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Slow-release melarsoprol microparticles

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Abstract

The present study compares two methods of preparation of microparticles of melarsoprol for the treatment of the human trypanosomiasis. Melarsoprol is poorly soluble in water and in organic media. Microparticles were formulated with modified O/W and W/O/W methods, Poly(ε-caprolactone) microparticles were prepared either with a suspension-in-oil-in-water (S/O/W) solvent evaporation method or by complexation of melarsoprol with methyl ß-cyclodextrin followed by a water-in-oil-in-water (W_{CD}/O/W) solvent evaporation method. Results showed a poor incorporation of melarsoprol (2.89 ± 0.20 μg mg^{-1}) using the W_{CD}/O/W process, while the S/O/W process allowed achieving 161 ± 5 μg mg^{-1} and seemed to be very effective for the preparation of a sustained release form of melarsoprol. Moreover S/O/W microparticles showed a slow release of the drug in 70% of phosphate buffer pH 7.4, 0.1 M and 30% of propylene glycol (about 50% in 2 h and 80% after 7 h). © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Melarsoprol; Microparticles; Polymer; Sustained release; Poly(ε-caprolactone)

The human African trypanosomiasis (HAT) or sleeping sickness, transmitted by tsetse flies, is characterized by an irregular fever, lymph nodes, a confusion and sometimes a lethargic state (Boa et al., 1988). Until now, the only effective drug available for late-stage treatment of HAT was the trypanocide melarsoprol. This drug is very effective but relapses can occur in up to 20% of cases in the current epidemics (Barrett, 1999).

Melarsoprol, poorly soluble in water, has been dissolved in propylene glycol to develop the ARSOBAL® commercial solution. This non-aqueous solution, poorly tolerated, must be always administered by slow intravenous injection, with a fine needle and extravasations must be avoided. Several therapeutic plans can be used but none of them seems to present decisive advantages on the others. They include either three series of three or four daily injections, with a 7-day interval between series (Pepin et al., 1995; Burri et al., 2000). Patients must be hospitalized and strictly followed up during the treatment to monitor the compliance and prevent an eventual acute reactive arsenical encephalopathy. In this setting, the development of a microparticulate suspension of melarsoprol could have numerous advantages. This formulation would avoid the injection of

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propylene glycol. Moreover, it could allow achieving a sustained release device, reduce the frequency of the intakes and improve the compliance.

The use of microparticulate carriers to obtain a sustained release form suitable for intramuscular administration is well known. Obviously, biodegradable polymers are requested. Solvent-evaporation processes are convenient to encapsulate drugs, but the major problem of some pharmaceutical compounds is their poor solubility in water and simultaneously in organic media. One approach for the formulation of poorly soluble drugs is the development of drug nanosuspensions (Grau et al., 2000). The formulation of nanoparticles or microparticles, as sustained release formulation could be an attractive alternative but the drug needs to be at least soluble in one solvent. Many hydrophobic drugs, dissolved in dichloromethane or chloroform can be incorporated in most of polymers by O/W solvent evaporation processes. These methods have been adapted to increase the incorporation of hydrophilic drugs: for example, by saturation of this phase with the drug (Bodmeier and McGinity, 1987), by modification of the pH of the discontinuous phase (Bodmeier and McGinity, 1987) or by addition of a cosolvent in the discontinuous phase (Bodmeier and McGinity, 1987). A double emulsion W/O/W method is often used to encapsulate hydrophilic drugs as proteins.

Nevertheless, melarsoprol is poorly soluble in water and simultaneously in organic media. Actually, Cristau et al. have assessed the [octanol/buffer (pH 7)] partition coefficient of melarsoprol which is 40 and corresponds to a high lipophilicity (Cristau et al., 1972; Keiser and Burri, 2000). Solvents usually chosen for O/W emulsion-evaporation method as chloroform or dichloromethane have given very low solubilities and the first attempts have led to very low incorporations.

In the present study, we have developed two modified methods called W_{CD}/O/W and S/O/W to incorporate poorly soluble drugs as melarsoprol. ß-Cyclodextrins and their hydrophilic derivates have been described in the literature as solubilizers capable of enhancing the loading capacity of liposomes (McCormack and Gregoriadis, 1994, 1996), nanoparticles (Duchene et al., 1999) and microcapsules (Loftsson et al., 1992). In our case the W_{CD}/O/W is a complexation of melarsoprol with a methyl ß-cyclodextrin followed by a double emulsion-evaporation method. S/O/W (solid-in-oil-in-water) methods have been used for protein drugs, because solid-state proteins retain their activity in organic conditions (Putney and Burke, 1998; Morita et al., 2000).

The W_{CD}/O/W microparticles were prepared as follows: melarsoprol (Aventis, France) was dissolved by complexation in methyl ß-cyclodextrin: 50 mg of melarsoprol was added to 10 ml of water and 500 mg of methyl ß-cyclodextrin. The complete dissolution of melarsoprol had been previously assessed showing a binding constant (K11) of 9413 mol^{-1}.

About 1 ml of the aqueous solution of the complex was added in 10 ml of a dichloromethane solution of PCL (Steinheim, Germany) (250 mg) under mechanical stirring (Ultraturax, 13500 rpm, 3 min). This first emulsion, called W_{CD}/O was poured into 800 ml of water containing 0.1% PVA under mechanical stirring (1500 rpm). The stirring was maintained for 2 h, leading to a total evaporation of the solvent. The microparticles were then recovered by filtration (HA filter, Millipore, 0.45 μm), washed three times with water and dried under vacuum during 24 h.

The S/O/W microparticles were prepared as follows: melarsoprol (50 mg) was added to a dichloromethane solution (10 ml) of PCL (250 mg). The poor solubility of this drug led to a suspension (S/O). A solution of polyvinyl alcohol 0.1% (800 ml) was prepared as previously described and the organic suspension was poured under mechanical stirring (1500 rpm) in the aqueous phase leading to the S/O/W emulsion. The stirring was maintained 2 h until a total evaporation of the solvent and the hardening of the microparticles. The microparticles were then recovered by filtration (HA filter, Millipore, 0.45 μm), washed three times with water and dried under vacuum during 24 h.

The microparticle size distribution was estimated by optical microscopy. About 5 mg of particles were vortexed in 1 ml of 0.1% PVA. A drop of the suspension was then poured onto a

Table 1
Incorporation of melarsoprol in the microparticles

	Melarsoprol microparticles prepared by $W_{CD}/O/W$ emulsion	Melarsoprol microparticles prepared by S/O/W emulsion
Amounts of melarsoprol (μg) incorporated per milligram of powder	161 ± 5 μm mg^{-1}	2.89 ± 0.20 μm mg^{-1}
Yield of incorporation (%)	$82.2 \pm 3.3\%$	$6.37 \pm 0.39\%$

Comparative table between the S/O/W process and the $W_{CD}/O/W$ emulsion process (mean of three experiments).

glass slide and observed at $\times 100$ magnification using a microscope equipped with a digital-camera and an image analysis software (Kappa Image base, Kappa Opto-Electronics, Gleichen, Germany). Microparticles obtained with $W_{CD}/O/W$ emulsion had sizes of 31.1 ± 16.9 μm and microparticles obtained with S/O/W had sizes of 34.0 ± 16.7 μm.

The amount of melarsoprol entrapped in the microparticles was assessed by reversed-phase high-performance liquid chromatography (HPLC). Accurately weighed samples of microparticles were dissolved in 100 ml of acetonitrile. Twenty μl were then injected onto a C_{18} column (5 μm, 4.6×25 cm, Macherey-Nagel, Eckbolsheim, France) using an autosampler (WISP 712, Waters). The mobile phase was a mixture of acetonitrile, water and acetic acid (23:72.4:4.6; V/V) at a flow rate of 1.5 ml min^{-1} (SP8800 pump, Spectra Physics, TSP, CA). Detection was performed by UV spectrometry at 286 nm (Waters 490E detector) using a SP-800 integrator (Spectra Physics). Results (Table 1) showed a poor incorporation of melarsoprol (2.89 ± 0.20 μg mg^{-1}) using the $W_{CD}/O/W$ process, while the S/O/W process allowed achieving 161 ± 5 μg mg^{-1} and seemed to be very effective for the preparation of a sustained release device.

As different phases were involved in the processes, the final aspect of microparticles was observed by scanning electron microscopy (SEM). The microparticles were fixed with carbon-glue and coated with gold–palladium under argon atmosphere. Samples were then observed with a Cambridge model S scanning electron microscope (Leica Cambridge Ltd., Cambridge, UK) at 20 kV. Microparticles obtained with $W_{CD}/O/W$ emulsion had a smooth aspect, but some of them partially collapsed, bringing evidence of the presence of cavities (Fig. 1a and b). Microparticles obtained with S/O/W emulsion had a rougher aspect (Fig. 2a and b) and crystals were observed on the polymeric surface (Fig. 2c).

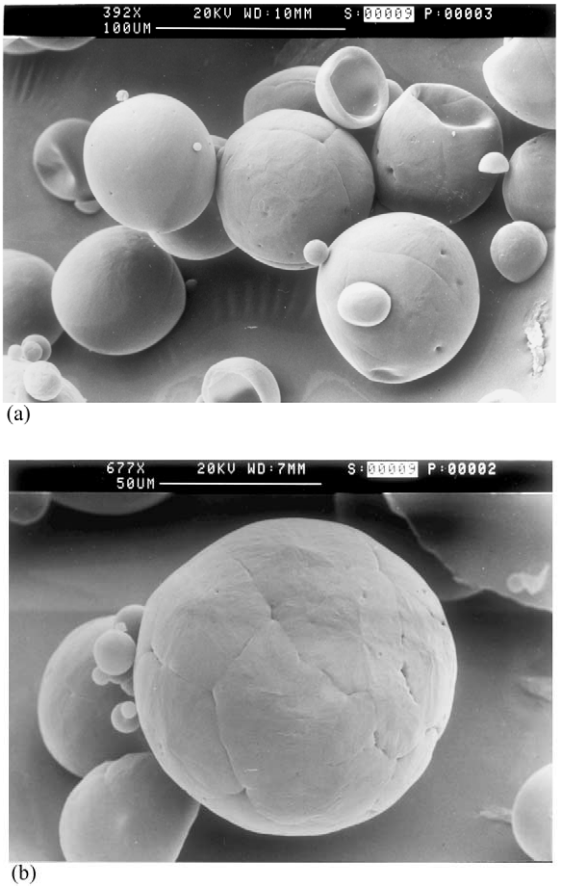

Fig. 1. Melarsoprol microparticles prepared by double emulsion $W_{CD}/O/W$. (a) global view, (b) details of one particle.

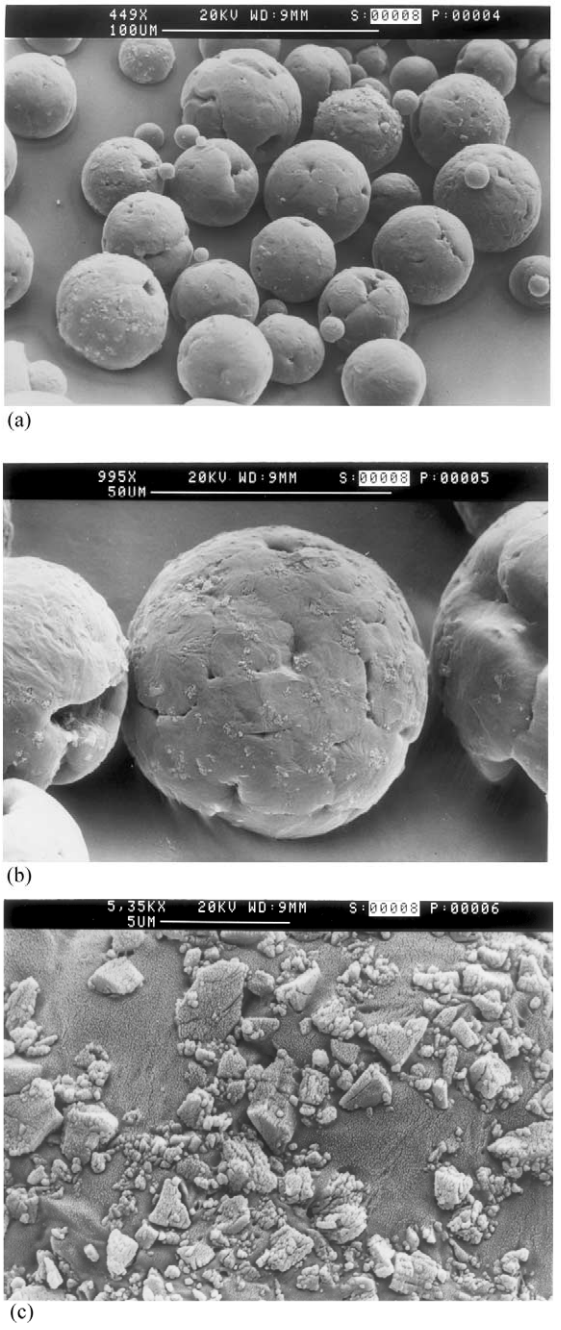

Fig. 2. Melarsoprol microparticles prepared by a S/O/W emulsion, (a) global view, (b) details of one microparticle, (c) details of crystals embedded in the polymeric surface of a microparticle.

X-ray examinations (D500 Siemens diffractometer) were conducted (Fig. 3). Pure melarsoprol exhibited several diffraction peaks which were typical of a crystalline pattern, while the semi-crystalline PCL showed only two peaks. Diffraction spectra of S/O/W microparticles showed peaks of melarsoprol confirming the hetreogeneous structure of these microparticles.

Finally, the release of melarsoprol from microparticles in a phosphate buffer 0.1 M; pH 7 mixed with propylene glycol (30%) were represented (Figs. 4 and 5). The release of the melarsoprol from W_{CD}/O/W microparticles is very rapid

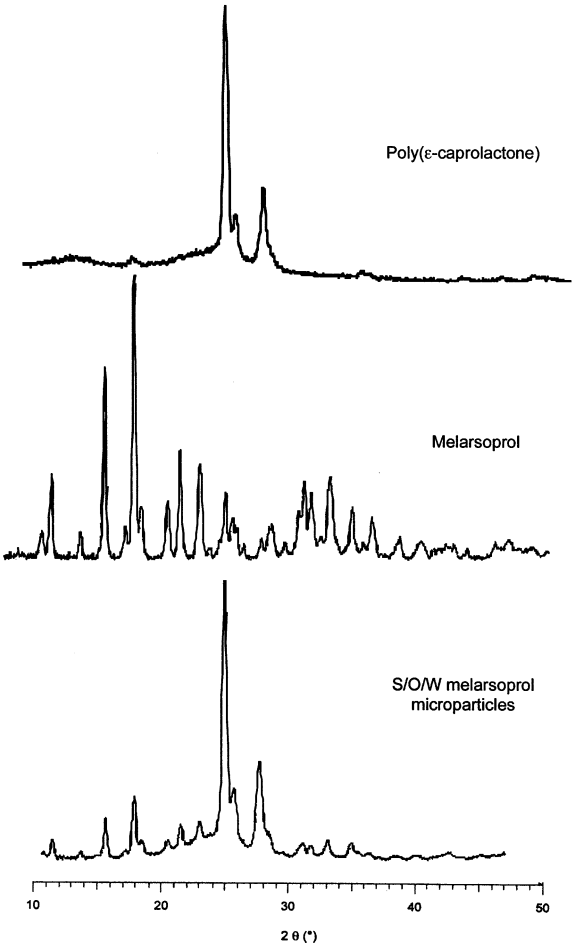

Fig. 3. X-ray diffraction spectra of poly(ε-caprolactone), melarsoprol, and melarsoprol microparticles prepared using the S/O/W method. Anticathode cobalt ($\lambda = 1.78897$ Å) 35 kV 20 mA.

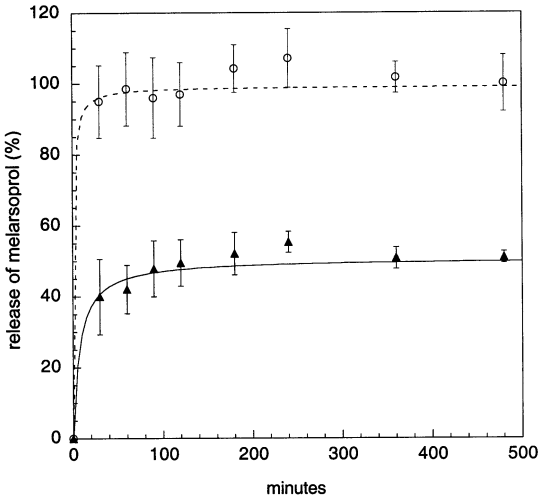

Fig. 4. Release of melarsoprol from microparticles prepared by the $W_{CD}/O/W$ process (–▲–) in a phosphate buffer (pH 7.4; 0.1 M) with 30% of propylene glycol. The release of melarsoprol from the complex melarsoprol/methyl ß-cyclodextrin (---○---) was represented as comparison.

and remains incomplete in our operating conditions. This 'burst effect', is probably due to the break of the aqueous cavities embedded in the polymeric structure. The proportion [melarsoprol]/[polymer] are very low and the adsorption of the drug to the fragments of polymer could explain a partial release.

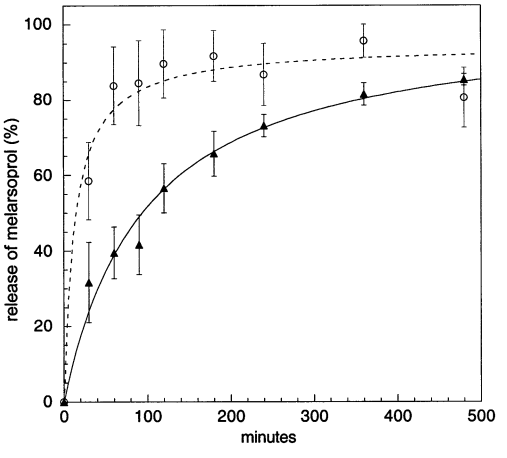

Fig. 5. Release of melarsoprol from microparticles prepared by the S/O/W process (–▲–) in a phosphate buffer (pH 7.4; 0.1 M) with 30% of propylene glycol. The release of melarsoprol from crystals of melarsoprol (---○---) was represented as comparison.

The release from the S/O/W microparticles was relatively prolonged (about 50% in 2 h) and reached 80% after 7 h. In this case, the proportion [melarsoprol]/[polymer] was very high and the adsorption of melarsoprol has become minor. Taking account of the propylene glycol in the media, S/O/W microparticles can be considered as slow-release device.

However, this formulation should be assessed in vivo to confirm the release profile.

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