Research Article

PREPARATION AND EVALUATION OF ONCE A WEEKLY BIODEGRADABLE MICROSPHERE CONTAINING A BIOLOGICALLY ACTIVE AGENT BY THE SOLVENT EVAPORATION TECHNIQUE

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ABSTRACT

The microencapsulation process in which the removal of the hydrophobic polymer solvent is achieved by evaporation has been widely reported in recent years for the preparation of microspheres based on biodegradable polymers. The purpose of our present study was to investigate the feasibility of prolonged delivery of a synthetic peptide, Pramlintide, with a biodegradable PLGA microsphere depot formulation. Pramlintide loaded PLGA microspheres were prepared by the O/W single emulsion-solvent evaporation method and the *in vitro* drug release profiles from PLGA microspheres were investigated. Pramlintide loaded PLGA microspheres with a suitable particle size (mean diameter about 40 – 100 µm based on the preparation conditions) and high entrapment efficiency (> 90%) were prepared. All the prepared microspheres were subjected to various physicochemical studies, such as drug-polymer compatibility by Thin Layer Chromatography (TLC) and Fourier Transform Infrared Spectroscopy (FTIR), surface morphology by Scanning Electron Microscopy (SEM), frequency distribution, encapsulation efficiency by High Performance Layer Chromatography, *in vitro* drug release characteristics.TLC and FTIR studies indicated no drug-polymer incompatibility. The *in vitro* release study showed sustained drug release over 7 d with obvious "burst release" in the first 24 h. Such "burst release" could be decreased by introducing high viscosity grade PLGA into the microsphere matrix and able to achieve close to zero-order kinetics without a significant burst effect. Pramlintide PLGA microspheres can be uniformly suspended in aqueous medium for subcutaneous injection, and may be used for sustained delivery of Pramlintide to Diabetic patients.

Keywords: PLGA, Microspheres, Pramlintide, Sustained release, Single emulsion-solvent evaporation.

INTRODUCTION

Diabetes mellitus is the only non-infectious disease designated as an epidemic by the World Health Organization[1]. The prevalence of all types of diabetes is estimated to be 2.3 % of the world's population, with the number of diabetic increasing by 4 to 5% per annum. It is projected that as many as 40 to 45% of people aged 65 or greater have either type 2 diabetes or its precursor state, impaired glucose tolerance (IGT). Diabetes can treat by a combination of lifestyle and dietary changes medication. The United Kingdom Prospective Diabetes Study (UKPDS), a long-term study of type 2 diabetics, showed that rigorous management of blood pressure substantially reduced the incidence of complications such as peripheral nerve damage, kidney damage, impaired blood circulation and damage to the retina[2,3].

Pramlintide (25,28,29-pro-h-amylin) is the synthetic analog of human amylin, which is a 37-amino acid peptide related to calcitonin gene-related peptide (CGRP) and calcitonin, and is co-secreted with insulin in response to elevated plasma glucose concentrations from pancreatic β-cells. Pramlintide is an injectable drug that lowers the level of sugar (glucose) in blood. It is used for treating type 1 and insulin-using type 2 diabetes[4-9].

An injectable, maltidose liquid formulation for Pramlintide drug product has been marketed to permit chronic self-administration by the anticipated patient population[10]. However, the complexity of the Pramlintide treatment regimen, including the frequency of administration and duration of treatment, negatively affects patient compliance. Reduction of the required frequency of administration is one strategy that might significantly enhance compliance.

Encapsulation with biodegradable polymers has been considered as one possibility to overcome various obstacles associated with the systemic delivery of peptide drugs. Biodegradable polymer microspheres using poly (lactide-co-glycolide) (PLGA) and poly(lactic acid) (PLA) as wall materials containing water-soluble bioactive substances (such as proteins, peptides) have received a great deal of interest in recent years due to their many advantages, such as improved patient compliance, increased bioavailability and reduced immunogenicity. PLGA microspheres have been widely investigated and used as injectable depot drug carriers[11-30].

The aim of our present study was to investigate the feasibility of developing an injectable depot formulation for chronic delivery of Pramlintide, as well as improving patient compliance and achieving better therapeutic efficiency by reducing the frequency of injections and decreasing the fluctuations in plasma drug levels. In this study, Pramlintide loaded PLGA microspheres were prepared by the conventional O/W emulsion-solvent evaporation method and the *in vitro* release profiles were investigated.

MATERIALS AND METHODS

Pramlintide (purity > 99%) was provided by Sun Pharmaceutical Ltd. Co (Vadodara, Gujarat, India). PLGA of different monomer ratios and intrinsic viscosity (IV) grades was purchased from Lakeshore biomaterial, USA. In this study, PLGA5050 (LA/GA = 50:50, IV = 0.78 dL/g), PLGA5050 (LA/GA = 50: 50, IV = 0.67 dL/g), PLGA5050 (LA/GA = 50: 50, IV = 0.48 dL/g), PLGA5050 (LA/GA = 50: 50, IV = 0.36 dL/g) and PLGA5050 (LA/GA = 50:50, IV = 0.28 dL/g) were used as wall materials for the microspheres. Polyvinyl alcohol (PVA, Mw: 30-70 kDa) was from (Merck, Germany). All other reagents were from commercial sources.

Preparation of microspheres

Pramlintide microspheres were prepared using oil-in-water (O/W) emulsion solvent evaporation. Briefly, 10 mg of Pramlintide were dissolved in 1.0 mL of dichloromethane and 1.0 mL of methanol containing 300 mg of PLGA. The Pramlintide oily solution was emulsified in continuous phase (W: 1%PVA in water for injection) at a W: O volume ratio of 25:1 by homogenization at 3000 rpm for 1 min (Lab Mixer, silverson). After emulsification, the dichloromethane and methanol were evaporated by stirring at 37°C for 3 h. The resultant microspheres were collected by centrifugation, washed three times with water for injection, and freeze dried (Fig. 1).
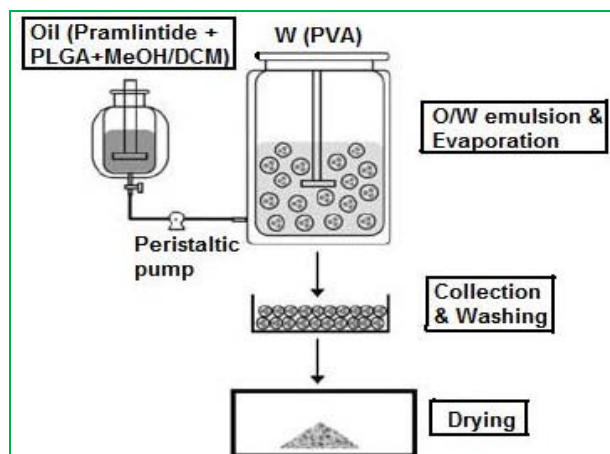

Figure 1: Schematic illustration of the solvent evaporation method used to prepare the Pramlintide microspheres.

In vitro drug release

A sample of accurately weighed microspheres (10 mg) was dispersed in 1 ml of a release test solution (10 mM HEPES, pH 7.5, 100 mM NaCl), and incubated at 37°C under mild stirring at 5 rpm. At intervals, the tubes were taken out and centrifuged at 5,000 r/min for 10 min, and the supernatants were removed and stored in a refrigerator until HPLC analysis, and 1 ml fresh release media was added to each tube. The microspheres were redispersed uniformly by vigorous vortexing before further release studies. Each experiment was performed in triplicate. The shape and surface morphology of the microspheres were observed after different periods in the *in vitro* release experiments.

Analysis of Pramlintide

Reversed phase high performance liquid chromatography (RP-HPLC) was used to determine the concentration of Pramlintide. The liquid chromatograph was equipped with a 215 nm detector and an YMC Pack Pro C18 column (250 × 4.0) mm, 3 µm. A mixture of phosphate buffer and acetonitrile (70: 30) was used as the mobile phase at a flow rate of 0.8 ml per minute.

Thin Layer Chromatography (TLC)

Thin Layer Chromatography was carried out in TLC chamber. The sample solution of pure drug and prepared microspheres were prepared by dissolving in methanol: water (80:20) and applied to silica gel G plates. The plates were then developed in the following solvent systems.

Solvent system 1: n-butanol: water: methanol: ammonia (20%) (14:0.2:0.2:2 %v/v/v/v)

Solvent system 2: Concentrated ammonia: alcohol (20:80 % v/v)

The R_f value of the pure drug as well as prepared microspheres were determined by placing the plates in an iodine chamber and the R_f value of pure drug was compared with the R_f value of prepared microspheres.

Fourier Transform Infrared Spectroscopy (FTIR)

Infrared (FTIR) spectrum of the Pramlintide loaded microspheres, and physical mixture were recorded using a FTIR (Model FTIR-8400S, Shimadzu, Japan) spectrometer using KBr pellets (400-4000^{-1}) with a scanning speed of 2mm/sec.

Scanning Electron Microscopy (SEM)

Scanning electron microscope (LEO, 430 surface controlled digital SEM) was performed to characterize the surface of formed microspheres. A small amount of microspheres were spread on glass stub. Gold palladium coating on the prepared stub was carried out by using sputter coater. Afterwards, the stub containing the sample was placed in the electron microscope. The scanning electron photomicrograph (Plate-1, Plate-2) was taken at acceleration voltage of 15 kV, chamber pressure of 0.3 Torr.

Frequency distribution analysis

Samples of microspheres were analyzed for frequency distribution with calibrated optical microscope fitted with a stage and an ocular micrometer. Small quantities of microspheres were spread on a clean glass slide and the average size of 200 particles, frequency distribution as determined in each batch using the calibration factor.

Determination of Percent Drug Entrapment (PDE)

Efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment (PDE) as per the following formula;

PDE = Practical drug loading*100/Theoretical drug loading

Theoretical drug loading

Theoretical drug loading was determined by the calculation assuming that the entire drug present in the polymer solution used gets entrapped in microspheres, and no loss occurs at any stage of preparation of microspheres[31].

Practical drug loading

Practical drug loading was analysed as follows:

20 mg of microsphere were added to 100 ml of acetonitrile and methanol in ratio of 3:2 and occasionally shaken for 30 min. The solution was centrifuge and 1 ml of clear supernatant was diluted to 10 ml of water, the supernatant liquid was filter through Watt mann filter paper and analysed for Pramlintide by High Performance Liquid Chromatography.

Preparation and characterization of microspheres

Microspheres prepared by the solvent evaporation method normally have a wide particle size distribution and a mean diameter that varies from batch to batch. The mean diameters of the microspheres made from different types of PLGAs or PLGA blends were between 40-100 μm. Normally, the higher the intrinsic viscosity of the PLGA used, the higher the mean diameter obtained (Table 1). The scanning electron micrographs showed that the Pramlintide microspheres were spherical in shape with a smooth surface. The typical entrapment efficiencies were more than 90% as shown in Table 1. In order to obtain a high trapping efficiency, an O/W single emulsion-solvent evaporation procedure was adopted.

Table 1: Particle size, Drug entrapment of Pramlintide microspheres

S. No.	Formulation code	Variability (PLGA Intrinsic Viscosity, dL/g)	Paticle Size (μm) Mean±S.D	Drug entrapment efficiency (%) Mean±S.D
1	S1	0.78	98.70±3.0	95.11±1.2
2	S2	0.67	70.83±2.4	91.32±2.2
3	S3	0.48	50.90±1.5	88.12±1.3
4	S4	0.36	45.33±3.2	80.33±3.2
5	S5	0.28	40.23±1.4	68.00±1.6

In vitro drug release

The typical drug release profiles of Pramlintide PLGA microspheres exhibited significant "burst" release followed by slow drug release for over 7 days (Fig. 2). Although, the microspheres with the high viscosity PLGA (IV = 0.78 dL/g) not showed even the lowest "burst" release (not more than 1% in the first 24 h), the drug release rate was also slow. Even after 7 d of *in vitro* release, the cumulative drug release was less than 50% of the total drug loaded. Hence, polymer blends of PLGAs with various viscosities were used as matrix materials for microspheres in further investigations in order to lowest "burst release". By using blends of different viscosity PLGAs as matrix material for the microspheres, the burst release in the first 24 h could be limited to less than 1% of the total drug loaded, but the disadvantage was that the drug release rate was inevitably decreased at the same time Figure 2 Sometimes, the burst release was caused by the rapid diffusion release of drug from the surface of the microspheres. We believe that the initial rapid release observed in present studies may due to some of the Pramlintide molecules migrating from the inner phase to the surface of the microspheres during the drying process. According to our present results, the intrinsic viscosity of the matrix materials had a significant effect on *in vitro* drug release, but further experiments should be carried out to obtain ideal release profiles.

Drug release from PLGA microspheres was generally considered as a polymer degradation-controlled process. The SEM observations of microspheres after different periods of *in vitro* drug release testing showed a gradual degradation of the microsphere matrix. As shown in Figure 3, with the degradation of PLGA, the microsphere morphology changed from an original smooth surface to a porous surface and, finally, the microspheres broke into pieces.
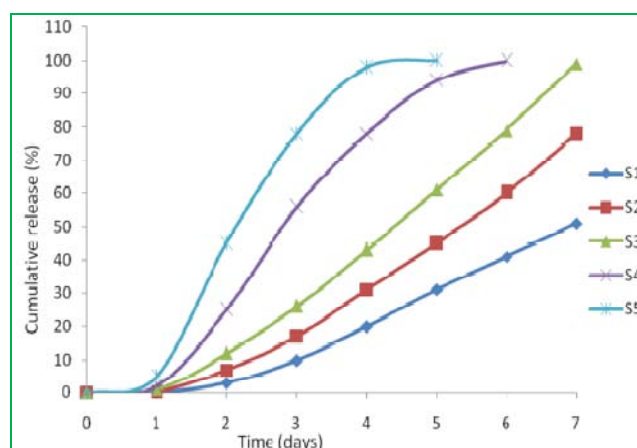

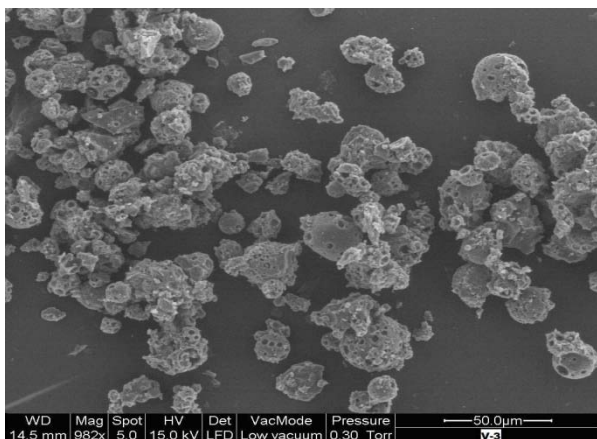**Figure 2:** *In vitro* release of Pramlintide Microsphers.

Figure 3: Microsphere showing porous surface during *in-vitro* release.

Compatibility studies

Chemical interaction between drug and the polymeric material, if any, during the preparation of microspheres was studied by using a TLC and FTIR. The comparable Rf values of microspheres in the TLC study indicated the compatibility of drug with polymer and other excipients used in the preparation of Pramlintide microspheres [32]. No difference in the IR patterns of a physical mixture (Figure 4), and Pramlintide loaded microspheres was observed (Figure 5). Therefore, the FTIR studies ruled out the possibility of any drug polymer interaction during the preparation of microspheres [33].
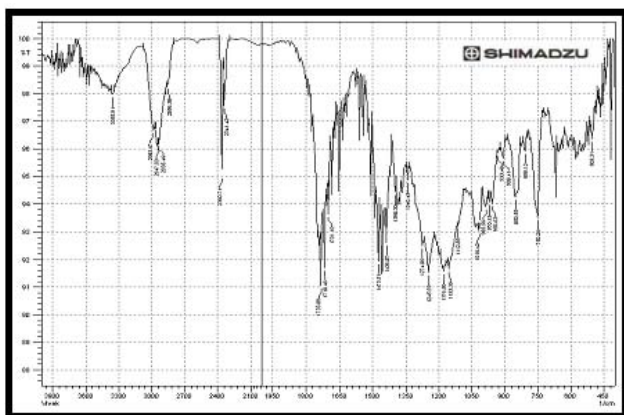

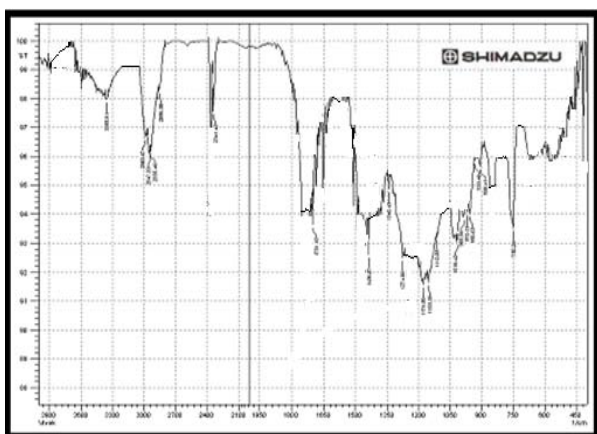**Figure 4:** FTIR Spectrum of Physical mixture.

Figure 5: FTIR Spectrum of Pramlintide loaded microsphere.

Morphological characteristics (SEM)

The surface morphology of Pramlintide loaded microspheres were studied by scanning electron microscopy (Figure 6). Surface smoothness of microsphere was increased by increasing the polymer concentration and viscosity, which was confirmed by SEM.
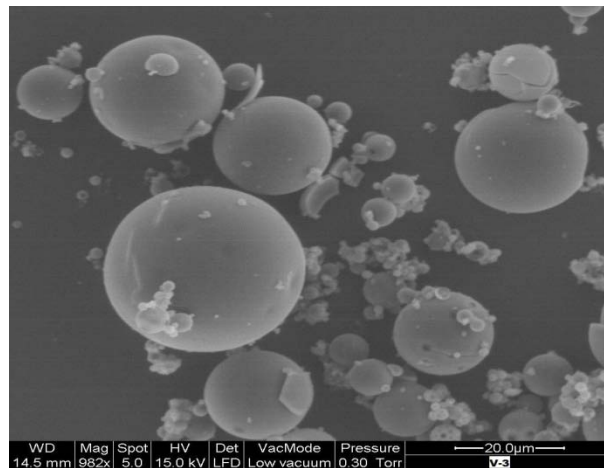

Figure 6: Microsphere showing spherical in shape with a smooth surface before *in-vitro* release.

Particle size distribution

The results of accuracy and precision of frequency distribution studies showed the normal frequency distribution of microspheres. As the viscosity of polymer was increased, the mean particle size of Pramlintide microspheres was also increased (Table 1). The significant increase may be because of the increase in the viscosity of the droplets (due to the increase in concentration of polymer solution). This increase is high enough to result in difficult dispersion and subdivision of droplets reported [34, 35].

Drug entrapment efficiency

The drug loading efficiency of Pramlintide microspheres was determined by HPLC method. A more than 90% of drug entrapment efficiency was obtained by the method employed (Figure 7). By increasing the polymer concentration the encapsulation efficiency was increased (Table 1).
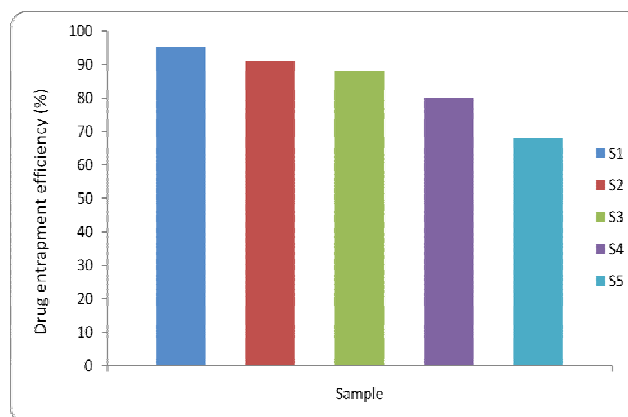

Figure 7: Drug entrapment efficienies in various microspheres.

CONCLUSION

Pramlintide loaded PLGA microspheres with a suitable particle size, high entrapment efficiency and relatively low "burst release" were prepared by the single emulsion- solvent evaporation method, and they could be readily suspended in appropriate medium for SC administration. Such Pramlintide PLGA microspheres may be used as an alternative dosage form of Pramlintide with sustained drug release properties and improved patient compliance, thereby offering an almost ideal therapeutic effect.

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