International Journal of Drug Delivery 4 (2012) 477-483
http://www.arjournals.org/index.php/ijdd/index

Original Research Article

ISSN: 0975-0215

Poly(DL-Lactide-co-caprolactone) as drug carrier for antifungal agent Amphotericin B

Paolo Yammine[1], Rima Kassab*[1], Dima Moussa[1], Rima Moussa[1]

*Corresponding author:

Rima Kassab

[1]Department of Chemistry, Faculty of Sciences, University of Balamand, Tripoli, Lebanon

Abstract

The objective of the present study is the preparation and characterization of Amphotericin B-loaded poly(DL-lactide-co-caprolactone) microspheres. The microspheres were prepared based on o/w emulsion solvent evaporation technique by varying the quantity of drug introduced. Characterization experiments included drug encapsulation efficiency, drug loading, particle size, morphology, FT-IR, stability, and drug release study. The drug encapsulation and drug loading increased with increasing drug mass. The microspheres exhibited homogeneous particle sizes with a spherical shape and porous surface. FT-IR spectrum of drug-loaded microspheres confirmed the absence of polymer-drug interaction. The stability study showed no major difference in the degradation of microspheres after 3 months of storage. Finally, the *in vitro* drug release behavior of the prepared formulations revealed a sustained release profile, with a burst effect for the formulations with the highest drug loadings.

Keywords: Drug Delivery System, Poly(DL-lactide-co-caprolactone), Amphotericin B, Microspheres

Introduction

Targeted drug delivery has gained a great importance in medical and pharmaceutical research. All Drug Delivery Systems (DDS) should include continuous regulation of drug levels with the therapeutic range, effective targeted delivery, reduction in the amount of drug needed, and, as consequence, a decrease in toxicity and side effects [1-6].

Microspheres and microcapsules based on biodegradable polyesters have received much attention in recent years as DDS. They are suitable for the controlled and sustained release of many pharmaceutical agents. Polymeric microspheres can be produced using several techniques such as solvent evaporation, spray drying, coacervation, etc. The most common technique for the formulation of microspheres is the emulsion solvent evaporation technique. It is widely used because it is a relatively simple and inexpensive method which enables the encapsulation of a broad variety of drugs [7, 8].

Many polymeric particulate carriers have been used for protecting the active molecules and for controlling their release in body fluids. Poly(lactic acid) (PLA) and its copolymers are well known biodegradable carriers in drug delivery, for bonding fractures, and as resorbable sutures [9-14]. Poly(-caprolactone) (PCL) is also an aliphatic polyester which is very suitable for controlled drug delivery, due to its high permeability to many drugs and non-toxicity, and very slow degradation rate [15]. Poly(DL-lactide-co-caprolactone) (PLC) is obtained by varying the composition of PLA and PCL copolymers, thus modifying the degradation profile of the co-polymer [16, 17, 21].

Many pharmaceutical agents have been microencapsulated within PLC showing promising interest for this polymer as a DDS. These include -lipoic acid for the prevention of neointimal formation in vivo [17], the anti-inflammatory agent Colchicine for the treatment of restenosis [18], the anti-cancer drug Cisplatin [19], and the non-steroidal drug Ibuprofen [20]. PLC has been also used to produce a substitute for various tissue repair applications [21].

The antifungal drug Amphotericin B (AmB) has showed a major importance in DDS through formulation of microspheres in order to decrease its side effects while providing a controlled release of it [3, 4]. These side effects have resulted from its insolubility and instability in water, as well as its great acute toxicity which could prohibit its direct therapeutic uses [22, 23]. In this context, the main objective of the present study is to investigate the possibility of formulating a new DDS based on AmB-loaded PLC microspheres. Characterization of the prepared model system then has been carried out.

Materials and Methods

Materials

Amphotericin B, Poly(DL-lactide-co-caprolactone) (86 mol% DL-lactide), the surfactant tween 80, and phosphate buffered saline (PBS) (0.2 M, pH 7.4) are purchased from Sigma-Aldrich, Chemie Germany. The solvents dichloromethane and methanol are of analytical grade.

Microspheres preparation

O/W emulsion solvent evaporation technique was used for the preparation of microspheres. Different formulations were prepared and referenced F1 to F5 by fixing the quantity of polymer and the surfactant, and changing the quantity of drug introduced.

Each synthesis consisted of dissolving 500 mg of the polymer PLC and a different mass of the drug in 20 ml dichloromethane/methanol at a ratio 14:6, forming the organic phase. This mixture was added into an aqueous solution containing 250 ml water and 45 g Tween 80 used as surfactant. The o/w emulsion was continually stirred for 6 hours at room temperature, and at 1400 rpm over a mechanical stirrer (MSP-1 Digital Overhead Stirrer, Jeiotech, Korea). The formed microspheres were filtered and washed with distilled water and 10 ml methanol. They were then dried at 40 C for 48 hours.

Microspheres characterization

Drug Encapsulation (%EE) and Drug Loading (%DL) :

%EE and %DL were evaluated using UV/vis spectrophotometry (Nicolet Evolution 300, Thermo-Scientific, UK). For this, 7 mg of each microsphere formulation were dissolved in 7:3 ml dichloromethane/methanol. They were then assessed for their AmB content at 409 nm. %EE and %DL were calculated according to the following equations:

$$\%EE = \frac{\text{Encapsulated drug mass}}{\text{Introduced drug mass}} * 100$$

$$\%DL = \frac{\text{Encapsulated drug mass}}{\text{Microspheres mass}} * 100$$

Particle size measurement:

A laser diffraction granulometer (LA-950A2 instrument, Horiba Ltd., France) was used to measure the particle size of microspheres. For this purpose, a small quantity of microspheres was suspended in water, with few drops of Tween 80, used as dispersing agent. The average particle size obtained was expressed in micrometers.

Morphology study

The morphological characteristics of the prepared microspheres were examined using Scanning Electron Microscopy (SEM) (LYRA3 XMU, TESCAN, Czech Republic). Microspheres were mounted on metal stubs with conductive silver paint, and then sputtered with a thin gold layer.

Fourier Transform-Infrared (FT-IR) study

Spectra for AmB, PLC, and AmB-loaded microspheres were recorded on a FT-IR spectrometer (Nicolet IS10, Thermo-Scientific, UK) using the Attenuated Total Reflectance Technique (ATR). In this method, the sample is held in contact with an optically dense crystal with a high refractive index for testing.

Stability study

The stability study was carried over a period of 3 months to follow the changes in the morphological state of the polymer inside the matrix material at different environmental conditions (4 C, 25 C, and 37 C). Every month, a certain quantity of the stored microspheres was examined by Optical Microscopy (LEICA DM LS2, Vashaw Scientific Inc., USA)

In Vitro drug release study

The drug release experiments were conducted in PBS. 25 mg microspheres were suspended in 25 ml PBS into a vial rotated at 150 rpm and maintained at 37 C. At pre-determined time intervals, 5 ml of the release medium was withdrawn and replaced by fresh solution. The drug content of this release medium was determined at 409 nm.

Results and Discussion

In this study, o/w emulsion solvent evaporation technique known for its simplicity, reproducibility and good results was chosen as the method for the formulation of AmB-loaded PLC microspheres.

Results concerning the %EE, %DL, and particle size are summarized in Table 1.

The %EE increased with increasing quantity of drug introduced, from 51% to 81%. %EE then decreased due to a saturated capacity of the polymeric matrix system. As for the % DL, the results revealed an increasing trend from 0.05% to 0.16% (Table 1). The high %EE could be explained by the hydrophobic nature of the drug and its great affinity to the amphiphilic copolymer used, that was able of solubilizing the drug inside the microspheres [3, 4]. Since AmB is insoluble in the aqueous phase, it has a higher tendency towards the organic phase during the microencapsulation process. As the quantity of drug introduced increased, more of the drug was able to be incorporated into the matrix system, which yielded high %EE and %DL values. In comparison with the other antifungal agent Nystatin, which was also encapsulated within PLC microspheres, the maximum %EE was found 20% [1, 2]. This relatively low encapsulation obtained with Nystatin could be attributed to the difference in solubility between the two antifungal drugs in the water-miscible co-solvent methanol.

Concerning the particle size of microspheres, it was homogeneous and had an average of 85 µm for all the formulations (Table 1). This result showed the suitability of AmB microspheres for oral administration.

SEM microphotographs of drug-loaded PLC microspheres revealed a spherical profile, with a rough and porous surface (Figure 1).

The physicochemical stability and compatibility studies were performed through FT-IR spectroscopy. FT-IR spectra were recorded for AmB, PLC and AmB-loaded microspheres. Comparison of the characteristic bands among the three spectra didn't reveal any large shift or deviation in the spectrum of the drug

when formulated into microspheres (Figures 2, 3, and 4). This result confirmed the absence of any drug-polymer interaction.

Table 1.Drug encapsulation, drug loading and average particle size of AmB-loaded PLC microspheres

Formulation Code	Quantity of AmB (mg)	Encapsulation Efficiency (%)	Drug Loading (%)	Average Particle Size (µm)
F1	35	51	0.05	86
F2	50	67	0.093	84
F3	60	74	0.14	80
F4	70	81	0.16	87
F5	75	57	0.12	84

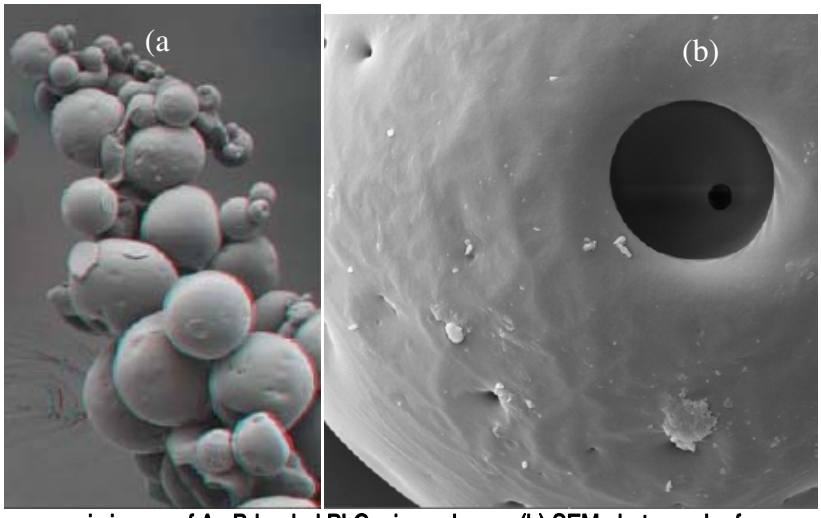


Figure 1. (a) SEM 3D stereoscopic image of AmB-loaded PLC microspheres, (b) SEM photograph of a porous microsphere (10,000x magnifications)

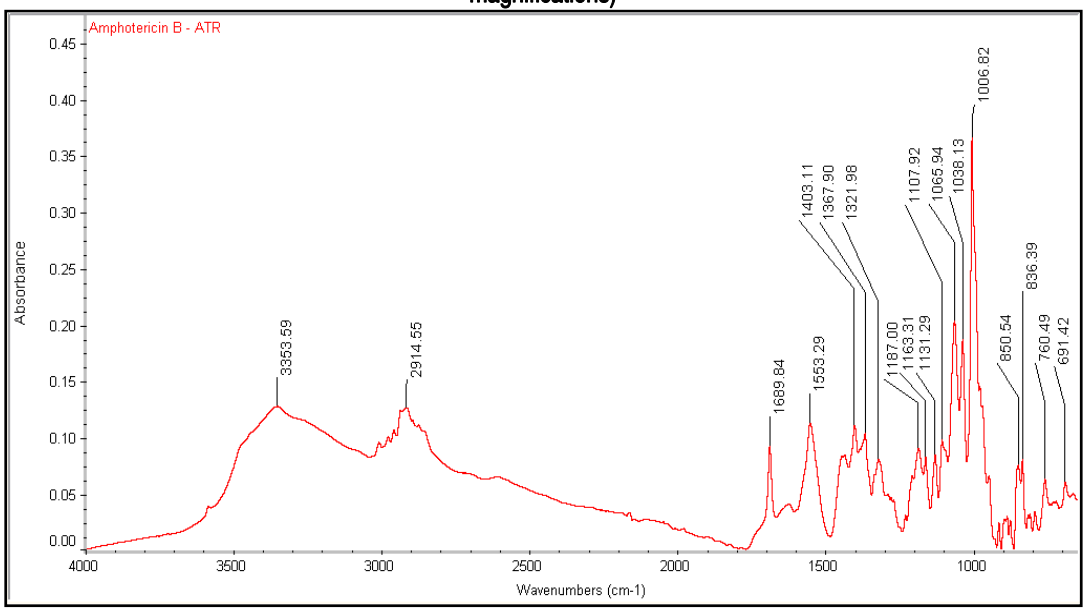


Figure 2.FT-IR spectrum for AmB

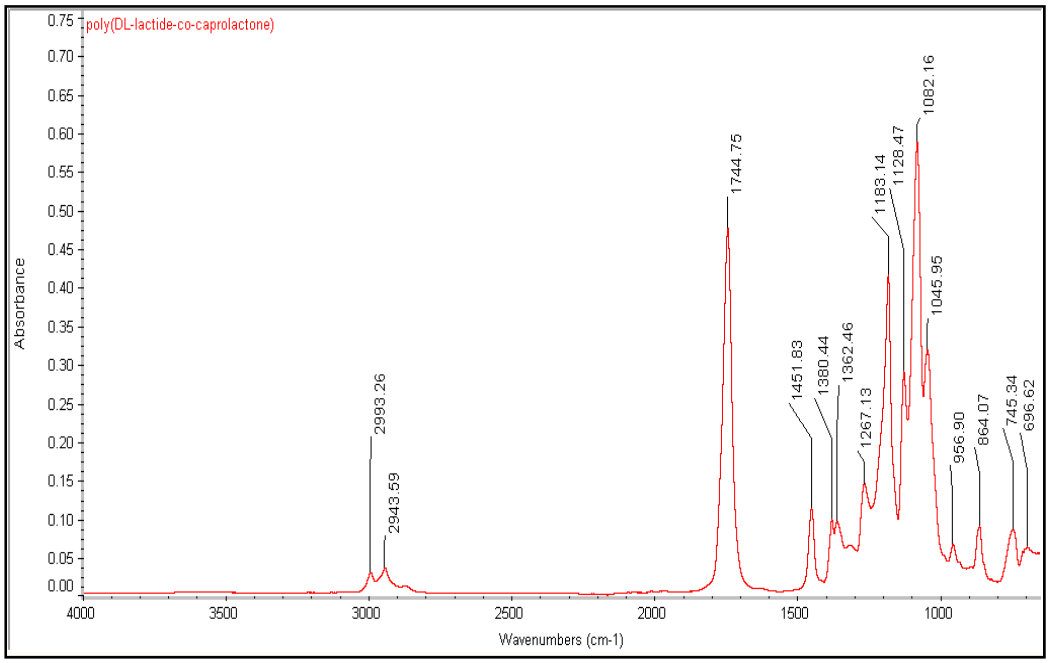


Figure 3.FT-IR spectrum for PLC

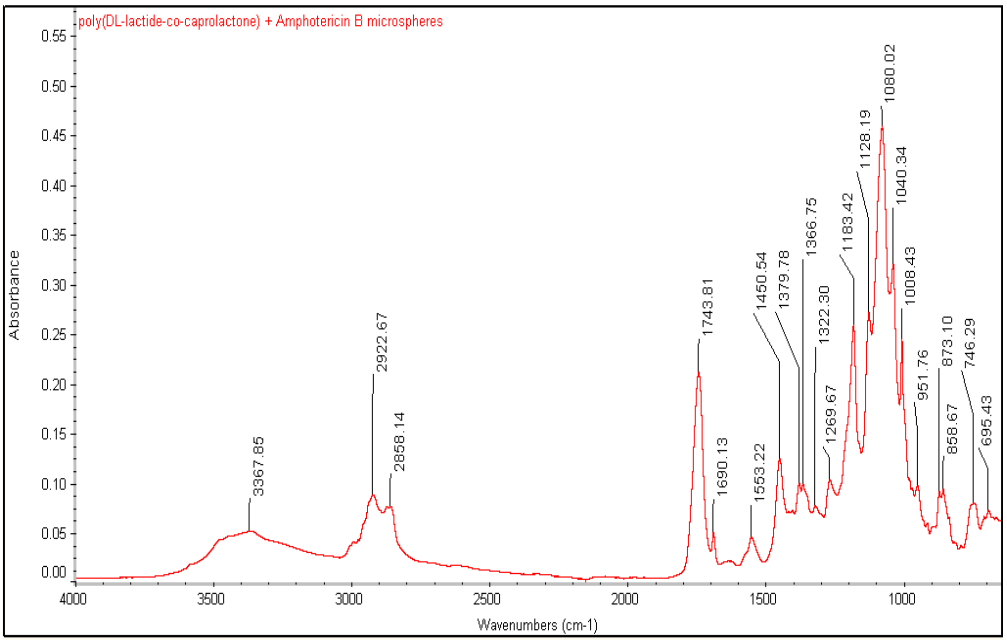


Figure 4.FT-IR Spectrum for AmB-loaded PLC microspheres

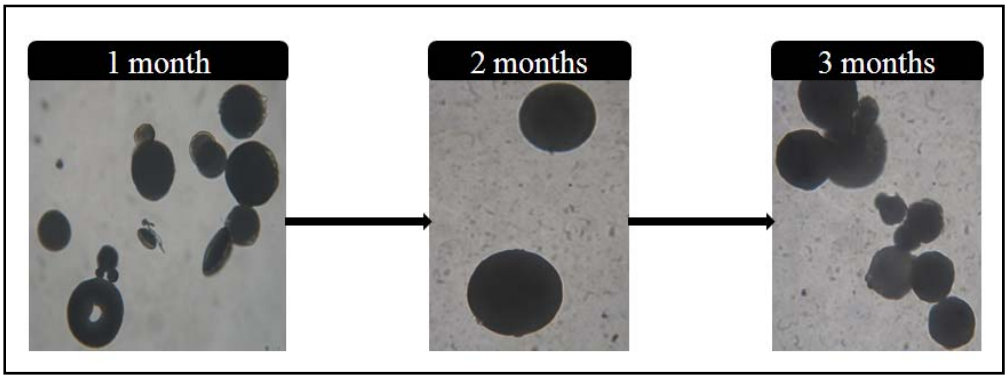


Figure 5.Microphotographs of microspheres after 3 months of storage

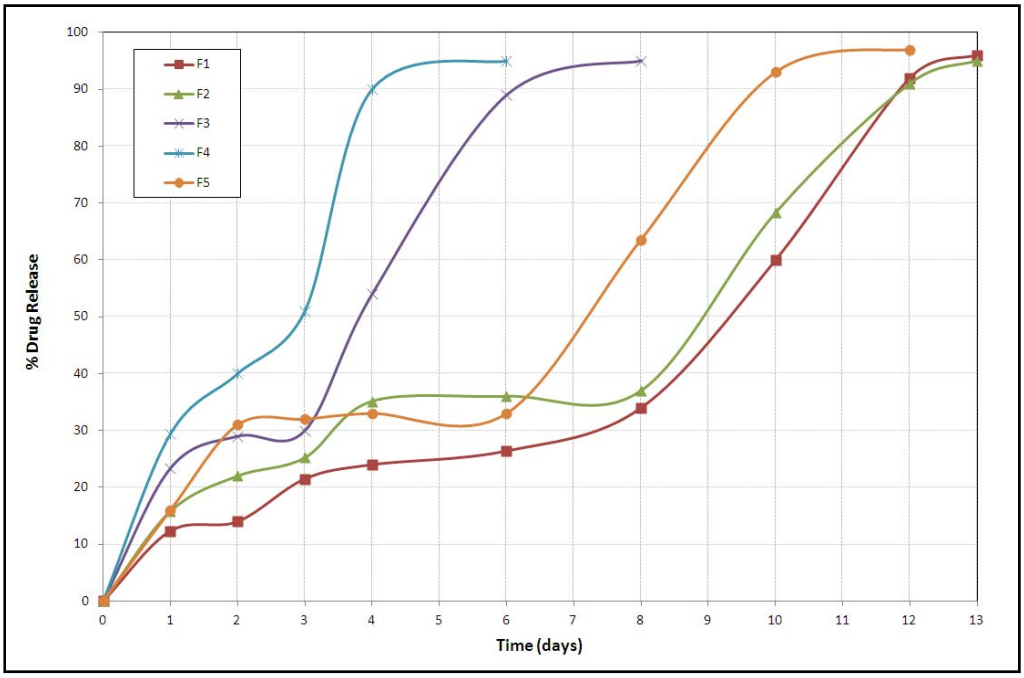


Figure 6.*In vitro* drug release profiles for AmB-loaded PLC microspheres formulations.

Several bands in the spectrum of drug-loaded PLC microspheres (Figure 4) showing the presence of both the drug and the polymer were observed. Bands at 1743(-C=O ketone), 1450 (-CH_3 aliphatic alkane), 1183 (-C-O ester) refer to the polymer. Bands at 2922 and 2858 cm^{-1} also corresponds to the stretching vibration of aliphatic –C-H of the polymer. As for the drug, it is characterized by the band at 1690 (C=C alkene), the broad band at 3300-3400 cm^{-1} of the –OH (alcohol) and –NH_2 (amine) groups.

The stability of microspheres was studied by examining the changes in their morphology over a storage period of 3 months. The microphotographs taken within this period revealed a spherical profile, which confirmed the resistance of microspheres to degradation (Figure 5). After that period, the degradation started to occur showing a non-spherical shape.

The *in vitro* release profile was found to be strongly dependent on the %DL of different AmB formulations (Figure 6).

The fastest release time was observed for F3 and F4 with the highest %DL. A burst release took place for these formulations after 2 to 3 days only. The complete release then needed approximately one week. As for the other formulations, a controlled extended release was observed during the first week, which was later followed by a less significant burst effect. The complete drug release required 12 to 13 days.

AmB release from PLC microspheres took place by a diffusion mechanism through the polymeric matrix, as droplets form the aqueous release medium (PBS) were percolating inside the microspheres [24, 25]. This drug has a poor water-solubility, thus a low affinity to the aqueous buffer medium. This low affinity could

explain the slow diffusion through the porous matrix system. This has led in turn to the slow drug release, which required a period of 1 to 2 weeks for most of the formulations.

Conclusion

The efficiency of the solvent evaporation technique in the formulation of a new DDS has been shown by the significant encapsulation percentages of drug within microspheres in this study. The AmB-loaded PLC microspheres exhibited a spherical profile, with a porous surface, as well as homogenous particle sizes and stability against degradation after three months of storage. The *in vitro* release of AmB from the PLC microspheres required several days due to the hydrophobic character of the drug. The copolymers of PLA and PCL have been found as promising materials in the formulation of microspheres for the sustained release of AmB.

References

[1]. Kassab R, Yammine P, Moussa D. New drug delivery system based on Nystatin loaded poly(DL-lactide-co-caprolactone) microspheres. Asian J Chem. 2011;23(7):3161-3164

[2]. Kassab R, Yammine P, Moussa D. Preparation and characterization of antifungal drug-loaded poly(DL-lactide-co-caprolactone) and poly(L-lactide-co-caprolactone-co-glycolide) microspheres. Int J Nov Drug Deliv Tech. 2011;1(4):213-219

[3]. Kassab R, Parrot-Lopez H, Fessi H, Menaucourt J, Bonaly R, Coulon J. Molecular recognition by *Kluyveromyces* of Amphotericin B-loaded, galactose-tagged, poly(Lactic Acid) microspheres. Bioorg Med Chem. 2002;10(6):1767–1775

[4]. Paul M, Durand R, Fessi H, Rivollet D, Houin R, Astier A, Deniau M. Activity of a new liposomal formulation of Amphotericin B against two strains of Leishmania infantum in a murine model. Antimicrob Agents Chemother. 1997;41(8):1731-1734

[5]. Alberto B, Kostas K, Charalambos DP, Maurizio P. Biomdeical applications for functionalized nanotubes. Chem Commun. 2005;5:571-577.

[6]. Alberto B, Kostas K, Maurizio P. Applications of carbon nanotubes in drug delivery. Expt Opin Drug Deliv. 2005;9(6):674-679

[7]. Li M, Rouaud D, Poncelet D. Microencapsulation by solvent evaporation technique: state of the art for process engineering approaches. Int J Pharm. 2008;363(1-2):26-39

[8]. Venkatesan P, Muralidaharan C, Manavalan R, Valliappan K. Selection of better method for the preparation of microspheres by applying analytic hierarchy process. J Pharm Sci Res. 2009;1(3):64-78

[9]. David WMM, Stephen JE, Eric JT. Colchicine and antineoplastic therapy for the prevention of restenosis following percutaneous coronary interventions. J Am Coll Cardiol. 1991;17(6) Supplement 2:126-131

[10]. Huayu T, Zhaohui T, Xiuli Z, Xuesi C, Xiabin J. Biodegradable synthetic polymers: preparation, functionalization and biomedical application. Prog Polym Sci. 2012;37(2):237-280

[11]. Nihant N, Grandfils C, Jerome R, Teyssie P. Microencapsulation by coacervation of PLGA. IV. Effect of the processing parameters on coacervation and encapsulation. J Controlled Release.1995;35(2,3):117-125

[12]. Taizo F, Yoshitaka M, Tsunoru Y, Yasuo S, Masaki O, Takashi N. Biodegradation behavior of ultra-high-strength hydroxyapatite/poly(L-lactide) composite rods for internal fixation of bone fractures. Biomaterials.2000;21(9):889-898

[13]. [13]. Isabel S, Carmen E, Matias L. Preparation and evaluation of insulin-loaded poly(DL-lactide) microspheres using an experimental design. Inter J Pharm. 1996;142(2):135-142

[14]. Tadashia I, Youshiharu D. Morphology and enzymatic degradation of poly(L-lactic acid) single crystals. Macromolecules. 1998;31(8):2461-2467

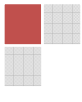
[15]. Sinha VR, Bansal K, Kaushik R, Kumria R, Trehan A. Poly(-caprolactone) microspheres and nanospheres: an overview. Inter J Pharm. 2004;278(1):1-23

[16]. Broz1 ME, VanderHart DL, Washburn NR. Structure and mechanical properties of poly(DL-lactic acid)/poly(-caprolactone) blends. Biomaterials. 2003;24(23):4181–4190

[17]. Hyo JL, Seung HC, Mun HN, Jeong OL, In KL. Fabrication of an -lipoic acid-eluting poly(DL-lactide-co-caprolactone) cuff for the inhibition of neointimal formation. Exp Mol Med 2009;41(1):25-32

[18]. [18]. Das GS, Rao GHR, Wilson RF, Chandy T. Colchicine encapsulation with poly(ethyleneglycol)-coated poly(lactic acid)/poly(-caprolactone) microspheres- controlled release studies. Drug Deliv. 2000;7(3):129-138

[19]. Chandy T, Wilson R, Rao GHR, Das GS. Changes in Cisplatin delivery due to surface-coated poly(lactic acid)/poly(-caprolactone) microspheres. J Biomater Appl. 2002;16(4):275-291



[20]. Zhu KJ, Li Y, Jiang HL, Yasuda H, Ichimaru A, Yamamoto K, Lecomte P, Jerome R. Preparation, characterization and in vitro release properties of Ibuprofen-loaded microspheres based on polylactide, poly(-caprolactone) and their copolymers. J Microencapsulation. 2005;22(1):25-36

[21]. Ranne T, Tirri T, Yli-Urpo A, Narhi TO. In vivo behavior of poly(-caprolactone-co-DL-lactide)/Bioactive Glass Composites (BAG) in rat subcutaneous tissue. J Bioact Compat Pol. 2007;22(3):249-264

[22]. Khatry S, Shastri N, Sadanandam M. Novel drug delivery systems for antifungal therapy. Int J Pharm Pharm Sci. 2010;2(4):6-9

[23]. Jay PJ, Neeraj K. Development of Amphotericin B- loaded polymersomes based on (PEG)3-PLA copolymers: factors affecting size and in vitro evaluation. Eur J Pharm Sci. 2010;40(5):456-465

[24]. Sunil A, Nadagouda M, Tejraj A. Recent advances on chitosan-based micro- and nanoparticles in drug delivery. J Controlled Release. 2004;100(1):5-28

[25]. Yoon Y, Kinam P. Control of Encapsulation Efficiency and Initial Burst in Polymeric Microparticle Systems. Arch Pharmacal Res. 2004;27(1):1-12