Etoposide-Loaded Microparticles Prepared with Poly (Hydroxybutyrate-Co-Hydroxyvalerate) and Poly(ε-Caprolactone) Blends: Formulation, Characterization and in vitro Drug Release

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ABSTRACT: The purpose of this research was to formulate and systematically evaluate etoposide-loaded microparticles. Etoposide microparticles containing poly(hydroxybutyrate-co-hydroxyvalerate) and poly(ε-caprolactone) were prepared by an emulsion/solvent evaporation process. Microparticles were discrete, spherical and free flowing. The microparticles showed high % of yield and drug entrapment efficiency. Etoposide-loaded microparticles demonstrated drug sustained releases (up to 200 hours). The drug release mechanism was dependent on the presence of PCL in the microparticles. The release of etoposide caused an increase in the surface area of the microparticles. A Fickian release was determined for the microparticles prepared exclusively with P(HBHV), while non-Fickian release behaviors were found for the P(HBHV)/PCL microparticles.

KEYWORDS: Etoposide, poly(hydroxybutyrate-co-hydroxyvalerate), poly(ε-caprolactone), release mechanism and SEM.

Introduction

The polymeric microparticles have been studied in the last 3 decade in order to carry drugs for oral administration (Freiberg, 2004). In general, these systems consist of polymeric materials, in which the drug is dispersed, entrapped, dissolved or adsorbed (Benita S, 1996, Wan LS, et al., 1992). Recently dosage forms that can precisely control the release rates and target drugs to a specific body site have created enormous impact in formulation and development of novel drug delivery systems. Microparticles form an important part of such novel drug delivery systems (Woo BH et al., 2001, Capan Y, et al., 2003, Gohel MC, et al., 1998). Additionally, those multiparticulated systems provide controlled release profiles and improve oral bioavailability of drugs. Different polymers can be used to formulate microparticles presenting specific morphological and release characteristics.(Kim C, 2000)

Poly(hydroxybutyrate-co-hydroxyvalerate) [P(HBHV)] is a biodegradable and biocompatible polyester produced by bacteria in a biotechnological process (Lee S, et al., 1999). The poly(hydroxybutyrate) and its copolymers, like P(HBHV), have been used to develop drug delivery systems due to their physico-chemical and biocompatible characteristics. (Pouton CW, 1996).

Poly(ε-caprolactone) (PCL) is a synthetic biodegradable polyester, which has been employed for the preparation of multiparticulated systems, either microparticles or nanoparticles (Pohlmann AR, et al., 2004, Cruz L, et al., 2006). Microcapsules composed by poly(butylene succinate) (PBS) and PCL blends containing indomethacin have been prepared by the emulsion/solvent evaporation process (Park S, et al., 2006). PCL has been used with polyhydroxyalkanoates to produce blends presenting lower crystallization rate than the materials prepared exclusively with polyhydroxyalkanoates (Chum YS, et al., 2000).

Microparticles prepared with P(HBHV)/PCL blends showed that the porosity observed by scanning electron microscopy increased with the decrease of the P(HBHV)/PCL ratio (Embleton K, et al., 1993). Moreover, the increase in the P(HBHV)/PCL ratio caused a proportional augmentation in the specific areas of the microparticles (Re MI, et al., 2004).

Etoposide is an anticancer agent used in the treatment of a variety of malignancies and has been demonstrated to be effective in the treatment of malignant lymphoma, brain stem gliomas, SCLC (small cell lung carcinoma), stomach cancer and ovarian cancer. It acts by inhibition of topoisomerase II and activation of oxidation reduction reactions to produce derivatives that bind directly to DNA and cause DNA damage (Chamberlain MJ, 1993, Ashley D, et al., 1996).
Materials and methods

Materials

Etoposide was a gift sample obtained from the Dabur Research Center Cipla Ltd, Mumbai, India. Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) \([\text{PHBHV}]\) was kindly gifted by PHB Industrial S.A., Brazil. Poly(\varepsilon\text{-caprolactone}) (PCL) was supplied by Aldrich Chemicals Strasbourg, France, poly(vinyl alcohol) from S. D. Fine Chemicals Ltd., Mumbai, India. All other chemicals and solvents were pharmaceutical grade and used as received.

Methods

Preparation of PHBHV and PHBHV/PCL microparticles

The microparticles were prepared by an emulsion/solvent evaporation method. Briefly, 30 mL of an aqueous phase prepared with 1% PVA (w/w) was slowly dropped into 30 mL of a chloroform solution of 0.3 g of polymers at the proportions of 10:0, 9:1 or 8:2 (w/w) of PHBHV/PCL. These organic solutions were also prepared with 10% (w/w) etoposide. The emulsification was performed at 45°C under stirring (1200 rpm) for 10 minutes. The chloroform was eliminated by evaporation under reduced pressure (Rotary Vaccum evaporator, super vertical Model PBV-7D). The microparticles were filtered, washed with 100 mL of distilled water and dried in a dessicator for 24 hours.

Drug content analysis

Drug content was determined in chloroform by spectrophotometry at 286 nm using a UV-1601 spectrophotometer Shimadzu ((Shimadzu UV 1601; Shimadzu, Kyoto, Japan)). The method was validated presenting linearity \((r = 0.999)\). The analyses were performed using samples from three different batches.

Scanning electron microscopy (SEM)

Scanning electron photomicrographs of unloaded and the etoposide-loaded microparticles were taken. A small amount of microparticles was spread on glass stub. Afterwards, the stub containing the sample was placed in the scanning electron microscope (JSM 5610 LV SEM, JEOL, Datum Ltd., Japan) chamber. Scanning electron photomicrograph was taken at the acceleration voltage of 20 KV, chamber pressure of 0.6 mm Hg, at different magnification.

Particle size and size distribution

The microparticle sizes and size distributions were determined by laser diffractometry (Malvern Instruments Ltd, Worcestershire, UK). The samples were previously dispersed in polysorbate 80 aqueous solutions and ultrasonicated. The analyses were carried out in triplicate. The particle sizes were expressed as \(d(4.3)\) and the size distributions (span) were calculated using the equation (1):

\[
\text{span} = \left( \frac{d(0.9) - d(0.1)}{d(0.5)} \right) \quad \ldots(1)
\]

where \(d(0.1), d(0.5)\) and \(d(0.9)\) are, respectively, the particle diameters at 10%, 50% and 90% of the undersized particle distribution curve.

Surface area

The specific surface areas were calculated using the Brunauer-Emmette-Teller (BET) method. Nitrogen adsorption isotherms were obtained in a homemade volumetric apparatus, connected to a turbo molecular Edwards (London, England) vacuum line system, employing an Hg capillary barometer. The microparticles were previously degassed under vacuum at room temperature for 2.5 hours. The results were compared to an alumina pattern.

Drug release studies

in vitro release of etoposide from microparticles was evaluated by the dialysis bag diffusion technique reported by Yang et al. The release studies of etoposide from different batches of microparticles were performed in phosphate buffer (pH 7.4). The aqueous microparticulate dispersion equivalent to 2 mg of etoposide was placed in a cellulose dialysis bag (cutoff 12 000; HIMEDIA, Mumbai, India) and sealed at both ends. The dialysis bag was immersed in the receptor compartment containing 50 mL of dissolution medium, which was stirred at 100 rpm and maintained at 37 ± 2°C. The receptor compartment was covered to prevent the evaporation of dissolution medium. Samples were withdrawn at regular time intervals, and the same volume was replaced by fresh dissolution medium. The samples were analyzed using a UV-visible spectrophotometer set at 286 nm. All the experiments were repeated 3 times, and the average values were taken.

Results and Discussion

Preparation of the microparticles

Prepared microparticles were discrete, spherical and free flowing. Unloaded and drug-loaded microparticles prepared using 0, 10 and 20% (w/w) of PCL were named, respectively, UA, UB, UC, LA, LB and LC. All
formulations presented microscopic aspect of powders, mean yields ranging between 75 and 83 % (Table 1) and encapsulation efficiencies between 84 and 90 % independently on the polymeric composition.

**Scanning electron microscopy**

Scanning electron microscopy images (Figures 1 and 2) demonstrated spherical shaped microparticles and presence of pores for all formulations. The augmentation of the PCL concentration increased the size of the pores independently of the presence of the drug. For LA, the photomicrography showed the presence of free crystals in the sample (Figure 2A), while for LB and LC, SEM images showed that the crystals were adhered at the microparticle surfaces (Figures 2B and 2C).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Etoposide</th>
<th>Yield mean (%) ± SD</th>
<th>Encapsulation efficiency mean (%) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>UA unloaded</td>
<td>80 ± 4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>UB unloaded</td>
<td>78 ± 2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>UC unloaded</td>
<td>75 ± 3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LA loaded</td>
<td>80 ± 2</td>
<td>85 ± 4</td>
<td>-</td>
</tr>
<tr>
<td>LB loaded</td>
<td>81 ± 4</td>
<td>90 ± 8</td>
<td>-</td>
</tr>
<tr>
<td>LC loaded</td>
<td>83 ± 3</td>
<td>84 ± 7</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 1.** Yield and encapsulation efficiencies of unloaded and etoposide-loaded microparticles.

**Fig 1.** Photomicrographs of UA, UB and UC microparticles.

**Fig 2.** Photomicrographs of LA, LB and LC microparticles.
Particle diameters, size distribution and specific surface areas

Particle size analyses demonstrated mean diameters between 79 and 113 μm (Table 2) and span values lower than 2. The mean diameters were influenced neither by the presence of the drug nor the PCL, as well as the PCL concentration did not influence the particle sizes or particle size distributions.

Unloaded microparticles (UA, UB and UC) showed surface areas of 16, 20 and 25 m² g⁻¹, respectively. The augmentation of the PCL content in the blend resulted in a slight increase in the surface area of the microparticles. These findings are in agreement with those reported for similar systems prepared by the emulsion/solvent evaporation process 15, 20. Drug-loaded microparticles (LA, LB and LC) showed surface areas of 40, 25 and 20 m² g⁻¹, respectively. Despite the size of the macropores increased with the augmentation of PCL concentration (Figures 1 and 2) the formulations prepared exclusively with P(HBHV) (UA and LA) presented different surface areas (Table II), while the formulations containing PCL (UB, UC, LB and LC) presented similar values.

Drug release profiles and mechanism of release

To verify if the drug release profiles are dependent on the PCL concentration in the microparticles an in vitro release experiment was carried out (Figure 3). The dissolution profile of a pure drug sample, recrystallized by following the same procedure used to prepare the microparticles, showed total dissolution in phosphate buffer (pH 7.4) after 15 hours (Figure 3). The drug-loaded microparticles presented different drug release profiles after 200 hours showing a burst followed by a sustained release (Figure 3).

Table 2. Particle sizes d(0.1), d(0.5), d(0.9), d(4.3), particle size distributions (span) and superficial specific area.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>d(0.1) (μm)</th>
<th>d(0.5) (μm)</th>
<th>d(0.9) (μm)</th>
<th>d(4.3) (μm)</th>
<th>span</th>
<th>Superficial specific area (m² g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UA</td>
<td>32</td>
<td>62</td>
<td>180</td>
<td>88</td>
<td>2.0</td>
<td>16</td>
</tr>
<tr>
<td>UB</td>
<td>35</td>
<td>64</td>
<td>122</td>
<td>79</td>
<td>1.2</td>
<td>20</td>
</tr>
<tr>
<td>UC</td>
<td>36</td>
<td>67</td>
<td>150</td>
<td>84</td>
<td>1.7</td>
<td>25</td>
</tr>
<tr>
<td>LA</td>
<td>31</td>
<td>64</td>
<td>151</td>
<td>84</td>
<td>1.5</td>
<td>40</td>
</tr>
<tr>
<td>LB</td>
<td>40</td>
<td>70</td>
<td>141</td>
<td>89</td>
<td>1.5</td>
<td>25</td>
</tr>
<tr>
<td>LC</td>
<td>32</td>
<td>101</td>
<td>199</td>
<td>113</td>
<td>1.2</td>
<td>20</td>
</tr>
</tbody>
</table>

Fig 3. Etoposide release profiles of recrystallized pure drug and LA, LB and LC microparticles.
To establish the mechanism of the drug release the Korsmeyer-Peppas model was used (Ritger PL, et al., 1987). A Fickian release ($n = 0.390 \pm 0.025$) was determined for the microparticles prepared exclusively with P(HBHV), while the P(HBHV)/PCL microparticles prepared with the proportions 90:10 and 80:20 (w/w) showed non-Fickian release behaviors ($n = 0.571 \pm 0.105$ and $n = 0.426 \pm 0.96$, respectively). The drug release mechanism was dependent on the presence of PCL in the microparticles.

**Conclusion**

The study was to prepare etoposide-loaded microparticles with poly(hydroxybutyrate-co-hydroxyvalerate) and poly(ε-caprolactone) blends. The microparticles prepared by an emulsion/solvent evaporation technique were discrete, spherical and free flowing. The microparticles showed high % of production yield and drug entrapment efficiency. Moreover, the formulations showed sustained release of etoposide from the microparticles. The release profiles fit biexponential model showing half-lives for the sustained released. The augmentation of the PCL concentration in the blend increased the drug release rate. In this way, the release can be controlled by varying the proportion of P(HBHV)/PCL in the microparticles. The drug release mechanism was dependent on the presence of the PCL in the microparticles.

**References**


