



Received on 09 October, 2013; received in revised form, 20 March, 2014; accepted, 03 May, 2014; published 01 June, 2014

A COMPARATIVE STUDY OF DOXYCYCLINE AND TETRACYCLINE POLYMERIC MICROSPHERES

Rima Kassab, Paolo Yammine*, Dima Moussa and Nahed Safi

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

Keywords:

Polylactic acid, Doxycycline, Tetracycline, microspheres, *in vitro* release

Correspondence to Author:

Dr. Paolo Yammine

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

E-mail:

paolo.yammine@balamand.edu.lb

ABSTRACT: Solvent evaporation technique is used for the preparation of Polylactic acid microspheres loaded with two antibiotics, Doxycycline and Tetracycline, by introducing different masses for each drug. They are characterized by calculation of drug encapsulation and drug loading, measuring the particle size, examining microspheres morphology, and performing FT-IR, stability and *in vitro* drug release studies. Moderate drug encapsulation values (38% and 24%) are observed for Doxycycline and Tetracycline respectively. Microspheres of both drugs show homogeneous size distribution varying between 80 and 110 μm . the FT-IR study has helped in verifying that both Doxycycline and the polymer are intact after microencapsulation. In the case of Tetracycline, it hasn't revealed any significant results due to the low loading values. After 6 months of storage at different conditions, no appreciable difference is observed concerning the degradation of microspheres. *In vitro* release of drugs from encapsulated Polylactic acid microspheres shows that Tetracycline release is slightly faster and reaches 98% in less than 5 hours, in comparison with Doxycycline which attains the same percentage after 7 hours.

INTRODUCTION: Targeted drug delivery to a specific body site is of great benefit in the treatment of several diseases such as cancer, viral diseases, bacterial infections, and others¹.

Considerable approaches for producing novel drug delivery systems to target body sites are wide, such as liposomes, micelles, microspheres, and nanospheres.

Among them, in recent years, much research has been focused on biodegradable polymeric microspheres².

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^{3,4,5}. Different kinds of agents such as active pharmaceutical materials, proteins, pesticides, etc. can be encapsulated with various polymeric coating materials, including chitosan, cellulose, polyesters, and others. One of the popular techniques for the encapsulation of a variety of hydrophilic as well as hydrophobic drugs is the solvent evaporation technique. It has many advantages over other

	<p style="text-align: center;">DOI: 10.13040/IJPSR.0975-8232.5(6).2452-57</p>
	<p style="text-align: center;">Article can be accessed online on: www.ijpsr.com</p>
<p>DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(6).2452-57</p>	

techniques because it requires only simple experimental conditions such as ambient temperatures and mild stirring.

Poly(lactic acid) (PLA) belongs to the family of thermoplastic aliphatic polyesters^{3,4,5}. It has been used extensively in medical implants, sutures, and drug delivery systems due to its high biodegradability and biocompatibility⁶⁻⁹. Drugs encapsulated with PLA include Paclitaxel¹⁰, Progesterone¹¹, Naltrexone¹², Methadone¹³, and others.

Doxycycline (DOXY) and Tetracycline (TETRA) are two antibiotics that belong to the same family of Tetracyclines. They have similar chemical structures but different chemical properties¹⁴.

Although more expensive, DOXY has a number of advantages over TETRA; it exhibits greater oral absorption, more prolonged half-life, and enhanced lipid solubility, which is more important for its bacterial action^{15,16,17}.

Microspheres of the two drugs, DOXY and TETRA, are formulated using the o/w emulsion solvent evaporation technique, with the same coating polymer, PLA. They are then characterized for drug loading and encapsulation efficiencies, particle size, morphology, microspheres stability, drug-polymer interaction, and in vitro drug release.

MATERIALS AND METHODS:

Materials: The polymer used, PLA (average MW 75,000-120,000), DOXY, TETRA, 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 solvent evaporation technique is used for the formulation of DOXY and TETRA microspheres. It 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 at 1300 rpm over a mechanical stirrer (MSP-1 Digital Overhead Stirrer, Jeiotech, Korea). The resulting solution is filtered in order to recuperate microspheres. These are then washed with distilled water and 5 ml MeOH, and dried for 48 hrs at 40°C.

Characterization:

1. **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 275 nm and 264 nm, for DOXY and TETRA respectively. %DE and %DL are calculated as follows:

$$\%DE = \frac{\text{Encapsulated drug mass}}{\text{Introduced drug mass}} \times 100 \dots \dots \dots \text{Eq. 1}$$

$$\%DL = \frac{\text{Encapsulated drug mass}}{\text{Microsphere mass}} \times 100 \dots \dots \dots \text{Eq. 2}$$

2. **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 microns. 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.

3. **Fourier Transform-Infrared study (FT-IR):** FT-IR spectra of PLA microspheres loaded with DOXY and TETRA, PLA, and pure drug are recorded on FT-IR spectrometer (Frontier, Perkin Elmer, USA) in order to investigate the possible chemical interactions between the drugs and the blend matrix.

4. **Stability study:** Microspheres are stored at different experimental conditions for few months to check for changes 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).

5. **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 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 DOXY and TETRA content at 347 nm and 357 nm respectively.

RESULTS AND DISCUSSION: Two types of microspheres are formulated with PLA using the o/w emulsion solvent evaporation, which is widely applied in pharmaceutical industry for encapsulation and controlled release of drugs. They are referenced as D1 to D5 for DOXY-loaded microspheres and T1 to T5 for TETRA-loaded microspheres. The %DE, %DL, and average particle size of the prepared microspheres are presented in **Table 1**.

TABLE 1: %DE, %DL, AND PARTICLE SIZE OF DOXY AND TETRA MICROSPHERES FORMULATIONS

Formulation Code	Drug Mass (mg)	% EE	% DL	Average Particle Size (µm)
D1	3	25	0.15	90
D2	8	38	0.16	88
D3	10	23	0.25	91
D4	20	6	0.20	87
D5	30	3	0.15	91
T1	0.7	4	0.006	97
T2	1	24	0.06	90
T3	3	12	0.09	101
T4	8	7	0.14	108
T5	30	1	0.10	96

The maximum % DE is 38% and 24% for formulations D2 and T2 respectively as shows Table 1. The low values observed in terms of %DE and %DL for both kinds of formulations are related to the properties of polymer and the two drugs.

According to Mehta *et al.*, the solubility of polymers in organic solvents determines the precipitation rate during the microencapsulation process¹⁸. 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 low drug encapsulation values.

Along with the polymer properties in this case, drug properties, as the molecular weight and the solubility have affected the drug entrapment. Drugs having an average molecular weight easily diffuse out of the microspheres^{18, 19}. In the case of DOXY and TETRA, their relatively average molecular weight, 513 g/mol and 481 g/mol respectively, enables their loss out of the polymeric matrix, by

diffusion during or after preparation, which results in low %DE and %DL for the different microspheres formulations. The solubility of the drug is also a key parameter in the encapsulation process^{18, 19}. The two drugs are amphiphilic in character.

Thus, after mixing the organic phase with the continuous phase, they have a tendency to migrate to the aqueous solution during the preparation of microspheres. This behavior is more obvious in the case of TETRA than DOXY, due to its higher hydrophilic character, which explains its lower percentage of encapsulation. Concerning the size of microspheres, both kinds of drugs 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, the two drugs, and drug-loaded microspheres. **Table 2** gives a summary of the characteristic bands in PLA, DOXY and TETRA.

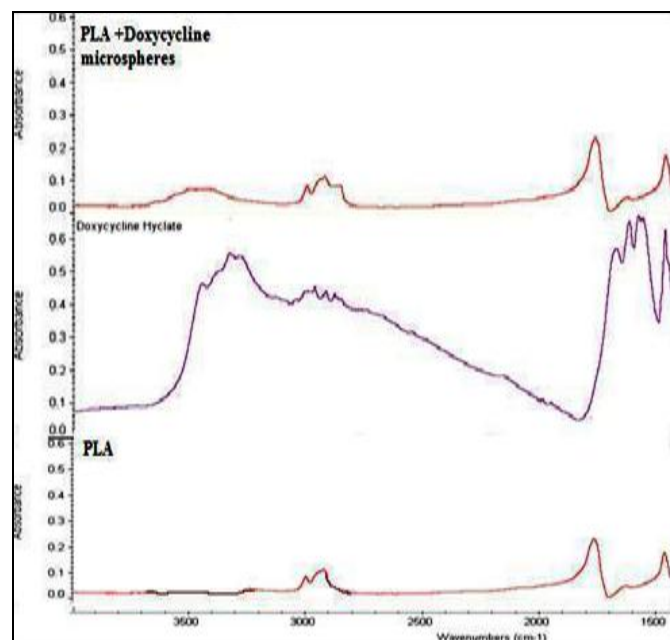
TABLE 2: CHARACTERISTIC PEAKS OF PLA, DOXY AND TETRA

PLA	Absorption Peak (cm⁻¹)	2942-1382	1748	2994-1451	1180
	Type of bond	-CH	-C=O	-CH ₃	-CO ester
DOXY	Absorption Peak (cm⁻¹)	3327	3277	1610	1459
	Type of bond	-OH	-NH	-C=O	-CH ₂
TETRA	Absorption Peak (cm⁻¹)	3359	3299	1580-1614	1448
	Type of bond	-OH	-NH	-C=O	-CH ₂

The comparative spectra of DOXY, PLA and DOXY-loaded microspheres are showing in **Fig. 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 -CH₃ of the polymer PLA.

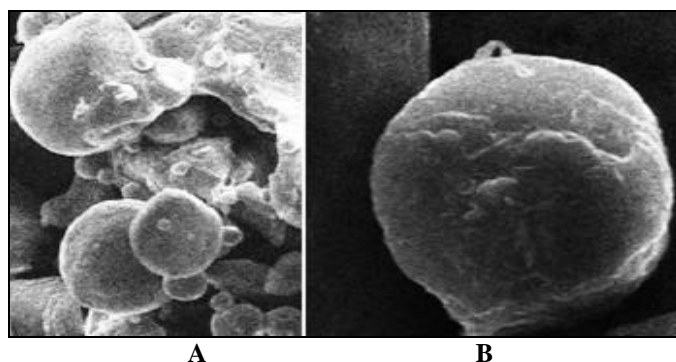
Peaks observed at 1210 and 691 cm⁻¹ could be assigned to -CH and -CN groups of the drug^{19, 20}. This result clearly indicates the stability of the drug during the microencapsulation process. Results also show small deviations of some polymer bands like from 1748 to 1756 cm⁻¹ and from 868 to 871 cm⁻¹, probably due to the presence of DOXY.

Because of the low loading values in the case of TETRA, the FT-IR spectrum didn't show any significant peak for Tetra in the microspheres.

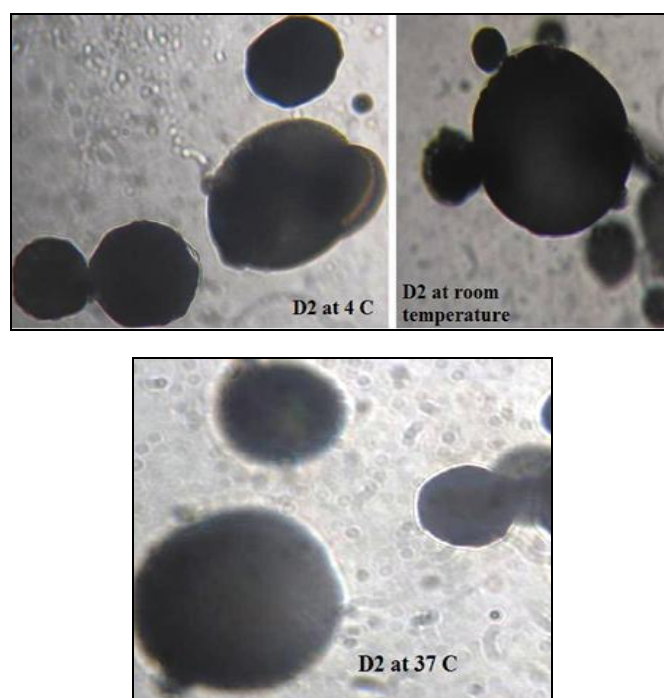
**FIG. 1: FT-IR SPECTRA OF DOXY, PLA, AND DOXY-LOADED MICROSPHERES**

The morphology of the microspheres is viewed by SEM. **Fig. 2** shows the SEM microphotographs of DOXY and TETRA microspheres. SEM reveals a spherical profile of the microspheres with a porous surface. Also microspheres form 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^{19, 20}.

**FIG. 2: SEM PHOTOGRAPHS OF MICROSPHERES TAKEN AT (a) 200x AND (b) 3200x 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 (**Fig. 3**).

**FIG. 3: MICROPHOTOGRAPHS OF STABILITY STUDY AT DIFFERENT TEMPERATURES**

PLA is known to be hydrolytically unstable, although insoluble in water. It undergoes degradation by hydrolytic cleavage of ester bonds^{19, 20, 21}. 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 (Fig. 4). Finally, the acidic media (pH 2) doesn't show an influence on PLA formulations (Fig. 5). This indicates that microspheres can be taken orally and remain intact in the gastro-intestinal tract.

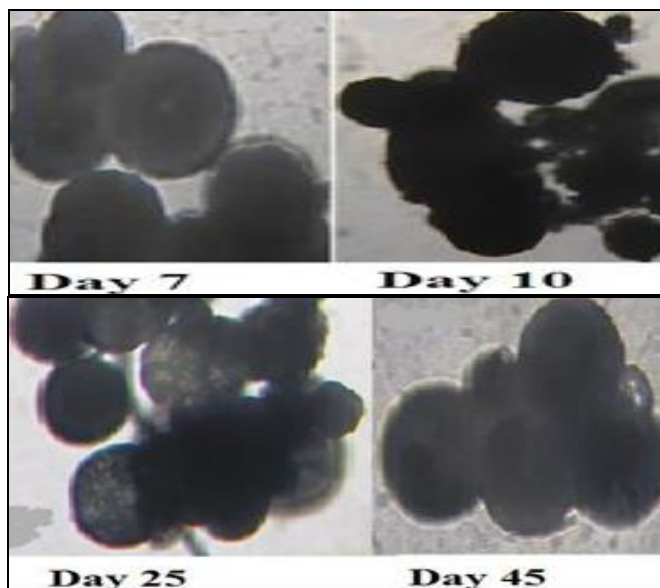


FIG. 4: MICROPHOTOGRAPHS OF STABILITY STUDY IN PBS AT 37°C

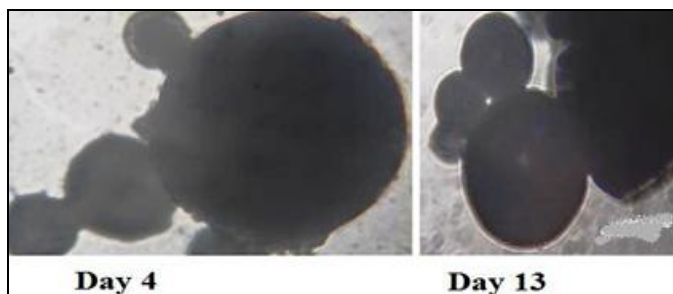


FIG. 5: MICROPHOTOGRAPHS OF STABILITY STUDY IN pH 2 AT 37°C

The *in vitro* drug release is studied for DOXY formulations D1, D2, and D3 and TETRA formulations T2, T3 and T4 (Fig. 6 and 7). These formulations are chosen because they have the highest %DE and % DL.

For both drugs the release is fast. It requires only few hours to go to completion. This can be

explained by the high affinity of these drugs towards PBS used as release medium.

The release rate is also higher for formulations having the lowest amount of drug (D1 and T2). It becomes slower when higher amount is present inside microspheres, thus the drug will take longer time to escape into the release medium. This result is particularly noticed in the case of formulations D2 and T4 characterized by the slowest release.

At higher drug loading, the chances of particles to contact 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 formulations D1 and T2 can be explained by the presence of surface associated drugs. 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 drugs¹⁸⁻²².

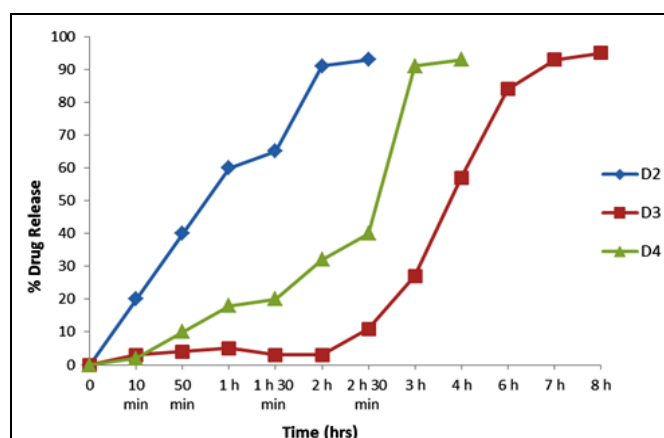


FIG. 6: IN VITRO RELEASE PROFILE OF DOXY MICROSPHERES

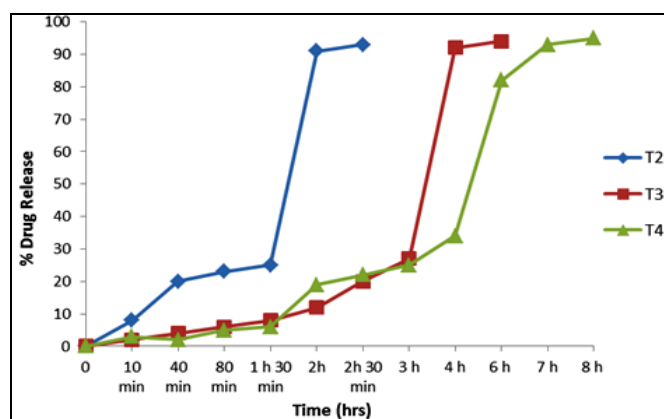


FIG. 7: IN VITRO RELEASE PROFILE OF TETRA MICROSPHERES

CONCLUSION: The o/w solvent evaporation technique has been used for the preparation of Doxycycline- and Tetracycline-loaded PLA microspheres. Microspheres have a spherical and porous profile, with an average size of 80 to 110 μm . Both drugs show average %DE and % DL due to their hydrophilicity; however, Doxycycline have higher encapsulation than Tetracycline. After carrying out the FT-IR study, results show that Doxycycline remains intact inside the microspheres after encapsulation.

Microspheres are stable after storage at different conditions. The *in vitro* release is fast for the two kinds of drugs; but it is faster in the case of Tetracycline. A controlled release followed by a burst release is observed for formulations with lowest %DL. This study has revealed that the prepared PLA-coated microspheres are suitable for the encapsulation of many drugs and biological molecules.

ACKNOWLEDGMENTS: The authors would like to thank the Chemistry Department at the University of Balamand for the help in funding the project, and allowing buying the necessary chemicals needed for the laboratory work.

REFERENCES:

1. Stevanovi M and Uskokovi D: Poly(lactide-co-glycolide)-based Micro and Nanoparticles for the Controlled Drug Delivery of Vitamins. *Curr Nanosci* 2009; 5(1): 1-15.
2. Hanwei Z, Jianzhong B, and Shenguo W: Preparation and drug release behaviors of 5-Fluorouracil loaded poly(glycolide-co-lactide-co-caprolactone) nanoparticles. *J Appl Polym Sci* 2007; 106(6): 3757-3767.
3. Heidi MM, Sohn MJ, Al-Ghananeem A and DeLuca PP: Materials for pharmaceutical dosage forms: molecular pharmaceutics and controlled release drug delivery aspects. *Int J Mol Sci* 2010; 11(9): 3298-3322.
4. Mohammad S: Pectin-based biodegradable hydrogels with potential biomedical applications as drug delivery systems. *J Biomater Nanobiotechnol* 2011; 2(1): 36-40.
5. Liu Q, Cai C and Dong CM: Poly(L-lactide)-b-poly(ethylene oxide) copolymers with different arms: hydrophilicity, biodegradable nanoparticles, in-vitro degradation, and drug release behavior. *J Biomedical Mater Res A* 2009; 88(4): 990-999.
6. Letizia MM: Chitosan and PLGA microspheres as drug delivery system against pulmonary mycobacteria infections. Doctoral dissertation, University of Cagliari, Italy 2007.
7. Lendlein A and Sisson A: Drug Delivery Systems. Handbook of Biodegradable Polymers: Synthesis, Characterization and Application. Wiley-VCH Verlag and Co., 2011: 1-27.
8. Lassalle V and Ferreira ML: PLA nano and micro particles for drug delivery: Overview of the methods of preparation. *Macromol Biosci* 2007; 7: 767-787.
9. Zhang Z and Huang G: Micro- and nano-carrier mediated intra-articular drug delivery systems for the treatment of osteoarthritis. *J Nanotechnol* 2012.
10. Pu X, Liu J, Guo Y, Yan X, Yang H and Yuan Q: Study progression in polymeric micelles for the targeting delivery of poorly soluble anticancer agents to tumor. *Asian J Pharm Sci* 2012; 7(1): 1-17.
11. Gavini E, Manunta L, Giua S, Achenza G and Giunchedi P: Spray-Dried Poly(D,L-Lactide) Microspheres Containing Carboplatin for Veterinary Use: In Vitro and In Vivo Studies. *AAPS PharmSciTech* 2005; 6(1): E108-E114.
12. Fan M, Guo QF, Luo JC, Luo F, Xie P, Tang XH, and Qian ZY: Preparation and in vitro characterization of dexamethasone-loaded poly(d,l-lactic acid) microspheres embedded in poly(ethylene glycol)-poly(ϵ -caprolactone)-poly(ethylene glycol) hydrogel for orthopedic tissue engineering. *J Biomater Appl* 2013; 28(2): 288-297.
13. Negrin CM, Delgado A, Labrés M and Evora C: Methadone implants for methadone maintenance treatment. In vitro and in vivo animal studies, *J Control Release* 2004; 95(3):413-421.
14. Giovagnoli S, Tsai T and DeLuca PP: Formulation and release behavior of doxycycline-alginate hydrogel microparticles embedded into pluronic F127 thermogels as a potential new vehicle for doxycycline intradermal sustained delivery. *AAPS PharmSciTech* 2010; 11(1): 212-220.
15. Mandell G and Bennett J: Tetracyclines and Chloramphenicol. Principles and Practice of Infectious Diseases. Churchill Livingstone, New York, 2009.
16. Pejic A, Kesic L, Obradovic R and Mirkovic D: Antimicrobial treatment of periodontal diseases. *Acta Fac Med Naiss* 2010; 27(2): 85-92.
17. Villagrana A and Clavel J: Antimicrobial or Subantimicrobial Antibiotic Therapy as an Adjunct to the Nonsurgical Periodontal Treatment, *ISRN Dentistry* 2012.
18. Yoon Y and Kinam P: Control of Encapsulation Efficiency and Initial Burst in Polymeric Microparticle Systems. *Archives of Pharmacal Research* 2004; 27(1): 1-12.
19. Belgacem M and Gandini A: Poly(lactic Acid): Synthesis, Properties and Applications. Monomers, Polymers, and Composites from Renewable Resources, Elsevier Ltd., 2008; 433-450.
20. Xudong S, Lei S and Zhihua G: Formation mechanism of solvent-induced porous PLA microspheres. *Acta Polym Sin*, 2011; 8: 866-873.
21. Song N, Liu W, Qin Tua, Liu R, Zhang Y and Wang J: Preparation and in vitro properties of redox-responsive polymeric nanoparticles for paclitaxel delivery. *Colloid Surface B* 2011; 87(2): 454-463.
22. Wang Y, Yu L, Han L, Sha X and Fang X: Difunctional pluronic copolymer micelles for Paclitaxel delivery: synergistic effect of folate-mediated targeting and pluronic-mediated overcoming multidrug resistance in tumor cell lines. *Int J Pharm* 2007; 337(1-2): 63-73.

How to cite this article:

Kassab R, Yammine P, Moussa D and Safi N: A comparative study of Doxycycline and Tetracycline Polymeric Microspheres. *Int J Pharm Sci Res* 2014; 5(6): 2452-57. doi: 10.13040/IJPSR.0975-8232.5(6).2452-57