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Preparation and Characterization of Paclitaxel-loaded PLDLA Microspheres

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Paclitaxel (Taxol®), is a drug used to treat ovarian, breast, lung and bladder cancer. However, the low solubility of this drug in water is a major limitation in its clinical use. One strategy to overcome this limitation would be to encapsulate paclitaxel in polymeric microspheres that are biocompatible and can be used as drug carriers. The aim of this study was to use the bioresorbable, biocompatible copolymer poly-L-co-D,L-lactic acid (PLDLA) in the 70:30 rate to produce and characterize microspheres containing paclitaxel. The simple emulsion technique was used to obtain spherical microspheres that were studied by scanning electron microscopy (SEM) and atomic force microscopy (AFM). The average size of PLDLA microspheres without and with paclitaxel was 10.3 ± 1.7 μm and 12.7 ± 1.3 μm, respectively, as determined by laser light scattering (LLS). Differential scanning calorimetry (DSC) showed that pure paclitaxel had an endothermic peak corresponding to a melting point of 220 °C, which indicated its crystalline nature. The same peak was observed in a physical mixture of PLDLA + paclitaxel in which both components were present in the same proportions used to prepare the microspheres . In contrast, this peak was not observed for the drug, indicating that paclitaxel did not crystallize in PLDLA microspheres. Differential scanning calorimetry (DSC) indicated that paclitaxel was homogeneously dispersed in the PLDLA microspheres, the incorporation of paclitaxel into the microspheres did not alter the thermal properties of PLDLA. The Fourier transform infrared spectroscopy (FTIR) analysis seems to indicate the absence of chemical interaction between polymer and drugs in microspheres and the presence of drugs as a molecular dispersion in the polymer matrix. The efficiency of paclitaxel encapsulation in PLDLA microspheres was 98.0 ± 0.3%, as assessed by high performance liquid chromatography (HPLC). A kinetic study of drug release *in vitro* using HPLC showed an initial burst release followed by a slower release characteristic of large diameter distribution systems. PLDLA microspheres released 90 ± 4% of the drug over a 30-day period. These findings indicate that PLDLA microspheres are promising carriers for paclitaxel, with a potential for future applications in drug delivery systems.

Keywords: *chemotherapy, microspheres, paclitaxel, PLDLA*

1. Introduction

Paclitaxel (Taxol®), (Figure 1) is one of the best anticancer drugs and is active against a wide spectrum of cancers, including breast cancer, ovarian cancer, colon cancer, small and non-small cell lung cancer and neck cancer[1]. However, the main limitation in the clinical use of paclitaxel is its low solubility in water and most pharmaceutical solvents. The formulation of paclitaxel used clinically is a mixture containing the adjuvant Cremophor EL. This adjuvant is associated with severe side effects, including hypersensitivity reactions, nephrotoxicity and cardiotoxicity. Alternative paclitaxel formulations have been suggested to eliminate the Cremophor EL-based vehicle and to improve the therapeutic efficacy of the drug[1-7]. Despite the existence of many pharmaceuticals, natural or synthetic, used in the treatment of cancer, the biological features inherent to each type of tumor, along with resistance to the chemotherapeutic agents that tumor cells can develop boost the search for new products with antineoplastic action[1,8]. A considerable research has been conducted on drug delivery by biodegradable polymeric devices. Amongst the different classes of biodegradable polymers, the thermoplastic aliphatic poly(esters) like poly(lactide) (PLA), poly(glycolide) (PGA), a have generated immense interest due to their favorable properties such as good biocompatibility, biodegradability, and mechanical strength. Also, they are easy to formulate into different devices for loading of paclitaxel[3-7].

Polymeric microspheres are small spherical particles, with diameters in the micrometer range (typically 1 μm to 250 μm) and are typically used in biomedical applications. Polymeric microspheres have several advantages with respect to other drug systems, including greater therapeutic efficacy, a gradual, controlled release of the drug during

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matrix degradation, a significant decrease in toxicity, an increase in circulating concentrations, safe administration (without local inflammatory reactions) and convenient dosage regimens (fewer doses required), as well as the possibility of targeting specific cells or tissues in the body[9-12].

Poly L-co-D,L lactic acid (PLDLA), (Figure 2) is a polyester polymer that has been studied for various applications in the medical field[13]. Bioreabsorbable polymers are routinely used as temporary prostheses for fractured bones and drug delivery system. Among the bioreabsorbable polymers the poly(L-co-D, L lactic acid), PLDLA, in the 70:30 rate has been studied by the group of Biomaterials at PUC-SP for various applications in the biomedical field. In this monomers rate, an amorphous polymer is obtained. Depending on the application it is desirable that polymeric devices should have a low degree of crystallinity in order to facilitate degradation and hasten removal of the remaining fragments of material from the site of implantation, particularly since the accumulation of crystalline polymeric fragments may produce inflammatory reactions[13]. PLDLA has excellent biodegradability, biocompatibility and controllable degradation. In addition, PLDLA microspheres are degraded into non-toxic products in the human body. These characteristics make PLDLA an ideal new drug delivery carrier system[14].

The use of polymeric microspheres for drug encapsulation provides an alternative for drug delivery that helps to maintain the physical and chemical properties of the drug without changing its chemical structure. Drugs encapsulated within polymer matrices are not as readily available to biological systems as when they are in solution such that the drug will be released only after the onset of polymer degradation. Consequently, the polymer used to prepare the microspheres must be biocompatible and susceptible to hydrolysis when in contact with the body[15,16].

The aim of this study was to produce PLDLA microspheres loaded with paclitaxel and to examine their properties for potential future applications in drug delivery systems.

2. Material and Methods

2.1. *Materials*

Paclitaxel was donated by the pharmaceutical company Libbs (Embu das Artes, São Paulo, Brazil). Poly-L-co-D,L-lactide (PLDLA, Mw = 70.000, Mn = 41.000 e I.P = 1,7) was prepared by ring-opening polymerization, as previously described by Motta and Duek[13] using a 70:30 (w/w) ratio of L-lactide and D,L-lactide monomers (Purac Biomaterials, Schiedam, The Netherlands). The average molar mass in weight (Mw) and number (Mn) and the index polydispersivity (I.P) of the PLDLA were determined by gel permeation chromatography (GPC) using a column of Ultrastyragel (Waters) coupled to a refraction index detector (Waters 2414) . Samples of 3 mg dissolved in 1 mL of tetrahydrofuran (Merck) and applied to the column, which was eluted with tetrahydrofuran at a rate of 1 mL/min. The molar mass and rate of polydispersivity were calculated using polystyrene as a standard.

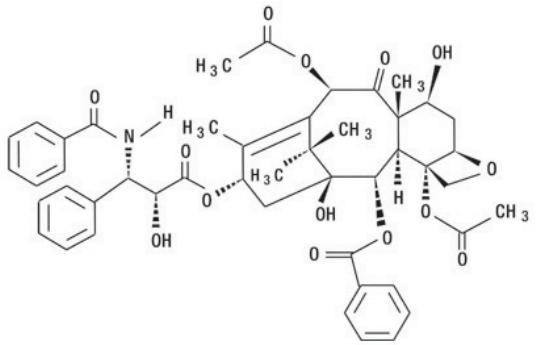

Figure 1. Molecular Structure of Paclitaxel Drug.

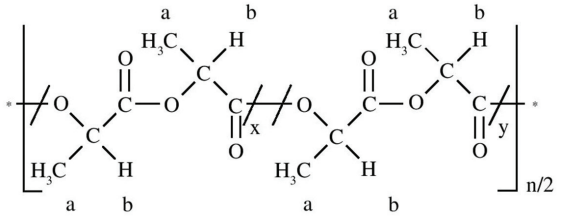

Figure 2. Molecular Structure of poly-L-co-D,L-lactic acid (PLDLA).

2.2. *Preparation of paclitaxel-loaded microspheres*

Paclitaxel-loaded microspheres were prepared by the simple emulsion solvent evaporation technique[8,17,18]. Briefly, 140 mg of PLDLA polymer and 14 mg of paclitaxel were added to 2 mL of chloroform (Merck) with stirring to ensure that all material was dissolved. This organic mixture was then slowly poured into a stirred aqueous solution of polyvinyl alcohol 1% (Merck) and homogenized for 20 min at 5200 rpm (Ultra-turrax Ika 25, Germany). The resulting on-in-water (O/W) emulsion was stirred gently at room temperature with a magnetic stirrer (Ika, Germany) for at least 4 h to evaporate the organic solvent.

2.3. *Microsphere size and morphology*

The size of PLDA microspheres with and without paclitaxel was measured by laser light scattering (Mastersizer 2000) and the morphology of these microspheres was studied by scanning electron microscopy (Jeol JXA 840A) and atomic force microscopy (Dimlutimode V).

2.4. *Fourier transform infrared spectroscopy (FTIR)*

Powders of paclitaxel, PLDLA, PLDLA microspheres, PLDLA microspheres loaded with paclitaxel were examined by FTIR, using a spectrophotometer model ATR-FTIR Perkin-Elmer 100S. Samples were taken in a KBr pellet, and scanned in the IR range from 600 to 4000 cm^{-1}.

2.5. *Differential scanning calorimetry (DSC)*

Powders of paclitaxel, PLDLA, PLDLA microspheres, PLDLA microspheres loaded with paclitaxel and a physical

mixture of paclitaxel and PLDLA were examined by DSC using a model MSDC2910 colorimeter (TA Instruments). The empty pan was used as a reference while another empty pan served as the sampling pan in which 10 mg samples were placed. A heating speed of 10 °C/min was used and the sample was kept in a nitrogen atmosphere. Samples were examined over the temperature range of 0 °C to 300 °C.

2.6. *Efficiency of encapsulation*

The efficiency of paclitaxel encapsulation in PLDLA microspheres was determined in triplicate by high performance liquid chromatography (HPLC)[2,8,19] (model UV 2487 integrated with Waters Breeze software; using a C18 column operated at 30 °C and a flow rate of 1 mL/min, with detection at 227 nm. The sample volume was 40 µl and the column was eluted with a mobile phase of acetonitrile:water (70:30, v/v). The column was calibrated with standard solutions of paclitaxel (1-160 µg/mL) dissolved in acetonitrile (correlation coefficient, $r^2 = 0.999$).

The efficiency of paclitaxel encapsulation after microsphere preparation (see Section 2.2) was assessed by determining the amount of paclitaxel in the resulting supernatant. This value was then subtracted from the amount of paclitaxel used to prepare the microspheres in order to obtain the amount of paclitaxel encapsulated. The efficiency of encapsulation, expressed as a percentage, was calculated as (Amount of paclitaxel incorporated into microspheres ÷ Amount of paclitaxel initially used)/100.

2.7. *Release of paclitaxel in vitro*

The release of paclitaxel from the microspheres was measured in triplicate in phosphate-buffered saline (PBS; pH 7.4). Paclitaxel-loaded microspheres were suspended in 100 mL of buffer solution in test tubes and placed in an orbital shaker at 37 °C with horizontal shaking (120 rpm). At predetermined intervals (1, 3, 5, 10, 15, 20, 25 and 30 days) the tubes were removed from the shaker and centrifuged at 3500 rpm for 10 min. The paclitaxel concentration in the supernatant was determined by HPLC as described in section 2.5.

3. Results and Discussion

3.1. *Morphological analysis and average size of microspheres*

Scanning electron microscopy (SEM) and atomic force microscopy (AFM) have been extensively used to examine the morphology and size of polymer-based microspheres[17,20,21]. Figures 3 and 4 show SEM and AFM

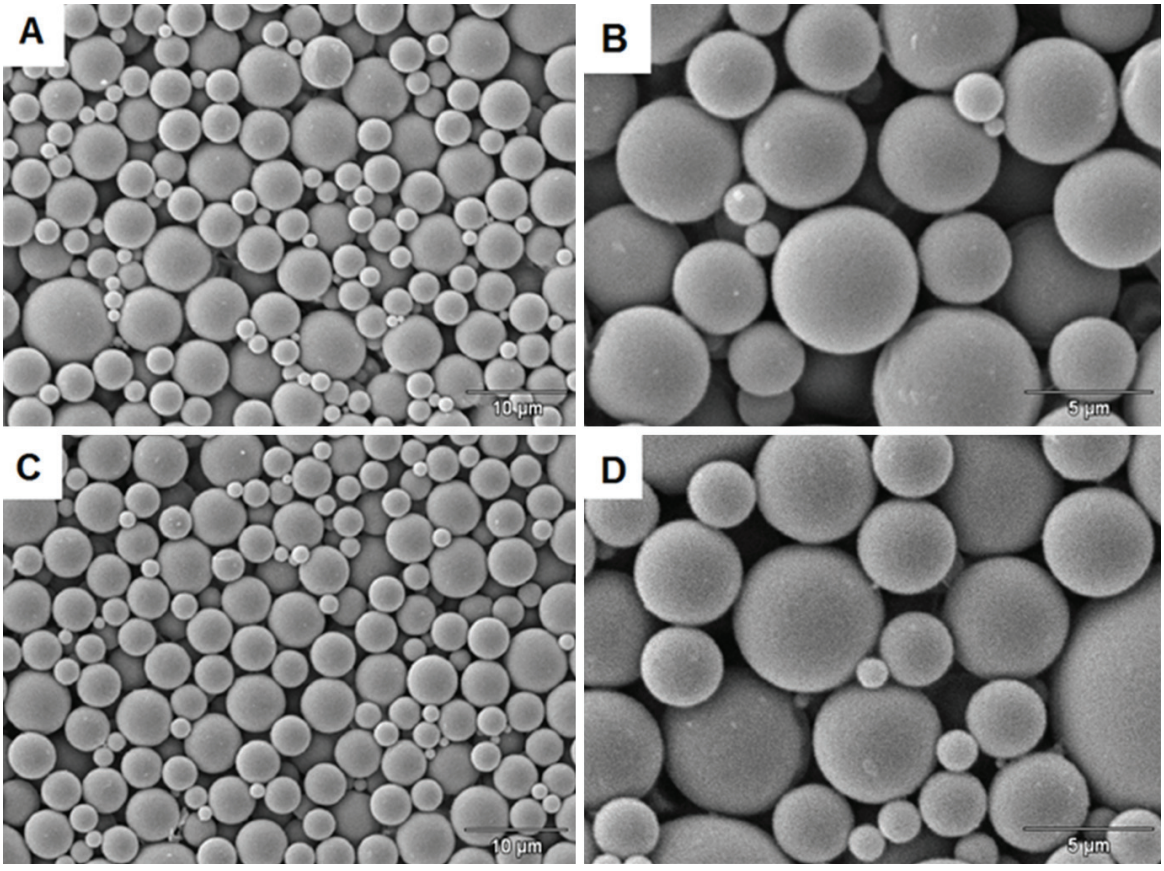

Figure 3. Scanning electron micrographs of microspheres. (A) and (B) – PLDLA microspheres without paclitaxel, (C) and (D) – PLDLA microspheres with paclitaxel.

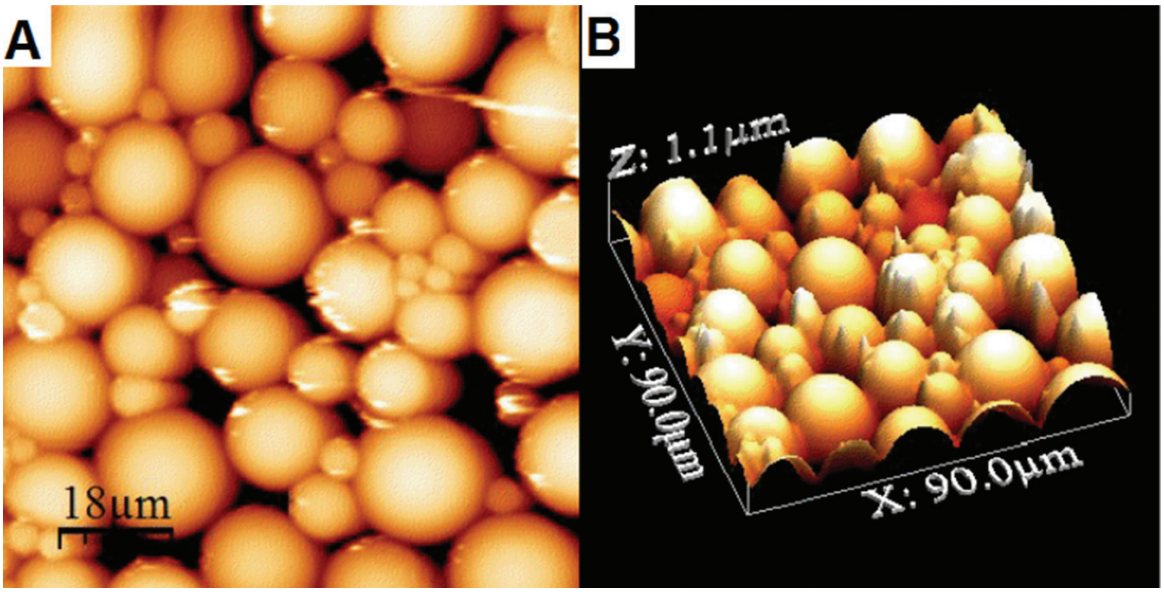

Figure 4. Two-dimensional (A) and three-dimensional (B) atomic force microscopy images of PLDLA microspheres containing paclitaxel at ambient conditions.

images, respectively, of PLDLA microspheres with and without paclitaxel. In all cases, the microspheres were spherical and no drug crystals were observed.

Polymeric devices have been successfully used to deliver a wide variety of drugs, including enzymes, hormones, peptides, antibiotics, anti-cancer agents, antifungals, anti-inflammatories and analgesics[22-24]. In an ideal drug delivery system, the average particle should be <250 µm in diameter in order to avoid the clogging of syringe needles. In the present study, the diameters of the PLDLA microspheres without and with paclitaxel were (in µm): 10.3 ± 1.7 and 12.7 ± 1.3 (mean ± SD; n=3), respectively, as determined by laser light scattering (LLS), i.e., well below the 250 µm limit indicated above.

3.2. *Drug state in the microspheres*

DSC has been used to investigate drug-polymer interactions in nano- and microparticles and can provide information on the melting temperature, crystallization temperature, glass transition temperature, melting enthalpy, enthalpy of crystallization and degree of crystallinity[2,25]. Figure 5 shows the DSC curves for pure paclitaxel, for a physical mixture of PLDLA + paclitaxel, for PLDLA microspheres with and without incorporation of paclitaxel and for the PLDLA polymer. Pure paclitaxel (Figure 5A) had an endothermic peak corresponding to a melting point of 220 °C, which indicated its crystalline nature. The same peak was observed in a physical mixture of PLDLA + paclitaxel in which both components were present in the same proportions used to prepare the microspheres (Figure 5B). In contrast, this peak was not observed for the drug, indicating that paclitaxel did not crystallize in PLDLA microspheres, i.e., the drug occurred as a solid dispersion in microspheres. These results agree with those of Yang et al.[2].

The incorporation of paclitaxel into the microspheres did not alter the thermal properties of PLDLA, as shown by

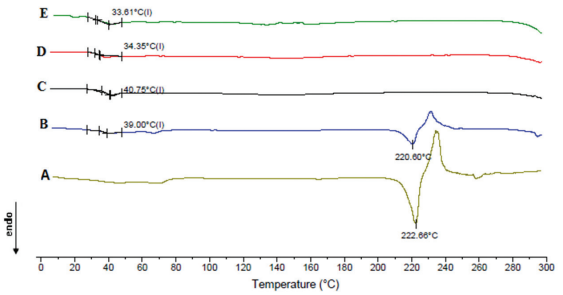

Figure 5. DSC thermograms of (A) pure paclitaxel, (B) a physical mixture of PLDLA + paclitaxel, (C) PLDLA microspheres with paclitaxel, (D) PLDLA microspheres without paclitaxel and (E) PLDLA polymer alone.

the glass transition temperature (Tg) of the polymer PLDLA at 33-40 °C (Figure 5B-E). Since PLDLA is an amorphous polymer no melting peak was observed. This property is important because it directly influences the degradation rate of the polymer. Depending on the application it is desirable that polymeric devices should have a low degree of crystallinity in order to facilitate degradation and hasten removal of the remaining fragments of material from the site of implantation, particularly since the accumulation of crystalline polymeric fragments may produce inflammatory reactions[13,14]. The results described here indicate that PLDLA is a suitable material for drug encapsulation and delivery.

3.3. *Fourier transform infrared spectroscopy (FTIR)*

FTIR spectroscopy is widely used by researchers to verify the chemical characteristics of drug and polymer used in the preparation of polymeric microspheres[26,27]. The

FTIR spectrum of paclitaxel is shown in Figure 6 A. The main infrared peaks of the Paclitaxel are as follows: N-H stretching vibrations at 3479-3300 cm^{-1}, CH_2 asymmetric and symmetric stretching vibrations at 2976-2885 cm^{-1}. The peak situated at 1734 assigned to C=O stretching vibration from the ester groups. The amide bound was located around 1647 cm^{-1}. Ester bond stretching vibrations and C-N stretching vibrations are situated at 1254 cm^{-1}, and 1276 cm^{-1} respectively. Absorption at 1647, 1074, 963 and 709 were assigned to the aromatic bonds[27].

Figures 6 B and C refers respectively the analysis of the spectra of PLDLA and PLDLA microspheres, which showed the following absorption bands (n, cm^{-1}): 2997-2965 (CH_2, CH_3), 1759 (C = O), 1360-1450 (CH_3), 750 (CH) that characterize the material[13].

The FTIR spectrum corresponding to loaded paclitaxel PLDLA microspheres (Figure 6 D) was identical to polymer spectra. These spectra did not display the characteristic intense bands of drugs, they may have been masked by the bands produced by the polymer. This seems to indicate the absence of chemical interaction between polymer and drugs in microspheres and the presence of drugs as a molecular dispersion in the polymer matrix.

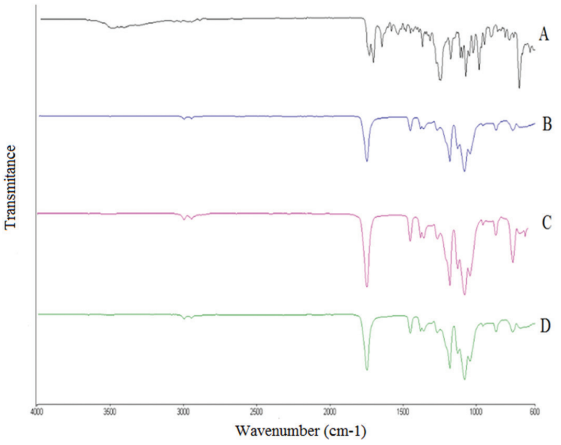

Figure 6. FTIR spectros of (A) pure paclitaxel, (B) PLDLA polymer alone, (C) PLDLA microspheres without paclitaxel, (D) PLDLA microspheres with paclitaxel.

3.4. *Efficiency of encapsulation and drug release in vitro*

HPLC is a versatile, safe and convenient technique for separating and quantifying drugs in polymeric nano- and microparticles[2,8,19]. As described in section 2.6, the efficiency of paclitaxel encapsulation in microspheres was calculated by subtracting the amount found in the supernatant from the total amount used to prepare the microspheres. HPLC analysis showed that the efficiency of drug encapsulation was high (98.0 ± 0.3%). These results agree with those of Ciftci et al.[26].

The formation of an emulsion followed by solvent evaporation is commonly used to immobilize therapeutic compounds in biodegradable and biocompatible polymeric microparticles[3,26]. This technique has been widely used to prepare microparticles of poly (lactic acid) (PLA) and poly (lactic-co-glycolic acid) (PLGA)[2,17,18]. There are several ways to immobilize a bioactive agent in polymeric microparticles using the technique of solvent evaporation. The choice of a particular method depends mainly on the solubility characteristics of the bioactive compound, with the aim being to ensure a high efficiency of drug encapsulation in the polymer matrix. The simple emulsion technique used here to prepare the PLDLA microspheres ensured a high efficiency of paclitaxel encapsulation in the polymer matrix[2, 17,18].Several methods and techniques are potentially useful for the preparation of microparticles in the field of controlled drug delivery. The size of the microparticles, the entrapment, and release characteristics of drug are dependent on the method used. One of the most common methods of preparing microparticles is the single emulsion technique. Poorly soluble, lipophilic drugs are successfully retained within the microparticles prepared by this method[2,17,18,26].

HPLC was used to study the release of paclitaxel incorporated into PLDLA microspheres. Figure 7 shows the release of paclitaxel versus time. During the first five

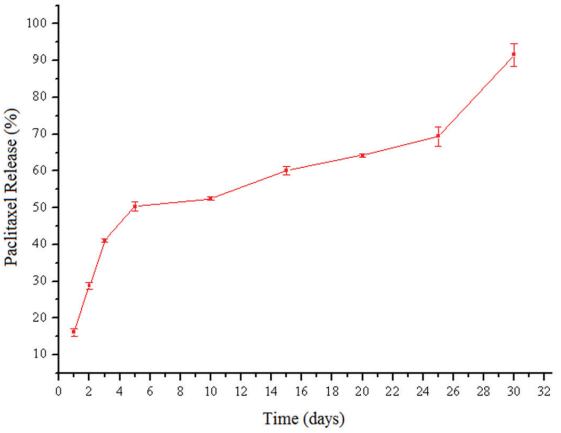

Figure 7. Time-dependent release of paclitaxel *in vitro*. The points are the mean ± SD of n = 3 determinations.

days there was an initial 'burst' of paclitaxel release that corresponded to ~50% of the amount of drug incorporated into the microspheres. This was followed by a slower release between days 10 and 25 of the study (~20% release) and an additional 20% release between days 25 and 30, when there was accelerated drug release.

Drug release by polymeric microspheres may occur in three phases: (a) rapid release (known as "burst release") that occurs within hours or days and is attributed to drug that is adhered to the wall of the microspheres, (b) slow release, which depends on the degradation kinetics of the polymer used in the microspheres, and (c) accelerated delayed release, which depends on the microsphere diameter[2,16-18]. The two main factors that influence drug release from polymeric microspheres are the degradation of the polymer matrix and diffusion, both of which are influenced by the polymer morphology[16-18].

The poly (L, co-D,L-lactic acid) (PLDLA, Mw = 70.000 g/mol^{-1}), in the 70:30 rate used in this study is an amorphous and bioresorbable material, has a structure

that combines the best characteristics of poly (L-lactic acid) and poly (D-lactic acid), i.e., the mechanical properties of the first and the shorter degradation time of the second; these properties have made PLDLA a compound of great relevance in the controlled release of drugs[13,14].

Poly (hydroxy acid) degradation in vitro is generally considered to be heterogeneous and is more rapid in the center than at the surface when the devices are in contact with an aqueous medium. Initially, degradation probably occurs mainly on the surface because of the absorption gradient of water, but as the concentration of carbonyl groups increases in the center these serve as catalysts for degradation. This self-catalyzing behavior is common during the degradation of aliphatic polyesters. However, the process depends on the chemical structure and configuration of the polymeric chains, as well as the morphology of the device involved[13,14].

The paclitaxel-loaded PLDLA microspheres used here were obtained from poly (L, co-D,L-lactic acid), the degradation of which involves the hydrolysis of ester bonds. The rate of hydrolysis depends on the chemical composition of the polymer, particularly the proportion of monomers and chain length, which can result in degradation times ranging from a few weeks to several months[2,9,13,14,16-18]. The kinetic profile of paclitaxel release from PLDLA microspheres was characteristic of systems containing carriers of different diameters, with the smaller diameter microspheres releasing the drug rapidly while larger microspheres release the drug more slowly[16-18]. The release behavior of paclitaxel from PLDLA microparticles is illustrated in Figure 7. At the burst release initial , PLDLA-based microspheres is the release of paclitaxel loosely bound on the surface of the microspheres. This loosely bound drug would be released by a mechanism of diffusion through the aqueous the surface of the microspheres created by the water uptake by PLDLA microspheres immediately after being exposed. At the later stage, the paclitaxel release was more slow, whose rate is determined by the diffusion of the polymer matrix[2,16-18]. As the PLDLA is the amorphous polymer, the diffusion of the paclitaxel occorrer through the amorphous region of polymer. PLDLA microspheres are spherical particles composed of biodegradable polymer; their release profile *in vitro* helps us to understand the behavior of these systems in terms of drug release, and therefore its efficacy.

4. Conclusion

In summary, the simple emulsion technique used here allowed us to produce PLDLA microspheres with a high efficiency of encapsulation (98%) for paclitaxel. The PLDLA microspheres were spherical and had a diameter well below the recommended limit of 250 μm. Differential scanning calorimetry (DSC) indicated that paclitaxel was homogeneously dispersed in the PLDLA microspheres, the incorporation of paclitaxel into the microspheres did not alter the thermal properties of PLDLA. The Fourier transform infrared spectroscopy (FTIR) analysis seems to indicate the absence of chemical interaction between polymer and drugs in microspheres and the presence of drugs as a molecular dispersion in the polymer matrix**.** The study of paclitaxel release *in vitro* indicated that PLDLA microspheres are potentially useful drug carriers that could have applications in drug delivery systems.

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