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POLYCAPROLACTONE AS DRUG CARRIER FOR AN ANTIFUNGAL AGENT

Kassab Rima, Moussa Dima, Yammine Paolo*

Department of Chemistry, Faculty of Sciences, University of Balamand, Tripoli, Lebanon

ABSTRACT

One of the most suitable and most used biodegradable polymers in particulate drug delivery is Polycaprolactone. This could be attributed to its several important characteristics including its low degradation rate, high permeability and low toxicity. In this study, Polycaprolactone microspheres loaded with the antifungal agent Amphotericin B were synthesized by means of the solvent evaporation method. The prepared microspheres had homogeneous particle sizes between 110 and 125 µm. they also showed a porous structure and a spherical profile. Satisfactory drug encapsulation percentages and drug loading values were obtained. They varied from 28 to 61 % and 0.22 to 1.13 % respectively. These observed results could be related to the properties of both drug and polymer such as hydrophobicity and solubility in organic solvents. In addition, the low degradation activity of the polymer Polycaprolactone had a direct impact on the release rate of Amphotericin B, yielding prolonged and slow release profiles for most of the formulations. Finally, Infrared study revealed that the method used for encapsulation of Amphotericin B with Polycaprolactone did not have any negative effect on the integrity and stability of the drug.

Key Words: drug delivery, solvent evaporation, Polycaprolactone, Amphotericin B, *In vitro* release

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***Address for Correspondence**

Dr. Paolo Yammine, PhD, Associate Professor, Department of Chemistry, Faculty of Sciences, University of Balamand, Tripoli, Lebanon, Phone: 00961-6-931 952 Ext: 3841, P.O. Box: 100 Tripoli, Lebanon. E-mail: paolo.yammine@balamand.edu.lb

INTRODUCTION

Drug Delivery Systems using biodegradable polymeric particles have become one of the most important topics in the pharmaceutical and medical work. They present many advantages including sustained delivery of drugs, their localized delivery, and their stabilization in order to avoid their major side effects. Biodegradable and biocompatible polyesters such as Polylactic acid (PLA), poly(lactic-co-glycolic acid) (PLGA), polycaprolactone and their copolymers are considered essential materials for successful and efficient drug delivery systems [1-4].

Polycaprolactone (PCL) is very suitable for controlled delivery due to its high permeability to a wide range of drugs, non-toxicity, and low degradation rate. PCL is a hydrophobic and semi-crystalline polymer[1-5]. Its good solubility in many solvents and its exceptional blend-compatibility has enabled it to be used extensively in several medical applications. This has a positive effect on the drug release rate [6, 7]. These advantages have made it widely used in preparation of polymeric microparticles and nanoparticles, scaffolds and tissues [1-6].

Degradation of PCL in comparison to the other polyesters is very slow, which is mainly needed for long-term delivery extending over a period of more than one year. In addition, the non-toxic products from its biodegradation also contribute in being favorable in delivery vehicles [8, 9]. Several drugs or biological molecules have been encapsulated into PCL microspheres or nanospheres such as Nifedipine, Indomethacin, Taxol, antigens, hormones, steroids, etc. [5-10].

In previous studies, PCL nanospheres loaded with the antifungal agent Amphotericin B (AmB) were prepared

using the solvent displacement method [11]. In this project, AmB will be encapsulated into PCL microspheres by means of oil-in-water solvent evaporation technique. Preparation of microspheres with this technique is very convenient in yielding particles with a large surface area to volume ratio. This makes them very efficient in drug delivery and targeting, mainly in cases where extended and prolonged release is required [12, 13].

MATERIALS AND METHODS

Chemicals used: The polymer, PCL (average MW 80,000), AmB, 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 (DCM) and Methanol (MeOH) are of analytical grade.

Formulation of Microspheres: o/w emulsion solvent evaporation technique is used for the formulation of AmB-loaded PCL microspheres. It consists of mixing the organic polymeric phase with an aqueous phase. Specific drug mass is dissolved in 14:6 ml DCM/MeOH containing 500 mg polymer, forming the organic phase. This mixture was added into 250 ml aqueous phase containing 1% w/v Tween 80. The whole combination is stirred for 6 hours at 1400 rpm over a mechanical stirrer (MSP-1 Digital Overhead Stirrer, Jeiotech, Korea). The resulting solution is filtered to collect the obtained microspheres. These are then washed with distilled water and MeOH, and oven dried.

Characterization:

1. Particle size and morphology: A Laser Diffraction Granulometer (LA950V2, Horiba Ltd., France) is used to determine the size of microspheres. A quantity of microspheres is suspended in water, with Tween 80 used as dispersant. The average particle size is measured in micrometers.

The morphological characteristics of microspheres are examined by Scanning Electron Microscopy (SEM) (LYRA3 XMU, TESCAN, Czech Republic). Microspheres are fixed to a carbon conductive tape. A coating of 10 nm of Platinum is applied using a sputter coater.

2. **Drug Encapsulation (%DE) and Drug Loading (%DL):** Microspheres are dissolved in 7/3 ml DCM/MeOH and the drug content of each formulation is measured using a UV/Vis Spectrophotometer (Microplate Spectrophotometer, Epoch Biotek, USA) at 409 nm. %DE and %DL are calculated as follows:

$$\%DE = \frac{\text{mass of drug encapsulated}}{\text{mass of drug introduced}} * 100$$

$$\%DL = \frac{\text{mass of drug in microspheres}}{\text{mass of microspheres}} * 100$$

3. *In vitro* drug release study: The *In vitro* release study is carried out in PBS solution (0.2 M, pH 7.4). 25 mg microspheres are introduced in small vials containing 25 ml PBS, used as release medium, and maintained at 37°C. At specific time intervals, 5 ml of the release medium is withdrawn and replaced with fresh solution. It is then evaluated for its drug content at 409 nm.

4. Fourier Transform-Infrared study (FT-IR): FT-IR spectra of AmB, PCL blank microspheres and AmB-loaded microspheres are determined using a FT-IR spectrometer (Frontier, Perkin Elmer, USA) in order to investigate the possible chemical interactions between the drugs and the polymer matrix.

RESULTS AND DISCUSSION

The solvent evaporation is one of the most common and successful methods for drug encapsulation. It presents many positive returns including the ease of its application and the reproducibility of its results. Also, a major factor for its effectiveness is in obtaining good encapsulation values for both hydrophobic as well as hydrophilic drugs [14].

Particle Size and Morphology:

AmB-loaded PCL Microspheres were homogeneous in size ranging between 110 µm and 125 µm (Table 1). As for the surface morphology of microspheres after examination by SEM, Figure 1 shows that they exhibited a spherical profile with a smooth and porous structure.

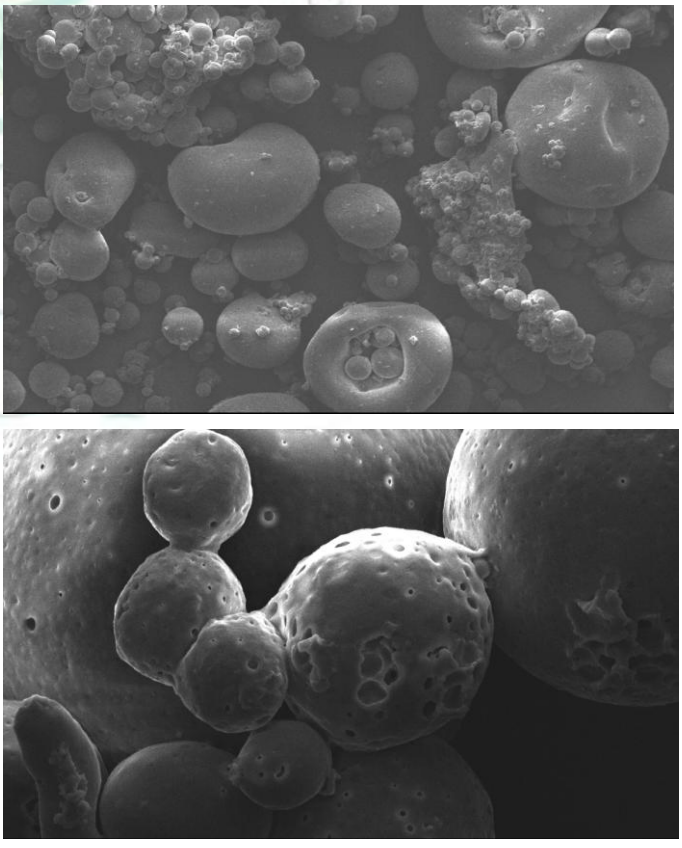

Figure 1: SEM photograph of the prepared microspheres at (a) x 100 and (b) x1000 magnifications

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

Good values for drug encapsulation and drug loading were obtained. Since AmB is hydrophobic in character,

it tends to remain in the organic polymer phase, leading to low loss of the drug into the aqueous phase and the satisfactory encapsulation values. In addition, using dichloromethane as major solvent in the microencapsulation procedure is advantageous compared to other organic solvents as Chloroform or Benzene. Dichloromethane has a high solubility in water which allows fast mass-transfer between the dispersed and continuous phases. This leads to fast precipitation of the polymer and good drug entrapment [15].

Regarding drug loading values, as more drug quantity is introduced, it is being more included into the polymeric microparticles. This explains the increasing %DL from 0.2 to 1% as the drug mass increases from 10 mg to 75 mg. In a previous study, the antifungal drug AmB was encapsulated into poly (DL-lactide-co-caprolactone) microspheres under the same conditions. Results showed lower drug loading compared to PCL. This could be explained by the more hydrophobic character of the polymer which enables it to retain more drug quantity [16].

Table 1: %DE, %DL and particle size of the prepared formulations

Formulation code	Drug Introduced (mg)	%DE	%DL	size (µm)
A1	10	55	0.22	114
A2	20	61	0.48	115
A3	35	46	0.63	110
A4	50	36	0.71	126
A5	75	31	1.08	126
A6	100	28	1.13	123

***In Vitro* Release:**

Conforming to the low degradation and crystallinity of PCL among known polyesters used in targeted delivery, water penetration into PCL polymeric matrices is lower. This leads to drug release by diffusion and not by polymer degradation [17].

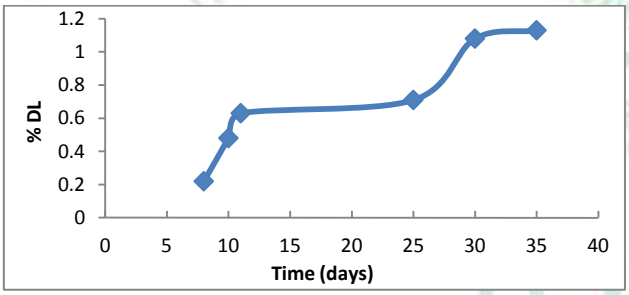

Figure 2: Variation of the release time according to %DL of AmB-loaded formulations

Figure 2 shows the release time of the different %DL of the prepared formulations. The release rate becomes slower at higher %DL to reach a maximum of one month for formulation A5. In fact, at higher loadings, the chances of drug particles inside the polymer matrix to contact each other are high. This causes the formation of aggregates that need more time to dissolve, and thus more time for the drug to be released [18].

Figure 3 presents the drug release profile of the available drug formulations. It is characterized by a burst release between days 5 and 10 where 30-40% of AmB was released. This is shown in particular for formulations with lower % DL. The initial burst could be due to the dissolution of drug molecules that are present at the surface of microspheres [14, 19].

In opposite, an extended prolonged release was shown for the other formulations and required one month to go to completion. Since AmB is a hydrophobic drug, it is retained inside the core of the polymeric matrix after encapsulation not near the surface of particles as in the case of hydrophilic drugs. As a result, it requires more time to diffuse to the aqueous buffer medium and to be released [8-10, 15].

Besides, the polymer PCL is more hydrophobic than other polyesters. This contributes in making its degradation very slow in aqueous medium, and in turn suitable for controlled and extended drug release [9, 10, 14-17].

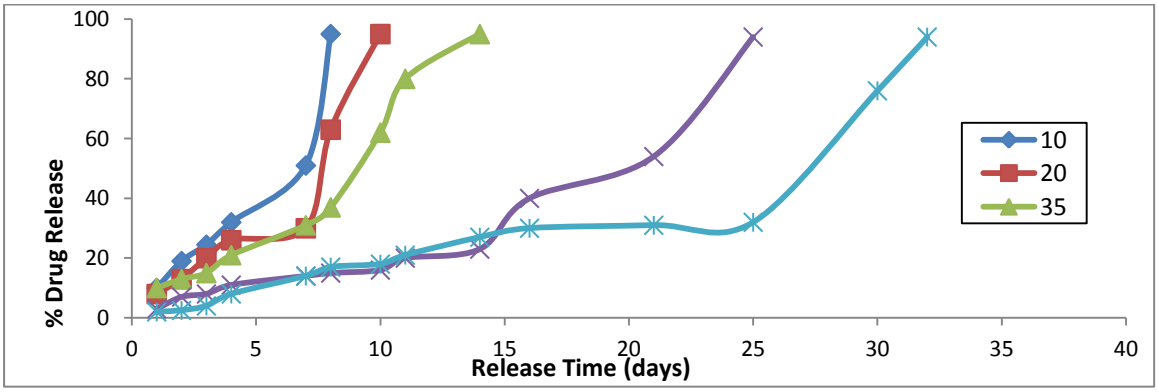

Figure 3: Drug release profile of the prepared AmB-loaded formulations

FT-IR Analysis:

Table 2: Characteristics Peaks of AmB and PCL [4, 16, 20, 21]

	Band (cm^{-1})	Type of bond
AmB	1560	-C=C alkene
	2900-3000	-C-H stretching alkane
	3300-3400	-OH alcohol -NH_2 amine
PCL	1160	-C-O ester
	1365	-C-H banding alkane
	1720	-C=O Ketone
	2900-3000	-C-H bending alkane

FT-IR study was carried out in order to identify the purity and chemical stability of drug and polymer following the microencapsulation process. Table 2 shows the characteristic peaks of AmB and PCL.

Figures 3, 4 and 5 illustrate the spectra recorded on the IR spectrometer. Comparison of bands among the three spectra showed no significant large shift or deviation in the spectra of the drug and polymer when formulated into microspheres. All the characteristic peaks of AmB and PCL are showing in the spectrum of AmB-loaded microspheres (Table 2). These results indicate that the solvent evaporation was efficient in encapsulating the drug into PCL microspheres while remaining stable and intact.

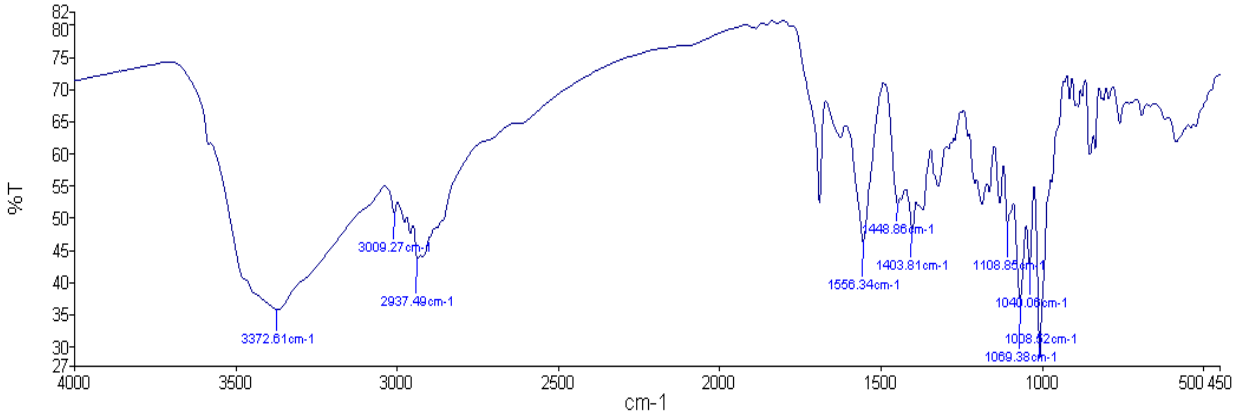

Figure 4: FT-IR spectrum of AmB

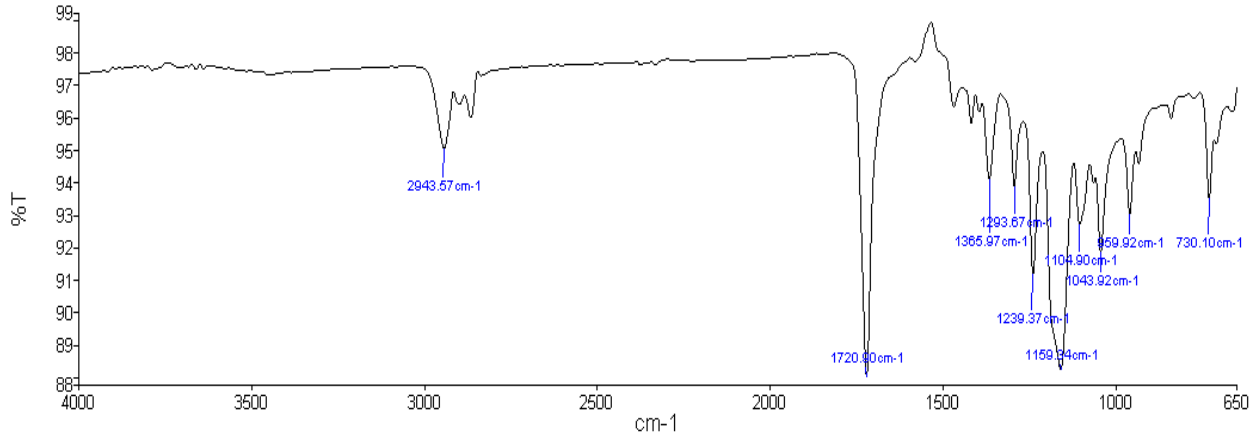

Figure 5: FT-IR spectrum of PCL blank microspheres

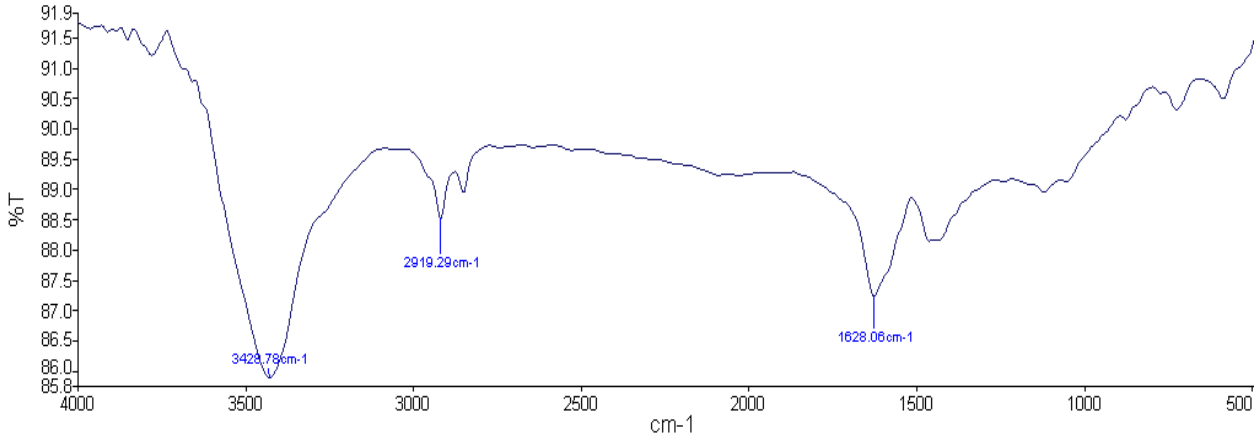

Figure 6: FT-IR spectrum of AmB-loaded microspheres

CONCLUSION

In this project, the biodegradable polymer Polycaprolactone was used for the encapsulation of the antifungal agent Amphotericin B into porous and spherical particles with homogeneous sizes. Several samples were prepared by fixing the polymer quantity and modifying the drug mass. Drug encapsulation and loading percentages were reasonable, ranging between 28 and 61% and 0.22 and 1.13% respectively. The release rate was slow for the most of the formulations, and was directly affected by the degradation rate of the polymer and hydrophobicity of both drug and polymer. Finally, the drug-polymer compatibility study by FT-IR revealed their stability with no change in any chemical composition.

Conflict of Interest

All contributing authors declare no conflicts of interest.

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