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Note

Preparation and in vitro evaluation of pyridostigmine bromide microparticles

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Abstract

Pyridostigmine bromide (PB) is an anticholinesterase agent whose aqueous solubility is high and which has a short elimination half-life. Its dosage rate in the treatment of myastenia gravis is frequent due to the short half-life and it exhibits side effects. Microparticles designed to deliver a pharmaceutical active ingredient efficiently at the minimum dose and also to enhance stability, reduce side effects and modify drug release were prepared in this study using an acrylic polymer (Eudragit) as the vehicle by the spray-drying technique. The drug was either dissolved or dispersed in the polymeric solution and following the preparation of microparticles using different ratios of ingredients, characterization studies including the determination of shape, particle size distribution, amount loaded, release and stability of PB were performed. The results obtained were compared to those of pure PB. Drug release from microparticles could be modified and was found to depend on the shapes of the microparticles. In vitro evaluation results indicate that the frequent dosage and side effects of pure PB may be reduced with the formulation of microparticles. © 2002 Published by Elsevier Science B.V.

Keywords: Pyridostigmine bromide; Microparticles; Spray-drying; Release

Pyridostigmine bromide (PB) is a carbamate type of reversible acetylcholinesterase inhibitor (Lintern et al., 1997) which is used to treat *myasthenia gravis* (Breyer-Pfaff et al., 1990) and to protect against exposure to nerve agents (Kluwe et al., 1990). Because of its quaternary amine structure, it is relatively poorly absorbed from the gastrointestinal tract (Kluwe et al., 1990). The elimination half-life of PB after a 60 mg single dose administration in healthy volunteers was found to be 200 min. This leads to the need for frequent administration. Ninety nine percent of acetylcholinesterase inhibitors, among which PB is included, are given orally (Sghirlanzoni et al., 1992).

The objective of this study was to prepare microparticles of PB using an acrylic polymer solution or dispersion, Eudragit RS, by the spray-drying technique and to characterize the microparticles prepared, and to evaluate the modification of the release rate.

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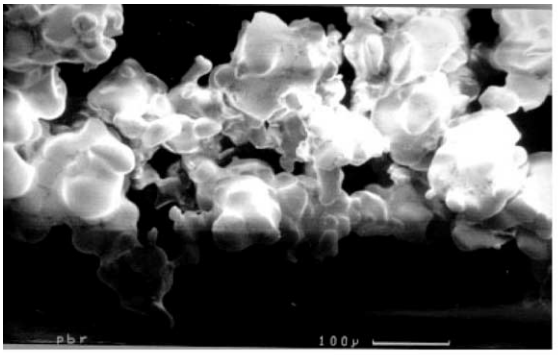
Pure PB was characterized by spectrophotometric (UV and IR) analyses (Shimadzu 160 A and Shimadzu IR 435), melting point determination (Electrothermal 1A90009), thermal analyses (Mettler Toledo DSC 821), X-ray diffraction analysis (Rigaku), UV-spectrophotometric quantification and the validation (applicability, linearity, repeatability and accuracy), particle size distribution (Malvern Mastersizer 2000) and microphotographing (Olympus BX50 and Cam-Scan S4) prior to formulation studies. UV-spectra of PB in distilled water, pH 1.2 and 7.4 were very similar to each other. All had λ_{max} at 270 nm. This is expected since the solubility of PB is independent of pH. IR spectrum of PB showed characteristic peaks due to its chemical structure. Melting point of PB determined by an apparatus and differential scanning calorimeter was 154.2 ($n = 3$). Intense peaks at 2ϕ were determined upon X-ray analysis of PB. Analysis of particle size distribution of PB demonstrated that 100% of the particles were below 2190 μm and the average particle size was 84 μm. A microphotograph of PB powder taken with scanning electron microscope is shown in Fig. 1.

Two methods were used for the preparation of microparticles: solubilization or dispersion of the active agent. Drug:polymer ratios of 1:1, 1:2, 1:3 and 1:4 were used to evaluate the incorporation and the release rates. Spray-drying technique was selected among the many preparation methods for microparticles, taking into account the high melting point of PB and solubilities of the active ingredient and the polymer (Pignatello et al., 1997). Preparation parameters were kept constant to follow the effect of active ingredient and polymer concentration on the preparation yield, loading capacity, particle size and in vitro release rate. Conte et al. (Conte et al., 1994) have reported that the preparation parameters affect the shapes, volume and in vitro release from the particles obtained.

Preparation yields of microparticles were calculated based on the amounts of materials used. The yield of microparticles obtained by dispersion in the solvent (53.8%) was found to be lower than the yield of microparticles obtained by solubilization in the solvent (66.0%) (Table 1). Preparation yields calculated in this study were higher than those of 40% as reported in previous similar studies (Bittner et al., 1998). Loss during the preparation process may be due to the design of the



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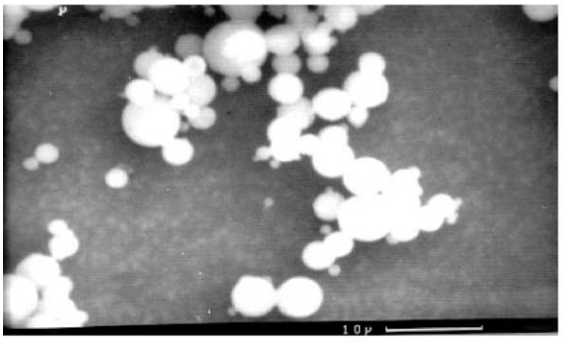

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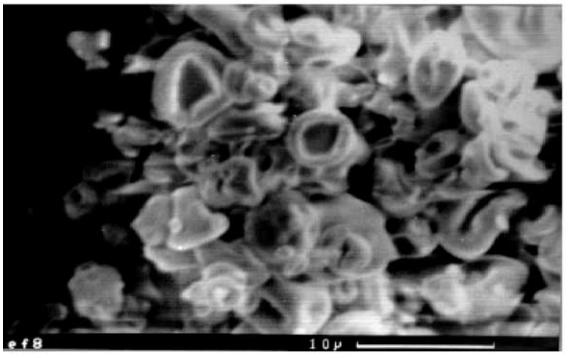

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Fig. 1. Microphotographs taken with scanning electron microscope of (A) PB Powder; (B) microparticles prepared by solvation of active agent; (C) microparticles prepared by dispersion of active agent.

Table 1
Percentage of preparation yield, loading capacity, entrapment capacity and standard errors of PB microparticles

Formulation	Preparation yield (%)	Loading capacity (% ± SE)	Entrapment capacity (% ± SE)
EF8	53.8	35.9 ± 2.2	31.3 ± 0.5
DF7	66.0	100.2 ± 1.3	19.4 ± 0.3

EF8, microparticles prepared by dispersion of active agent; DF7, microparticles prepared by solvation of active agent. $n = 5$.

spray-drying apparatus. Embryonic microparticles which can not be dried completely may lead to the formation of film on the surface of the drying component, thus reducing the preparation yield. The amount of drug loaded was calculated using the experimental results for PB on the surface and entrapped. Loading capacity was determined to be related to microparticle size (Sánchez et al., 1993) and dependent on active material-polymer ratio (Giunchedi and Conte, 1995). Amounts of PB loaded and entrapped into microparticles (Table 1) in our study showed correlation with the variable parameters.

IR-spectrophotometric, thermal and X-ray analyses of all microparticles were performed. Characteristic peaks of PB were observed in the IR spectra of the microparticles obtained. At the 500–1100 cm^{-1} region, peaks owing to PB structure were broadened and reduced in number. Spectra obtained showed that there was no chemical interaction or changes during microparticle preparation. In all thermal analyses studies, no interactions or incompatibilities were determined. No PB peaks were seen in the thermograms since the melting point of PB is higher than the polymer.

Peaks were sharp as the peaks of pure PB, with the microparticles prepared by dispersion of the active material, upon examination of X-ray analysis. However, no peaks of PB could be seen with the microparticles prepared by solvation of the active material. This shows that the active agent is in the amorphous state in the microparticles prepared by solvation of the active material while it is crystalline in microparticles prepared by dispersion of the active material (Guo and Bodmeier, 1997).

Microscopic examination of microparticles (Fig. 1) showed that the particle shapes were different and the high aggregation was relatively reduced in comparison to pure PB. Microparticles obtained by dispersion of the active agent were microcapsules with irregular and disorganized shapes while those obtained by solvation of the active ingredient showed matrix structure with spherical and regular shapes.

Particle size distribution analyses of microparticles were determined by a laser diffraction apparatus. Mean particle sizes of the microparticles obtained by both methods showed values between 3.7 and 10.3 μm, as found in previous studies (Bittner et al., 1998; Wan et al., 1992). The range of the particle sizes of microparticles prepared by dispersion of PB was 3.7–6.9 μm and was 5.9–10.3 μm for the microparticles prepared by solvation of the active ingredient.

Drug release from the microparticles was determined by an incubation method (Conti et al., 1995). High initial release was observed in all formulations: 65% for the microparticles obtained by dispersion of the active agent and 70% for the microparticles obtained by solvation of the active agent (Fig. 2). This may be due to two reasons:

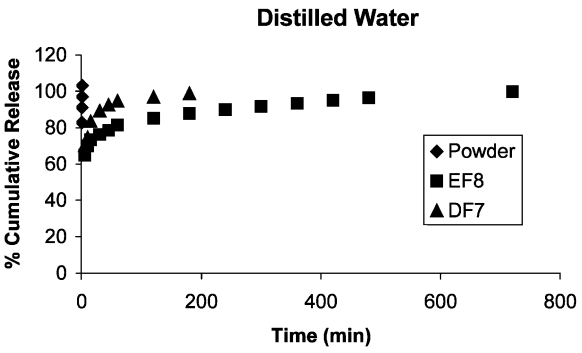

Fig. 2. Percentage cumulative release profiles of PB (Powder: Pure PB; EF8: microparticles prepared by dispersion of active agent; DF7: microparticles prepared by solvation of active agent).

the first is the active ingredient near or on the surface of the microparticles; the second is that no complete encapsulation may have occurred (Conte et al., 1994). Dissolution rate studies of microparticles obtained by both methods demonstrated the effects of mean particle size and entrapment capacity.

When the release profiles of the microparticles obtained by the two methods were compared, percentage of release from the microparticles prepared by dispersion of the active agent was found to be lower than the microparticles prepared by solvation of the active agent. It was observed that the cumulative percentage of release from the microparticles prepared by solvation of PB reached 100% at the 3rd h, while it reached 100% at the 12th h from the microparticles prepared by dispersion of the active agent. This value was 45 min for pure PB.

As a conclusion, release rate of PB could be reduced by either dispersion or solvation of the active agent during microparticle preparation. This reduction in the release rate indicates that sustained release can be achieved by modifying preparation parameters in further studies.

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