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Short communication

Optimization of entrapment of metronidazole in amphiphilic β-cyclodextrin nanospheres

Malika Lahiani-Skiba [a], Frederic Bounoure [a], Sozan Shawky-Tous [b], P. Arnaud [a], Mohamed Skiba [a,*]

[a] *Université de Rouen UFR de Médecine & Pharmacie, A.D.E.N. EA 3234, Laboratoire de Pharmacie Galénique, 22 Bd Gambetta, 76000 Rouen, France*
[b] *Assiut University, Faculty of Pharmacy, Department of Pharmaceutics, Assiut, Egypt*

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Abstract

Several aspects of the manufacture of nanospheres containing metronidazole were studied. Nanospheres made of amphiphilic β-cyclodextrin containing metronidazole were prepared by adding an acetone solution of amphiphilic cyclodextrin to an aqueous solution of metronidazole with or without Pluronic PE/F68® as the surfactant. An optimized formulation with high encapsulation efficiencies and with an appropriate particle size for intravenous administration, was developed.

The entrapment of metronidazole was strongly dependent of the method of preparation and drug concentrations. These nanospheres prepared by nanocrystallization are promising carriers for metronidazole in the treatment of hepatic abscess.

Keywords: Amphiphilic β-cyclodextrin; Nanosphere suspension; Metronidazole

1. Introduction

Metronidazole is a nitro-imidazole which has been used as an antiprotozoal and antimicrobial agent for many years. It is the first-line drug used in the treatment of extra-intestinal involvement of amoeba (hepatic abscess). However, due to its toxicity, which can lead to such complications as convulsive seizures and neuropathies, gastrointestinal irritation, leukopenia, potentiation of an anticoagulation therapy and hypersensitivity reactions, metronidazole dosing must be carefully regulated. Dosing regimens often include sets of daily doses extending over periods of several weeks [1].

Alternate dosage forms in which metronidazole is sustain released from a drug carrier have been suggested as a means to reduce dosing frequency and systemic toxicity.

The potential use of nanoparticles made of amphiphilic cyclodextrin as drug carriers has been exploited with success to reduce the toxic side effects of several drugs, thus improving their therapeutic indexes [2] and amphiphilic cyclodextrin nanospheres present no toxic reaction [3]. Furthermore, the preferential uptake of nanospheres by liver macrophages opens up important therapeutic perspectives in the particular case of hepatic abscess.

A colloidal carrier system prepared from modified cyclodextrins was described [4,5]. These nanospheres have been characterized and visualized by freeze-fracture electron microscopy [6,7]. The self-assembling structural properties of several amphiphilic cyclodextrins and the internal organization of the amphiphilic cyclodextrin nanospheres have been described [8,9]. These modified cyclodextrins will be hereinafter called β-CD-C_6.

The most suitable operative conditions for the entrapment of metronidazole in β-CD-C_6 nanospheres were determined as a preliminary step for their use as pharmaceutical carriers in the treatment of hepatic abscess. β-CD-C_6 nanospheres containing metronidazole were prepared by adding an acetone solution of amphiphilic cyclodextrin to an aqueous solution of

* Corresponding author at: Université de Rouen, Faculté de Médecine & Pharmacie, Laboratoire de Pharmacie Galénique et Biopharmacie, 22, boulevard Gambetta, 76183 Rouen Cedex, France. Tel.: +33 2 35 14 85 96; fax: +33 2 35 14 85 94.
E-mail address: mohamed.skiba@univ-rouen.fr (M. Skiba).

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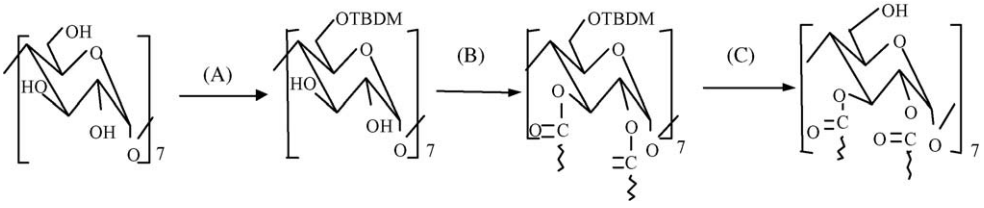
metronidazole with or without Pluronic PE/F68® as surfactant. An optimized formulation with high encapsulation efficiencies, drug concentrations and particle size appropriate for intravenous administration, was developed.

2. Materials and methods

2.1. Materials

The β-cyclodextrin was purchased from Wacker Chimie S.A. (Lyon, France) and was previously recrystallized. Hexanoyl-β-cyclodextrin ester (β-CD-C_6) was obtained by a synthetic route [10]. 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*-butyldimethylchlorosilane (TBDM) (Fluka Chemie AG, St-Quentin Fallavier, France) in dry pyridine. After, the esterification of the secondary hydroxyl groups at O2 and O3 positons 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 sylil 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 ^{1}H RMN. For each step, an extraction and a preparative column purification procedures is realized.



A, protection; B, grafting; C, deprotection.

Metronidazole (Fig. 1) was supplied by Sigma (St Quentin, France) and Pluronic PE/F68®, (poloxamer: ethylene oxide/propylene oxide block copolymer) was provided by ICI (Clamart, France).

2.2. Preparation of β-CD-C_6 nanospheres

The nanocrystallization method consisted of injecting an acetonic solution (15 ml) of β-CD-C_6 (2 mg/ml) into an aqueous phase (15 ml) containing the non-ionic surfactant Pluronic PE/F68® surfactant (30 mg), under stirring. Nanospheres of β-CD-C_6 precipitate spontaneously. We compared this method with the reverse method of injecting an aqueous phase containing the same surfactant into an acetonic solution of β-CD-C_6.

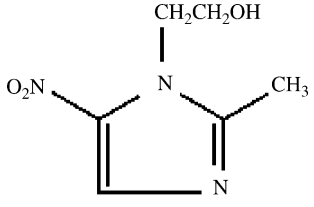


Fig. 1. Chemical structure of métronidazole.

Preparation of drug-loaded nanospheres can be carried out in the same manner, with metronidazole, a hydrosoluble drug, dissolved in the organic solvent because more soluble in acetone than in water.

The added quantities of metronidazole were: 0, 1, 5, 20 and 200 mg. These quantities correspond respectively to the following concentrations: 0, 0.07, 0.33, 1.00, 3.33 and 13.33 mg/ml with a final volume of aqueous phase of 15 ml. The aqueous phase was constituted of phosphate buffer solutions at different pH: 4.6, 7 and 9.6. The nanocristallization method was compared with the reversed method.

In both cases, the water-miscible solvent was removed completely using rotary evaporation.

2.3. Particle size determination

The particle mean diameter and the size distribution of the nanospheres were determined by the quasi-elastic light scattering method (QELS) with a nanosizer N4MD apparatus (Beckman–Coulter) ($n = 3$).

2.4. Determination of drug loading

Metronidazole content was determined by a reversed-phase high performance liquid chromatography (HPLC) method already described [11]. The chromatographic analysis was performed under the following conditions: spectrophotometric detector set at 320 nm, column μBondex C_{18} (300 × 4.6 mm, SFCC, France); mobile phase: acetate buffer 0.05 M/methanol (70/30, v/v) at a pH of 4.5, a flow rate of 0.8 ml/min. Under these conditions the retention time of métronidazole was 4 min and the detection limit was 0.1 μg/ml. The metronidazole 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 120 000 × *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 metronidazole per milligram of β-CD-C_6, was calculated.

The metronidazole entrapment efficiency (%) was then estimated from the drug content found in the nanospheres and the initial drug content added in the formulations.

2.5. In vitro metronidazole release

Metronidazole release from nanospheres was carried out at 37 °C under mechanical stirring after dilution of the colloidal

suspensions. These dilutions were performed in an isotonic phosphate buffer solution at pH 7.4. In order to separate the particles from the medium, centrifugal ultrafiltration technique was employed. A volume of 400 μl of the diluted suspensions was deposited in the Ultrafree MC unit (100 000 NMWL, Polysulfone membrane type, Millipore, France) and subjected to centrifugation at 5000 × *g* for 5 min. The released metronidazole (%) was determined by the HPLC method as described above.

3. Results and discussion

Preparation of β-CD-C_6 Nanospheres: Influence of the pH of the buffer on the percentage of metronidazole associated and on the particle size of nanospheres.

Inclusion complexation of drugs with cyclodextrins was useful to solve various pharmaceutical formulation problems and permitted to improve the solubility, the dissolution and the chemical stability of various drugs. Metronidazole benzoate, a prodrug of metronidazole, was found to form an inclusion complex with β-CD in aqueous solution and in solid phase. This inclusion complexation with β-CD protected the drug against photochemical degradation and increased its physical stability [12].

In the present work, we extended the field of pharmaceutical applications of cyclodextrins with an original carrier system from modified cyclodextrins, a non-polymeric material. This new type of nanospheres are capable of combining with water-soluble drugs (such doxorubicin hydrochloride) and insoluble drugs (progesterone and indomethacin) [7,13] and could have therapeutic value.

In a previous study [2], we investigated the role of encapsulation of indomethacin, a non-steroidal anti-inflammatory drugs (NSAID) within modified β-cyclodextrin (β-CD-C_6) nanocapsules. The use of this drug is limited by their ulceronecrotic effects on the gastro-intestinal mucosa. The encapsulation of indomethacin within orally administered β-CD-C_6 nanocapsules protected against both gastric and intestinal ulceration and the relative bioavailability was also increased.

In the present work, several aspects of the manufacture of β-CD-C_6 nanospheres containing metronidazole were studied.

The influence of the pH of the buffer on the percentage of metronidazole associated and on the particle size of the nanospheres is shown in Fig. 2. The results suggest that the pH of the buffer used for the preparation influence neither the percentage of metronidazole associated nor the particle size of nanospheres.

3.1. Preparation of β-CD-C_6 nanospheres: influence of the metronidazole initial content on the percentage of encapsulation in cyclodextrin nanospheres

Metronidazole loading in the amphiphilic β-CD-C_6 cyclodextrin nanospheres was influenced by the initial content of the drug added to organic phase (Fig. 3). The maximum association of metronidazole with the cyclodextrin nanospheres was reached when 50 mg of the drug was added in the formulations. In this case, the entrapment efficiency was almost 84%.

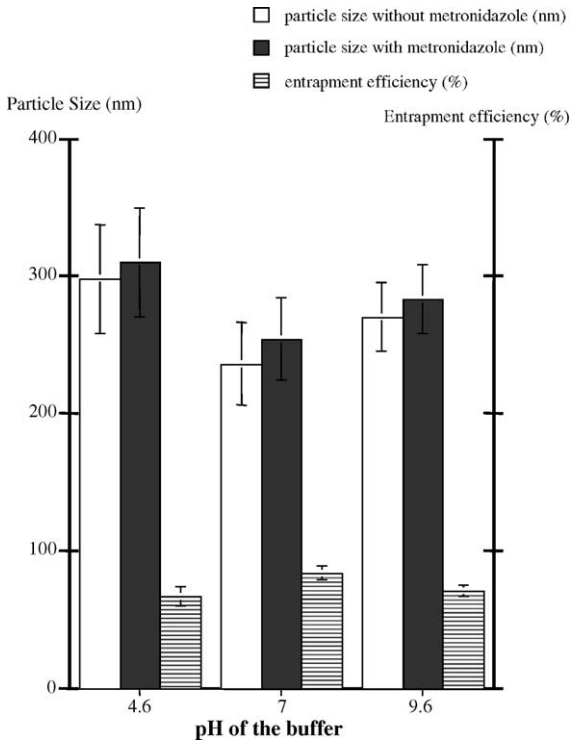


Fig. 2. Influence of the pH of the buffer on the percentage of metronidazole associated and on the particle size of nanospheres ($n = 3$).

The excess metronidazole formed a precipitate with the evaporation of the organic solvent, which was then eliminated by filtration. The metronidazole loading of β-CD-C_6 nanospheres is much higher than that which was obtained for nanopar-

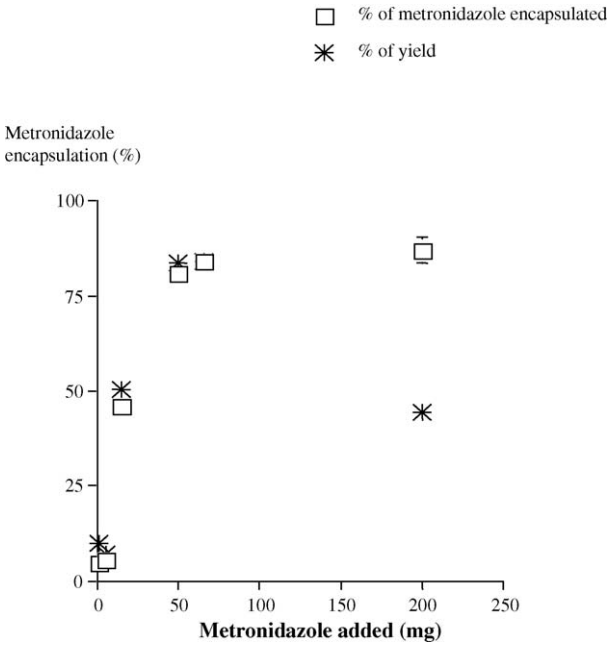


Fig. 3. Influence of the metronidazole initial content on the percentage of encapsulation in amphiphilic cyclodextrine nanospheres ($n = 3$).

cles produced by desolvation of gelatin, polyglutaraldehyde nanoparticles, albumin nanoparticles produced by denaturation at high temperature or nanoparticles produced by emulsion polymerization using acrylamide [14].

3.2. Role of the general preparation procedure

With a view to investigate the role of the general preparation procedure, we compared the nanocrystallization process already described (injecting an acetone solution of β-CD-C_6 into an aqueous phase containing Pluronic F68 surfactant at several concentrations (Fig. 6)) with another process (injecting an aqueous phase containing Pluronic F68 surfactant into an acetone solution of β-CD-C_6). Metronidazole was added to the organic phase for each process, these hydrosoluble molecule being more soluble in acetone than in water.

For the second procedure, as soon as the first drops of the aqueous phase are injected into the organic phase, the nanospheres are formed instantaneously and all the mass of cyclodextrins in acetone is used to form the matrix of the nanoparticles. A small quantity of drug is trapped in the matrix during the formation of nanospheres and the majority of metronidazole remains in the organic medium where it is more soluble. This process led to lower drug encapsulation (Fig. 4) probably because the solubility limit of β-CD-C_6 was reached before the whole volume of the aqueous phase had been injected. As the volume remaining of aqueous phase is injected, metronidazole will be adsorbed preferentially on the surface of the nanoparticles. For the other process, the nanospheres are progressively formed as the organic phase is injected into the aqueous phase. Metronidazole being a drug more soluble in acetone than in water, it will be completely trapped during the formation of the nanoparticles. More the organic phase is added, more the nanospheres are formed and this process thus leads to higher rates of encapsulation.

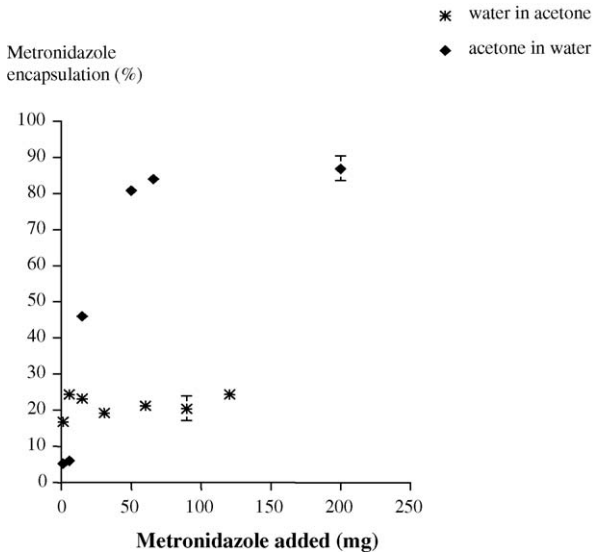


Fig. 4. Percentage of encapsulation of metronidazole in amphiphilic cyclodextrine nanospheres prepared by the two different procedures (acetone in water or water in acetone) ($n = 3$).

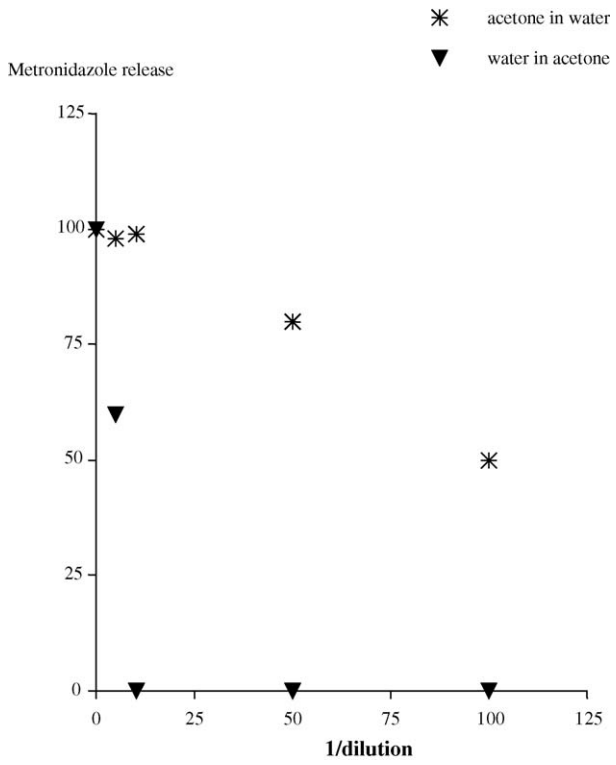


Fig. 5. Percentage of release of metronidazole from nanospheres prepared by the two different procedures (acetone in water or water in acetone) ($n = 3$).

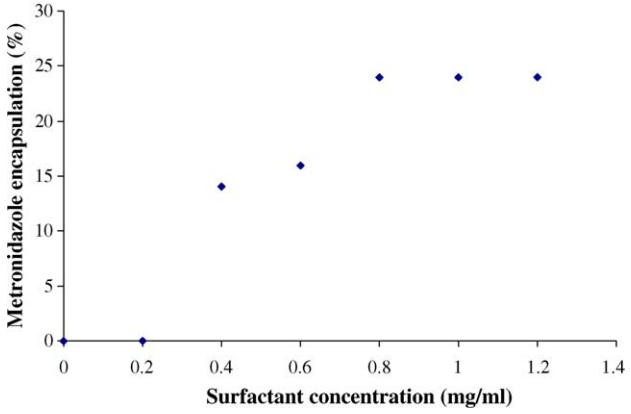


Fig. 6. Influence of the surfactant at several concentrations of encapsulation in amphiphilic cyclodextrine nanospheres ($n = 3$).

We also studied the release of metronidazole from nanospheres prepared by the two different procedures. When the acetonic phase was added to the aqueous phase, metronidazole was released only progressively with dilution, showing a strong association with the particles. In contrast, when nanospheres were prepared in the reverse method, the drug was completely dissociated by a 10-fold dilution, suggesting that it was simply adsorbed on the surface (Fig. 5).

4. Conclusion

In the present study, the most suitable manufacturing parameters for the entrapment of metronidazole in nanospheres were

determined as a preliminary step for their use as pharmaceutical carriers in the treatment of hepatic abscess. Nanospheres made of amphiphilic β-cyclodextrin, a non-polymeric material, containing metronidazole were prepared by nanocrystallization in injecting an acetone solution of amphiphilic cyclodextrine and metronidazole to an aqueous solution with Pluronic PE68® as the surfactant. This process permited to obtain an optimized formulation with high encapsulation efficiency, with the drug inside the nanosphere matrix and a particle size appropriate (<1 μm) for intravenous administration. Comparatively, the second procedure in injecting an aqueous phase containing Pluronic PE68® surfactant led to lower drug encapsulation with a simple adsorption of the drug on the surface of nanospheres.

The entrapment of metronidazole was strongly dependent of the method of preparation, and drug concentration, but was independent of the pH of the hydration medium.

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