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PREPARATION AND CHARACTERIZATION OF POLY (D, L-LACTIDE-CO-GLYCOLIDE) NANOPARTICLES CONTAINING CLOPIDOGREL BISULFATE

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ABSTRACT

Clopidogrel bisulfate, a potent oral antiplatelet agent, is a drug of first choice used in the treatment of coronary artery disease, peripheral vascular disease, and cerebrovascular disease. PLGA nanoparticles loaded with clopidogrel bisulphate were prepared with 5 ratios and then characterized with respect to size, surface morphology, entrapment efficiency, *invitro* drug release profile and stability studies under specific conditions. The diameter of particles was ranging from 120 nm to 600 nm. The highest cumulative percentage of drug release (98.01 %) was observed with F_1, whose entrapment efficiency was 48.18 % and contains Clopidogrel bisulfate/PLGA in the ratio of 15/85 %.

Keywords: Platelet, Clopidogrel, Nanoparticles, PLGA.

INTRODUCTION

The drug delivery technology has certainly infused new interests in seemingly traditional old drugs by providing them new life especially through their therapeutic targets. Many drugs, particularly chemo-therapeutic agents have narrow therapeutic window (low therapeutic indices) and their clinical use is limited and compromised by dose limiting toxic side effects. Several advancements resulted in the development of new techniques for drug delivery, which are capable of controlling the rate of drug delivery, sustaining the duration of therapeutic activity and targeting the delivery of drugs to a cell or tissue [1, 2].

Carriers are drug vectors, which sequester, transport and retain drug on route, while elute or deliver it within or in the vicinity of target. Nanotechnology has the potential to produce low-cost, self-replicating systems that could revolutionize the scientific landscape. Nanoparticle drug delivery, utilizing degradable and absorbable polymers, provides a more efficient, less risky solution to many drug delivery challenges [3-6]. Thrombosis is the formation of a blood clot (thrombus) inside a blood vessel, obstructing the flow of blood through the circulatory system [7, 8]. Platelet aggregation and adhesion act together to form the platelet plug. Oral agents often used to alter/suppress platelet function are aspirin, clopidogrel, cilostazol and ticlopidine. Clopidogrel, a potent oral antiplatelet agent and a novel ADP receptor antagonist, is a drug of first choice used in the treatment of coronary artery disease, peripheral vascular disease, and cerebrovascular disease. Currently it is also used in the prophylaxis of sub-acute stent thrombosis and post-ischemic stroke treatment [9-11]. It exerts its antiplatelet effect by inhibiting adenosine diphosphate—induced platelet aggregation pathway. The aim of this study was to conceptualize an ideal drug delivery system for clopidogrel bisulfate. The formulated PLGA nanoparticles deliver clopidogrel bisulfate to specific site within the body and also control the delivery rate. Thus, using the system for the controlled and balanced release of medicaments, opposing to standard and conventional methods, constant and uniform concentration of clopidogrel bisulfate is achieved for longer period of time in the body.

MATERIALS AND METHODS

Materials

Clopidogrel bisulfate was obtained as gift sample from Aravind Pharmaceuticals, Chennai. PLGA was procured from Sigma Aldrich Chemical Pvt Ltd, Bangalore. Others excipients like polyvinyl alcohol, methanol, acetone, hydrochloric acid, potassium dihydrogen phosphate and sodium hydroxide were procured from Hi-Pure Fine Chem Industries, Chennai.

METHODS

Preparation of Calibration Curve

Calibration curve of clopidogrel bisulfate was prepared using 0.1 N HCl in the concentration range from 5 to 50 µg/ml. The drug was analyzed spectrophotometrically (UV 1600 Shimadzu, Japan) at 270 nm.

Preparation of Nanoparticles

PLGA nanoparticles containing clopidogrel bisulfate were produced by using nanoprecipitation method with solvent/nonsolvent systems. PLGA and the drug were dissolved in small quantity of acetone and distilled water respectively. After 2 hrs methanol was added and the polymeric solution thus obtained was poured slowly into aqueous PVA solution (0.02 %w/w) while continuous stirring at 1200 rpm. Then the solution was centrifuged at 4000 rpm for 120 min and decanted. Clopidogrel bisulfate was thus encapsulated into the polymeric matrix in different concentrations by means of homogenization of aqueous and organic phases [12-16]. The various compositions of the formulations are shown in Table 1.

Table 1. Composition and Characterisation of Clopidogrel bisulfate loaded PLGA Nanoparticles

S.No	Formulation Code	Clopidogrel bisulfate (mg)	PLGA (mg)	0.02% PVA (ml)	Methanol (ml)	Drug Content (%)	Entrapment Efficiency (%)
1	F_1	15	85	20	2	69.15	48.18 (± 0.07)
2	F_2	30	70	20	2	55.89	43.17 (± 0.06)
3	F_3	50	50	20	2	45.94	35.86 (± 1.03)
4	F_4	70	30	20	2	29.05	21.92 (± 0.92)
5	F_5	85	15	20	2	17.67	19.26 (± 0.11)

Drug-excipient interaction studies

Fourier Transform Infrared (FTIR) Spectroscopy

FT-IR spectra for drug, polymer and other excipients were obtained by KBr pellet technique using powder diffuse reflectance on a FT-IR spectrophotometer in the wave number region of 400-4000cm^{-1}. Thus, the identity of drug and other excipients were identified through the interpretation of IR spectra.

Differential Scanning Calorimetry (DSC, Perkin Elmer)

5-10mg of sample was weighed, hermetically sealed in flat-bottomed aluminium-pans and heated over a temperature range of 50-550^{0}C in an atmosphere of nitrogen (50ml/min) at a constant heating rate of 20^0 C per min with alumina being the reference standard [17, 18].

Characterization of PLGA Nanoparticles

Determination of surface morphology

5ml of the clopidogrel bisulfate nanoparticulate suspension was transformed to a cover slip, which in turn was mounted on a specimen tab. The samples were allowed to dry at room temperature. Then the surface of the particles was viewed and photographed using Scanning Electron Microscope (SEM). Clopidogrel bisulfate loaded PLGA nanoparticles were coated with platinum by using vacuum evaporator. Thus, the coated samples were viewed and photographed in JEOL JSM-6701F Field Emission SEM.

Particle size distribution Analysis

The prepared nanoparticulate suspension was subjected to laser particle counting using Nanosizer for characterization of its size distribution. Here the sample was injected into the sample delivery port which reaches the controlling chamber and then suitable solvent was pumped through the chamber. Now, a beam of laser light was allowed to fall on the sample cell. After required number of runs, they were directed towards the detector. From this the particle size range and the average mean particle size of the formulation can be studied [19, 20].

Drug Content Analysis

For determination of drug content, 1ml of nanoparticulate suspension was swirled with 1ml of 0.1 % Triton X-100 for 1 hr and kept aside at room temperature for 24 hrs. Then, the resulted solution was analyzed spectrophotometrically at 270 nm after suitable dilution with distilled water.

Drug Entrapment Efficiency

The loading efficiency of clopidogrel bisulfate in PLGA nanoparticles was determined by ultracentrifugation at 20,000 rpm for 30min at 5^{O}C. Then the resulting supernatant solution was decanted and dispersed into phosphate buffer saline pH 7.4. Thus the procedure was repeated to remove the unentrapped drug molecules completely. The amount of drug entrapped in the nanoparticles was determined as the difference between the total amount of drug used to prepare the nanoparticles and the amount of drug present in the aqueous medium.

***In vitro* diffusion studies**

The diffusion studies were performed in triplicate for all the batches in a Franz diffusion cell apparatus using 0.1 N HCl at 37 ± 0.2ºC. The donor and receptor compartments are partitioned using sigma dialysis membrane. Periodically 5ml aliquots were withdrawn and after each withdrawal, same volume of fresh diffusion medium was replaced. Then the samples were analyzed UV spectrophotometrically at 270nm using 0.1N HCL as blank.

Stability studies

The purpose of stability testing is to provide evidence on how the quality of a drug product varies with time under the influence of variety of environmental conditions such as temperature, humidity and light. The formulation which showed best *in vitro* release was selected for stability studies. The accelerated stability studies were conducted according to the ICH guidelines based on Q1C for a period of 6 months.

RESULTS AND DISCUSSION

Novel drug delivery system using various carriers like nanoparticles results in successful transportation of the loaded drug moiety. Several advancements resulted in the development of new techniques for drug delivery are capable of controlling the rate of drug delivery, sustaining the duration of therapeutic activity and targeting the delivery of drugs to a cell or tissue. Thus, it produces constant effective drug level in the body with concomitant minimization of undesirable side effects and thereby improves patient compliance due to reduction in dosing frequency.

The Clopidogrel bisulfate nanoparticles were prepared by nanoprecipitation method. Calibration curve of clopidogrel bisulfate was found to be linear in 0.1 N HCl at the wavelength of 270 nm between the concentration range of 5 and 50 µg/ml. The correlation coefficient was found to be 0.9984.

Compatibility studies were carried out among the drug, polymer and other excipients by FTIR and DSC techniques. There were no new bands or shift in characteristic functional peaks produced by FTIR and no considerable change was observed in melting endotherm by DSC. The results revealed that the drug and the excipients used in the formulation are compatible with each other and were shown in the Figure 1 and 2.

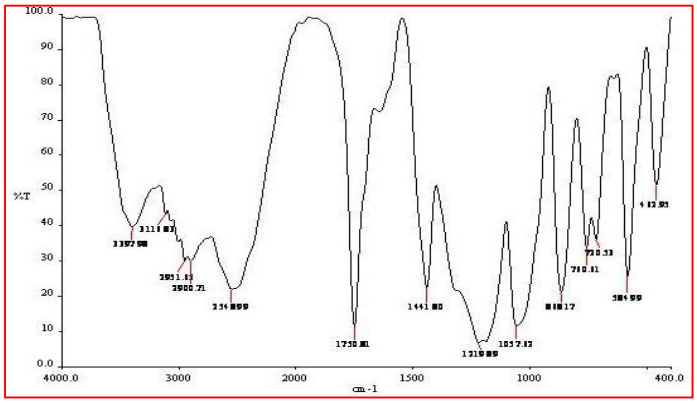

Figure 1. FT-IR Spectra of mixture of Clopidogrel bisulfate, PLGA and PVA

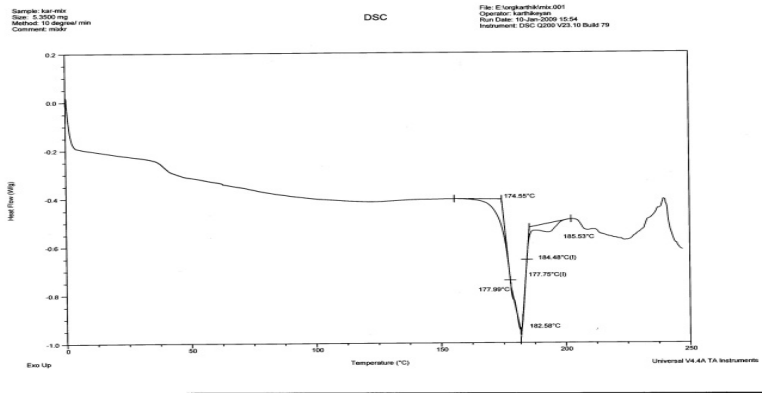

Figure 2. DSC chromatogram of mixture of Clopidogrel bisulfate, PLGA and PVA

The formulated nanoparticles were evaluated for various parameters like surface morphology, average mean particle size, percentage drug content and entrapment efficiency. The surface of the prepared nanoparticles was smooth and spherical (Figure 3) and the particle size ranges between 18.50 to 352 nm and is given in Figure 4.

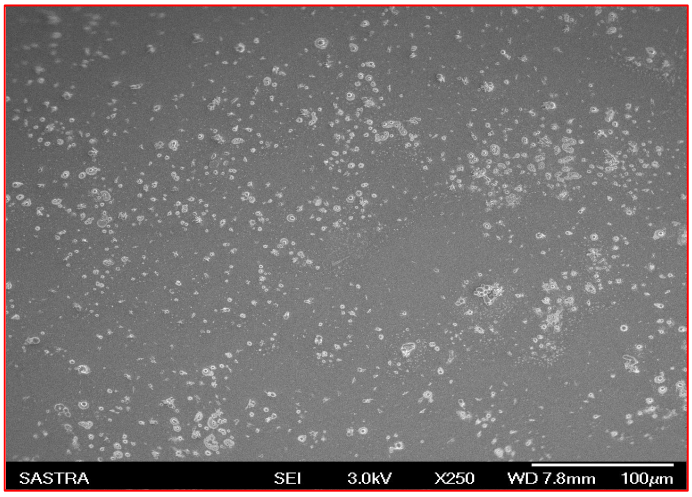

Figure 3. SEM Photograph of PLGA nanoparticle suspension F_1

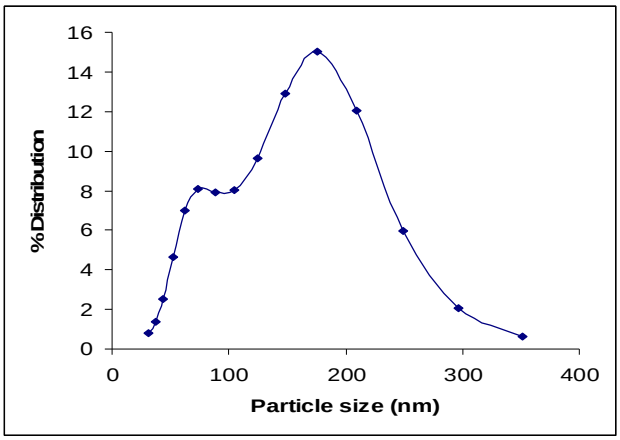

Figure 4. Particle Size Distribution of PLGA nanoparticle suspension

Drug content and entrapment efficiency of clopidogrel bisulfate from all the formulations was found to be in the range of 17.67 to 69.15 % and 19.26 to 48.18 % respectively and is given in Table 1. The formulation F1 was tested for its stability at three different conditions viz. refrigerated condition, room temperature and as per ICH guidelines at 40°C ± 2 °C & RH 75 % ± 5% and found to be stable with the results of 97.07%, 88.30% and 82.18 % respectively after the study period of 6 months.

For Free Drug and Clopidogrel bisulfate tablets, the cumulative percentage of drug released was 99.33 % at the end of 150 minutes and 99.30 % at the end of 20 minutes respectively and is given in Figure 5.

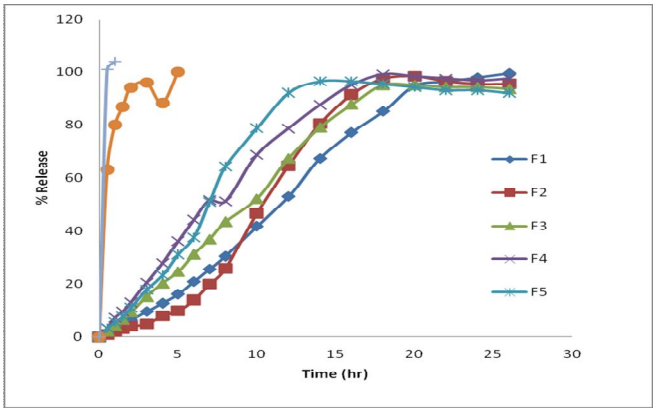

Figure 5. Comparative *Invitro* Diffusion Studies for F_1 - F_5

CONCLUSION

Nanoparticulate drug delivery system was employed effectively for the improved efficacy of the drug, clopidogrel bisulfate. The prepared clopidogrel bisulfate nanoparticulate formulation has found to have better efficacy than the existing conventional dosage forms. In

this emerging nanoworld, it would cause a real revolution in the field of medicine and pharmacy and all in favour of better medical treatments to the patients.

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