Available online on 15.01.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research



Open Access Research Article

# Encapsulation of metronidazole in polycaprolactone microspheres

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## ABSTRACT

Non-aqueous oil-in-oil solvent evaporation technique is used for the preparation of polycaprolactone microspheres loaded with the antibiotic metronidazole by introducing different masses for the drug. The prepared microspheres are characterized by calculating drug encapsulation and drug loading percentages, measuring the corresponding particle size, performing FT-IR polymer-drug compatibility study and in vitro drug release. Moderate drug encapsulation values with a maximum of 34% are observed due to the low molecular weight of the drug. Microspheres had a particle size ranging between 130 and 280 µm with a spherical profile and porous structure. FT-IR study showed no interactions between the drug and the polymer. Drug release studies showed fast release rates for all the formulations with the slowest release for the highest drug loading.

**Keywords:** polycaprolactone, metronidazole, targeted drug delivery, solvent evaporation.

**Article Info:** Received 02 Dec 2018; Review Completed 05 Jan 2019; Accepted 07 Jan 2019; Available online 15 Jan 2019

**Cite this article as:**

Rima K, Dima M, Cherine S, Paolo Y, Encapsulation of metronidazole in polycaprolactone microspheres, Journal of Drug Delivery and Therapeutics. 2019; 9(1):190-194 **DOI: http://dx.doi.org/10.22270/jddt.v9i1.2306**

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## INTRODUCTION

Targeted drug delivery is a not a new concept in therapy, it dates back to 1906. The challenge is to create a medicine that targets only specific sites in the human body. This drug must be effective, and carried to the specific site without avoiding the immunogenic system [1-4]. Using biodegradable and biocompatible polymers in targeted drug delivery has many advantages such as providing sustained delivery of the drug, its localized delivery and its stability. Different kinds of drugs can be encapsulated in various types of polymers depending on their chemical and physical characteristics. One of the most common techniques used for microencapsulation is the solvent evaporation used for both hydrophobic and hydrophilic drugs. It has many advantages because it requires only simple experimental conditions like heat and stirring [5, 6].

Metronidazole (MTZ) is an antibiotic used against anaerobic bacteria and certain parasites. Metronidazole has a broad spectrum of protozoal and antimicrobial activity, both locally and synthetically [7]. Treatment using MTZ could induce some reported side effects such as epigastric pain, mouth dryness, nausea and others. In order to decrease these adverse effects, in addition to deliver the drug to the site of infection for a longer period and reach the best antibiotic efficiency, MTZ polymeric microspheres could be formulated[8].

Polycaprolactone (PCL) belongs to the family of polyesters. It is highly miscible in organic solvents. In addition, it adheres well to a large number of surfaces, which enabled it to be used widely in several medical applications [9-11]. PCL is nontoxic and semicrystalline with high organic solvent solubility. Due to the polymer very low degradation rate and high drug permeability, it has been extensively used in the medical field in implant devices in tissue engineering and in drug delivery devices [12, 13].

In this study, MTZ microspheres formulations are prepared using non-aqueous o/o emulsion solvent evaporation technique with PCL as the coating polymer. Microspheres are then characterized for drug loading, drug encapsulation, particle size, drug-polymer interaction and *in vitro* drug release.

## EXPERIMENTAL

Materials used include the polymer, Poly-ε-caprolactone (average MW 80,000), the drug Metronidazole, the surfactant Span 80, and Phosphate Buffered Saline (PBS) (0.2 M, pH 7.4). They are purchased from Sigma-Aldrich, Chemie, Germany. Paraffin Oil, dichloromethane (DCM), Methanol (MeOH) and n-hexane are of analytical grade.

**Method description:** non-aqueous oil-in-oil (o/o) solvent evaporation technique is used for the formulation of MTZ microspheres. Several formulations were prepared by fixing the PCL mass to 500 mg and changing drug masses between

50 and 200 mg. The polymer and the drug are dissolved in a mixture of DCM/MeOH forming the first organic phase. The mixture is then added into a second organic phase formed of 250 ml Paraffin oil containing 1% surfactant Span 80. After mixing the solution for 6 hours using a mechanical stirrer (MSP-1 Digital Overhead Stirrer, Jeiotech, Korea) to allow the solvent evaporation and microencapsulation of the drug, microspheres are recuperated by filtration after washing with hexane and few drops of methanol. Microspheres are then dried for 48 hrs at 40°C.

**Characterization:**

1- **Drug encapsulation (%DE) and Drug Loading (%DL):** microspheres are dissolved in 10 ml DCM/MeOH (7/3) and the drug content of each formulation is measured using UV/Vis spectrophotometer (Epoch Bioteck, USA) at 311 nm and calculated as follows:

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

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

2- **Particle size:** Laser Diffraction Granulometer (LA950V2, Horiba Ltd., France) is used to determine the size of microspheres. A quantity of microspheres is suspended in water, with few drops of Tween 80 for better dispersion. The average particle size is measured in micrometers.

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

4- ***In vitro* drug release study:** *in vitro* release study is carried out in PBS solution (0.2 M, pH 7.4). 25 mg microspheres are introduced in dialysis bags having a molecular weight cut off of 12000-14000. These were suspended in 15 ml PBS at 37°C. At different time intervals, 1 ml of the release medium is withdrawn and replaced with fresh solution and tested for its drug content at 311 nm.

## RESULTS AND DISCUSSION

Non-aqueous solvent evaporation method was applied for the preparation of MTZ-loaded PCL microspheres. Since MTZ is water soluble, the oil-in-oil method was chosen. It consists of adding the organic active phase containing the drug into another organic phase containing the hydrophobic surfactant Span 80. This method is preferred in the case of MTZ in order to avoid the loss of drug during microencapsulation step and improve drug encapsulation and bioavailability compared to hydrophobic drugs where an aqueous phase is used instead [14].

*Morphology of microspheres:*

SEM microphotographs of the prepared samples showed a homogeneous reproducible spherical profile with a porous structure of the polymeric matrix (Figure 1).

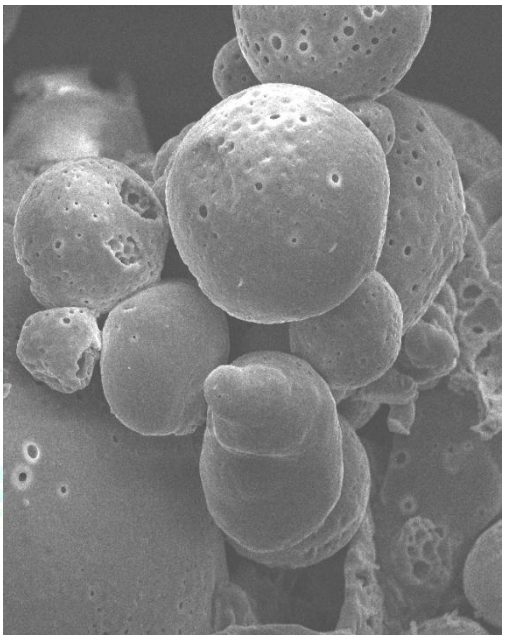


**Figure 1: SEM microphotographs of microspheres at 500x magnifications**

*Drug content and size of microspheres:*

Microspheres formulations were prepared by changing the drug mass between 50 and 200 mg. For each mass, %DE and %DL were calculated along with the average particle size. Results are presented in Table 1.

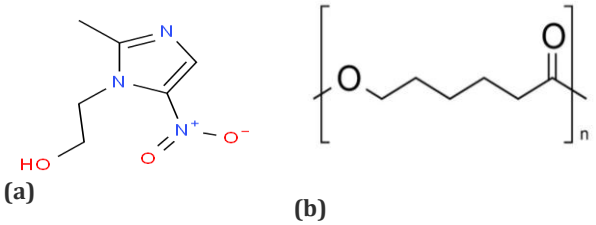
**Table 1: %DE, %DL and particle size of the different prepared formulations**

| Formulation Code | Drug Introduced (mg) | % DE | % DL | Size (µm) |
|---|---|---|---|---|
| $M_1$ | 50 | 13 | 0.26 | 280 |
| $M_2$ | 75 | 14 | 0.4 | 131 |
| **$M_3$** | **100** | **34** | **1.5** | **142** |
| $M_4$ | 150 | 24 | 1.4 | 165 |
| $M_5$ | 200 | 11 | 0.9 | 242 |

PCL microspheres loaded with MTZ had a particle size ranging between 131 and 280 µm (Table 1). In comparison to other drugs encapsulated in PCL such as Amphotericin B, and prepared using an aqueous phase and a hydrophilic surfactant, microspheres had a particle size ranging between 110 µm and 125 µm[11]. This difference could be attributed to the fact that the organic solvent DCM used in preparing MTZ microspheres has a higher solubility in Paraffin oil than in water. This leads to a faster mass transfer between the two dispersed and continuous phases, leading to rapid precipitation of microspheres and thus larger particles [15, 16].

Concerning the drug content of microspheres, the maximum %DE and % DL were 34% and 1.5% respectively. They were attributed to formulation $M_3$ containing 100 mg drug. Drug

molecular weight may have an effect on %DE and %DL. Drugs with low and average molecular weights easily diffuse out of the porous polymeric matrix [17].




(a) (b)

**Figure 2: (a) Metronidazole (MTZ), $C_6H_9N_3O_3$, (b) Polycaprolactone (PCL),$(C_6H_{10}O_1)_n$**

In the case of Metronidazole, it has a low molecular weight, which is about 171 g/mol. Thus, diffusion out of the microspheres could have occurred yielding low %DE and %DL [18, 19].

*Drug-polymer compatibility study:*

FT-IR study could confirm the chemical stability of MTZ encapsulated in PCL microspheres. This is done by measuring the characteristic peak shifts of the loaded microspheres in comparison with these of pure drug MTZ and polymer PCL. Recorded spectra are presented in Figure 3.

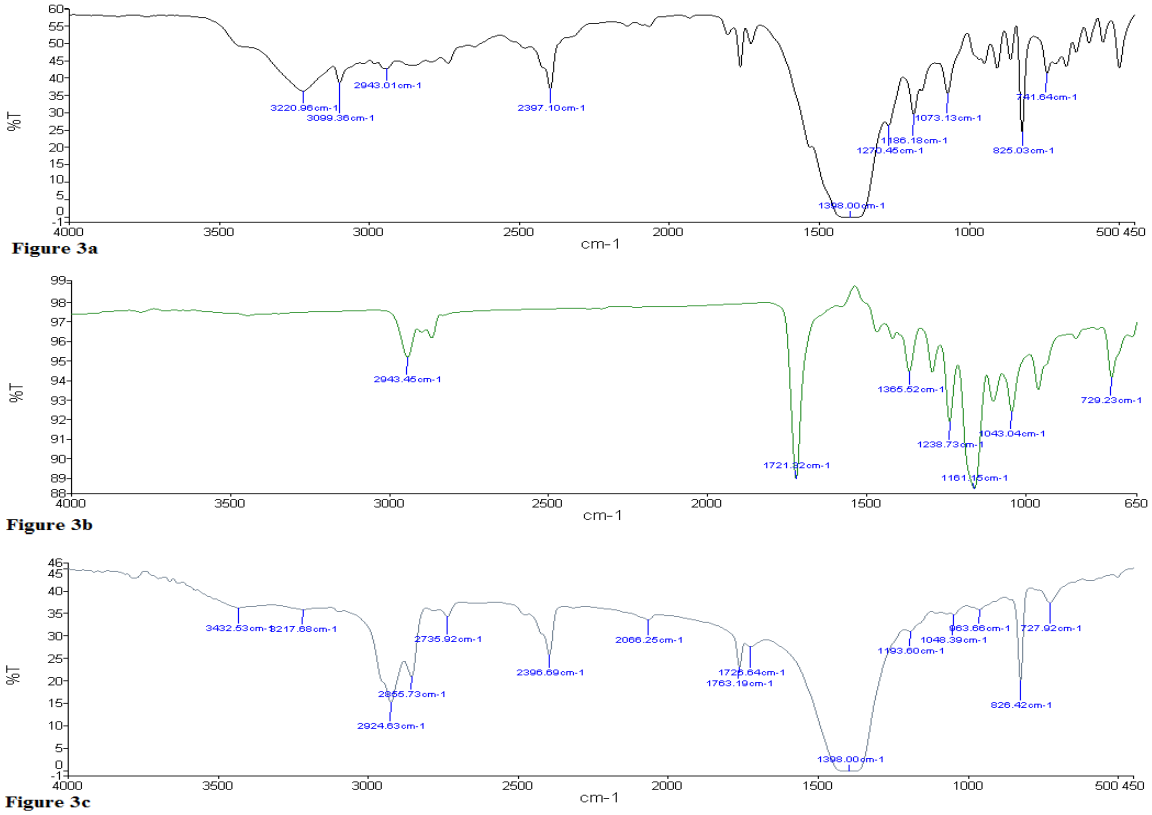



**Figure 3: FT-IR spectra of MTZ (a), PCL (b) and PCL MTZ-loaded microspheres (c)**

MTZ characteristic peaks are showing at 3220 $cm^{-1}$ and assigned to stretching –OH, 3099 $cm^{-1}$ to stretching =CH, 1073 $cm^{-1}$ to stretching –C-O of –C-OH (Figure 3a) [20].

Important bands are also showing in the spectrum of PCL (Figure 3b). Peak at 2943 $cm^{-1}$ is assigned to stretching of –C-H bending alkane. The peak at 1721 $cm^{-1}$ corresponds to stretching of –C=O carbonyl[21].

The representative peaks of MTZ and PCL didn't show any significant shifting and are present in the spectrum of loaded microspheres (Figure 3c). This indicated that the microencapsulation process didn't change the chemical structure of the drug and there were no chemical interactions observed between the drug and the polymer.

*In vitro release study:*

Figure 4 shows the *In vitro* release profiles of the different formulations prepared. The study reveals that the release rate is fast for all the formulations and requires minutes to go to completion. This could be explained by the fact that the drug MTZ used is water soluble. It has a good affinity towards the PBS release medium, and thus it tends to escape easily and be diffused out of the porous polymeric matrix within a short time [22]. In addition, the sample containing 100 mg drug, which is characterized by the highest %DL, has the slowest release rate and required 80 minutes to get completely released.

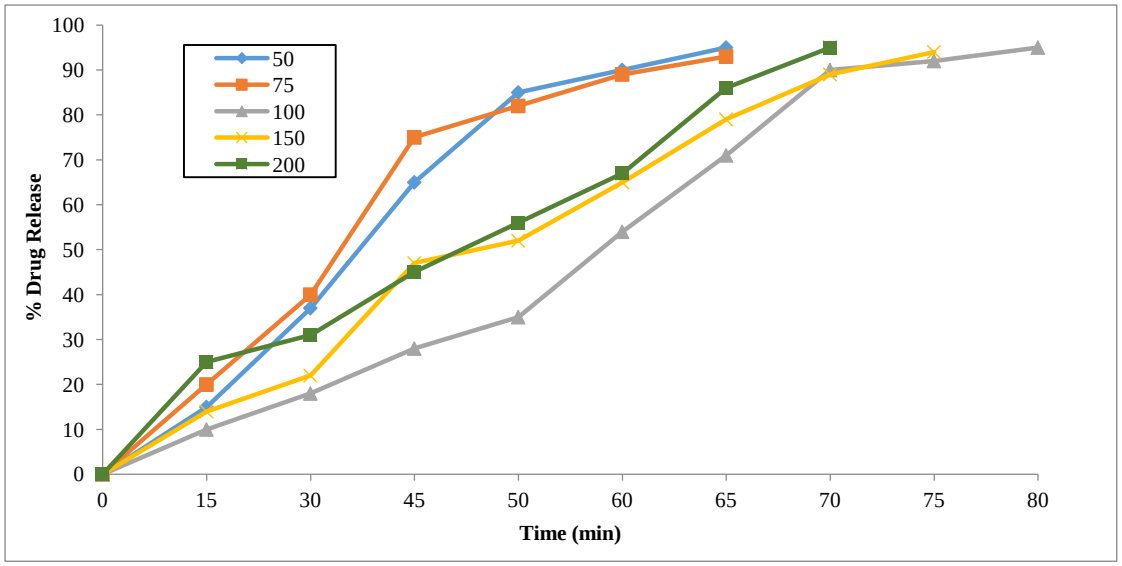



**Figure 4: *In Vitro* release profile of MTZ prepared formulations**

Figure 5 show that the release rate becomes slower with the increase in drug content. 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 [23].

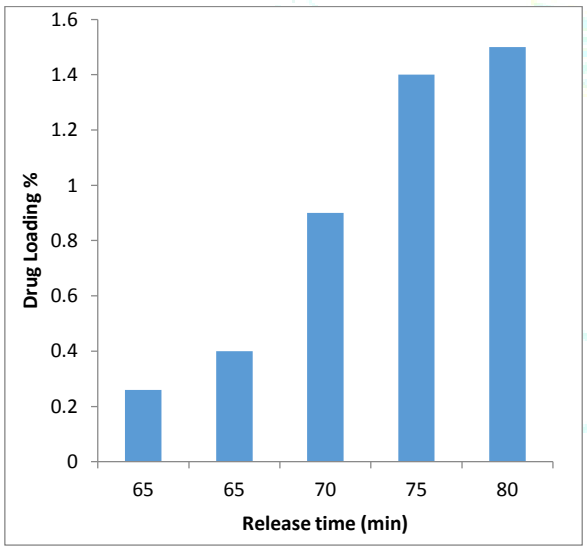



**Figure 5: Variation of release time with %DL of MTZ prepared formulations**

## CONCLUSION

The non-aqueous o/o solvent evaporation method was used to prepare metronidazole-loaded Polycaprolactone microspheres. Moderate drug encapsulation values with a maximum of 34% are observed. Microspheres had a particle size ranging between 130 and 280 µm with a spherical profile and porous structure. FT-IR study showed no interactions between the drug and the polymer. Drug release studies showed fast release rates for all the formulations with the slowest release for the highest drug loading.

## CONFLICT OF INTEREST

The authors who contributed in this project declare no conflicts of interest.

## REFERENCES

1- Sudhakar K, Madhusudana KR, Sudhakar P, Babu AC, Babu PK, Subha MCS, Rao KC, Development of pH-sensitive polycaprolactone-based microspheres for *in vitro* release studies of Triprolidine Hydrochloride, Designed Monomers and Polymers, 2014; 17(7):617-623.

2- Erdemli O, Ozen S, Keksin D, Usanmaz A, Batu ED, Atilla B, Tezcaner A, *In vitro* evaluation of effects of sustained anti-TNF release from MPEG-PCL-MPEG and PCL microspheres on human rheumatoid arthritis synoviocytes, Journal of Biomaterials Applications, 2014; 29(4):524-542.

3- Mohammad S, Pectin-based biodegradable hydrogels with potential biomedical applications as drug delivery systems, Journal of Biomaterials and Nanobiotechnology, 2011; 2(1):36-40.

4- Yammine P, Maarawi T, Moussa D, Abdel-Massih R, Kassab R, Effects of different surfactants on Indomethacin microspheres formulations, Journal of Advances in Chemistry, 2015; 11(4):3453-3462.

5- Ferreira SI, Bettencourt AF, Gonçalves MD, Kasper S, Bétrisey B, Kikhney J, Moter A, Trampuz A, Almeida AJ, Activity of Daptomycin- and Vancomycin-loaded poly-epsilon-caprolactone microparticles against mature staphylococcal biofilms, International Journal of Nanomedicine, 2015; 10(1):451-4366.

6- Dash TK, Konkimalla VB, Poly-ϵ-caprolactone based formulations for drug delivery and tissue engineering: a review, Journal of Controlled Release, 2012; 158(1):15-33.

7- Choughury PK, Murthy PN, Tripathy NK, Panigraphy R, Behera S, Investigation of drug polymer compatibility: formulation and characterization of metronidazole microspheres for colonic delivery, Pharmaceutical Sciences, 2012; 3(5):1-20.

8- Emara L, Abdo A, El-Ashmawy A, Mursi N, Preparation and evaluation of metronidazole sustained release floating tablets, International Journal of Pharmacy and Pharmaceutical Sciences, 2014; 6(9):198-204.

9- Dash TK, Konkimalla VB, Polycaprolactone based formulations for drug delivery and tissue engineering: a review, Journal of Controlled Release, 2012; 158(1):15-33.

10- Miladi K, Ibraheem D, Iqbal M, Sfar S, Fessi H, Elaissari A, Particles from preformed polymers as carriers for drug delivery, Excli Journal 2014; 13:28-57.

11- Kassab R, Moussa D, Yammine P, Polycaprolactone as drug carrier for an antifungal agent, Journal of Drug Delivery and Therapeutics, 2018; 8(1):81-85.

12- Mondal D, Griffith M, Venkatraman S, Polycaprolactone-based biomaterials for tissue engineering and drug delivery: current scenario and challenges, International Journal of Polymeric Materials and Polymeric Biomaterials, 2016; 65(5):255-265.

13- Shi R, Xue J, He M, Chen D, Zhang L, Tian W, Structure, physical properties, biocompatibility and in vitro-vivo degradation

behavior of anti-infective polycaprolactone-based electrospun membranes for guided tissue/bone regeneration, Polymer Degradation and Stability, 2014; 109:293-306.
14- Lokhande AB, Mishra S, Kulkarni RD, Naik JB, Influence of different viscosity grade ethylcellulose polymers on encapsulation and in vitro release study of drug loaded nanoparticles, Journal of Pharmacy Research, 2013; 7(5):414-420.
15- Yuce M, Canefe K, Indomethacin-loaded microspheres: preparation, characterization and in-vitro evaluation regarding ethylcellulose matrix material, Turkish Journal of pharmaceutical sciences, 2008; 5(3):129-142.
16- Maji R, Ray S, Ras B, Nayak AK, Ethylcellulose microparticles containing metformin HCl by emulsification-solvent evaporation technique: effect of formulation variables, ISRN Polymer Science, 2012, article ID 801827.
17- Jelvehgari M, Valizadi H, Rezapour M, Nokhodchi A, Control of Encapsulation Efficiency in Polymeric Microparticle System of Tolmetin, Pharmaceutical Development and Technology, 2010; 15(1):71-79.
18- Kassab R, Yammine P, Moussa D, Safi N, A comparative study of Doxycycline and Tetracycline polymeric microspheres, International Journal of Pharmaceutical Sciences and Research, 2014; 5(6):2452-2457.
19- Cetin M, Atila A, Sahin S, Vural I, Preparation and characterization of metformin hydrochloride loaded-Eudragit RSPO and Eudragit RSPO/PLGA nanoparticles, Pharmaceutical Development and Technology, 2013; 18(3):570–576.
20- Zupancic S, Potrc T, Baumgartner S, Kocbek P, Kristl J, Formulation and evaluation of chitosan/polyethylene oxide nanofibers loaded with metronidazole for local infections, European Journal of Pharmaceutical Sciences, 2016; 95:152-160.
21- Natarajan V, Krithica N, Madhan B, Sehgal PK, Formulation and evaluation of Quercitin polycaprolactone microspheres for the treatment of rheumatoid arthritis, Journal of Pharmaceutical Sciences, 2011; 100(1):195-205.
22- Kassab R, Yammine P, Moussa D, Safi N, Microspheres containing Doxycycline: properties and *in vitro* study, International Journal of Drug Delivery, 2013; 5(3):264-269.
23- Yoon Y, Kinam P, Control of Encapsulation Efficiency and Initial Burst in Polymeric Microparticle Systems, Archives of Pharmacal Research, 2004; 27(1):1-12.