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Note

Preparation of 3,4-diaminopyridine microparticles by solvent-evaporation methods

Stéphane Gibaud *, Aurélie Bonneville, Alain Astier

Laboratoire de Pharmacie Clinique, UPRES EA 2399, Faculté de Pharmacie, 5, rue Albert Lebrun, 54000 Nancy, France

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Abstract

The present study compares two methods of preparation of microparticles of 3,4-diaminopyridine (3,4-DAP) for the treatment for multiple sclerosis and Lambert-Eaton myasthenia syndrome. Poly(ε-caprolactone) microparticles were prepared with a solvent-evaporation W/O method. The 3,4-DAP was dispersed in dichloromethane, leading to a suspension. The dispersion and the solidification of the dichloromethane droplets in an aqueous phase have led to microparticles of 55.3 ± 34.7 μm. The incorporation of the drug by milligram of powder was very low (1.91 μg/mg) and the scanning electron microscopy (SEM) did not show any crystal but marks of dissolved crystals were observed on the polymeric surface. Eudragit®RS microspheres containing 3,4-DAP were prepared by a solvent-evaporation technique using light mineral oil as continuous phase. The drug and the polymer were completely dissolved in an acetone solution, used as discontinuous phase. This formulation have led to a higher incorporation of the drug (88.25 μg/mg). The particle size was 91.8 ± 44.3 μm. The observation, by SEM, shows many crystals on the surface and inside the microparticles. A slow-release of the drug in a phosphate buffer pH 7.4 was observed (50% in 60 min and about 70% in 4 h).

Keywords: 3,4-Diaminopyridine; Microparticles; Polymer; Sustained release; Poly(ε-caprolactone)

3,4-Diaminopyridine (3,4-DAP) is used for the treatment of neuromuscular diseases as Lambert-Eaton myasthenic syndrome (Sanders, 1998) and multiple sclerosis (Bever et al., 1990; Polman et al., 1994; Aisen et al., 1995, 1996; Bever et al., 1996; Sheean et al., 1998). This potassium channel blocker is able to restore a nerve conduction block by prolonging the duration of the action potential (Durant and Marshall, 1980; Thesleff, 1980). The highest plasma concentrations, observed from 20 to 60 min (Leslie and Bever, 1989) after oral administration, can be correlated to a gastrointestinal toxicity (Bever, 1994). A slow-release device with a daily administration could be convenient for these patients treated during months. This formulation would allow reaching the therapeutic concentrations while reducing the toxicity (Bever, 1994).

* Corresponding author. Tel.: +33-3-83-17-88-40; fax: +33-3-83-17-88-79

E-mail address: stephane.gibaud@pharma.u-nancy.fr (S. Gibaud).

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The solvent-evaporation method was used first and foremost for its simplicity. This method had been rather developed for lipophilic drugs. Actually, it can allow the encapsulation of hydrophilic drugs if the method of preparation is slightly adapted to reduce the diffusion of the drug in the continuous phase. Different methods can be used: for example, the saturation of this phase with the drug (Bodmeier and McGinity, 1987), the modification of the pH of the discontinuous phase (Bodmeier and McGinity, 1987) or the addition of a cosolvent in the discontinuous phase (Bodmeier et al., 1997). In the present study, we have used two modified methods. Poly(ε-caprolactone) (PCL)-based microparticles were prepared by a solvent-evaporation method using a dichloromethane suspension of 3,4-DAP as discontinuous phase. This formulation had been successfully developed to encapsulate high amounts of fludrocortisone (Gibaud et al., unpublished results). However, highly hydrophilic drugs had not been tested yet.

The second formulation, Eudragit®RS-based microparticles, was prepared by a modified emulsion–solvent-evaporation method in an oily phase previously describe to encapsulate 5-fluorouracil (Zinutti et al., 1994). The microparticles were characterized with respect to drug loading, drug release, and morphological properties.

PCL microparticles were prepared as follows: 150 mg of 3,4-DAP (Pharmacie Centrale des Hôpitaux, Paris) and 500 mg of PCL (Aldrich, Steinheim, Germany) were added in 10 ml of dichloromethane (Acros, NJ) under magnetic stirring. A suspension of 3,4-DAP was obtained. This organic phase was poured into 800 ml of water containing poly(vinyl alcohol) (0.1%) (Sigma, St. Louis, MO) under mechanical stirring (1500 rpm). The stirring was maintained for 2 h, leading to a total evaporation of the solvent and a solidification of the microparticles. The microparticles, called 3,4-DAP/PCL, were then recovered by filtration (HA filter, Millipore, 0.45 μm), washed three times with water and dried under vacuum during 24 h. Three independent batches were prepared. The particle size, measured by optical microscopy, was 55.3 ± 34.7 μm. The amount of 3,4-DAP entrapped in the microparticles was determined by reversed-phase ion-pair high performance liquid chromatography after dissolution of 10 mg of microparticles in dichloromethane and a liquid extraction in chlorohydric acid (0.05 N, 2 ml). One milligram per milliliter of 2-amino 4-picoline was added in the dichloromethane as internal standard. The column was a C18 Bondasorb; 5 μm, 4.6×25 cm^2 (Macherey-Nagel, Eckbolsheim, France). The mobile phase was a mixture of acetonitrile and phosphate buffer 50 mM with sodium octane sulfonate (3 g/l) (50/50; v/v) at a flow rate of 1 ml/min and the detection was performed by UV spectrometry at 286 nm.

The results (Table 1) showed that 3,4-DAP incorporated by milligram of powder was very low (1.91 μg/mg). This could be explained by the high solubility of this drug. The observation of 3,4-DAP/PCL by scanning electron microscopy (SEM) (Leica Cambridge Ltd, Cambridge, UK) at 20 kV (Fig. 1) did not show any crystal but marks of dissolved crystals were seen on their surface. The crystals of 3,4-DAP, which were added in the organic phase, were probably dissolved in the aqueous phase during the solidification of the polymer.

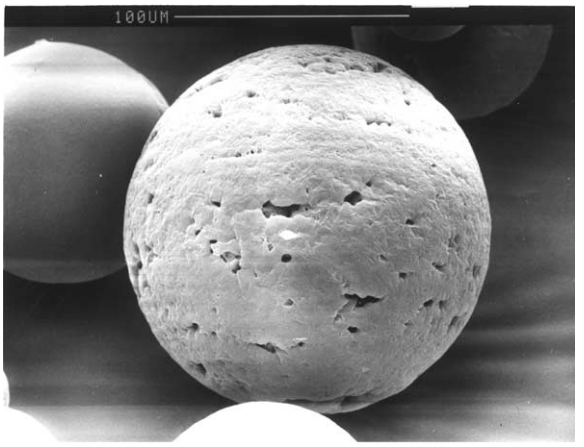
Eudragit®RS microparticles of 3,4–DAP were prepared as follows: 3,4-DAP (150 mg) and Eudragit®RS (500 mg) were completely dissolved in a mixture of ethanol (2.5 ml) and acetone (7.5 ml)

Table 1
Drug loading of 3,4-DAP microparticles

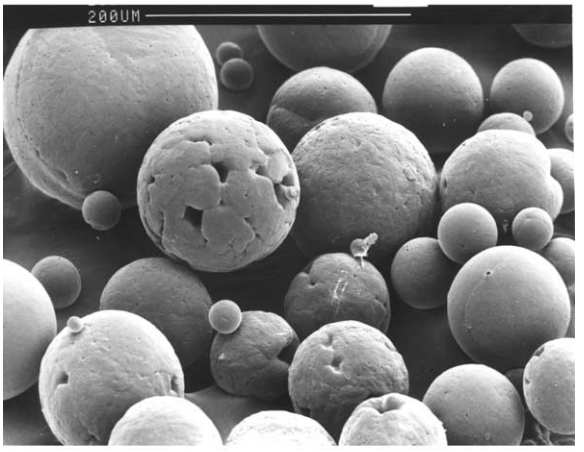
	3,4-DAP/PCL	3,4-DAP/Eudragit®RSPO
Q_{OP} (μg/mg)	230	230
Q_{PWD} (μg/mg)	1.91 ± 0.39	88.25 ± 2.23
% IC	0.83 ± 0.17%	38.37 ± 1.16%

Q_{OP}: amount of 3,4-DAP added in the dispersed phase, expressed in microgram per milligram of solid components (PCL + 3,4-DAP) dissolved in the organic phase. Q_{PWD}: amount of 3,4-diaminopyridine, expressed in milligram per milligram of the final powder and determined by HPLC. % IC: percentage of incorporation of 3,4-diaminopyridine, calculated as follows:

$$\%\ \mathrm{IC} = \left[\frac{Q_{PWD}}{Q_{OP}}\right] \times 100.$$



(a)



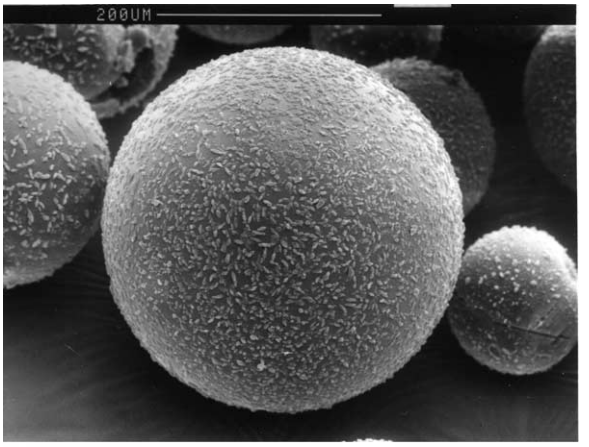
(b)

Fig. 1. Microparticles of 3,4-diaminopyridine 3,4-DAP/PCL observed by SEM. (a) Morphologic study of one microparticle. (b) Global aspect of the microparticles.

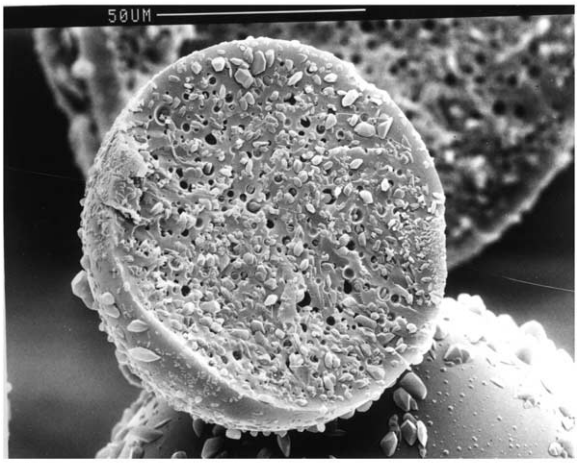
and the external phase was a mineral oil (200 ml) containing trioleate sorbitane (Montane® 85; 2.5% w/w).

The particles size was 91.8 ± 44.3 μm. This formulation leads to a higher incorporation of the drug (88.25 μg/mg). The observation of 3,4-DAP/Eudragit®RS by SEM (Fig. 2) show many crystals embedded in the polymeric structure. In order to determine the physical state of the microparticles and the drug, X-ray examinations (D500 Siemens diffractometer) were conducted (Fig. 3). It was obvious that the pure drug exhibited crystalline characteristics, while the polymer showed amorphous pattern. The peaks of the drug which were present in the case of 3,4-DAP/Eudragit®RS, confirmed the heterogeneous structure of the microparticles. Crystallization probably occurs during the solidification of the microparticles.

A slow-release of the drug in a phosphate buffer pH 7.4 (Fig. 4) was observed (50% in 60 min and about 70% in 4 h) whereas the pure 3,4-DAP dissolves immediately in an aqueous



(a)



(b)

Fig. 2. Microparticles of 3,4-diaminopyridine 3,4-DAP/Eudragit®RSPO observed by SEM. (a) Morphologic study of one microparticle. (b) Micoparticule cut. Crystals can be seen in the core of the microparticle.

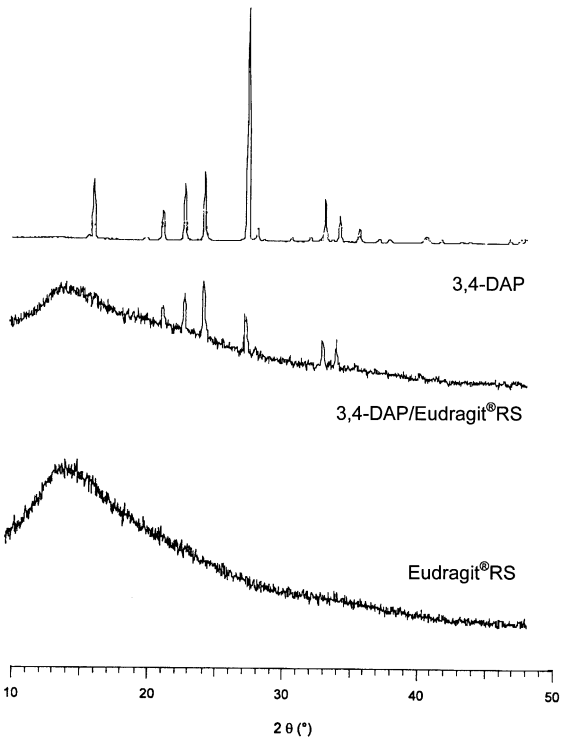


Fig. 3. X-ray diffraction spectra of 3,4-diaminopyridine (3,4-DAP), Eudragit®RS unloaded microparticles and 3,4-DAP-loaded microparticles (3,4-DAP/Eudragit®RS). Anticathode cobalt ($\lambda = 1.78897$ Å) 35 kV 20 mA.

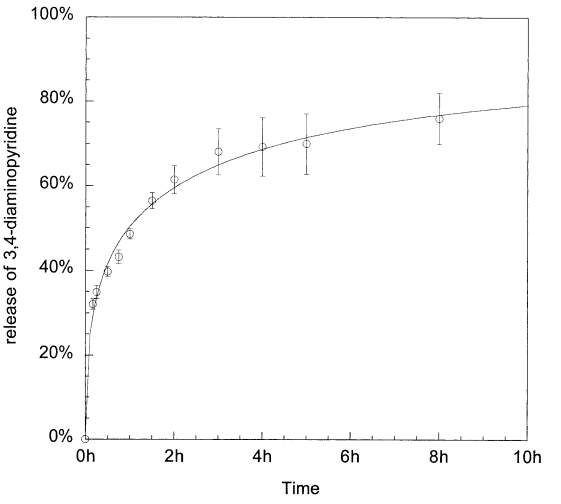


Fig. 4. Release of 3,4-DAP from 3,4-DAP/Eudragit®RS microparticles in phosphate buffer (0.1 M, pH 7.4, 37 °C).

phase. This was consistent with the localisation of crystals in the core of the microparticles. Eudragit®RS is not biodegradable and the solvent has to diffuse in polymer to dissolve the drug. Thereafter, the solution of 3,4-DAP can leave the core of the microparticles.

In conclusion, our results showed low levels of drug incorporation into PCL-based microparticles prepared by solvent-evaporation method. This poor incorporation was obviously unfavourable both for clinical purposes, requiring the administration of high amounts of microcarriers to obtain sufficient quantities of 3,4-DAP, and for manufacturing aspects, considering the loss of an expansive active substance. The use of an aqueous solution as continuous phase has not been chosen to encapsulate this highly hydrophilic drug, and the use of mineral oil has been a good alternative. Indeed, the evaporation of the acetone is not associated to a diffusion of the drug towards the oily continuous phase and the crystallisation of the drug probably occurs before the solidification of the polymer. The 3,4-DAP/Eudragit®RS microparticles obtained with an acceptable waste of the active substance, could necessitate less administered amounts of polymer to achieve the clinical goals without high peaks of concentration.

Finally, the low release rates of 3,4-DAP from 3,4-DAP/Eudragit®RS microparticles suggests that the modified solvent-evaporation method in an oily phase could be a useful way to prepare Eudragit®RS-based slow-release microcarriers for oral administration.

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