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Preparation of Biodegradable Microspheres Containing Repaglinide

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ABSTRACT

The objective of the present study is to prepare and evaluate poly(ε-caprolactone) microspheres of repaglinide by using the solvent evaporation technique. The microspheres were prepared with different drug-to-carrier ratios: F_1 (1:3), F_2 (1:4), F_3 (1:5), and F_4 (1:6). The microspheres were then evaluated for particle size, SEM, FT-IR study, percentage yield, drug entrapment, stability studies, and for *in vitro* release kinetics. Scanning electron microscopy (SEM) revealed that microspheres were spherical with a nearly smooth surface morphology. The percentage yield and drug entrapment efficiency were high for all the formulations. Fourier Transform-Infrared spectroscopy (FT-IR) showed that there was no chemical interaction between the drug and the polymer. No appreciable difference was observed in the stability study, in the extent of degradation of the product for 60 days in the microspheres that were stored at various temperatures. The *in vitro* release study showed that replaglinide release from all the formulations was slow and sustained over 12 h. Application of the *in vitro* drug release data to various kinetic equations indicated zero order release from repaglinide microspheres.

Key words: Biodegradable, microspheres, poly(ε-caprolactone), repaglinide

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INTRODUCTION

Microspheres are matrix systems that contain drug throughout their structure and are potential candidates for oral controlled release. Microspheres can be defined as solid spherical particles ranging from one to 1000 μm in size.[1-5] These particles consist of the drug which is the core material, and a coating material. The coating material can be of various types ranging from natural polymers such as albumin, gelatin, chitosan, and synthetic polymers such as poly(vinyl alcohol), poly(lactide-co-glycolide), and poly(ε-caprolactone).[6,7] Among the various coating materials used for the development of sustained release formulations, poly(ε-caprolactone) has been reported to be advantageous as it is biodegradable, biocompatible, and semicrystalline and has a very low glass temperature.[8,9] Poly(ε-caprolactone) is an aliphatic polyester polymer that is suitable for long-term delivery extending over a period of more than one year. This has led to its application in the preparation of different delivery systems in the form of microspheres, nanoparticles, and implants.[10]

Repaglinide is a fast, short-acting meglitinide analog with a very short half-life (one h) and low bioavailability (50%).[11] The aim of the study was to prepare poly(ε- caprolactone) microspheres containing repaglinide to achieve a controlled drug release profile suitable for peroral administration.

MATERIALS AND METHODS

Materials

Repaglinide was received as a gift sample from M/S Torrent Pharmaceuticals, Ahmedabad, India. Poly(ε-caprolactone) was procured from Fulka Cemika, Sigma-Aldrich Chemie, Switzerland. Dichloromethane was purchased from Loba Chem. Pvt. Ltd., Mumbai, India. All other reagents used were of analytical grade.

Preparation of microspheres

Solvent evaporation method was used for the preparation of repaglinide microspheres.[12] The drug-to-carrier ratios for different formulations were: 1:3 (F1), 1:4 (F2), 1:5 (F3), and 1:6 (F4). An accurately weighed quantity of the poly(ε- caprolactone) was dissolved in 10 mL of dichloromethane and 200 mg of repaglinide was dissolved in this polymer phase. This solution was poured into 100 mL of liquid paraffin containing 1.3% Tween 80 and stirred continuously for five hours at 1100 rpm. The microspheres formed were filtered and washed three times with 50 mL of *n*-hexane and dried at room temperature for 12 h. The dried microspheres were weighed and the yield of the microspheres preparation was calculated using the formula:[13]

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

Determination of percentage drug entrapment

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 spectrophotometrically at 243 nm for drug content (UV-1601 Shimadzu, Japan). Corresponding drug concentrations in the sample were calculated from the calibration plot and the drug entrapment efficiency was calculated using the following formula:

Percentage drug entrapment = Calculated drug concentration/Theoretical drug concentration × 100

Determination of the mean particle size and surface morphology

Particle size analysis was carried out by using optical microscopy.[14] About 200 microspheres were selected randomly and their size was determined using an optical microscope fitted with a standard micrometer scale. Morphology and surface topography of the microspheres were examined by scanning electron microscopy (SEM- Jeol, JSM-840A, Japan). The samples were mounted on the SEM sample stab using a double-sided sticking tape and coated with gold (200 Å) under reduced pressure (0.001 Torr) for five min to improve the conductivity using an ion sputtering device (Jeol, JFC-110 E, Japan). The coated samples were observed by SEM and photomicrographs of suitable magnification obtained.

FT-IR Study

FT-IR spectra of repaglinide and microspheres were recorded in an FT-IR spectrophotometer (IR-470, Shimadzu, Japan) to check the drug-polymer interaction and chemical integrity of the drug in the microspheres.

Stability studies

All the formulations were studied for their stability profiles for 60 days under different environmental conditions such as room temperature (27 ± 2°C/65% RH), oven temperature (40 ± 2°C/75% RH), and in the refrigerator (5-8°C).[15] The microspheres were analyzed for drug content.

In vitro release studies

Drug release studies were carried out using a USP type II dissolution apparatus (Model No TDT-08L, Electro lab, Mumbai). 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 absorbance was measured at 243 nm.

Kinetic modeling of drug release

The dissolution profiles of all batches were fitted to zero order, first order,[16] and Higuchi's model[17] to ascertain the kinetic modeling of drug release. The regression coefficient value (r^2) and K values were calculated for the linear curve obtained by regression analysis of the above plots.

RESULTS AND DISCUSSION

Repaglinide microspheres with varying proportions of poly(ε-caprolactone) 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 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 82.68 ± 2.40 to 87.68 ± 2.51%. The microspheres exhibited an increase in drug entrapment with an increase in the polymer ratio, up to a particular concentration. A decrease in drug entrapment was observed after that point due to the saturation capacity of the polymer.

The FT-IR spectra of replaglinide-loaded poly (ε- caprolactone) 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 and 3].

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 *in vitro* release profiles of all formulations have been shown in Figure 3. The release of replaglinide 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. Replaglinide release from all the formulations was found to be slow and sustained over 12 h. By the end of 12 h, formulations F_1, F_2, F_3, and F_4 were found to release 87, 76.36, 61.23, and 50.76% of the loaded drug respectively. The data obtained for *in vitro* release were fitted into equations for zero order, first order, and the Higuchi release model [Figures 4 and 5]. The interpretation of data was

Table 1: Yield, drug entrapment, and average particle size of replaglinide-loaded poly(ε-caprolactone) microspheres

Formulation code	Drug: polymer	Percent * Yield (%)	Drug * entrapment % w/w	Average * particle size (μm)
F_1	1: 3	82.68 ± 2.40	84.01 ± 3.23	36.70 ± 5.66
F_2	1: 4	84.18 ± 3.28	89.36 ± 2.58	47.73 ± 6.93
F_3	1: 5	87.68 ± 2.51	86.75 ± 2.78	57.54 ± 7.27
F_4	1: 6	85.10 ± 3.09	85.20 ± 3.55	65.44 ± 10.33

Average of three Preparation ± SD

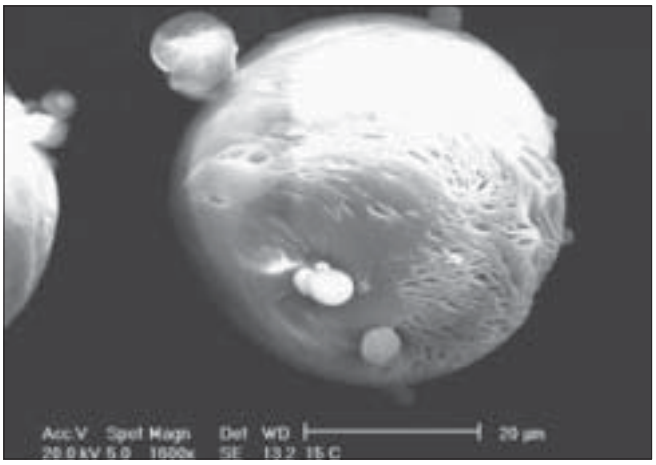

Figure 1: Scanning electron microphotograph of replaglinide-loaded poly(ε-caprolactone) microspheres

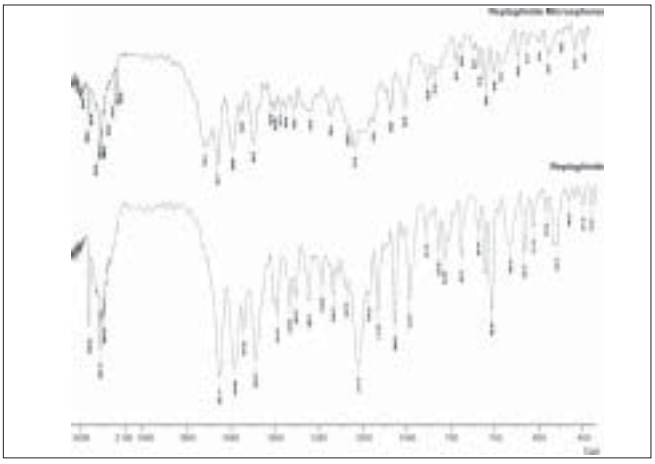

Figure 2: FT-IR spectra obtained for pure replaglinide and replaglinide -loaded poly(ε-caprolactone) microspheres

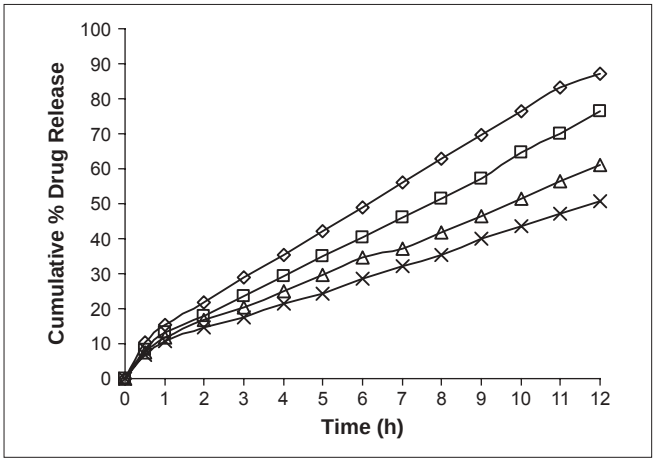

Figure 3: *In vitro* drug release profiles of replaglinide from poly (ε-caprolactone) microspheres formulations F_1(-◊-), F_2(-◊-), F_3(-Δ-) and F_4(-×-)

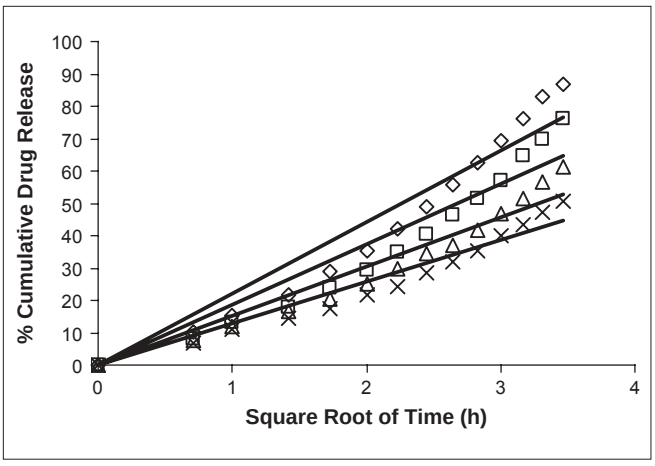

Figure 4: Diffusion-controlled release profiles of replaglinide from poly (ε-caprolactone) microspheres formulations F_1(-◊-), F_2(-◊-), F_3(-Δ-) and F_4(-×-)

based on the value of the resulting regression coefficient [Table 2]. The *in vitro* drug release from the microspheres showed that the regression coefficient value was close to one for zero order, indicating that drug release followed zero order kinetics.

CONCLUSION

This method of preparation of poly (ε-caprolactone) microspheres of replaglinide was found to be simple and reproducible. The carrier used, poly (ε-caprolactone), is biocompatible and biodegradable. From the above data, it may be concluded that drug-loaded microspheres appear to be a suitable delivery system for replaglinide and may help to reduce the dose of the drug and frequency of administration.

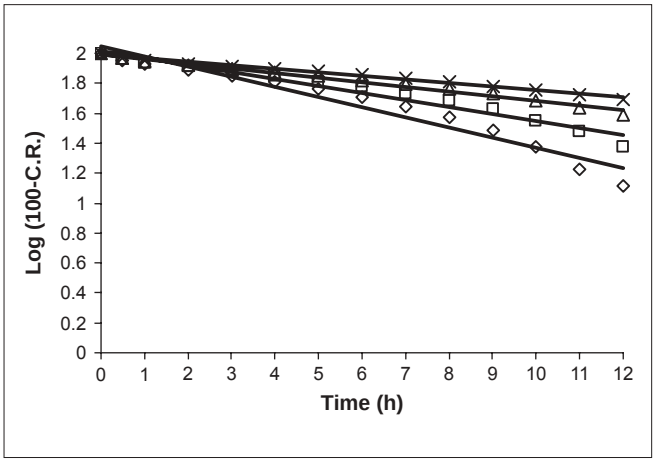

Figure 5: First order release profiles of replaglinide from poly(ε-caprolactone) microspheres formulations F_1(-◊-),F_2(-◊-), F_3(-Δ-) and F_4(-×-)

Table 2: Value of r^2 and K from release data of various formulations for different models of mechanisms of drug release

Formulation	Zero order		First order		Higuchi	
	r^2	K_o	r^2	K_1	r^2	K_H
F_1	0.994	6.966	0.951	0.155	0.961	26.175
F_2	0.995	5.954	0.964	0.107	0.510	22.242
F_3	0.990	4.666	0.985	0.071	0.963	17.58
F_4	0.988	3.875	0.991	0.054	0.965	14.626

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