

Preparation and evaluation of *in-vitro* release of amphiphilic β -cyclodextrin or PLA nanospheres containing a hydrophobic drug

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ABSTRACT

Mebendazole (MBZ) is an anthelmintic which belongs to the benzimidazoles. It is used against many intestinal diseases, and can also be efficient against extra-intestinal diseases. MBZ has poor oral bioavailability resulting from its insolubility and its extensive first pass metabolism in the liver

In the present work several formulations of amphiphilic β -cyclodextrin (β -CD-C₆ and β -CD-C₁₂) and poly(lactic acid) (PLA) nanospheres containing mebendazole (MBZ) were prepared according to the nanospherical crystallization and the nanoprecipitation techniques. These formulations differed in the type of polymer used to form the core of the nanospheres. Analysis of particle size distribution and encapsulation efficiency of the nanospheres revealed that the type and molecular weight of amphiphilic β -cyclodextrin used were the main factor influencing these properties. β -CD-C₁₂ had the highest encapsulation efficiency with the best reproducibility. From *in-vitro* release studies, a small amount of MBZ release was observed at pH 10. However, in gastric medium, an important burst effect occurred and was highest with β -CD-C₆ and PLA and lowest with β -CD-C₁₂, suggesting that drug release from these systems is affected by the type of cyclodextrins and the environmental conditions. The two formulations of MBZ-loaded nanospheres should be evaluated based on β -CD-C₆, β -CD-C₁₂ and PLA *in-vivo* in order to determine to what extent they are able to reduce the local side effects of this drug.

Keywords: Amphiphilic β -cyclodextrin; Nanosphere; Mebendazole; PLA

1 INTRODUCTION

Mebendazole (MBZ) (Fig.1) is a benzimidazole anthelmintic used in the treatment of ascariasis, oxyuriasis, trichuriasis and more than one worm infection at a time. It's active against intestinal nematodes and hydatid disease

when administered in high doses (2.4 g/day, for 1 to 6 months) [1].

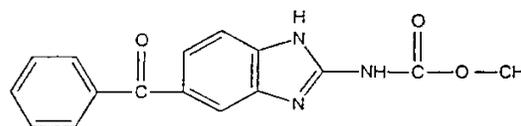


Figure.1

Nevertheless, its poor aqueous solubility limits its bioavailability and in case of a desirable systemic effect, enhancing its solubility could be an alternative to the use of high doses, generally associated to adverse side effects (gastro-intestinal disturbances, alopecia, reversible bone marrow depression ...). Recently, a new colloidal carrier system prepared from modified cyclodextrins was described [2]. These nanospheres have been characterized and visualized by freeze-fracture electron microscopy [3]. The self-assembling structural properties of several amphiphilic cyclodextrins and the internal organization of the amphiphilic cyclodextrin nanospheres have been described [4]. These modified cyclodextrins will be hereinafter called β -CD-C₆ and β -CD-C₁₂. The most suitable parameters for the entrapment of MBZ in PLA, β -CD-C₁₂ and β -CD-C₆ nanospheres were determined as a preliminary step for their use as pharmaceutical carriers in the treatment of hepatic abscess. Nanospheres containing MBZ were prepared by adding an acetone solution of amphiphilic cyclodextrin or PLA to an aqueous solution of MBZ with Pluronic PE/F68[®] as surfactant. The aim of this work was to prepare and characterize MBZ nanospheres made from amphiphilic-cyclodextrin and PLA according to the nanocrystallization and nanoprecipitation techniques. The influence of these cyclodextrins and polymer on the entrapment efficiency, and the capacity of a nanospheres system to retain the MBZ are discussed.

2 MATERIALS AND METHODS

2.1 Materials

MBZ ($C_{16}H_{13}N_3O_3$; MM: 295.3 g mol⁻¹; melting point 290 °C) was obtained from Janssen Cilag (France). poloxamer (PE/F68), neutral surfactant by LCI, hydrochloric acid and sodium hydroxyde, used to adjust pH by Cooper (France) and PLA (molecular weight 150 000) were purchased from PHUSIS (Le Versoul, France)

All organic solvents were of HLPC grade and were used without further purification.

The β -cyclodextrin was purchased from Wacker Chimie S.A. (Lyon, France) and was previously recrystallized. Amphiphilic- β -cyclodextrin ester (β -CD-C₆, β -CD-C₁₂) was obtained by a synthetic route [5]. The synthesis was realized in three steps and briefly consists in the protection of the primary hydroxyl groups at the O6 position by the t-butyltrimethylchlorosilane (TBDM) (Fluka Chemie AG, St-Quentin Fallavier, France) in dry pyridine. After, the esterification of the secondary hydroxyl groups at O2 and O3 positions is performed with n-hexanoyl chloride (Aldrich, St-Quentin Fallavier, France) in the presence of dimethylaminopyridine as acylation catalyst. In our case, this reaction was realized in dry pyridine at 60 °C during 24 h. The last step consists in the removing of the silyl protecting group by use of the borontrifluoride ethyletherate in free ethanol chloroform to give the amphiphilic β -cyclodextrin derivative. The reaction are monitored by CCM and ¹H RMN. For each step, an extraction and a preparative column purification procedures is realized.

2.2 Methods

2.2.1 Preparation of MBZ-loaded nanospheres

Nanospheres of made from β -CD-C₆, β -CD-C₁₂ and PLA were loaded with MBZ according to conventional technique[3], organic phase consisting of β -CD-C₆, β -CD-C₁₂ or PLA and MBZ dissolved in acetone was added with an eppendorf syringe under constant stirring at room temperature to the aqueous phase consisting of deionized water. Ratio of organic phase to aqueous phase was optimized to 1:2. After an equilibrium of the system for 30 min, organic solvent was evaporated under vacuum at 50°C and nanospheres was obtained

3 CHARACTERIZATION OF NANOSPHERES

3.1 Particle size determination

Mean particle size (diameter, nm \pm S.D.) and polydispersity index of nanospheres were determined by the quasi-elastic light scattering method (QELS) with a nanosizer N4MD apparatus (Beckman-Coulter) Measurements were realized in triplicate at a 90° angle at 20°C. A dispersion of intensity between 10⁴ and 10⁶ cps were analyzed for 90 s freshly after preparation of nanospheres.

3.2 Determination of drug loading

MBZ content was determined by a reversed-phase High Performance Liquid Chromatography (HPLC) method already described [7]. The chromatographic analysis was performed under the following conditions: spectrophotometric detector set at 289 nm, column μ Bondex C₁₈ (300 x 4.6 mm, SFCC, France); mobile phase : acetate buffer 0.25 M / acetonitrile (50/50, v/v) at a pH of 5.7, a flow rate of 1 ml/min. Under these conditions the retention time of MBZ was 6 min. and the detection limit was 0.1 μ g/mL. The MBZ concentration was determined in all the suspensions (total drug) after dissolution of the nanospheres in acetonitrile and in the supernatants (free drug) after ultracentrifugation at 120000 g for 1 h at 4°C. The association of the drug (%) in the nanospheres was calculated from the difference between the total and free drug. The drug loading, expressed as micrograms of fixed MBZ per milligram of PLA, β -CD-C₆, β -CD-C₁₂, was calculated. The MBZ entrapment efficiency (%) was then estimated from the drug content found in the nanospheres and the initial drug content added in the formulations.

3.3 Zeta potential

The charge of nanospheres was measured with a Malvern Zetasizer 2C instrument equipped with a tubular cell of 2.6 mm. The operating principle of this instrument is based on the Doppler shift caused by the movement of nanospheres across interference fringes which are produced by the intersection of two laser beams. The nanospheres were suspended in KCl (10⁻³ M), and the measurement was made at 25 °C.

3.4 Transmission electron microscopy

In the various staining procedures available for imaging nanovesicles [7], the drop method was adopted here as the standard procedure [7]. In this method a single drop

of freshly prepared nanospheres was placed onto a carbon-coated copper grid, left to stand for 1 min, and then dried using a wick of filter paper (Whatman No. 1). The stain, representative of an aqueous solution of 2% (w/v) phosphotungstic acid, was then placed onto the grid and left for an additional minute. A fresh filter paper wick was used to remove excess stain from the grid. The sample was then immediately imaged with a transmission electron microscope (Philips EM 301 G) operating at an acceleration voltage of 100 kV with zero tilt in order to avoid the artefacts known to occur as a consequence of prolonged drying [8].

3.5 In vitro MBBZ release

Skin preparation

Pig ears were obtained from a slaughterhouse. The skin was carefully removed leaving the fat tissue behind, then examined for damage conditions and any skin in which the barrier was disrupted was removed. The skin was cut into 2 cm · 2 cm samples for permeation studies. Its thickness was lower than 1,300 µm.

In vitro skin permeation studies

The excised skin was mounted between the donor and the receptor chambers of Franz type diffusion cells with epidermal side facing the donor fluid. The volume of donor and receptor chambers were respectively 2 ml and 13.5 ml and the effective surface area available for permeation of drug was 2 cm². About 2 ml of test nanospheres was placed on the donor side. The receptor chamber was filled with 13.5 ml of buffers solutions (pH 1.2; pH 6.9 and pH 10). Sink condition was obtained by the use of this buffer solution. The contents in the receiver compartment were stirred with magnetic bar. The diffusion cell was immersed in a water bath maintained at 37 ± 0.5°C on magnetic stirrer. At predetermined times, 0.5 ml samples were withdrawn from the receiver compartment and replaced with an equivalent amount of drug free solvent to maintain a constant volume. The samples were assayed for MBZ by HPLC and experiments repeated six times.

Data analysis and statistical

The cumulative amount of MBZ permeated per unit skin surface area was plotted against time. Slope of the linear portion of the plot was estimated as steady-state flux (JSS). Statistical data analyses were performed using the Student's test with P < 0.05 as the minimal level of significance.

4 RESULTS AND DISCUSSION

Preparation and characterization of PFC-NC

The nanospheres were prepared in a single step by colloidal nanocrystallization of amphiphilic β-Cyclodextrins (β CD-C₆ and β CD-C₁₂) and by nanoprecipitation of PLA. These nanospheres showed an

homogenous size distribution with a mean diameter of 178 ± 43 nm, 105 ± 10 nm and 253 ± 45 nm respectively. The profile corresponded to a normal distribution and the sample could be considered monodisperse as shown by the cumulative results and polydispersity index (Table 1). The morphological examination of nanospheres formed by chemically modified cyclodextrins (β CD-C₆ and β CD-C₁₂) or by Poly (D,L-Lactide), was performed by TEM and showed a spherical and homogenous system. We observed that nanospheres having a mean diameter were successfully prepared by the colloidal nanocrystallization and by precipitation method (Figure 3). In order to indirectly determine the surface charge of nanospheres, zeta potential values of nanospheres formed by chemically modified cyclodextrins (β CD-C₆ and β CD-C₁₂) or by Poly (D,L-Lactide) were analysed. We obtained a zeta-potential of -73 mV this charge probably arose from chemical interactions at the surface of the particles with the external medium and may depend on the ionic strength of the aqueous phase (Table 1). Both amphiphilic - cyclodextrin and PLA allow to obtain a good encapsulation of the MBZ (Table 2)

Nanosphères	Mean diameter (nm)	μ ² /γ	Zeta Potential mV
NS-PLA	253 ± 45	0,085	-72
NS β-CD C ₆	178 ± 43	0,040	-74
NS β-CD C ₁₂	105 ± 10	0,040	-72

μ²/γ : Polydispersity index

Table 1: Influence of polymer on the mean diameter size and zeta potential of nanospheres (n=3).

Nanosphères	Entrapment efficacy (%)
NS-PLA	97
NS β-CD C ₆	90
NS β-CD C ₁₂	100

Table 2: Efficiency of MBZ encapsulated

The solubility of MBZ depends on the pH from which its release is fast at a pH 1,2 (release of about 30% of MBZ in 24h and release of about 30 to 45% of MBZ in 90h). (Figure 1 & Figure 2). The release of MBZ is more important for the nanospheres of β-CD C₆, certainly because those nanospheres have a larger affinity for the skin use

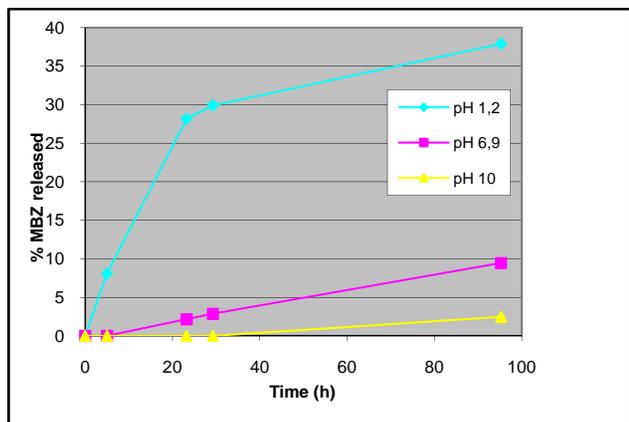


Figure 1 : Release of MBZ encapsulated in PLA nanospheres

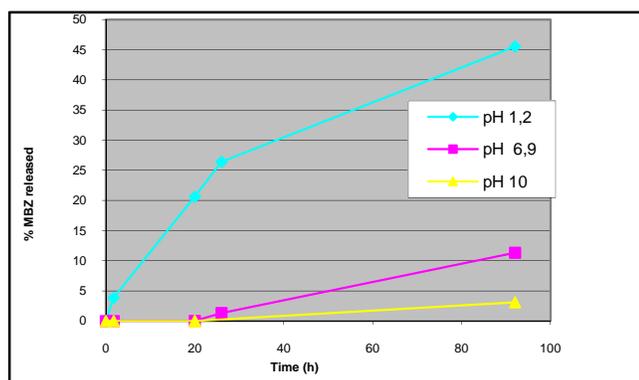


Figure 2 : Release of MBZ encapsulated in β -CD-C6 nanospheres

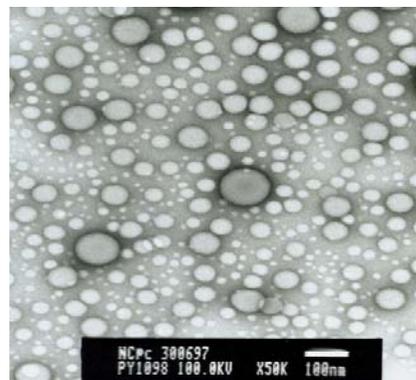
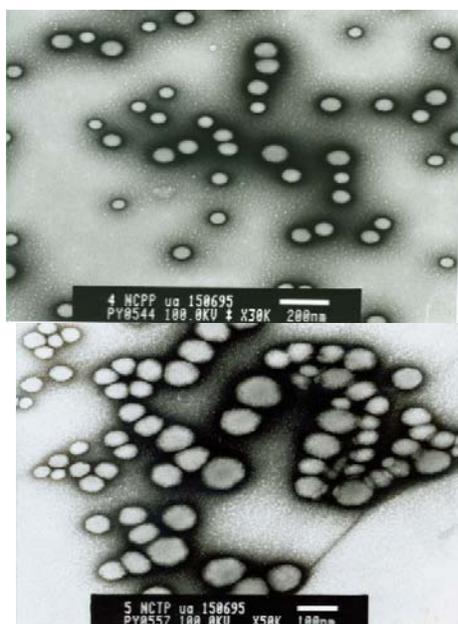


Figure 3: Electron transmission microphotography of nanospheres made from amphiphilic β -cyclodextrin and PLA

5 CONCLUSION

In this paper, we demonstrated that a molecularly dispersed hydrophobic drug incorporated in amphiphilic cyclodextrin nanospheres is released rapidly. The release was governed by a partition phenomenon and depends only of the solubility of the drug in the medium. This release profile can be interesting when an improvement in bioavailability of the drugs is desired.

6 REFERENCES

- [1] N. De Silva, H. Guyatt and D. Bundy, *Drugs* 53, 769 (1997).
- [2] M. Skiba, D. Wouessidjewe, A.W. Coleman, H.Fessi, J-P. Devissaguet, D. Duchêne and F. Puisieux. (1993). PCT Applications FR 93/00594.
- [3] Skiba, M., Wouessidjewe, D., Puisieux, F., Duchêne, D. and Gulik, A. *Int. J. Pharm.* 142 (1996) 121-124.
- [4] A. Gulik, H. Delacroix, D. Wouessidjewe and M. Skiba, *Langmuir*, 14 (1998) 1050-1057.
- [5] P. Zhang, C.C. Ling, A.W. Coleman, H. Parrot-Lopez and H. Galons. *Tetrahedron Lett.*, 32 (1991) 2769-2770.
- [6] M. Lahiani-Skiba, A. Coquard, F. Bounoure, P. Verite, P. Arnaud, M. Skiba, *J. OF Inclusion Phenomena and Macrocyclic Chemistry*, vol 57; number 1-4, pages 197-201, 2007
- [7]. S. Lukac and A. Perovic: *J. Colloid Interface Sci.* **103**,586 (1985).
- [8] Y. Talmon: *J. Colloid Interface Sci.* **93**, 366 (1983)