

# EFFECT OF DIETHYL PHTHALATE ON THE CHARACTERISTICS AND *IN VITRO* RELEASE FROM BIODEGRADABLE MICROSPHERES

Concepción Martínez Sancho; Rocío Herrero Vanrell; Sofía Negro Álvarez

Departamento de Farmacia y Tecnología Farmacéutica. Facultad de Farmacia. Universidad Complutense de Madrid. 28040 Madrid.

## Abstract / Resumen

The effect of a water-insoluble plasticizer (diethyl phthalate, DEP) was studied on biodegradable microspheres containing aciclovir. Microspheres were prepared by the solvent evaporation technique and analysed for their morphology, particle size distribution, loading efficiency and *in vitro* release assay. The microspheres morphology, particle size distribution and loading efficiency were not influenced by the incorporation of DEP. The aciclovir *in vitro* release was improved compared to DEP-free microspheres.

Se estudió el efecto de un plastificante insoluble en agua (dietilftalato, DEF) sobre microesferas biodegradables conteniendo aciclovir. Las microesferas se prepararon por el método de evaporación del solvente y fueron analizadas en cuanto a su morfología, tamaño de partícula, eficiencia de encapsulación y cesión *in vitro*. La incorporación de DEF no tuvo influencia sobre la morfología de las microesferas, el tamaño de partícula ni la eficiencia de encapsulación. La cesión *in vitro* de aciclovir se mejoró con respecto a las microesferas sin DEF.

## Introduction

Long-term delivery is necessary for drugs, which require frequent administrations. It has been reported that the release of aciclovir from poly (D,L-lactide-co-glycolide) microspheres is practically negligible at the first stages. This

low release rate would imply an increase in the amount of microspheres to be administered in order to reach effective concentrations. The incorporation of substances in the matrix has demonstrated to be an easy way to modify the release rate and/or profile of active compounds [1,2]. In this study, to modify the release of aciclovir from PLGA microspheres, a substance included in the FDA Inactive Ingredients Guide was incorporated into the inner phase of the emulsion, Diethyl phthalate (DEP). DEP is a non-fatty plasticizer widely used in pharmaceutical formulations. It is generally regarded as a non-toxic and non-irritant material at the levels employed as an excipient (10-30% by weight of polymer) [3].

The presence of compounds, such as plasticizers, has been reported to increase diffusion rate through PLA and PLGA polymeric systems [4]. Thus, the objective of the current work was to study the influence of DEP on the characteristics and the *in vitro* drug release from biodegradable microspheres.

## Materials and Methods

### Materials

Aciclovir (9[2-(hydroxyethoxy) methyl]-guanine) was obtained from Reig Farma, S.A. (Spain). PLGA 50/50 with an inherent viscosity of 0.2 dl/g (Resomer®RG502) was purchased from Boehringer Ingelheim Chemicals Division (Germany). Polyvinyl alcohol (PVA) MW 72000 Dalton (Fluka Chemie AG, Germany) and diethyl phthalate

(DEP) (Guinama, Spain) were used as received.

Methylene chloride (CH<sub>2</sub>Cl<sub>2</sub>) and sodium hydroxide solution, analytical grade, were obtained from Merck (Spain). Distilled and deionized water (Millipore Corporation, USA) was used in the preparation of buffer and solutions.

### **Preparation of microspheres**

Microspheres were prepared by the solvent evaporation technique from an O/W emulsion [5]. The starting drug:polymer ratio was 1:10 (w/w), being the amount of polymer processed 400 mg. The DEP:polymer ratio was 2:10 for DEP microspheres,.

Briefly, the organic phase was prepared by dissolving the polymer in 1 mL CH<sub>2</sub>Cl<sub>2</sub> by agitation in a vortex mixer (IKA Labortechnik, Germany) and then 40 mg aciclovir were suspended in the polymer solution. The aqueous phase consisted of a PVA solution (0.1%). The organic phase was slowly poured into the aqueous phase to form an emulsion and the mixture was continuously stirred for 3 h at room temperature to allow complete evaporation of the organic solvent. After evaporation of CH<sub>2</sub>Cl<sub>2</sub>, the microspheres were vacuum-filtered through a 5 µm filter, washed three times with water and lyophilised (Flexy-Dry™, FTS Systems, USA). These microspheres were kept in a desiccator until use.

DEP was added to the internal phase of the emulsion. Microspheres batches were prepared in triplicate.

### **Microsphere morphology**

The morphology of microspheres was examined by scanning electron microscopy (SEM, Jeol, JSM-6400, Japan). The microspheres were dried and gold sputter-coated before observation at 20 kV.

Granulometric analysis was performed by a Galai Cis-1 computerised inspection system (Galai Production Ltd., Israel) in the 2-300 µm range.

### **Loading efficiency**

10 mg of microspheres were dissolved in 1 ml CH<sub>2</sub>Cl<sub>2</sub>. Drug was extracted three times from this organic solution with 9 ml of

10<sup>-4</sup> M sodium hydroxide solution. The mixture was centrifuged (Eba 12R, Hettich, Germany) at 6000x g for 5 minutes and the supernatant was filtered through a 0.45 µm syringe filter (Tracer, Spain). Drug content of the extracted aqueous solutions was measured in a spectrophotometer at 254 nm (DU-6, Beckman, USA). The total amount of aciclovir was calculated from the aliquots of each extract. No component interfered at this wavelength.

### **In vitro release assay**

Replicate samples of microspheres (10 mg) from each batch were suspended in 3 ml of isotonic phosphate buffer saline (PBS) pH 7.4 (isotonic conditions) and placed in a water shaker bath (NE-5, Clifton, UK) at 37°C with constant agitation (100 strokes/min). Samples for analysis were taken with a syringe at previously established time intervals. They were filtered through a 0.45 µm filter and analysed for aciclovir by monitoring their absorbance at 251 nm. After sample removal, the same volume of fresh medium was replaced to continue the release test. The assay was performed in duplicate from each batch.

## **Results and Discussion**

The effect of DEP on the characteristics and *in vitro* release of aciclovir PLGA microspheres was evaluated. Table 1 shows the results obtained for the different batches in terms of mean loading efficiency and mean diameter of microspheres.

<i>Batch</i>	<i>Loading efficiency % (S.D.)</i>	<i>Diameter particle µm (S.D.)</i>
<i>DEP</i>	64.08 (4.25)	27.94 (4.91)
<i>DEP-free</i>	62.80 (9.04)	43.82 (17.88)

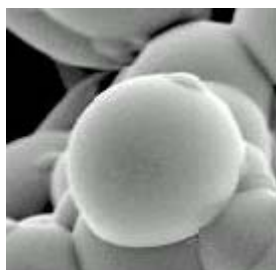
Table 1. Mean yield of production, loading efficiency and mean diameter of PLGA microspheres.

The yield of production was significantly influenced by the presence of DEP in the inner phase. It decreased from 57.00±6.85% (DEP-free microspheres) to 39.52±6.28% (DEP-loaded microspheres).

Loading efficiency increased very slightly when DEP was added. The mean percentage of incorporated aciclovir in these microspheres was  $64.08 \pm 4.25\%$ .

The particle size distribution of microspheres was influenced by the incorporation of this agent. DEP-free microspheres resulting in a lower polydispersion. The mean diameter of microspheres varied from  $27.94 \pm 4.91 \mu\text{m}$  (DEP microspheres) to  $43.82 \pm 17.88 \mu\text{m}$  (DEP-free microspheres).

SEM examination showed that microspheres were spherical and homogeneous in shape. This structure did not change when DEP was added, but DEP-loaded microspheres tended to stick together probably due to the presence of a residual of the plasticizer on the microspheres surface (Figure 1).



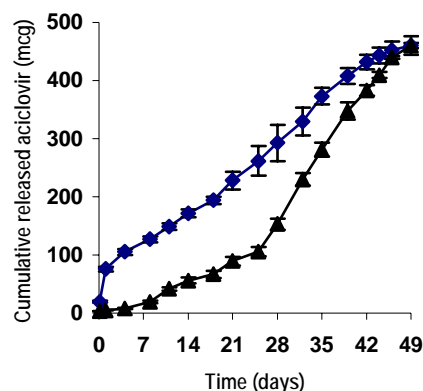
**Figure 1.** SEM photograph of DEP microspheres

The aciclovir release profiles from DEP-loaded microspheres (with respect of mean amount of incorporated drug) were compared with those obtained with DEP-free microspheres. Microspheres released the drug for 49 days.

Initially, DEP-free microspheres released a slight amount of drug within 24 hours of incubation ( $0.328 \pm 0.036 \mu\text{g}/\text{mg}$  microspheres). This initial burst was less than 5%. The release rate of the drug was carried out in three phases. For approximately eight days the drug was released slowly ( $0.223 \pm 0.038 \mu\text{g}/\text{day}/\text{mg}$  microspheres). On the following days (8-24) an increasing in the release rate was observed ( $0.461 \pm 0.036 \mu\text{g}/\text{day}/\text{mg}$  microspheres). From the twenty-four day to the end of the release assay the major release phase occurred. This sequence of events was consistent with literature reports

on the release behaviour of PLGA microspheres.

DEP addition changed the release of the drug with respect of DEP-free microspheres all over the release assay, indicating that DEP modified the aciclovir diffusion through the polymer matrix. When DEP was incorporated into the microspheres, the release patterns of the drug, within the first days (1-8 days) were increased. DEP-loaded microspheres showed a typical burst effect that results from rapid dissolution of the drug at the surface of the microspheres and a constant release until the end of the assay (Figure 2).



**Figure 2.** Aciclovir release profiles from DEP-free microspheres (▲) and DEP-loaded microspheres (●).

The *in vitro* release profile from 1-46 days for DEP-loaded microspheres was found to follow a zero-order release kinetic ( $r=0.9962$ ) with a mean release constant of  $0.865 \pm 0.04 \mu\text{g}/\text{day}/\text{mg}$  microspheres. This release constant was 3.88 times upper to that calculated for DEP-free microspheres during the first 8 days of the assay, which supposed a reduction in the amount of aciclovir microspheres to be administered.

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***Autor de contacto:***

*Concepción Martínez Sancho*

*soneal@farm.ucm.es*

*Departamento de Farmacia y Tecnología  
Farmacéutica. Facultad de Farmacia. UCM.*

*Plaza de Ramón y Cajal s/n  
28040 Madrid*

*Tel.: 913941739*

*Fax: 913941736*