Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 268 (2003) 47–57
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Encapsulation and release of the hypnotic agent zolpidem from biodegradable polymer microparticles containing hydroxypropyl-β-cyclodextrin

Giuseppe Trapani [a,*], Angela Lopedota [a], Giancarlo Boghetich [b], Andrea Latrofa [a], Massimo Franco [a], Enrico Sanna [c], Gaetano Liso [a]

[a] *Dipartimento Farmaco-Chimico, Facoltà di Farmacia, Università degli Studi di Bari, Via Orabona 4, 70125 Bari, Italy*
[b] *Dipartimento di Ingegneria Civile ed Ambientale-Sezione di Chimica Applicata-I, Facoltà di Ingegneria, Via Orabona 4, 70125 Bari, Italy*
[c] *Dipartimento di Biologia Sperimentale, Cattedra di Farmacologia, Università degli Studi Cagliari, 09123 Cagliari, Italy*

Received 8 May 2003; received in revised form 15 July 2003; accepted 30 August 2003

Abstract

The goal of this study was to design a prolonged release system of the hypnotic agent zolpidem (ZP) useful for the treatment of insomnia. In this work, ZP alone or in the presence of HP-β-CD was encapsulated in microparticles constituted by poly(DL-lactide) (PDLLA) and poly(DL-lactide-co-glycolide) (PLGA) and the drug release from these systems was evaluated. ZP alone-loaded microparticles were prepared by the classical O/W emulsion–solvent evaporation method. Conversely, ZP/HP-β-CD containing microparticles were prepared by the W/O/W emulsion–solvent evaporation method following two different procedures (i.e. A and B). Following procedure A, the previously produced ZP/HP-β-CD solid complex was added to the water phase of primary emulsion. In the procedure B, HP-β-CD was added to the aqueous phase and ZP to the organic phase. The resulting microparticles were characterized about morphology, size, encapsulation efficiency and release rates. FT-IR, X-ray, and DSC results suggest the drug is in an essentially amorphous state within the microparticles. The release profiles of ZP from microparticles were in general biphasic, being characterized by an initial burst effect and a subsequent slow ZP release. It resulted that co-encapsulating ZP with or without HP-β-CD in PDLLA and PLGA the drug release from the corresponding microparticles was protracted. Moreover, in a preliminary pharmacological screening, the ataxic activity in rats was investigated and it was found that intragastric administration of the ZP/HP-β-CD/PLGA microparticles prepared according to procedure B produced the same ataxic induction time as the one induced by the currently used formulation Stilnox®. Interestingly moreover, there was a longer ataxic lasting and a lower intensity of ataxia produced by the ZP/HP-β-CD/PLGA-B-formulation already after 60 min following the administration. However, a need for further pharmacokinetic and pharmacodynamic studies resulted to fully evaluate the utility of this last formulation for the sustained delivery of ZP.

Keywords: Zolpidem; Encapsulation; Microparticles; Biodegradable polymers

1. Introduction

Insomnia is one of the most common disorders in medical practice and, for its treatment, there are, nowadays, a lot of pharmacological agents (Charney et al.,

* Corresponding author. Tel.: +39-080-5442-764; fax: +39-080-5442-754.
E-mail address: trapani@farmchim.uniba.it (G. Trapani).

0378-5173/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2003.08.016

2001). In this regard, some benzodiazepines are the drugs of choice because they possess negligible side effects and toxicity. However, the long-term treatment of insomnia with benzodiazepines is problematic since it results in the development of tolerance and dependence (Doble, 1999). Further improvement in the management of insomnia and other sleep disorders resulted after the recent introduction of non-benzodiazepine hypnotics such as zolpidem, zoplicone, and zaleplon (Charney et al., 2001). In particular, zolpidem, *N*,*N*-dimethyl-[2-(4-tolyl)-6-methylimidazo[2,1-*a*]pyridin-3-yl]acetamide (ZP), exhibits strong hypnotic and sedative actions with negligible anxiolytic, muscle relaxant, or anticonvulsant properties, is widely prescribed for the treatment of the insomnia and sleep disorders (Martindale, 1999). In the management of short-term insomnia, ZP has a rapid onset of action but a short elimination half-life (about 2.5 h) (Martindale, 1999). In this context, researchers have demonstrated that withdrawal of but not long-term exposure to ZP resulted in change of $GABA_A$ gene expressions (Follesa et al., 2002). Therefore, to elucidate the potential of ZP in the treatment of different insomnia categories, it could be useful to develop formulations enabling sustained-release of this drug.

In the past two decades, cyclodextrins (CDs) have been widely used to modify drug solubility or improve drug stability, bioavailability or toxicity profiles. Recently, considerable interest have been focused on the incorporation of these pharmaceutical excipients into polymeric drug delivery systems such as microspheres, nanospheres, and polymeric films (Bibby et al., 2000). Thus, the preparation and characterization of β-cyclodextrin/poly(acrilic acid) (Bibby et al., 1999) and β-cyclodextrin/chitosan microspheres (Filipovic-Grcic et al., 1996, 2000) have been reported. Similarly, the use of poly(isobutylcyanoacrylate) nanoparticles containing CDs has been described (Duchene et al., 1999). It is now clear that drug–CD mixtures or their complexes when incorporated into polymeric matrices can improve hydration of the polymer matrix, promote its erosion (Song et al., 1997) and modify drug solubility and diffusivity, leading to a modified drug release (Bibby et al., 2000) from the polymeric system.

In a previous paper, we investigated the effect of complexation with some chemically modified CDs on ZP aqueous solubility and dissolution rate (Trapani et al., 2000). The pharmacological study demonstrated that ZP complexation with hydroxypropyl-β-cyclodextrin (HP-β-CD) or methyl-β-cyclodextrin results in longer ataxic induction times than those of both drug alone and the currently used formulation Stilnox®, but the duration of ataxia remains approximately unmodified.

The aim of the present work was to evaluate the potential of utilizing HP-β-CD to control drug release from zolpidem-loaded-poly(DL-lactide) (PDLLA) or -poly(DL-lactide-co-glycolide) (PLGA) microparticles. PDLLA and PLGA have been widely used for producing sustained-release systems, microspheres, and microcapsules (Yamakawa et al., 1999; Cohen et al., 1991; Jalil and Nixon, 1990). Incorporation of physical mixtures or drug–cyclodextrin complexes into such polymeric matrices for modifying drug release has been previously investigated (Sinisterra et al., 1999). In this study, PDLLA- and PLGA-based microparticles were prepared encapsulating ZP with or without HP-β-CD. The effect of cyclodextrin on microparticle properties such as size, morphology, loading, and drug release were studied. Finally, in a preliminary pharmacological study, the ataxic activity in rats of these new ZP-containing formulations was also evaluated and compared with that of Stilnox®.

2. Materials and methods

2.1. Materials

ZP was extracted from tablets of Stilnox® purchased from a local drugstore as follows. Thirty Stilnox® tablets were powdered in a mortar and the powder was dissolved in 10% aqueous $NaHCO_3$ (50 ml). The solution was transferred to a shake flask and extracted with ethyl ether (3×30 ml). The organic layer was dried (Na_2SO_4) and evaporated. The solid residue was the pure ZP identified by spectroscopic methods (IR,^{1}H NMR, and mass spectroscopy) and comparison with reported data (Trapani et al., 2000). Reagents used for preparations of buffers were of analytical grade. 2-Hydroxypropyl-β-cyclodextrin with a degree of substitution, 5.88 (calculated by means of ^{1}H NMR; Pitha et al., 1986) was obtained as a gift from Roquette (Italy). Poly(DL-lactide) (PDLLA) (Resomer® R 202 H, MW 15,000 Da, inherent

viscosity 0.2 dl/g) and poly(DL-lactide-co-glycolide) (50:50) (PLGA) (Resomer® RG 503 H MW 34,000 Da, inherent viscosity 0.32–0.44 dl/g) were purchased from Boehringer Ingelheim (Germany). Polyvinylalcohol (PVA) (polymerization degree 500, viscosity of a 4% aqueous solution at 20 °C: 5 mPa s) was supplied from Fluka. Analytical grade methylene chloride and methanol were obtained from Baker (Milan, Italy).

2.2. Preparation of microparticles

ZP-loaded PDLLA microparticles (referred to as ZP/PDLLA microparticles) were prepared by the O/W emulsion–solvent evaporation method (Huang et al., 1997).

PDLLA (150 mg) and ZP (15 mg) were solubilized in 6 ml of CH_2Cl_2 at room temperature. The solution was rapidly poured into 30 ml of an aqueous PVA solution (0.4%, w/v) and then the mixture was emulsified at room temperature using an Ultraturrax homogenizer (Janke and Kunkel, Germany) model T25 equipped with an S25N dispersing tool, at 13,500 rpm for 10 s. The resulting emulsion was stirred at room temperature using a propeller type stirrer at 900 rpm for 4 h to allow the evaporation of the organic solvent. The microparticles were harvested by centrifugation, washed three times with deionized water, freeze–dried for 13 h (0.0101 bars, −50 °C, Lio5Pascal model freeze-dryer) and then stored under vacuum until their further use.

PDLLA microparticles containing ZP and HP-β-CD were prepared according to the W/O/W emulsion–solvent evaporation method (Huang et al., 1997) and following the procedures A and B reported in the following. At first, however, the solid ZP/HP-β-CD-complex was prepared according to the reported method (Trapani et al., 2000). Briefly, ZP (0.66 mmol) and the equimolar quantity of HP-β-CD in 5 ml of deionized water were equilibrated under stirring at room temperature for 4 days, filtered through a 0.22 μm membrane filter, and the clear filtrate subjected to freeze-drying. The composition of the ZP/HP-β-CD-complex (i.e. the incorporation degree) was found to correspond to 24 mg of ZP per gram of solid complex. This last value was determined by dissolving 100 mg of the complex in 5 ml of deionized water and the resulting ZP content determined by HPLC.

2.2.1. Procedure A

ZP/HP-β-CD solid complex previously prepared (620 mg) was dissolved in 2 ml of water and the resulting solution poured into 6 ml of a CH_2Cl_2 solution containing PDLLA (150 mg) at room temperature and then emulsified by using an Ultraturrax homogenizer (Janke and Kunkel, Germany) model T25 equipped with an S25N dispersing tool, at 13,500 rpm for 10 s. The resulting primary emulsion was rapidly poured into 30 ml of an aqueous PVA solution (0.4%, w/v) and then, working up as above, ZP/HP-β-CD/PDLLA-A microparticles were obtained.

2.2.2. Procedure B

HP-β-CD (74 mg, 0.049 mmol) was solubilized in 2 ml of water. Then, the resulting solution was poured into 6 ml of a CH_2Cl_2 solution containing PDLLA (150 mg) and ZP (15 mg, 0.049 mmol) at room temperature and emulsified using an Ultraturrax homogenizer (Janke and Kunkel, Germany) model T25 equipped with an S25N dispersing tool, at 13,500 rpm for 10 s. The resulting primary emulsion was rapidly poured into 30 ml of an aqueous PVA solution (0.4%, w/v) and then, working up as above, ZP/HP-β-CD/PDLLA-B microparticles were obtained.

Similarly, following the procedures above described and employing PLGA instead of PDLLA the corresponding ZP/PLGA, ZP/HP-β-CD/PLGA-A, and ZP/HP-β-CD/PLGA-B microparticles were prepared.

2.3. Quantitative analysis of zolpidem

High-performance liquid chromatography (HPLC) analyses were performed with a Waters Associates Model 600 pump equipped with a UV-Vis detector (MicroUVis, Fisons Instruments) set at the wavelength of 245 nm and a 20 μl loop injection valve (Rheodyne, 7725(i) model). For analysis, a reversed phase Symmetry C_{18} (15 cm × 4.6 mm; 5 μm particles; Waters) column in conjunction with a precolumn insert was eluted with mixtures of methanol and deionized water (75:25). The flow rate of 0.8 ml/min was maintained. All analyses were performed under isocratic conditions. Quantification of the compounds was carried out by measuring the peak areas in relation to those of standards chromatographed under the same conditions. Standard calibration curves were prepared at a wavelength of 245 nm using methanol as the sol-

vent and were linear ($r^2 > 0.999$) over the range of concentrations of interest.

ZP content was determined by dissolving completely known amounts (5–10 mg) of microparticles into 10 ml of CH_2Cl_2. The mixture was agitated vigorously on a vortex for 1 min, filtered through 0.22 μm cellulose filter and analyzed by HPLC. The results are expressed as percentage of ZP encapsulation efficiency defined as 100 × (recovered mmol ZP per gram microparticles/loaded mmol ZP per gram polymer) (Schlicher et al., 1997).

2.4. Size and morphology of microparticles

The surface morphology and shape of the microparticles were examined by scanning electron microscopy (SEM) (Philips XL 20) on the samples gold-sputtered for 120 s at 14 mA, under argon atmosphere. The size of microparticles was determined by direct observation, using a light stereomicroscope (Leica Galen III) equipped with a Panasonic (WV CP 230) camera and an image analysis program (Leica Qwin v. 2.4 software). The arithmetic mean diameter of microparticles was determined by averaging the individual values of over 200 microparticles.

2.5. Solid state characterization

2.5.1. Fourier transform infrared spectroscopy

Fourier transform IR spectra were obtained on a Perkin-Elmer 1600 FT-IR spectrometer. Samples were prepared in KBr disks (2 mg sample in 200 mg KBr). The scanning range was 450–4000 cm^{-1} and the resolution was 1 cm^{-1}.

2.5.2. X-ray analysis

Powder X-ray powder diffraction (XRPD) patterns were recorded on a Philips PW 1830 powder X-ray diffractometer using Cu Kα radiation, a voltage of 30 kV and a current of 55 mA.

2.5.3. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) curves were obtained by a Mettler Toledo DSC 822e Star^e 202 System (Mettler Toledo, Switzerland) equipped with a thermal analysis automatic program.

Aliquots of about 5 mg of each sample were placed in an aluminium pan of 40 μl capacity and 0.1 mm thickness, press-sealed with a perforated aluminium cover of 0.1 mm thickness. An empty pan sealed in the same way was used as reference. Conventional DSC measurements were performed by heating the sample up to 250 °C at a rate of 5 °C/min, under a nitrogen flow of 50 cm^3/min. The starting temperature was 25 °C. Indium (99.99% of purity) was used as standard for calibrating the temperature. Reproducibility was checked running the sample in triplicate.

2.6. In vitro release studies

In vitro release of ZP from each formulation was determined at 37 °C in a paddle apparatus operating at a rotation speed of 60 rpm. Samples of each formulation equivalent to about 2 mg of ZP were added to 40 ml of dissolution medium (0.05 M phosphate buffer pH 7.4 containing 0.2% of Tween 20). At appropriate time intervals, 500 μl of the mixture were withdrawn and filtered through a 0.22 μm membrane filter (Millipore® cellulose acetate) in thermostated test tubes. The initial volume of dissolution medium was maintained by adding 500 μl of 0.05 M phosphate buffer pH 7.4 containing 0.2% of Tween 20. The clear filtrate was allowed to stand in bath at 37 °C until analyzed by HPLC.

2.7. Pharmacological studies

Male Sprague–Dawley rats (Charles River, Como, Italy) weighing 120–150 g were kept under a 12-h light:12-h dark cycle at a temperature of 23 ± 2 °C and 65% humidity. On arrival at the animal facilities there was a minimum of 7 days of acclimatization during which the animals had free access to food (until about 12 h from starting experiments) and water. Rats (five for each experimental group) received equimolar doses (10 mg/kg) of ZP by intragastric administration of the ZP with an appropriate needle. The different formulations (i.e. tablets powdered of Stilnox®, ZP/PDLLA, ZP/HP-β-CD/PDLLA-B, ZP/PLGA, ZP/HP-β-CD/PLGA-B, and ZP-unloaded PDLLA-, PLGA-microparticles) were suspended, in equimolar concentration of ZP (10 mg/kg), in distilled water containing five drops of Tween 80 per 5 ml. The drop of surfactant was added to suspensions since it was present in the control ZP suspension. Rats were observed for the following 60 min and the time of

ataxic induction, which was defined as the time from drug administration to the status of profound sedation characterized by motor incoordination of all four legs, was recorded. Ataxic effect was subdivided into three distinct levels of intensity, including (i) "ataxia level 1" occurring when the animal shows motor incoordination of posterior legs; (ii) "ataxia level 2" occurs when motor incoordination involves both anterior and posterior legs; and (iii) "ataxia level 3" when animals are unable to walk and lay on their abdomen; however, no loss of the righting reflex was observed if the animals were laid on their back.

2.8. Statistical analysis

The statistical significance of differences in behavioral data and mean size, yields, drug loading were analyzed utilizing analysis of variance (ANOVA) followed by the Schette and Tukey post hoc tests (GraphPad Prism version 3 for Windows, GraphPad Software, San Diego, CA,USA), respectively. Differences were considered statistically significant at $P < 0.05$. Differences in drug release profiles were evaluated utilizing the ZP released at 24, 48, and 72 h, respectively and considered statistically significant at $P < 0.05$ using ANOVA test followed by Tukey post hoc test.

3. Results and discussion

Management of insomnia requires the use of hypnotics which should be given at the lowest effective dose for as short time of treatment as possible by the oral administration. A sustained-release dosage form of hypnotics may provide increased clinical value over conventional formulations as a result of improved patient compliance, a decreased incidence, and/or intensity of the side effects and a more prolonged therapeutic effect. The goal of this study was to design a sustained-release system of ZP, potentially useful for the treatment of different insomnia categories. We decided to use PDLLA and PLGA to encapsulate the drug since ZP, like other hypnotics, requires chronic administration and a biodegradable drug carrier may offer the advantage of obviating the need to remove the drug-depleted device. The primary reason for the incorporation of HP-β-CD into these polymeric matrices was to evaluate if it could play a role as drug release modulator.

As reported above, ZP alone-loaded microparticles were prepared by the classical O/W emulsion–solvent evaporation method (Huang et al., 1997). Conversely, ZP/HP-β-CD containing microparticles were prepared by the W/O/W emulsion–solvent evaporation method (Huang et al., 1997) and, in particular, following two different procedures (i.e. A and B). It was our aim to evaluate the CD's influence on both microparticle characteristics and drug release. Following procedure A, the previously produced ZP/HP-β-CD solid complex was added to the water phase of primary emulsion. In the second case (procedure B), HP-β-CD was added to the aqueous phase and ZP to the organic phase. It should be pointed out that only a little amount of water is required by the procedure A to prepare the primary emulsion and hence, complete dissociation of the drug–CD complex should not result by the limited dilution effect (Pistel and Kissel, 2000). Thus, the formulation prepared by procedure A contains a ZP/HP-β-CD association complex, whereas the formulation prepared by procedure B contains ZP and CD in two different phases (ZP dispersed in the polymer and CD in the water phase). Importantly, procedure A (already described in the literature; Sinisterra et al., 1999), is more time-consuming because it requires preparation of the solid complex prior to polymer encapsulation.

3.1. Microparticles size and morphology

The size distributions of all prepared microparticles are shown in Table 1. PDLLA and PLGA microparticles containing ZP alone exhibited similar average diameter (i.e. 2.81 and 2.87 μm), whereas the ones containing also HP-β-CD were characterized by a larger size. In particular, PDLLA and PLGA microparticles prepared according to procedure B exhibited average diameters of 231 and 145 μm, respectively. The size of the ZP/HP-β-CD/PLGA-A microparticles was much larger (467 μm) while that of ZP/HP-β-CD/PDLLA-A microparticles was not determined because only few of them showed well-defined and spherical shape and it did not allow a reliable measure. SEM micrographs on ZP/PDLLA and ZP/PLGA microparticles revealed that they have a spherical shape and smooth surface while SEM analysis on the systems prepared

Table 1
Size distribution of PDLLA and PLGA microparticles containing ZP with or without HP-β-CD

Microparticle type	Mean size[a] (μm)	Yield[b] (%)	Encapsulation efficiency[c] (%)
ZP/PDLLA	2.81 ± 2.00	71 ± 7.5	56 ± 6
ZP/HP-β-CD/PDLLA-A	ND	24.6 ± 8.8	53 ± 6
ZP/HP-β-CD/PDLLA-B	231 ± 99	52 ± 2.8	56 ± 4
ZP/PLGA	2.87 ± 1.70	74 ± 6.9	50 ± 4
ZP/HP-β-CD/PLGA-A	467 ± 141	21 ± 3.5	26 ± 3
ZP/HP-β-CD/PLGA-B	145 ± 66	59.4 ± 4.8	61 ± 7

ND: not detected.
[a] Data are mean diameters ± S.D. of three different batches.
[b] Percent yield: weight of microparticles/weight of drug + weight of polymer (Singh and Udupa, 1997). Data are means ± S.D. of three determinations.
[c] Data are means ± S.D. of five determinations.

following procedure B confirmed they were larger and highly porous (Fig. 1). SEM micrographs on ZP/HP-β-CD/PDLLA-A microparticles showed that they were composed mainly of broken microparticles together with very few almost intact.

The presence of holes within microparticles containing HP-β-CD could reasonably be explained following the suggestions of some authors who reported that a high osmotic pressure inside microparticles prepared with double emulsion–solvent evaporation method can give rise to surface porosity (Zannou et al., 2001; Pistel and Kissel, 2000; Freytag et al., 2000). Besides, the osmotic pressure characterizing the internal water phase can also explain the increase of the mean size of HP-β-CD-containing microparticles. It has been suggested, indeed, that in the double emulsion–solvent evaporation method, a higher osmotic pressure within the dispersed water phase can give rise to an influx of water toward this aqueous phase with a subsequent increase of the droplet volume and surface porosity (De Rosa et al., 2002; Crotts and Park, 1995). In this view, the organic phase acts as a diffusional barrier between the internal and external aqueous phases.

3.2. Yields and drug loading

The yields in microparticles and encapsulation efficiencies are also shown in Table 1. It is apparent from the reported data that the use of ZP/CD complex (procedure A) within the internal aqueous phase affected the obtained yields. Microparticles prepared with ZP/CD complex were obtained in much lower yields than those prepared with the drug alone. Intermediate yields were observed when procedure B was followed. Encapsulation efficiencies were in the range of 50–61% and no significant differences in drug loading among the various preparations was observed except for the ZP/HP-β-CD/PLGA-A system where the lowest drug incorporation efficiency occurred. This last finding could be due to the fact that droplets of internal aqueous phase, containing ZP as an association complex with HP-β-CD, can be taken up by the more hydrophilic polymer PLGA during microparticle preparation. Then, ZP diffusion in the external aqueous phase could take place with consequent decrease in overall drug loading. On the other hand, it was somewhat surprising to observe that microparticles with higher size were characterized by a decrease in encapsulation efficiency (Table 1; De Rosa et al., 2002). However, it has been shown that the encapsulation of drug in microparticles is affected by different factors including drug/polymer interactions, emulsion stability, and microparticle size and all may be taken into account to fully explain the encapsulation efficiency (De Rosa et al., 2002).

3.3. Solid state studies

FT-IR spectra of pure ZP and of all the loaded microparticles were recorded (data not shown). The spectrum of ZP showed a strong absorption band of carbonyl stretching at 1633 cm^{-1}, whereas the spectra of microparticles displayed a broad band at 1760 cm^{-1}. This absorption is attributable to the carbonyl group of the polyester. IR spectra of the

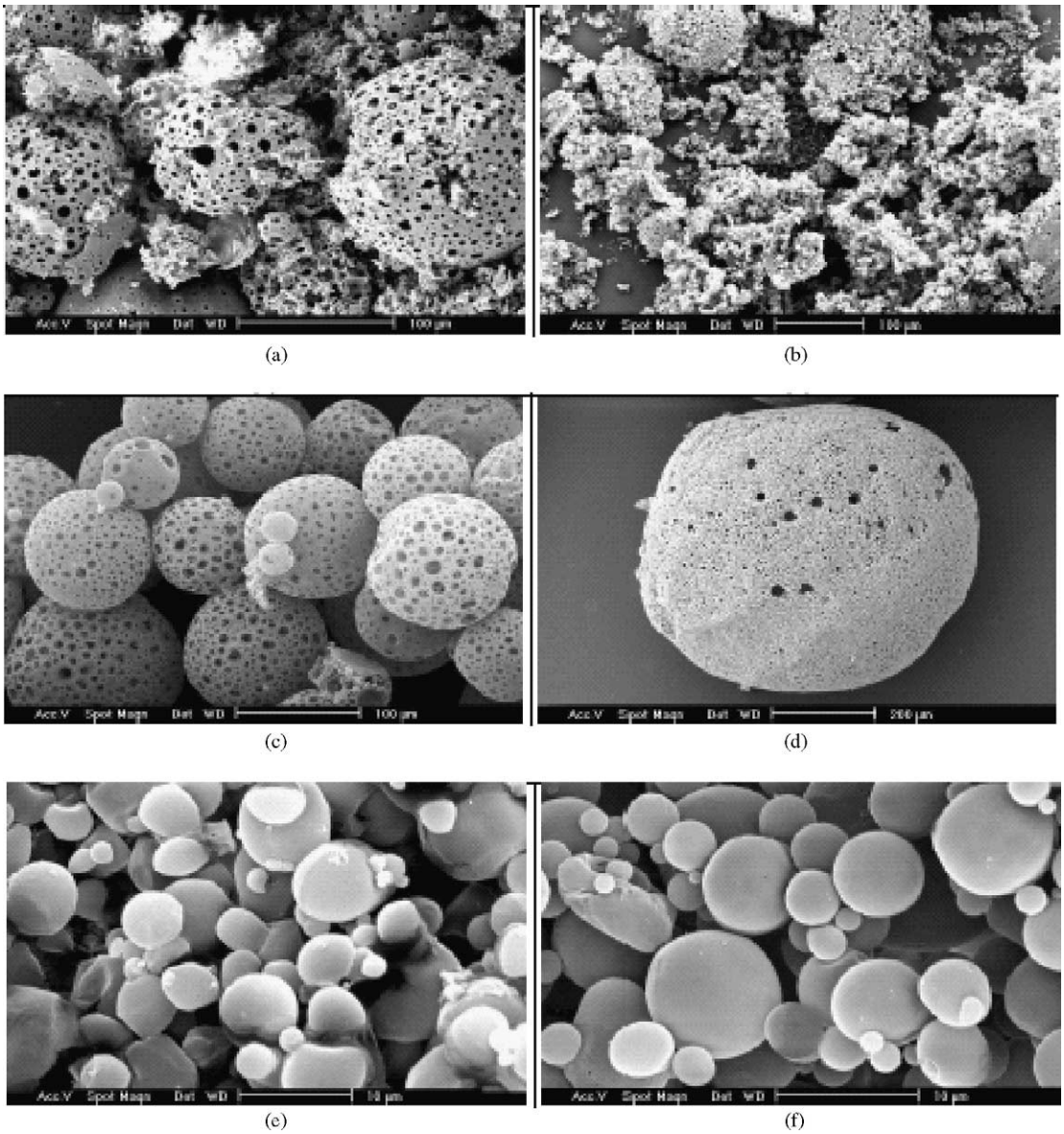

Fig. 1. SEM images of ZP/HP-β-CD/PDLLA-B (a), ZP/HP-β-CD/PDLLA-A (b), ZP/HP-β-CD/PLGA-B (c), ZP/HP-β-CD/PLGA-A (d), ZP/PDLLA (e), and ZP/PLGA (f) microparticles.

microparticles were essentially similar to those of the pure polymer. Fig. 2 shows the XRPD patterns of ZP and of selected CD-loaded microparticles. In the X-ray spectra of the latter microparticles the characteristic peaks of crystalline ZP disappeared and a halo pattern resulted. DSC patterns of ZP microparticles and those of the pure polymers are reported in Fig. 3. The DSC curves of the pure ZP showed a single endothermic peak at 193 °C corresponding to the melting of the drug. In the DSC profiles of all the loaded microparticles, the ZP melting peak was not present, while the endothermic peaks attributable to the Tg-relaxation enthalpy of the corresponding polymers in the range of 51–57 °C are well detectable.

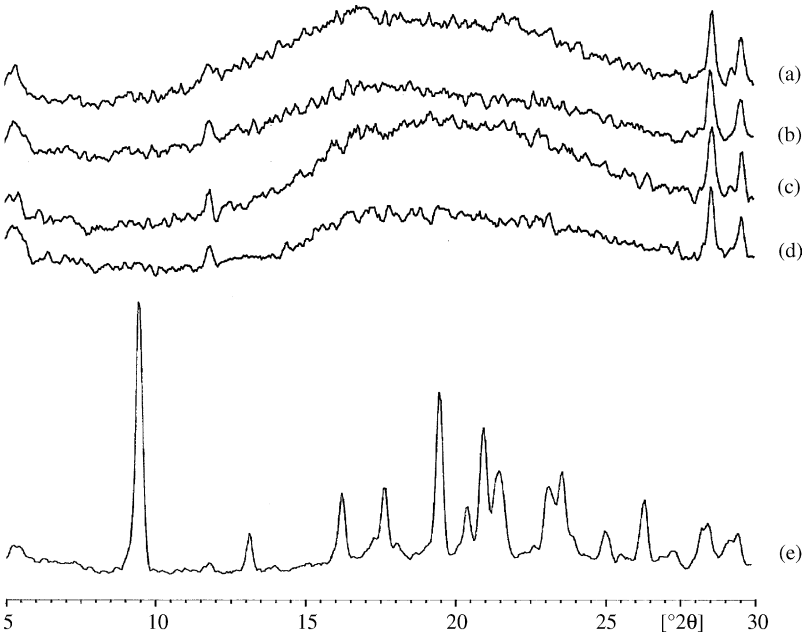

Fig. 2. XRPD patterns of ZP/PDLLA (a), ZP/HP-β-CD/PDLLA-B (b), ZP/PLGA (c), ZP/HP-β-CD/PLGA-B (d), and microparticles and pure ZP (e).

These results, taken together, suggest the drug is in an essentially amorphous state within the microparticles and it may influence, in some extent, its release kinetics (Dash et al., 2002; Baker, 1987).

3.4. Release of zolpidem from PDLLA and PLGA microparticles

In general, drug release from PDLLA/PLGA-based microparticles is depending on several factors including polymer composition, microparticles morphology, and encapsulation efficiency. In order to obtain useful information from the in vitro release kinetics of ZP/PDLLA-/PLGA-based microparticles, we decided to evaluate the release up to 24 h. Formulations allowing ZP release for several hours may be appropriate for the long-term treatment of insomnia. The release profiles of ZP from PDLLA and PLGA microparticles with or without HP-β-CD are shown in Fig. 4. Comparing these data with the dissolution previously observed for the drug alone (Trapani et al., 2000), it is apparent that the encapsulation in microparticles produces, in each case, a slower release. In general, the ZP release profiles from microparticles were biphasic, characterized by an initial ZP burst effect followed by a slow release (Fig. 4a and b). The burst effect period (up to 6 h) was almost identical in the systems examined and the amounts released during this period are reported in Table 2. On the basis of the percent of ZP released at 24 h, it resulted that release from ZP/PLGA and ZP/HP-β-CD/PLGA-B microparticles was faster than that observed for the corresponding PDLLA-based ones (Fig. 4). The amounts released beyond 6 h from all the prepared systems were better fitted according to the square-root equation ($t^{1/2}$). This square-root-of-time dependency should indicate a

Table 2
ZP release during the initial burst effect for PDLLA and PLGA microparticles

Microparticle type	Burst release[a] (%)
ZP/PDLLA	26 ± 3.6
ZP/HP-β-CD/PDLLA-A	74 ± 2.6
ZP/HP-β-CD/PDLLA-B	32 ± 3.2
ZP/PLGA	27 ± 3.7
ZP/HP-β-CD/PLGA-A	85 ± 1.0
ZP/HP-β-CD/PLGA-B	74 ± 7.0

[a] Burst release was measured at 6 h. Data are mean ± S.D.

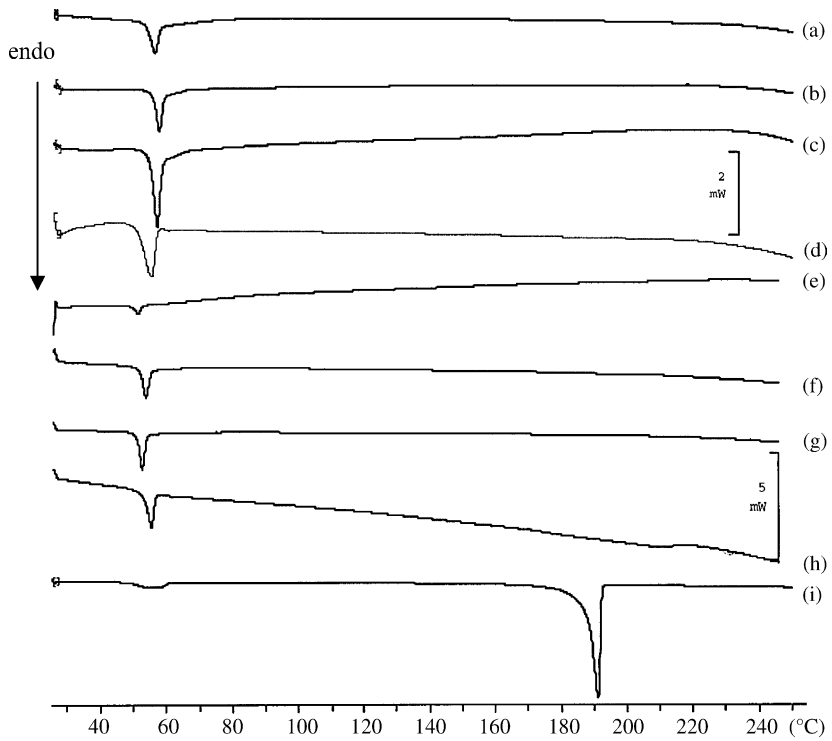

Fig. 3. DSC curves of the systems ZP/HP-β-CD/PDLLA-A (a), ZP/HP-β-CD/PDLLA-B (b), ZP/PDLLA (c), pure PDLLA (d), ZP/HP-β-CD/PLGA-A (e), ZP/HP-β-CD/PLGA-B (f), ZP/PLGA (g), pure PLGA (h), and pure ZP (i).

mass transport by simple diffusion mechanism. However, a biodegradable system may give zero-order release if the matrix degradation can compensate for the rate decline due to simple diffusion.

The initial burst effect was ascribed to the release of the drug on or close to the particle surface. After the rapid component of release is ended, there is a slower, much more controlled release of the drug owing to either diffusion through the matrix or polymer bulk erosion. It is apparent from the reported data that the ZP release from microparticles containing HP-β-CD was faster than that from microparticles containing ZP alone. This effect could be ascribed to both the presence of a porous structure and to the increased hydrophilicity (Bibby et al., 2000; De Rosa et al., 2002) of the polymer matrix due to the presence of HP-β-CD.

3.5. *In vivo screening of hypnotic activity*

As observed in both PDLLA and PLGA system, co-encapsulating ZP in the presence or in absence of HP-β-CD prolonged the duration of release from the corresponding microparticles. To explore whether these new formulations may lead to a sustained pharmacological activity, we investigated the ataxic action in rats following oral administration of ZP as ZP/PDLLA, ZP/HP-β-CD/PDLLA-B, ZP/PLGA, ZP/HP-β-CD/PLGA-B, and Stilnox® given in equimolar doses (10 mg/kg). Times of ataxic induction and duration of ataxia following the intragastric administration of each formulation were recorded and the results are summarized in Table 3. In these experiments, at the dose of ZP administered, rats treated with PDLLA- and PLGA-based formulations displayed, for the time considered (1 h), a lower level of ataxia (level of ataxia 1) with respect to the Stilnox®-treated animals (level of ataxia 2 and 3). The time indicated in Table 3 for these groups is thus relative to this lower level of ataxia. As shown, ataxic induction times subsequent to intragastric administration of ZP/PDLLA and ZP/PLGA microparticles were longer than those of the reference formulation,

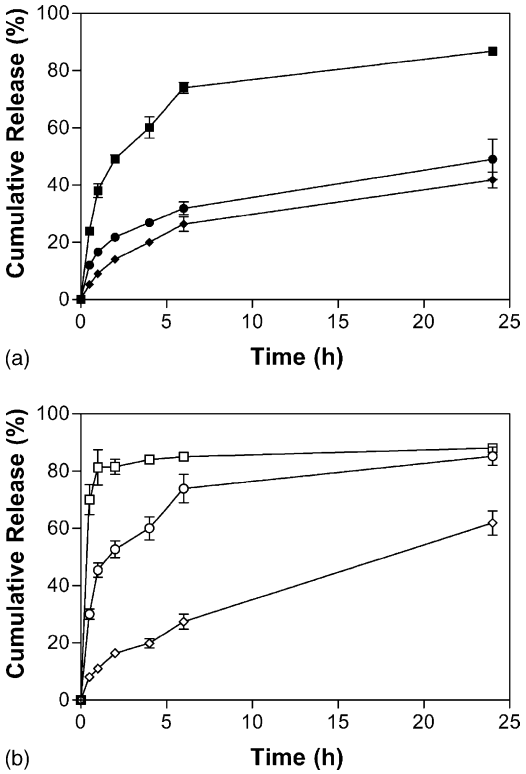

Fig. 4. (a) Release of zolpidem from ZP/PDLLA (◆), ZP/HP-β-CD/PDLLA-B (●), and ZP/HP-β CD/PDLLA-A (■) microparticles; (b) release of zolpidem from ZP/PLGA (◇), ZP/HP-β-CD/PLGA-B (○), and ZP/HP-β-CD/PLGA-A (□) microparticles. Each point represents the means of three different experiments ± S.D.

albeit the duration of ataxia was not modified. For HP-β-CD-containing microparticles the ataxic induction times were also not significantly different from the Stilnox® but, interestingly, administration of ZP/HP-β-CD/PLGA-B microparticles produced almost 65% increase ($P < 0.05$) in ataxic duration time than control. It is worth to note that, as previously reported (Trapani et al., 2000), intragastric administration of ZP-HP-β−CD complex give rise to almost two-fold longer ataxic induction times than control without any effect on the duration of ataxia. Therefore, it is apparent that the ZP/HP-β-CD/PLGA-B system allows to prolong the pharmacological efficacy of the drug in rats to a significant extent. Moreover, as an additional important finding, this prolonged ataxic effect occurs at a lower level of ataxia.

Looking at the data in Table 3, we could infer that the pharmacological effect showed by the ZP/HP-β-CD/PLGA-B system may be related to its initial release characteristics. Hence, in this preliminary pharmacological screening, we only characterized the initial diffusion phase of drug release when no polymer degradation occurred. Interestingly, there was a longer ataxic lasting and a lower intensity of ataxia produced by the ZP/HP-β-CD/PLGA-B-formulation already after 60 min following the administration (i.e. during the diffusion-controlled phase). However, to fully evaluate the utility of this last system for the sustained delivery of ZP, more appropriate pharmacokinetic and pharmacodynamic studies, including the use of repeated doses, different animal models and the implications of the erosion-controlled phase, are required.

Table 3
Evaluation of the ataxic effect in rats in the presence of PDLLA- and PLGA-microparticles containing ZP alone and those prepared according to procedure B

Zolpidem formulation	Time to ataxia (min)	Duration of ataxia (min)
Stilnox®	4.1 ± 0.4	10.8 ± 1.5
ZP/PDLLA	12.6 ± 1.0[a]	11.7 ± 2.0
ZP/HP-β-CD/PDLLA-B	7.5 ± 0.6	12.5 ± 1.8
ZP/PLGA	10.0 ± 1.6[a]	11.0 ± 1.4
ZP/HP-β-CD/PLGA-B	7.1 ± 0.5	17.8 ± 2.5[a]

[a] $P < 0.05$ versus Stilnox®-treated animals.

4. Conclusions

In this work, ZP alone or mixed with HP-β-CD was encapsulated in microparticles constituted of PDLLA and PLGA, and it was shown that the CD in these systems has potential to modulate the drug release. Preliminary pharmacological studies led us to single out the ZP/HP-β-CD/PLGA-B system which, following intragastric administration, produced the same ataxic induction time as the one induced by Stilnox® but, there were a longer ataxic lasting and a lower intensity of ataxia. This last feature may be favorable for the treatment of different insomnia categories. However, the development of the ZP/HP-β-CD/PLGA-B system as ZP's sustained-release product requires further investigations. Finally, the obtained results provide further support to the feasibility of using

CDs incorporated into polymeric delivery systems to modify drug release.

Acknowledgements

Thanks are due to Roquette Italia for the kind gifts of HP-β-CD. This work was supported by a grant from Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST).

References

Baker, R., 1987. Controlled Release of Biologically Active Agents. Wiley, New York.

Bibby, D.C., Davies, N.M., Tucker, I.G., 1999. Investigations into the structure and composition of β-cyclodextrin/poly(acrylic acid) microspheres. Int. J. Pharm. 180, 161–168.

Bibby, D.C., Davies, N.M., Tucker, I.G., 2000. Mechanisms by which cyclodextrins modify drug release from polymeric drug delivery systems. Int. J. Pharm. 197, 1–11.

Charney, D.S., Mihic, S.J., Harris, R.A., 2001. Hypnotics and sedatives. In: Hardman, J.G., Limbird, L.E., Gilman, A.G. (Eds.), Goodman & Gilman's The Pharmacological Basis of Therapeutics, 10th ed. McGraw-Hill, New York, pp. 399–427.

Cohen, S., Yoshioka, T., Lucarelli, M., Hwang, L.H., Langer, R., 1991. Controlled delivery system for protein based on polylactic/glycolic acid) microspheres. Pharm. Res. 8, 713–720.

Crotts, G., Park, T.G., 1995. Preparation of porous and nonporous biodegradable polymeric hollow microspheres. J Control. Release 35, 91–105.

Dash, A.K., Khin-Khin, A., Suryanarayanan, R., 2002. X-Ray powder diffractometric method for quantitation crystalline drug in microparticulate systems. I. Microspheres J. Pharm. Sci. 91, 983–990.

De Rosa, G., Quaglia, F., La Rotonda, M.I., Appel, M., Alphandary, H., Fattal, E., 2002. Poly(lactide-co-glycolide) microspheres for the controlled release of oligonucleotide/polyethylenimine complexes. J. Pharm. Sci. 91, 790–799.

Doble, A., 1999. New insights into the mechanism of action of hypnotics. J. Psychopharmacol. 13, S11–S20.

Duchene, D., Wouessidjewe, D., Ponchel, G., 1999. Cyclodextrins and carrier systems. J. Control. Release 62., 263–268.

Filipovic-Grcic, J., Becirevic-Lacan, M., Skalko, N., Jalsenjak, I., 1996. Chitosan microspheres of nifedipine and nifedipine-cyclodextrin inclusion complexes. Int. J. Pharm. 135, 183–190.

Filipovic-Grcic, J., Voinovich, D., Moneghini, M., Becirevic-Lacan, M., Jalsenjak, I., 2000. Chitosan microspheres with hydrocortisone-hydroxypropyl-β-cyclodextrin inclusion complex. Eur. J. Pharm. Sci. 9, 373–379.

Follesa, P., Mancuso, L., Biggio, F., Cagetti, E., Franco, M., Trapani, G., Biggio, G., 2002. Changes in $GABA_A$ receptor gene expression induced by withdrawal of, but not by long-term exposure to, zaleplon or zolpidem. Neuropharmacology 42, 191–198.

Freytag, T., Dashevsky, A., Tillman, L., Hardee, G.E., Bodmeier, R., 2000. Improvement of the encapsulation efficiency of oligonucleotide-containing biodegradable microspheres. J. Control. Release 69, 197–207.

Huang, Y.-Y., Chung, T.-W., Tzeng, T., 1997. Drug release from PLA/PGA microparticulates. Int. J. Pharm. 156, 9–15.

Jalil, R., Nixon, J.R., 1990. Biodegradable poly(lactic acid) and poly(lactide-co-glycolide)microcapsules: problems associated with preparative techniques and release properties. J. Microencapsul. 7, 297–325.

Martindale 1999. In: Parfitt, K., Sweetman, S.C., Blake, P.S., Pearson, A.V. (Eds.), The Complete Drug Reference, 32nd ed. The Pharmaceutical Press, London, pp. 698–699.

Pistel, K.F., Kissel, T., 2000. Effects of salt addition on the microencapsulation of proteins using w/o/w double emulsion technique. J. Microencapsul. 17, 467–483.

Pitha, J., Milecki, J., Fales, H., Pannell, L., Uekama, K., 1986. Hydroxypropyl-β-cyclodextrin: preparation and characterization effects on solubility of drugs. Int. J. Pharm. 29, 73–82.

Schlicher, E.J.A.M., Postma, N.S., Zuidema, J., Talsma, H., Hennink, W.E., 1997. Preparation and characterization of poly-(D,L-lactic-co-glycolic acid) microspheres containing desferrioxamine. Int. J. Pharm. 153, 235–245.

Singh, U.V., Udupa, N., 1997. In vitro characterization of methotrexate loaded poly(lactic-co-glycolic) acid microspheres and antitumor efficacy in Sarcoma-180 mice bearing tumor. Pharm. Acta Helv. 72, 165–173.

Sinisterra, R.D., Shastri, V.P., Najjar, R., Langer, R., 1999. Encapsulation and release of rhodium (II) citrate and its association complex with hydroxy-β-cyclodextrin from biodegradable polymer microspheres. J. Pharm. Sci. 88, 574–576.

Song, C.X., Labhasetwar, V., Levy, R.J., 1997. Controlled release of U-86983 from double-layer biodegradable matrices: effect of additives on release mechanism and kinetics. J. Control. Release 45, 177–192.

Trapani, G., Latrofa, A., Franco, M., Pantaleo, M.R., Sanna, E., Massa, F., Tuveri, F., Liso, G., 2000. Complexation of zolpidem with 2-hydroxypropyl-β-, methyl-β-, and 2-hydroxypropyl-γ-cyclodextrin: effect on aqueous solubility, dissolution rate, and ataxic activity in rat. J. Pharm. Sci. 89, 1443–1451.

Yamakawa, I., Kawahara, M., Watanabe, S., Miyake, Y., 1999. Sustained release of insulin by double-layered implant using poly(DL-lactic acid). J. Pharm. Sci. 79, 505–509.

Zannou, E.A., Streng, W.H., Stella, V.J., 2001. Osmotic properties of sulfobutylether and hydroxypropyl cyclodextrins. Pharm. Res. 18, 1226–1231.