed quantity of the microspheres was crushed and suspended in phosphate buffer, pH 7.4 to extract the drug from the microspheres. After 24 h, the filtrate was assayed by HPLC with mobile phase of methanol: ammonium acetate buffer (pH-4) (80:20*)* at 242 nm for drug content. Corresponding drug concentrations in the sample were calculated from the calibration plot and the drug entrapment efficiency was calculated using the formula:

% Entrapment Efficiency = Quantity of drug in Microspheres/Theoretical drug loading × 100

FT-IR Study: FT-IR spectra of repaglinide and microspheres were recorded in an FT-IR spectrophotometer to check the drug-polymer interaction and chemical integrity of the drug in the microspheres.

Stability studies: For the purpose of stability studies all the formulations were packed in 0.044 mm laminated aluminum foil and subjected to storage at elevated temperature and humidity conditions of 40°±2°C/75±5% RH in an environment chamber. Samples were withdrawn at the end of 1, 3 and 6 months and evaluated for physical properties, encapsulation efficiency, drug content, particle size and *In-Vitro* drug release. [14]

***In vitro Drug* release studies:** Drug release studies were carried out using a USP type II dissolution apparatus and the dissolution vessel was filled with 900 mL of 0.1 N HCl and the temperature was kept constant at 37±0.5°C. Samples were withdrawn at predetermined time intervals with the same volume of fresh medium being added after each withdrawal. The sample was suitably diluted and assayed by HPLC method using PDA detector at 242 nm.

Kinetic modeling of drug release: The dissolution profiles of selected formulations were fitted to zero order, first order, Higuchi's and Kresmeyar-Peppas model to ascertain the kinetics of drug release. The regression coefficient (r^2) was calculated for the curves obtained by regression analysis of the above plots. [15-17]

Results and Discussion: Repaglinide microspheres with varying proportions of drug & polymer were prepared by the solvent evaporation method. The particle size was determined by optical microscopy and was found to increase with increasing polymer proportions. The % entrapment efficiency, % Drug content and mean particle size of the microspheres is shown in Table 1. Electron microscopy revealed that the microspheres were spherical with a nearly smooth surface [Figure 1]. The yield obtained for all batches was good and in the range of 83.54 ± 2.42 to 88.28 ± 2.54%. The microspheres exhibited an increase in drug entrapment with an increase in the polymer ratio. As the stirrer speed goes high the particle size was lower and the PVP showed the better result as compare to polysorbate 80 in terms of release profile also.

The FT-IR spectra of repaglinide-loaded microspheres showed characteristic absorption peaks that were identical with the drug's reference spectrum. This clearly indicated the stability of the drug during the microencapsulation process and revealed the absence of any drug-polymer interaction [Figures 2].The stability studies did not reveal any remarkable change in the drug content. This indicated that the formulation was stable in medium storage conditions.

The release of repaglinide mainly depended upon the polymer concentration. The release rate of the drug from the microspheres was found to decrease drastically on increasing the polymer concentration. Repaglinide release from all the formulations was found to be slow and sustained over 7 days. By the end of 7^{th} day, the formulations RMS6 and RMS24 were found to release 49.6±1.06 and 43.7±1.31 of the loaded drug respectively.

Release pattern from all the formulations showed an initial burst release followed a sustained drug release. The cumulative percentage drug release has been observed at the end of day 7. Various kinetic models applied, revealed that drug release was initially zero order followed by first order in both the formulations. The mechanism of drug release from RPG microspheres was studied by using Higuchi and Krsemeyer-peppas models. The r^2 value shows that drug release was initially zero order (diffusion controlled release system) followed by first order (Diffusion and erosion controlled release). The *in vitro* release kinetics profiles of selected formulations have been shown in table 2 and Figure 3, 4, 5 and 6.

Conclusion: Present results suggest that biodegradable microspheres of Repaglinide can be rationally employed as long acting formulation. The prepared microparticulate system ensures the sustained delivery of the drug for extended period of time. From the above data, it may be concluded that drug-loaded microspheres appear to be a suitable delivery system for repaglinide and may help in reducing the frequency of medication, improving patient compliance, cost of therapy and reducing side-effects.

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Table 1: Characterization of Repaglinide loaded PLGA Microspheres

Fln Code	*Formula*	*EE (%)*	*DC (%)*	*PS (µm)*
RMSA1	*RPG:PLGA[1:2]+Methanol+500 Rpm + PVA*	*15.35±1.25*	*3.12±1.23*	*Not Done*
RMSA2	*RPG:PLGA[1:2]+Dichloromethane+500 Rpm + PVA*	*32.45 ±2.72*	*12.89 ± 2.32*	*29.54±8.84*
RMSA3	*RPG:PLGA[1:2]+Methanol+1000 Rpm + PVA*	*21.31±2.01*	*5.23±1.14*	*Not Done*
RMSA4	*RPG:PLGA[1:2]+Dichloromethane+1000 Rpm + PVA*	*46.12 ±1.83*	*17.41±1.52*	*36±5.24*
RMSA5	*RPG:PLGA[1:2]+Methanol+1500 Rpm + PVA*	*28.45±1.57*	*9.21±1.26*	*Not Done*
RMSA6	*RPG:PLGA[1:2]+Dichloromethane+1500 Rpm + PVA*	*58.7±1.2*	*26.84±2.2*	*12.68±8.26*
RMSA7	*RPG:PLGA[1:1]+Methanol+500 Rpm + PVA*	*20.14±1.25*	*7.11±1.2*	*Not Done*
RMSA8	*RPG:PLGA[1:1]+Dichloromethane+500 Rpm + PVA*	*39.28±1.47*	*16.06±1.31*	*33±10.13*
RMSA9	*RPG:PLGA[1:1]+Methanol+1000 Rpm + PVA*	*37.13±1.56*	*14.13±1.27*	*Not Done*
RMSA10	*RPG:PLGA[1:1]+Dichloromethane+1000 Rpm + PVA*	*50.35±1.48*	*21.76±1.21*	*32±9.37*
RMSA11	*RPG:PLGA[1:1]+Methanol+1500 Rpm + PVA*	*42.15±1.35*	*19.46±1.32*	*39±11.26*
RMSA12	*RPG:PLGA[1:1]+Dichloromethane+1500 Rpm + PVA*	*61.98 ± 1.65*	*29.81 ± 2.76*	*26.9 ±8.46*
RMSA13	*RPG:PLGA[2:1]+Methanol+500 Rpm + PVA*	*23.34±1.54*	*10.44±1.34*	*Not Done*
RMSA14	*RPG:PLGA[2:1]+Dichloromethane+500 Rpm + PVA*	*68.41±1.21*	*30.41±1.24*	*Not Done*
RMSA15	*RPG:PLGA[2:1]+Methanol+1000 Rpm + PVA*	*35.15±1.32*	*13.41±1.45*	*Not Done*
RMSA16	*RPG:PLGA[2:1]+Dichloromethane+1000 Rpm + PVA*	*75.43±1.76*	*33.51±1.37*	*29±8.48*
RMSA17	*RPG:PLGA[2:1]+Methanol+1500 Rpm + PVA*	*41.34±1.26*	*15.21±1.23*	*42±13.18*
RMSA18	*RPG:PLGA[2:1]+Dichloromethane+1500 Rpm + PVA*	*79.81 ± 2.24*	*37.56 ± 3.84*	*24.5±8.25*
RMSA19	*RPG:PLGA[1:2]+Methanol+500 Rpm+ Polysorbate 80*	*11.43±1.54*	*1.69±1.24*	*Not Done*
RMSA20	*RPG:PLGA[1:2]+Dichloromethane+500 Rpm+ Polysorbate 80*	*30.65±1.43*	*9.93 ±1.32*	*48 ±11.34*
RMSA21	*RPG:PLGA[1:2]+Methanol+1000 Rpm+ Polysorbate 80*	*15.45±1.25*	*3.78±1.13*	*Not Done*
RMSA22	*RPG:PLGA[1:2]+Dichloromethane+1000 Rpm+ Polysorbate 80*	*31.45±1.23*	*12.13±1.34*	*22.5±11.19*
RMSA23	*RPG:PLGA[1:2]+Methanol+1500 Rpm+ Polysorbate 80*	*39.11±1.23*	*20.43±1.65*	*31±10.13*
RMSA24	*RPG:PLGA[1:2]+Dichloromethane+1500 Rpm+ Polysorbate 80*	*47.44±1.22*	*22.84±1.32*	*14.24±10.22*
RMSA25	*RPG:PLGA[1:1]+Methanol+500 Rpm+ Polysorbate 80*	*14.11±1.53*	*1.96±1.43*	*Not Done*
RMSA26	*RPG:PLGA[1:1]+Dichloromethane+500 Rpm+ Polysorbate 80*	*30.43±1.35*	*10.31±1.31*	*32±15.32*
RMSA27	*RPG:PLGA[1:1]+Methanol+1000 Rpm+ Polysorbate 80*	*30.74±1.36*	*9.64±1.34*	*Not Done*
RMSA28	*RPG:PLGA[1:1]+Dichloromethane+1000+Rpm+ Polysorbate 80*	*40.34±1.74*	*15.23±1.56*	*31±13.84*
RMSA29	*RPG:PLGA[1:1]+Methanol+1500 Rpm+ Polysorbate 80*	*36.65±1.31*	*13.12±1.13*	*Not Done*
RMSA30	*RPG:PLGA[1:1]+Dichloromethane+1500 Rpm+ Polysorbate 80*	*52.21±1.65*	*21.43±1.68*	*23±8.14*
RMSA31	*RPG:PLGA[2:1]+Methanol+500 Rpm+ Polysorbate 80*	*21.32±1.52*	*11.62±1.32*	*Not Done*
RMSA32	*RPG:PLGA[2:1]+Dichloromethane+500 Rpm+ Polysorbate 80*	*67.38±1.21*	*31.16±1.28*	*33±10.14*
RMSA33	*RPG:PLGA[2:1]+Methanol+1000 Rpm+ Polysorbate 80*	*34.52±1.28*	*12.86±1.48*	*Not Done*
RMSA34	*RPG:PLGA[2:1]+Dichloromethane+1000 Rpm+ Polysorbate 80*	*76.22±1.46*	*34.18±1.38*	*30.6±12.28*
RMSA35	*RPG:PLGA[2:1]+Methanol+1500 Rpm+ Polysorbate 80*	*42.48±1.22*	*15.88±1.24*	*28.8±14.18*
RMSA36	*RPG:PLGA[2:1]+Dichloromethane+1500 Rpm+ Polysorbate 80*	*78.94 ± 2.42*	*38.16 ± 3.68*	*26.5± 11.25*

Table 2: Release rate kinetics of Repaglinide loaded PLGA Microspheres

Formulation Code	*Zero Order Release Rate*		*First Order Release Rate*		*Higuchi kinetics*		*Krsemeyer- Pappas Kinetics*	
	Equation	*R^2*	*Equation*	*R^2*	*Equation*	*R^2*	*Equation*	*R^2*
RMSA6	*y = 6.0241x + 2.25*	*0.9064*	*y = -0.0377x + 1.95*	*0.9495*	*y = 18.505x + 2.08*	*0.9854*	*y = 0.3772x + 1.37*	*0.9983*
RMSA24	*y = 6.0033x + 5.15*	*0.9797*	*y = -0.0347x + 1.98*	*0.9931*	*y = 17.802x - 4.08*	*0.9924*	*y = 0.668x + 1.09*	*0.9967*

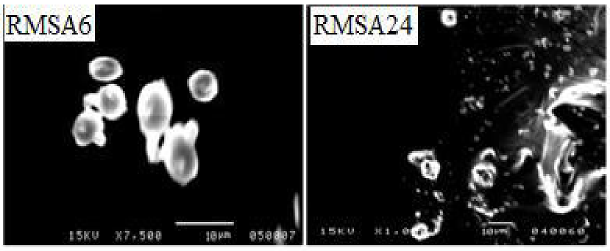

Fig.1 SEM Microphotograph of RMSA6 and RMSA24

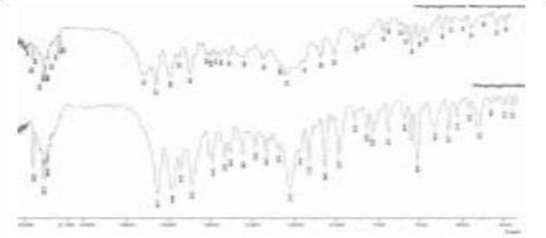

Figure 2: FT-IR spectra obtained for pure Repaglinide and Repaglinide -loaded microspheres

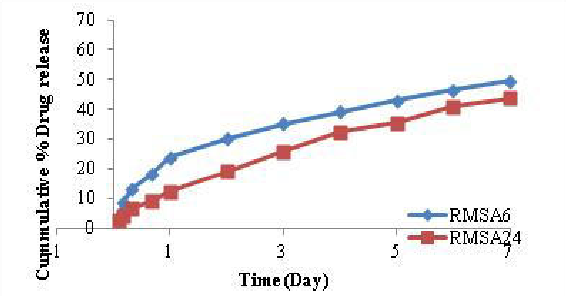

Fig.3: Zero Order drug release Kinetics of RPG loaded Microspheres with PLGA

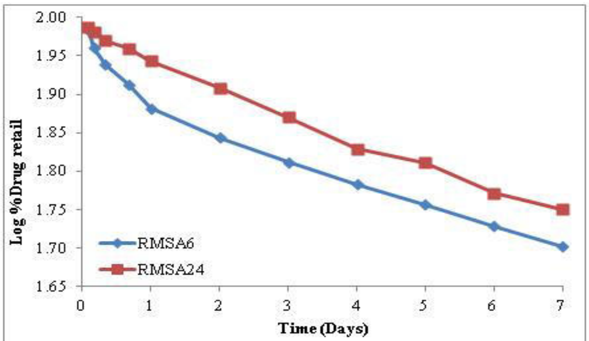

Fig.4: First Order drug release Kinetics of RPG loaded Microspheres with PLGA

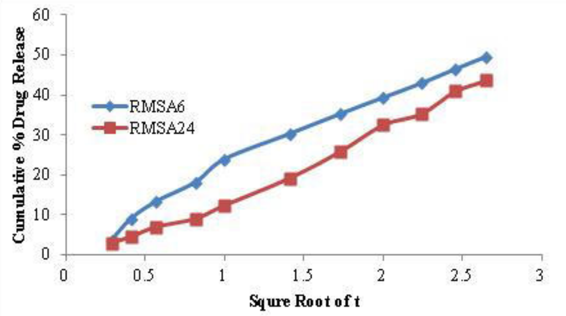

Fig.5: Higuichi Model drug release Kinetics of RPG loaded Microspheres with PLGA

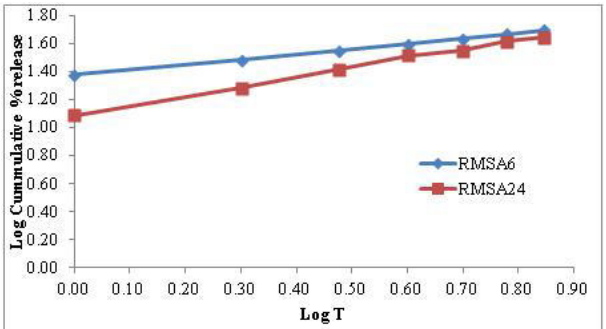

Fig.6: Krsemeyer- Pappas Model drug release Kinetics of RPG loaded Microspheres with PLGA