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Development and characterization of Poly (lactide-co-glycolide) microspheres loaded with Flutamide, an anticancer drug for controlled drug delivery

P. Kumara Babu[1], Y. Maruthi[1], A. Parandhama[1], C. Madhavi[1], H. Sudhakar[1], U. Sajankumarji Rao[2], K.V. Sekharnath[2], M.C.S.Subha[2] and K. Chowdoji Rao[1]*

[1]Department of Polymer Science & Technology, Sri Krishnadevaraya University, Anantapuramu, Andhra Pradesh, India – 515 003
[2]Department of Chemistry, Sri Krishnadevaraya University, Anantapuramu, Andhra Pradesh, India – 515 003

***Corresponding author e-mail:** chowdojirao@gmail.com

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ABSTRACT

Poly (Lactide-co-glycolide) (PLGA) microspheres were prepared through Oil in Water (O/W) emulsion-solvent evaporation method using dichloromethane as solvent. Flutamide (FLT), an anti Cancer drug, was used for encapsulation within PLGA microspheres. Morphology, size, encapsulation efficiency and drug release from these microspheres were evaluated. The Differential scanning calorimetry (DSC) confirmed the molecular level dispersion of Flutamide in the microspheres. Scanning electron microscopy (SEM) studies confirmed the spherical nature and smooth surface of the microspheres produced. X-ray diffraction studies (X-RD) was performed to understand the crystalline nature of drug after encapsulation into the microspheres. *In-vitro* release studies indicated a dependence of release rate on the concentration of polymer, the amount of drug loading, but slow release rates was extended up to 14 h.

Keywords: Poly (Lacide-co-glycolide), Flutamide, microspheres, SEM, X-RD.

INTRODUCTION

Polymer microspheres can be employed to deliver medicine in a rate-controlled and sometimes targeted manner. Micro and nanospheres fabricated from biodegradable polymers for drug delivery systems have become increasingly important owing to the fact that such systems enable controlled drug release at desired sites by active agents [1-2]. Carrier matrices are usually formed from biocompatible polymeric materials such as solid lipid microspheres [3-5], inorganic materials [6-7] or spheres fabricated from biodegradable polymers [8-9].

Biodegradable materials are natural or synthetic in origin and are degraded in vivo, either enzymatically or non-enzymatically or both, to produce biocompatible, toxicologically safe by-products which are further eliminated by the normal metabolic pathways. The number of such materials that are used in or as adjuncts in controlled drug delivery has increased dramatically over the past decade. Polyesters based on polylactide (PLA), polyglycolide (PGA), polycaprolactone (PCL), and their copolymers have been extensively employed as systems for controlled drug delivery [10-13]. PLGA and PLA have been approved by the FDA for numerous clinical applications, such as sutures, bone plates, abdominal mesh, and extended-release pharmaceuticals [14-15].

Now a days, poly(lactic-co-glycolic acid) (PLGA) is widely applied in controlled drug delivery systems due to its biodegradability, toxicological safety, and good biocompatibility [16-20]. Specifically, PLGA based carriers have been used in long-term drug

delivery systems because they have the potential to control drug release from a few days up to several months [21-22]. In general, PLGA can degrade into water soluble, non toxic products of normal metabolism through hydrolysis which is important for its practical application [23]. Moreover, PLGA is one of the few synthetic polymers which have been approved for human clinical use. Several products such as Lupron Depot based on PLGA microparticles are available in the market [20, 24]. However, PLGA carriers are mostly solid particles including nanoparticles, microparticles, and microcapsules, which have the advantages of low drug loading capacity, easy aggregation, and polydispersable particle sizes [25].

Flutamide(FLT) is an oral, non-steroidal antiandrogen drug used for the treatment of prostate cancer [26]. This drug has quite extensive fast pass metabolism, shorter elimination half life and poor bioavailability, which reduces testosterone only when administered on a continuous basis. Moreover high dose of Flutamide produces hepatotoxicity [27]. Flutamide is a prodrug that is rapidly metabolized to hydroxyflutamide, it's major active metabolite [28]. The recommended dose is associated nausea, diarrhea, vomting and increased appetite [29]. The chemical structure of Flutamide is as shown in Fig.1

In continuation of our controlled release studies [30-32] and as there were no reports in literature on the Flutamide release studies through PLGA, here we are presenting the details of the preparation of Flutamide loaded PLGA microspheres, their characterization and drug release results.

MATERILAS & EXPERIMENTAL METHODS

Materials:

Poly (D,L-lactide – co – glycolide)(M.Wt. 70,000 – 90,000), Flutamide, an anticancer drug, dichloromethane was purchased from Sigma Aldrich Chemicals (St. Louis), USA. Poly (ethylene glycol), Potassium mono hydrogen phosphate, Potassium dihydrogen phosphate and Hydrochoric acid were purchased from S.D.fine chemicals, Mumbai, India. Double distilled water was used throughout the research work.

Preparation of PLGA/Flutamide microsphere formulations:

The Flutamide loaded PLGA microspheres were prepared by the o/w emulsion solvent evaporation method. In this technique hydrophobic polymers and water-insoluble drugs are commonly used. PLGA (0.5g) was first dissolved in 10 mL of dichloromethane and then various concentrations (10%, 20% and 30% of polymer weight) of Flutamide was added to it. This solution was poured into 200 mL of purified water containing 1% poly (ethylene glycol) as emulsifier. The dichloromethane was removed by stirring at 1,000 rpm at room temperature for 2h. When evaporation was complete, the microspheres were collected by filtration on a filter paper (Whatman filter paper No. 40), washed three times with distilled water and air-dried overnight at room temperature. Each formulation was prepared at least thrice and resulting values along with % of encapsulation efficiency values are presented in Table 1.

Estimation of drug and encapsulation efficiency:

Flutamide loaded microspheres equivalent to 100 mg was stirred in 20ml of phosphate buffer solution (pH 7.4) and the drug content was analyzed by UV spectrophotometer (Lab India, Mumbai, India) at a λ_{max} of 200 nm. Encapsulation efficiency (EE) was calculated as the percentage (w/w) of the theoretical drug content (Equation 1). Results were based on triplicate and the average values are compiled in Table 1.

$$\% \text{ of Encapsulation efficiency} = \frac{\text{Actual loading}}{\text{Theoretical loading}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots(1)$$

In vitro release studies:

Dissolution was carried out using Tablet dissolution tester (Lab India, Mumbai, India) equipped with eight baskets. Dissolution rates were measured at 37 ± 0.5^{o} C at constant speed of 100 rpm. Drug release from the microspheres was studied in 7.4 pH phosphate buffer solution. At regular intervals of time, sample aliquots were withdrawn and analyzed using UV spectrophotometer (Lab India, Mumbai, India) at the fixed λ_{max} value of 270 nm. After each sample collection, the same amount of fresh medium at the same temperature was added to the release medium to maintain the sink condition. All measurements were carried out in triplicate, and values were plotted with standard deviation errors.

Differential Scanning Calorimetry (DSC):

Differential scanning calorimetric (DSC) curves were recorded on a TA instruments (Model: ST A , Q600 USA). The samples were weighed between 10 and 12 mg. The samples were heated from 50 to 400^{o}C at a heating rate of 10^{o}C/min in nitrogen atmosphere (flow rate of 100 mL/min).

X-ray diffraction (X-RD):

X-RD measurement of plain drug, plain

microspheres, and drug loaded microspheres were recorded using a Rigaku Geiger flex Diffractometry (Tokyo, Japan) equipped with Ni-filtered Cu Ka radiation (k = 1.548 A°). The dried microspheres of uniform thickness were mounted on sample holder, and the patterns were recorded in the range 0 –50^o at the speed of 50/min.

Scanning Electron Microscopy (SEM) studies:
To determine the particle size and size distribution, ~100–200 microspheres were taken on a glace slide and their sizes were measured using an optical microscope under regular polarized light. Scanning electron microscope (SEM) micrographs of microspheres were obtained under high resolution (Mag 3009 5kv) Using JOEL MODEL JSM 840A, SEM, equipped with phoenix energy dispersive analysis of X-ray (EDAX).

RESULTS AND DISCUSSIONS

Differential Scanning Calorimetry studies:
DSC studies were performed to understand the nature of the encapsulated drug in the matrix. The physical state of FLT in the polymer matrix would also influence its release characteristics. To probe this effect, DSC analysis was performed on (a) Pure drug (FLT), (b) Placebo PLGA Microspheres, (c) Flutamide loaded PLGA microspheres (Fig. 2). The melting endotherm peak of pure FLT is observed at 98^{o}C in Fig 2(a), which indicates the crystalline nature of the drug. The thermal analysis of PLGA revealed its characteristic glass transition temperature (T_g) at 56^{o}C (Fig. 2b). The T_g values was the same for the inert microspheres which indicates that dichloromethane had completely evaporated and the microencapsulation method employed did not alter the thermal characteristics of the polymer. The DSC curve (Fig. 2c) for the microspheres loaded with FLT was also endothermic showing a lower T_g for PLGA (47.2^{o}C), but the thermogram did not show the characteristic FLT peak, indicating a change in the crystallinity of the drug. The absence of detectable crystalline domains in the microspheres along with the presence of FLT degradation exotherm clearly indicates that drug was molecularly dispersed in the PLGA microspheres.

SEM Studies:
The SEM micrographs of Placebo PLGA (a) and FLT loaded PLGA(b) microspheres are shown in Fig.3. As seen in Fig 3, they were spherical in shape and exhibited rough surfaces due to higher concentration of drug in the microspheres. The mean particle size of PLF-3 formulation is around 100 - 200 μm. The size distribution is normal distribution showing 1μm. The particle size analysis also supports the formation of microspheres.

X-ray diffraction studies:
X-RD study is an important characterization technique in case of drug delivery applications, to study the crystallinity of drug present in the polymer matrix. XRD patterns of pure FLT (a), drug loaded microspheres (b) and pure PLGA microspheres (c) are shown in Fig.4. XRD pattern of pure FLT provides the clues about the crystallinity of drug in the microspheres. Here, the FLT drug peaks are observed at 2θ of 9^o to 25.3^o which are due to crystalline nature of FLT. But, in the case of drug loaded microspheres the drug peaks are observed with low intensity which indicate that the drug particles are dispersed in amorphous state in the polymer matrix. This was in agreement with the results observed by DSC analysis. This fact confirmed an interaction between polymer and FLT, when the latter was dissolved in the polymeric matrix.

In-vitro release studies:
The FLT loaded PLGA microspheres release behavior was examined in order to revealed their potential drug delivery system.

Effect of Drug content:
Fig. 5 shows the release profiles of Flutamide loaded PLGA microspheres at different amounts of drug loading in pH 7.4 phosphate buffer solution. The release data showed that the formulations containing highest amount of FLT (30 wt %) displayed higher release rates than those containing lower of amount of FLT. Formulation containing highest amount of FLT released 70.1 % (pH 7.4) of the total encapsulated drug. On the other hand, formulations containing lower amount of FLT (10 wt%) have released only 61.8 %. Thus, sustained release was observed for the formulation containing lower amount of FLT. Thus the release rates are slower for lower amount FLT in the matrix, probably due to the availability of more free void spaces through which a lesser number of drug molecules will transport.

Kinetics of In vitro release studies:
Drug-release kinetics was analyzed by plotting the cumulative release data versus time by fitting the data to a simple exponential equation (eqn. (2)) [33].

$$(M_t/M_\infty) = kt^n \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where M_t/M_∞ represents the fractional drug release at time t, k is a constant characteristic of the drug-

polymer system and 'n' is an empirical parameter characterizing the release mechanism. Using the least square procedure, we have calculated the values of n, k and r (correlation coefficient) for all the formulations and these values are given in **Table 2.** If n= 0.5, the drug diffuses and release from the polymer matrix following a Fickian diffusion. If n > 0.5, anomalous or non-Fickian drug diffusion occurs. If n = 1, a completely non-Fickian or case-II release kinetics is operative. The intermediary values ranging between 0.5 and 1.0 can be attributed to an anomalous type diffusive transport [34].

In the present study, the values of 'k' and 'n' showed a dependence on the % of drug loading are given in Table 2. The values of 'n' for microspheres prepared by using PLGA (0.5gm) while keeping various amounts of FLT (10, 20, 30 wt%) in pH 7.4 are ranged from 0.347 to 0.567. Further it is noticed that the 'n' values indicates a shift of transport from Fickian to the Non Fickian or anomalous type. Correlation coefficients, 'r' obtained in the present study varied from 0.746 to 0.963.

CONCLUSIONS

Flutamide loaded PLGA microspheres were developed by Oil/Water emulsion solvent evaporation method to study the controlled release of Flutamide drug. SEM, particle size analysis gave surface morphology and particle size of microspheres. DSC and XRD analysis of FLT loaded microspheres have shown molecularly dispersed drug in the microspheres. Based on *In vitro* release studies the FLT was released in a controlled manner by influencing the variation of drug percentage composition for more than 14 h.

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Table 1: Formulation Parameters and % Of Encapsulation of the Flutamide Loaded Microspheres.

Formulation code	PLGA(gm)	% of Flutamide	% of PEG solution	% of Encapsulation
PLF - 1	0.5	10	1	65.24 $\pm$ 0.24
PLF – 2	0.5	20	1	68.91 $\pm$ 0.15
PLF - 3	0.5	30	1	72.36 $\pm$ 0.23
PLF - 0	0.5	0	1	-

Table 2: Release kinetics parameters (k, n and correlation coefficient (r) values) for different formulations

Formulation Code	pH 7.4		
	n	k	Correlation coefficient (r)
PLF -1	0.347	2.9154	0.746
PLF -2	0.426	3.0159	0.859
PLF -3	0.567	3.1247	0.958

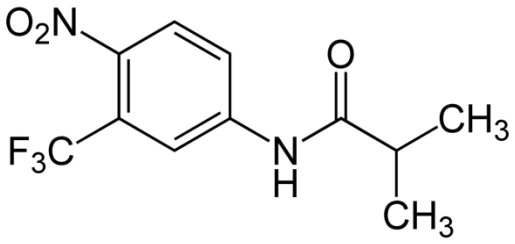

Fig. 1: Chemical structure of Flutamide

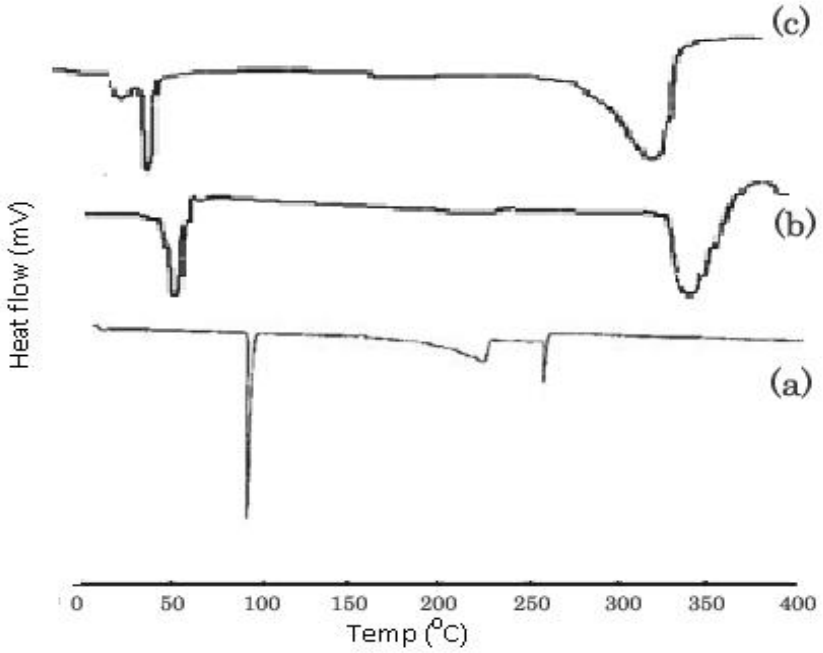

Fig. 2: DSC thermo grams of (a) pure drug (FLT) (b) PLGA microspheres (Without drug) (c) PLGA microspheres (with drug).

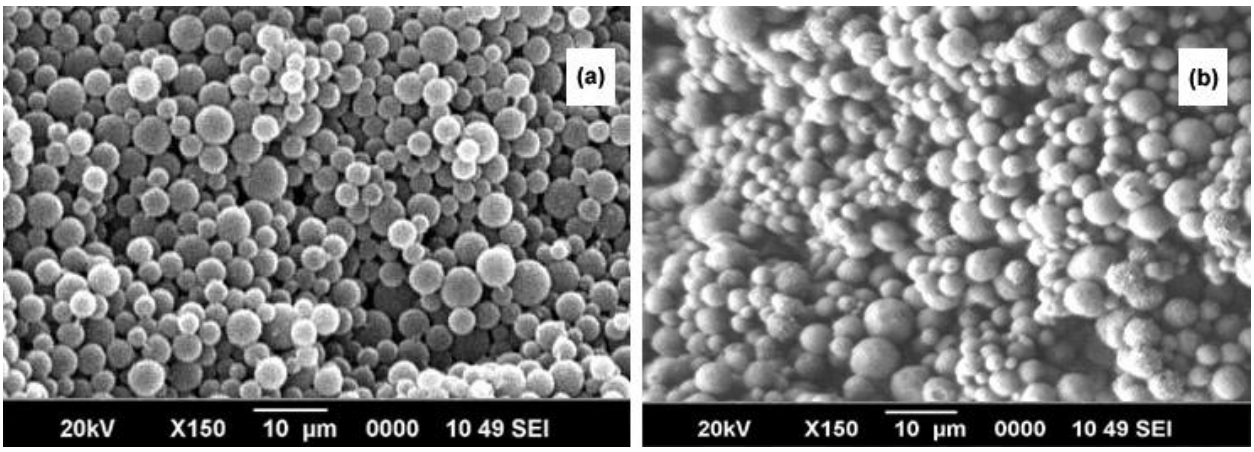

Fig. 3: SEM micrographs of (a) Placebo PLGA and (b) Flutamide loaded PLGA microspheres

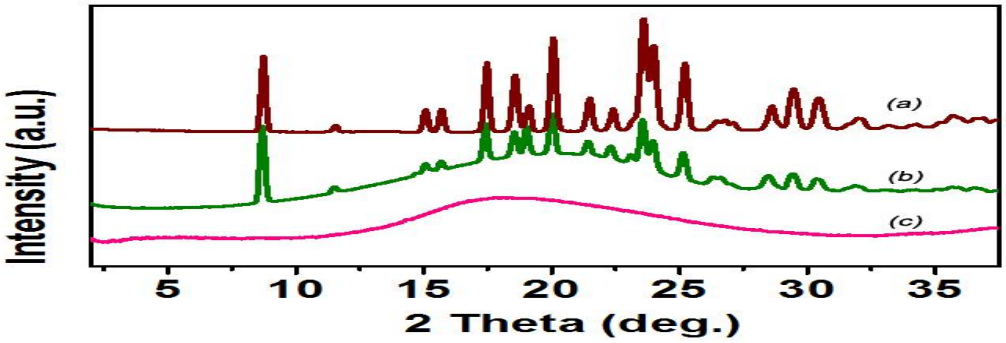

Fig. 4: XRD patterns of pure Flutamide (a), drug loaded PLGA microspheres (b) and pure PLGA microspheres (c).

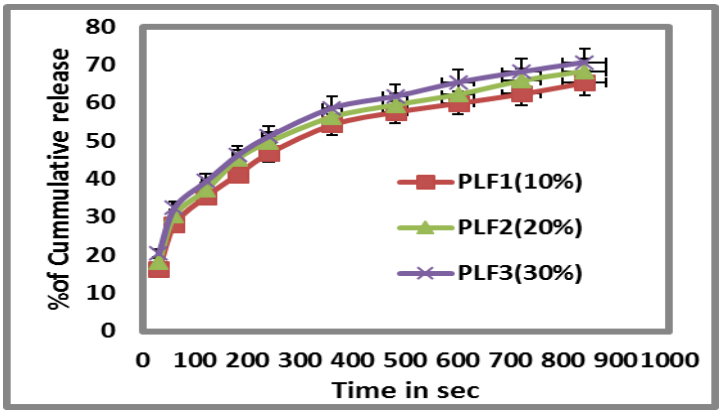

Fig. 5: % Cumulative release of Flutamide loaded microspheres containing different amounts of drug PLF 1 (10%), PLF 2 (20%), and PLF 3 (30%) in pH 7.4.

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