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New Drug Delivery System Based on Nystatin Loaded Poly(DL-lactide-co-caprolactone) Microspheres

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In this study, the usage of biodegradable polymer for microencapsulation of nystatin using solvent evaporation technique is investigated. For this purpose, poly(DL-lactide-co-caprolactone) microspheres containing nystatin were prepared with different drugs masses. The microspheres were then evaluated for particle size, morphology, Fourier transform-infrared spectroscopy (FT-IR) and differential scanning calorimetry (DSC) studies, percentage yield, drug entrapment, stability and *in vitro* studies. Optical microscopy revealed that the microspheres have a spherical shape. The drug entrapment increased with increasing drug content up to a particular concentration. FT-IR and DSC showed that there was no chemical interaction between the drug and the polymer. Stability study showed no appreciable difference concerning the degradation of the microspheres after 60 days of storage. The *in vitro* release study showed that nystatin release from all the formulations was slow for one week followed by an increase to reach 90 % for the formulation containing the highest quantity of nystatin.

Key Words: Biodegradable polymers, Microspheres, Poly(DL-lactide-co-caprolactone), Nystatin.

INTRODUCTION

Microencapsulation is a well-known method used to modify and delay drug release from pharmaceutical dosage forms[1-5]. A large number of microencapsulation techniques are available for the formulation of sustained release microparticles drug delivery systems[6,7]. One of the popular methods for the encapsulation of drugs within water insoluble polymers is the solvent evaporation technique[8-10]. Biodegradable polymers are the potential drug delivery systems[11,12]. Their major advantage over non-biodegradable delivery system polymers hinges on their fact of their auto-degradation in human body. Poly(DL-lactide-co-caprolactone) derivatives are widely used in a variety of medical applications, such as for sutures[13] and for encapsulation of drugs as doxocycline[14]. In addition, this polymer was used as an implant carrying different types of drugs such as: dexamethasone, etidronate, diclofenac sodium[15]. Nystatin is a polyene antifungal drug[16,17] to which many molds and yeast infections are sensitive. Nystatin may be given orally as well as its topical usage due to its minimal absorption through mucocutaneaous membranes such as the skin.

The aim of this study is to prepare poly(DL-lactide-co-caprolactone) microspheres containing nystatin as a model to achieve a controlled drug release profile suitable for pre-oral administration.

EXPERIMENTAL

Nystatin and the polymer poly(DL-lactide-co-caprolactone) (86 mol % DL-lactide) were purchased from Sigma-Aldrich Chemie Germany and were used as received. Tween 80 was also purchased from the same company. All other reagents used were of analytical grade. Solvent evaporation method was used for the preparation of nystatin microspheres.

General procedure: Different formulations were prepared by fixing the quantity of polymer to 500 mg and changing the quantity of the drug: F_1 (20 mg), F_2 (25 mg), F_3 (30 mg), F_4 (35 mg) and F_5 (40 mg). 500 mg of poly(DL-lactide co-caprolactone) was dissolved in 20 mL of dichloromethane/methanol (14/6) and different masses of nystatin were dissolved in this polymer phase. This mixture was poured into an aqueous solution containing 250 mL of distilled water and 45 g of Tween 80 and stirred continuously for 7 h at 1300 rpm. The formed microspheres were filtered and washed four times with 25 mL distilled water and 5 mL methanol and dried at room temperature for 48 h.

The dried microspheres were weighed and the yield of the microspheres preparation was calculated.

Detection method: In order to determine the percentage of drug entrapment, a weighed quantity of the microspheres

(7 mg) was dissolved in 10 mL dichloromethane/methanol (7/3). The samples were assayed for nystatin content by a UV-spectrophotometer (Nicolet Evolution 300, Thermo UK) at 320 nm. Percentage of drug entrapments were calculated as follows:

$$\text{Drug entrapment}\,(\%) = \frac{\text{Encapsulated drug mass}}{\text{Introduced drug mass}} \times 100$$

The microspheres were suspended in water and Laser diffraction technique (Horiba instrument Ltd., France) was used to study the size distribution of the microspheres. A quantity of microspheres was suspended in water, with the Tween 80 as dispersant. The average particle size was calculated and expressed in microns.

The morphological characteristics of microspheres were examined by optical microscopy (LEICA DM LS2, Vashaw Scientific Inc., USA) and the microphotographs were taken with a digital camera (PowerShot S70, Cannon, 7.1 megapixels).

FTIR spectra of microspheres loaded with nystatin and blank microspheres of the polymer were recorded on an FT-IR spectrophotometer (Nicolet IS10, Thermo-Scientific) in order to confirm the absence of drug-polymer interaction and chemical integrity of the drug in the microspheres.

The DSC thermograms were obtained using (Sensys Evo, Setaram) TGDSC. The samples were prepared by physical mixture drug to polymer ratio (50/50). 5-10 mg sample of pure nystatin and a mixture of drug and polymer (ratio 50:50) were prepared. They were accurately weighed and sealed in aluminum pans. Thermograms were obtained by heating at a constant rate of 15 ºC/min over a temperature range 200-500 ºC with an air flow of 50 mL/min.

In order to carry out the stability study, microspheres were stored under different environmental conditions at 5, 25 and 37 ºC. Every 20 days a certain quantity of the stored microspheres was used for evaluation. The study was carried out for 60 days. The microspheres were evaluated for their physical appearance.

Microspheres *in vitro* drug release experiments were carried out in 0.2 M phosphate buffer (pH = 7.4). 25 mg of microspheres loaded with nystatin were introduced in a small vial containing 25 mL of phosphate buffer, used as the release medium. The vial was rotated at 100 rpm and maintained at 37 ºC in a thermostat water bath. At the pre-determined time intervals, 5 mL of the release medium was evaluated for its drug content using UV spectrophotometry at 320 nm. The 5 mL phosphate buffer was replaced with fresh solution.

RESULTS AND DISCUSSION

Poly(DL-lactide-co-caprolactone) microspheres with varying proportions of nystatin were prepared by the solvent evaporation method. The particle size was determined by laser diffraction technique and was found to be homogeneous for all formulations in the range of 80-110 µm. The mean particle size of the microspheres is shown in Table-1. The microphotographs taken for the microspheres, as shows in Fig. 1, revealed a spherical profile.

TABLE-1
YIELD, DRUG ENTRAPMENT AND AVERAGE PARTICLE SIZE OF NYSTATIN-LOADED POLY(DL-LACTIDE-CO-CAPROLACTONE) MICROSPHERES

Formulation code	Quantity of nystatin (mg)	Yield (%)	Drug entrapment (%)	Average particle size (µm)
F1	20	78	9	110
F2	25	78	11	82
F3	30	76	13	90
F4	35	82	20	110
F5	40	88	15	96

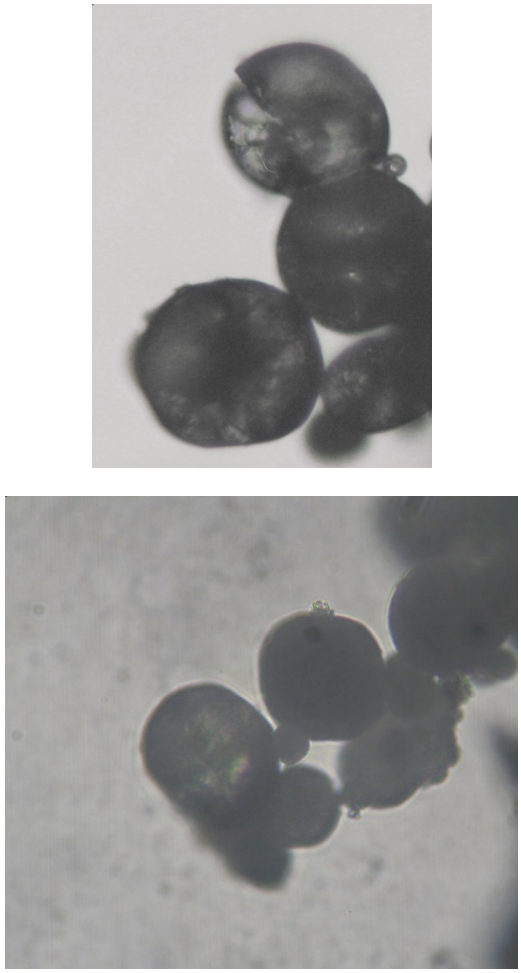

Fig. 1. Microphotographs of microspheres loaded with nystatin

The yield obtained for all formulations was satisfactory. It was in the range of 78-88 %. The microspheres exhibited an increase in drug entrapment with increase of the drug content up to a particular concentration of 35 mg of nystatin. A decrease in drug entrapment was observed after that point due to the saturated capacity of the polymer (Table-1).

FT-IR spectra of microspheres loaded with nystatin and blank microspheres of poly(DL-lactide-co-caprolactone) showed the same characteristic absorption peaks at the exception of two characteristic band values for loaded microspheres. The two bands at 3502 and 3649 cm^{-1} are attributed to the OH and NH_2 group of the drug. This result clearly indicated the stability of the drug during the microencapsulation process and revealed the absence of any drug polymer interaction (Fig. 2a-b).

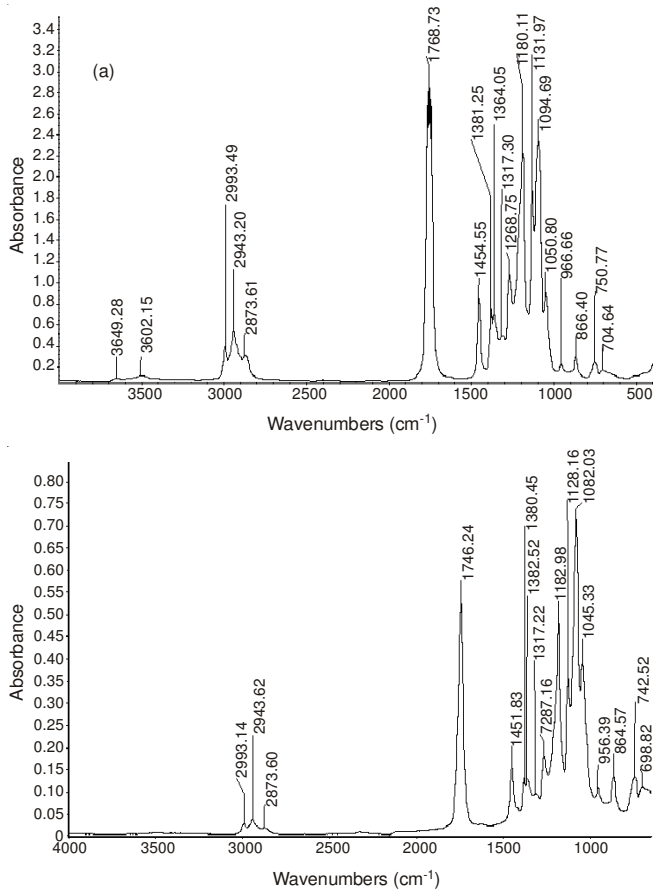

Fig. 2. (a and b): FTIR spectra for poly(DL-lactide-co-caprolactone) microspheres loaded with nystatin and poly(DL-lactide-co-caprolactone) microspheres, respectively

To confirm the absence of drug-polymer interaction during encapsulation a DSC study was carried out. Thermograms were generated for pure nystatin and a mixture polymer-nystatin. The two DSC thermograms revealed superimposition but a slight preshift was observed at 82 ºC instead of 85 ºC. No change in the melting endotherms of both the drug and the polymer was detected, as well as no other endotherms and exotherms were observed (Fig. 3).

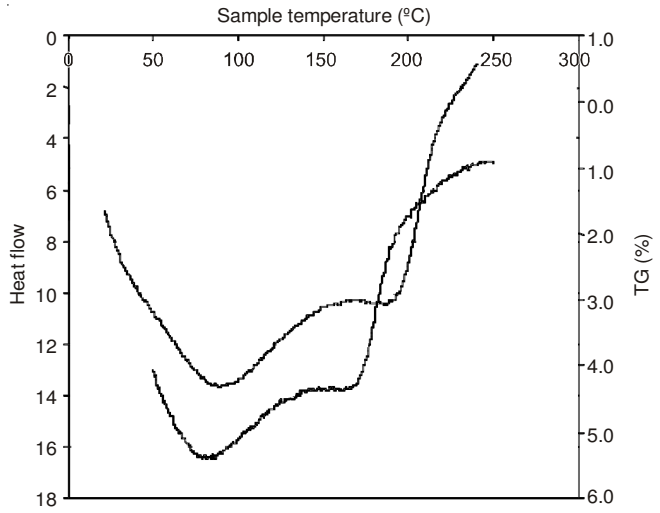

Fig. 3. DSC thermograms of pure nystatin (green) and physical mixture of polymer and nystatin (red)

The stability studies did not reveal any remarkable change in the morphology of microspheres. This result showed that the formulation was stable in medium storage conditions.

Formulations containing the lowest quantities of nystatin (F_1 and F_2) and the highest quantities of nystatin (F_4 and F_5) were subjected to *in vitro* studies (Fig. 4).

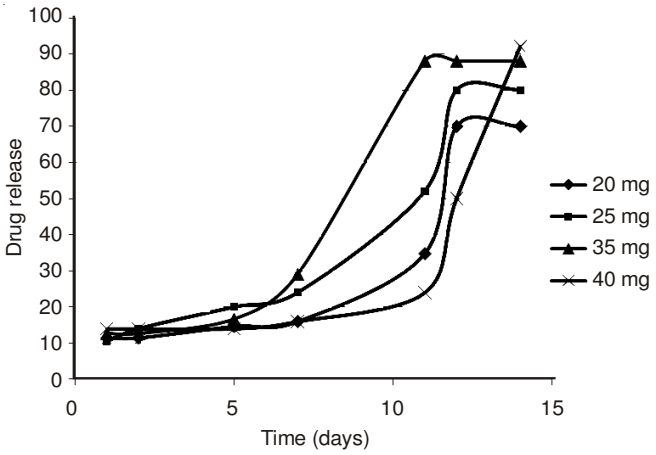

Fig. 4. *In vitro* drug release profiles of nystatin from poly(DL-lactide-co-caprolactone) microsphere formulations

The release profile depends upon the quantity of nystatin in the different microsphere formulations. During the first week, a slow release of the drug was observed from the different formulations. During the second week, a rapid release of the drug was detected. In fact, 92 % of nystatin is released from the formulation F_5 after 14 days. The percentage of release becomes constant after 2 weeks. This release profile could be related to the nature of the polymer which becomes more hydrated with time, leading to a faster release of nystatin.

Conclusion

Poly(DL-lactide-co-caprolactone) microspheres containing nystatin were prepared using the solvent evaporation technique. The percentage yield was high and the drug entrapment efficiency reached 20 % for the microspheres containing 35 mg of nystatin. Microspheres were spherical with a smooth surface having an average size between 80 and 110 µm. FTIR and DSC studies showed no interaction between the drug and the polymer during microencapsulation. The release of the drug depended on the quantity of nystatin encapsulated within the microspheres. The release profile was slow during the first week, then rapid during the second week to reach a maximum close to 90 % for the formulation F_5 containing the highest quantity of nystatin. The microspheres were stable and no degradation was observed during the period of study of 2 months.

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