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Microspheres containing Doxycycline: Properties and *in vitro* study

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Abstract

PLA microspheres loaded with the antibiotic Doxycycline are prepared using solvent evaporation technique (o/w) by varying the drug masses. They are evaluated for drug encapsulation, drug loading, particle size, morphology, FT-IR, stability and *in vitro* release. Microspheres loaded with Doxycycline show a maximum drug encapsulation of 38%. They exhibit homogeneous size distribution varying between 80 and 110 µm, with a spherical profile and porous surface. FT-IR study hasn't revealed any drug-polymer interaction. After 6 months of storage at different conditions, no appreciable difference is observed concerning the degradation of microspheres. *In vitro* release study of PLA microspheres loaded with Doxycycline reveals a rather fast release which requires only few hours to go to completion.

Keywords: Poly(DL-lactide), doxycycline, microspheres, solvent evaporation technique, drug release

Introduction

Drug delivery technology offers an intelligent approach in the medical field. It is based on encapsulating a drug into a carrier particle such as microspheres, nanospheres, and liposomes. Despite the several advancements in this technology, the oral route remains the most common route for the administration of therapeutic agents due to the low cost of therapy and ease of administration; thus leading to higher levels of patience compliance [1].Drug delivery systems possess many advantages including ease of application, site-specific action, prolonged delivery periods, and decreased body drug dosage with concurrent reduction in possible undesirable side effects [2, 3, 4]. In recent years, much research has been focused on the usage of biodegradable polymeric microspheres as novel drug delivery systems.Using biodegradable and biocompatible polymeric matrices in microspheres has many benefits for developing successful drug delivery systems. They provide sustained delivery of the drug, its localized delivery, and its stabilization [5, 6, 7]. Among the different classes of biodegradable polymers, the thermoplastic aliphatic esters as poly(lactic acid)) (PLA) and its glycolic copolymer poly(lactic-co-glycolic acid) (PLGA) are most commonly used as drug carriers due to their excellent biocompatibility, biodegradability, and mechanical strength [8]. They can degrade by non-enzymatic hydrolysis of the ester backbone in body fluids yielding metabolic compounds [9]. PLA was extensively studied in medical implants, sutures, and drug delivery systems [10]. A large number of drugs have been delivered using this polymer, such as the anti-cancer drugs Paclitaxel [11] and Aclacinomycin [12], the steroid hormone Progesterone [13], the opioid antagonist used to treat alcohol dependence Naltrexone [14], the non-steroidal anti-inflammatory drugs Ibuprofen [15] and Indomethacin [16].Doxycycline is described as an antibiotic belonging to the family of Tetracyclines. It is used against many intracellular and extracellular pathogens [17, 18, 19]. Microspheres loaded with Doxycycline will be formulated using the o/w emulsion solvent evaporation technique, with the same coating polymer, poly(DL-lactide) (PLA). Then, they will be characterized for drug loading and encapsulation efficiencies, particle size, morphology, stability, drug-polymer interaction, and *in vitro* drug release.

Materials and methods

PLA (MW 10, 000 g/mol), Doxycycline, Tween 80, and Phosphate Buffered Saline (0.2 M, pH 7.4) are all purchased from Sigma-Aldrich, Chemie, Germany. Dichloromethane and Methanol used as solvents are of analytical grade. Doxycycline-loaded PLA microspheres are synthesized using the o/w solvent evaporation technique. This method consists of mixing an organic phase with an aqueous phase. X mg drug and 500 mg polymer are dissolved in a mixture of 14:6 ml. Dichloromethane/Methanol (DCM/MeOH) forming the organic phase. The continuous phase is made of 250 ml water and 45 g Tween 80. The mixture is stirred continually for 6 hours over a mechanical stirrer (MSP-1 Digital Overhead Stirrer, Jeiotech, Korea). The resulting solution is filtered in order to recuperate microspheres. They are washed with distilled water and MeOH, and then dried.

Characterization

Encapsulation Efficiency (%EE) and Drug Loading (%DL):Microspheres are dissolved in 7/3 ml DCM/MeOH and the drug content of each formulation is measured by UV/Vis Sectrophotometry (Microplate Spectrophotometer, Epoch Biotek, USA) at 275 nm. The percentage drug entrapment (%EE) and drug loading (%DL) are calculated as follows:

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

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

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.

Fourier Transform Infrared (FT-IR) study

FT-IR spectra of Doxy –loaded PLA microspheres, PLA, and pure drug are recorded on FT-IR spectrometer (Frontier NIR, Perkin Elmer, USA) in order to investigate the possible chemical interactions between the drug and the blend matrix.

Stability study

Microspheres are stored at different experimental conditions for few months to check for any change in their physical appearance: In sealed tubes at different temperatures (4 C, 25 C, and 37 C). In Phosphate Buffered Saline (PBS) solution at 37 C.In acidic medium (PBS, pH 2) at 37 C Every 20 days, a certain quantity of the stored microspheres is evaluated by Optical Microscopy (LEICA DMLS2, Vashaw Scientific Inc., USA)

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 vial containing 10 ml PBS, used as release medium, and maintained at 37 C. At different time intervals, 1 ml of the release medium is withdrawn and replaced with fresh solution. This is evaluated for its drug content at 347 nm.

Results and Discussion

O/w emulsion solvent evaporation, which is widely applied in pharmaceutical industry for controlled release of drugs, is used to prepare Doxy-loaded PLA microspheres. Different formulations were prepared by fixing the quantity of polymer, and changing the quantity of drug each time. They were indexed $D_1$to D_5. The %EE, %DL, and average particle size of the prepared microspheres are presented in Table 1.

Table 1.% EE, % DL, and average particle size of Doxy microspheres formulations

Formulation Code	Drug Mass (mg)	%EE	% DL	Average Particle Size (µm)
D_1	3	25	0.15	90
D_2	8	38	0.16	88
D_3	10	23	0.25	91
D_4	20	6	0.20	87
D_5	30	3	0.15	91

Moderate % EE values were observed, with a maximum of 38%, which corresponds to formulation D_2 as shows Table 1. Obtained values for %EE and %DL of the different formulations are related to the properties of both the polymer and the drug. According to Mehta et. al., the solubility of polymers in organic solvents determines the precipitation rate during the microencapsulation process [20, 21]. PLA shows a high solubility in DCM; thus it has a tendency to remain in the organic layer. Its solidification rate will in turn decrease, leading to moderate drug encapsulation values. In addition, drug properties, such as the molecular weight and the solubility also affect the drug entrapment. Drugs having an average molecular weight easily diffuse out of the microspheres [20]. In the case of Doxy, its average molecular weight, that is 513 g/mol, enables its loss out of the polymeric matrix, by diffusion during or after preparation, which generates these observed % EE and % DL for the microspheres formulations. The solubility of the drug is also a key parameter in the encapsulation process [22]. Doxy is amphiphilic in character. It shows a good solubility in DCM; however, after mixing the organic phase with the continuous phase, it has a tendency to migrate to the aqueous solution during the preparation of microspheres, due to its high solubility in water. The prepared microspheres show a homogeneous particle size ranging between 80 and 110 µm (Table 1). The drug-polymer interaction study is carried out by FT-IR spectroscopy. Spectra are recorded for PLA, Doxy, and drug-loaded microspheres. Table 2 gives a summary of the characteristic bands in PLA and Doxy.

Table 2.Characteristics Peaks of PLA and Doxycycline

Absorption Peak (cm^{-1})	Type of bond
PLA	
2994-2942	$-CH_3$ and -CH stretching
1748	-C=O
1451-1382	$-CH_3$ and -CH bending
1180	-C-O
Doxycycline	
3327	-OH
3277	-NH
1610	-C=O
1459	$-CH_2$ bending

The comparative spectra of Doxy, PLA and Doxy-loaded microspheres are shown in Figure 1. The drug can be easily

identified by the broad peak of -OH alcohol at 3300-3500 cm^{-1}. Peaks observed around 2900-3000 cm^{-1} correspond to aliphatic -CH and $-CH_3$ of the polymer PLA. This result clearly indicates the stability of the drug during the microencapsulation process. Results also show small deviations of some polymer bands like from 1748 to 1756 cm^{-1} and from 868 to 871 cm^{-1}, probably due to the presence of Doxy.

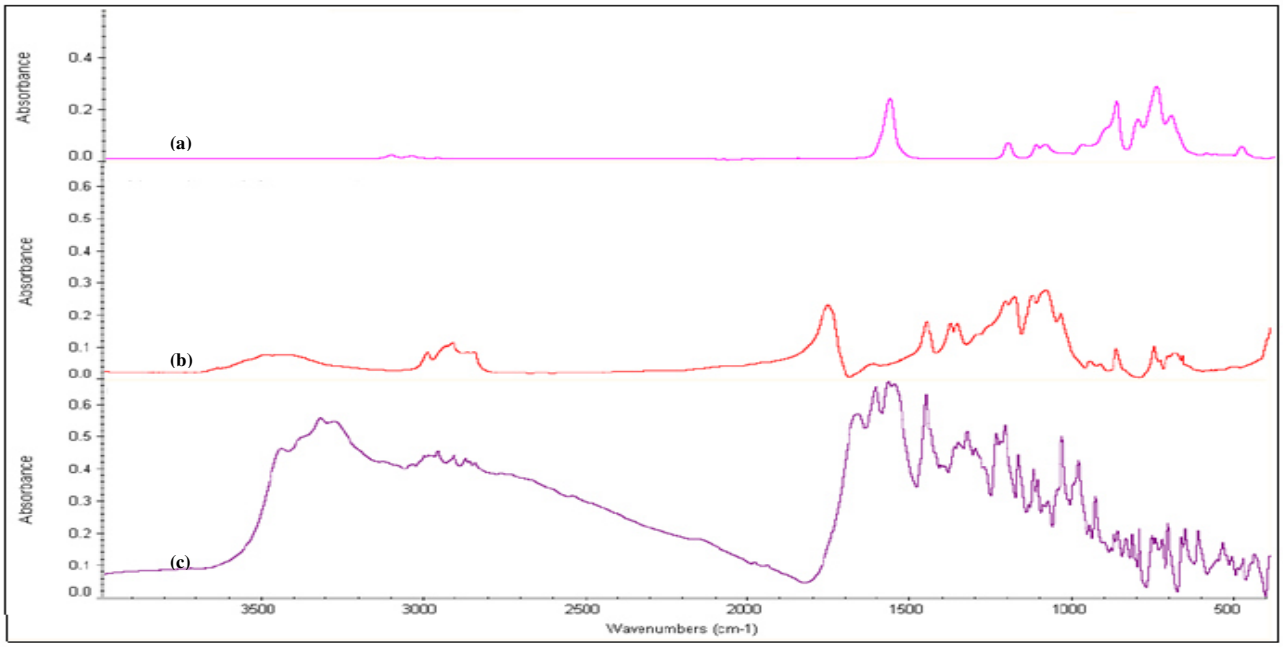
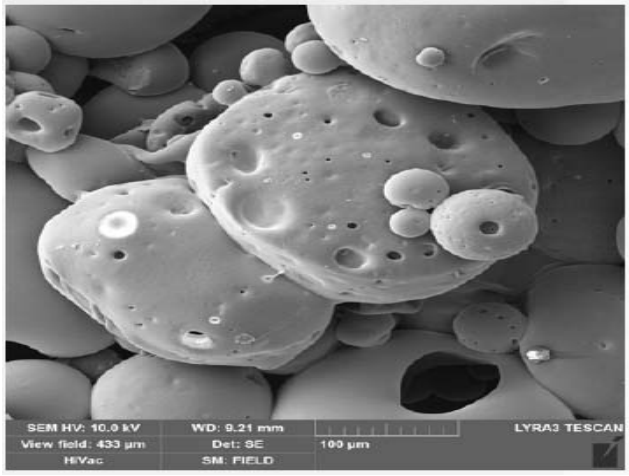


Figure1.FT-IR Spectra of (a) PLA, (b) Doxycycline-Loaded Microspheres, and (c) pure Doxycycline

The morphology of the microspheres is examined by SEM. Figure 2 shows the SEM microphotographs of Doxy-loaded micropheres. SEM reveales a spherical profile of the microspheres with a porous surface. Also microspheres forms aggregates. This is explained by Weihert et. Al. who reported that the surface of low-molecular weight PLA particles is more adhesive than that of particles from polymers with higher molecular weight, which causes an aggregation character [23].



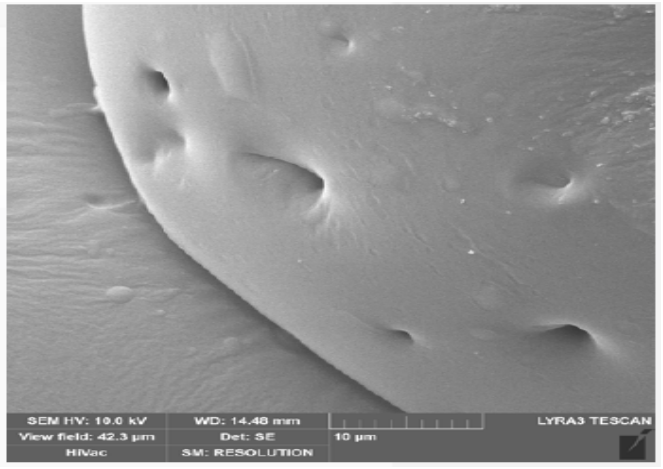


Figure 2. SEM Photographs of Microspheres taken at (a)1000x and (b)10000x magnifications

The stability study performed at different temperatures (4 C, 25 C, and 37 C) for 6 months shows spherical microspheres with no degradation and physical changes (Figure 3).

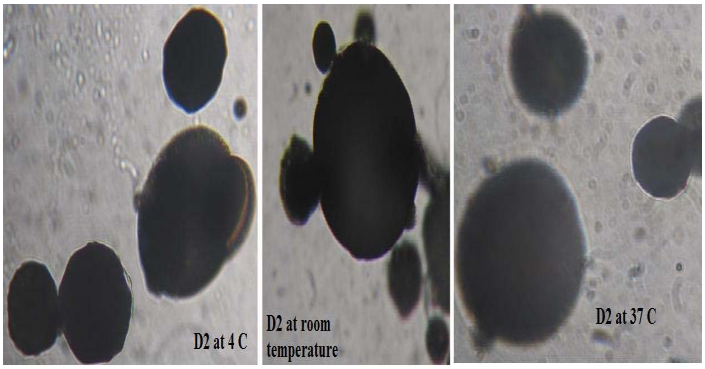


Figure 3.Microphotographs of Stability Study after 6 Months at Different Temperatures

PLA is known to be hydrolytically unstable, although insoluble in water. It undergoes degradation by hydrolytic cleavage of ester bonds [22]. This breakdown becomes fast at higher temperature; but PLA can resist in PBS at 37 C for almost 2 months. Therefore, it is stable in biological media (Figure 4). Finally, the acidic media (pH 2) doesn't show an influence on PLA formulations (Figure 5). This indicates that microspheres can be taken orally and remain intact in the gastro-intestinal tract.

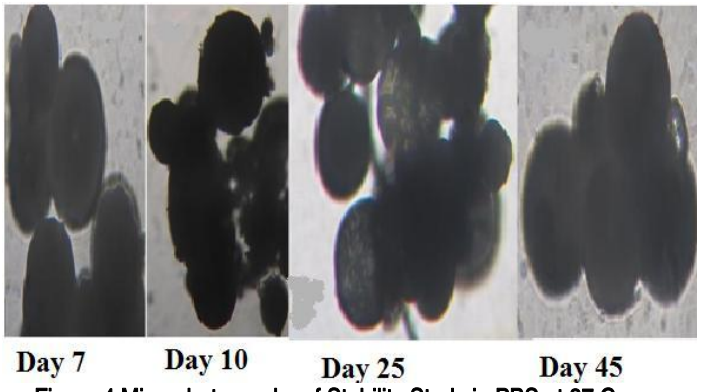


Figure 4.Microphotographs of Stability Study in PBS at 37 C

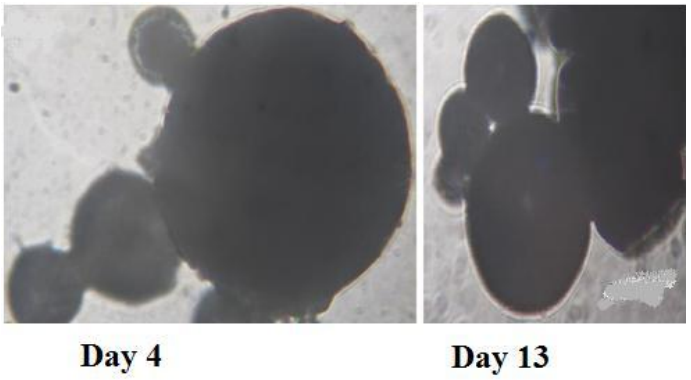


Figure 5. Microphotographs of Stability Study in pH 2 at 37 C

The *in vitro* release profiles of Doxy formulations D_1, D_2, and D_3, are shown in Figure 6. These formulations are chosen because they have the highest %EE. Results show that the drug release is fast and requires only few hours to go to completion. This can be explained by the high affinity of the drug towards PBS used as a release medium. The release rate is also higher for formulations having the lowest amount of drug (formulation D_1). It becomes slower when higher amount is encapsulated in microspheres, thus the drug will take longer time to escape into the release medium. This result is particularly noticed in the case of fomulation D_2 characterized by the slowest release. At higher drug loading, the chances of particles to interact with each other are high. This causes the formation of particular aggregates, which extends into the polymer matrix and needs more time to dissolve. The initial burst release observed for D_1 can be explained by the presence of surface associated drug. Besides, the absence of aggregates due to the small quantity of drug leads to fast dissolution into the release medium during the first few minutes, especially in the case of hydrophilic drugs [20].

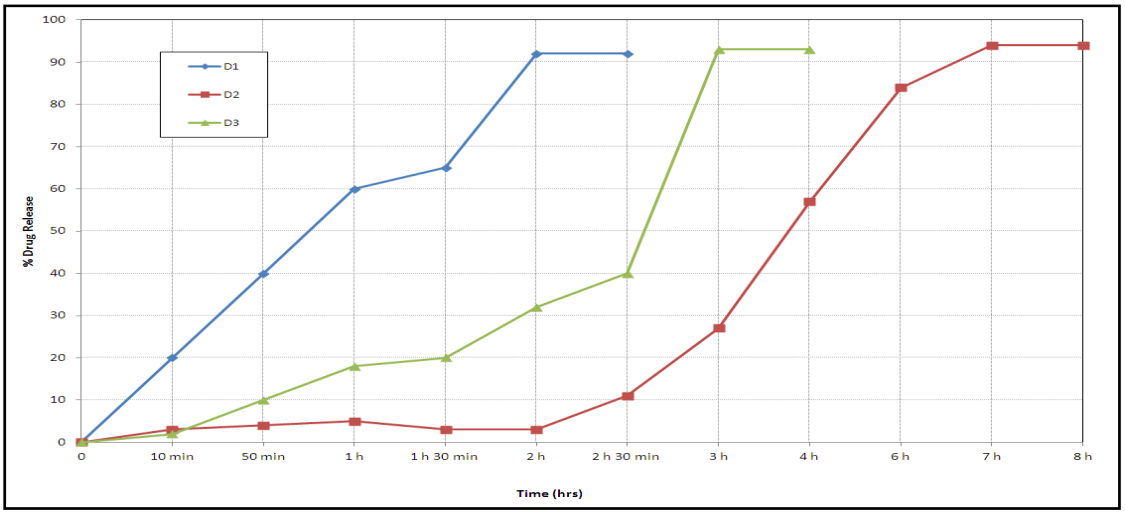


Figure 6. *In Vitro* Release Profile of Doxy Microspheres

Conclusion

Doxycycline has been successfully encapsulated in PLA microspheres using the o/w emulsion solvent evaporation technique. Microspheres have a spherical and porous profile, with an average size of 80 to 110 µm. They show good encapsulation and drug loading values despite their hydrophilicity. FT-IR study reveals that the drug remains intact following the microencapsulation process. Microspheres are stable after storage at different conditions. The *in vitro* release is fast and needed few hours to reach the maximum. A controlled release followed by a burst release is observed for formulations with highest drug amounts. This study has showed that PLA could be used as a promising coating carrier for the encapsulation of pharmaceutical agents.

Acknowledgments

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Author's contribution

All authors have been involved in accomplishing the project. Nahed Safi carried out the preparation of the different microspheres formulations. Dima Moussa performed the microscopic and stability studies. Paolo Yammine has been interested in FT-IR, size determination, and has drafted the manuscript. Rima Kassab carried out the loading capacities, the *in vitro* studies, and also helped in writing the manuscript. All authors have read and approved the final manuscript.

Conflict of interest

Manuscript Title: Microspheres containing Doxycycline: Properties and *in vitro* study. The authors whose names are listed immediately below certify that they have no affiliation with, or involvement in any organization or entity with any financial interest in the subject matter or materials in this manuscript. Author's names: Rima Kassab, Paolo Yammine, Dima Moussa, and Nahed Safi.

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