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Optimisation of aciclovir poly(D,L-lactide-co-glycolide) microspheres for intravitreal administration using a factorial design study

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Abstract

The purpose of this work was to obtain an optimised long-term aciclovir PLGA microspheres formulation for intravitreal administration to minimise, as much as possible, the dose of microspheres to be administered with a suitable particle size for its injection through a 27G needle in a single dose. Microspheres were prepared by the solvent evaporation method. To obtain the optimum formulation a two-factor five-level central rotatable composite 2^2 + star design was employed. The independent variables were aciclovir and gelatin (added to the external phase of the emulsion). The dependent variables were the yield of production (%), the encapsulation efficiency (%), the initial burst release (%), the cumulative amount released from 1 to 14 days and the amount of aciclovir at the end of the release assay (μg aciclovir/mg microspheres). The best formulation according to the studied variables was (0,0), prepared with 80 mg of aciclovir and 80 mg of gelatin. This formulation showed good yield of production (70.14 ± 3.72%) and encapsulation efficiency (70.77 ± 2.62%), and released the drug at a constant rate for 63 days with a mean release constant of 1.73 ± 0.08 μg/day per mg microspheres. The selected formulation reduces a 40% the dose of microspheres to be administered through a 27G needle with respect to previous studies.

Keywords: Aciclovir; PLGA; Microspheres; Experimental design; Controlled release; Gelatin

1. Introduction

Response surface methodology (RSM) is a rapid technique used to empirically derive a functional relationship between an experimental response and a set of input variables. RSM reduces the number of experimental runs that are necessary to establish a mathematical trend in the experimental design region allowing to determine the optimum level of experimental factors required for a given response. Thus, a screening phase that allows the key factors to be established is advisable (Gotti et al., 2000).

RSM is widely used to optimise process parameters, especially in determining optimum conditions for investigations and maximising yields in large-scale chemical synthesis (McCarron et al., 1999). It has been applied to pharmaceutical systems such as the preparation of particulate carriers as microspheres. Particles present obvious advantages for the administration of drugs. However, it is necessary to have a clear understanding of how preparation conditions determine particle characteristics and in particular,

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how these characteristics are influenced by potential interactions between preparation factors. RSM may provide an useful tool to analyse such interactions.

Especially, micro- and nanoparticles are widely investigated for the controlled release of classical drugs as well as peptides and proteins. They can be made of poly(D,L-lactic-co-glycolic acid) (PLGA), a biodegradable and biocompatible polymer with the advantage of being degraded and eliminated from the body once it has achieved its goal. This polymer has been used for the preparation of these particulate systems, sustained release preparations, implants and inserts for their administration through parenteral, oral, dermatological, pulmonary, nasal and ocular routes (Vandervoort and Ludwig, 2002).

Intravitreal administration of aciclovir has demonstrated to be more effective than intravenous administration for the treatment of some ocular pathologies such as herpes simplex virus retinitis and acute retinal necrosis, a virus infection characterised by necrosis of retinal cells that can lead to irreversible blindness. Nevertheless, due to its short vitreous half-life (2.98 h) (Hughes et al., 1996) it is necessary to administer relative high doses with the disadvantage of the side effects or to administer several doses frequently to maintain therapeutic drug concentrations in the site of action. But successive intraocular injections are poorly tolerated with risks such as endophthalmitis, cataract, retinal detachment and vitreous haemorrhage. These inconveniences could be overcome by the use of drug-delivery systems able to promote prolonged release of the drug into the vitreous cavity such as biodegradable microspheres. Microspheres, however, would constitute a poor delivery device if the release control of the core material were impossible. By modifying the microspheres preparation parameters, it is possible to exert control on the in vitro release profile. Thus, in a previous study carried out by the authors, aciclovir-containing PLGA microspheres (1:10 drug:polymer ratio) with several additives were prepared by the solvent evaporation method to identify a potential microspheres formulation which would provide controlled and predictable release kinetics including a minimal burst effect and a long-term release by the administration of a single intravitreous injection with the minimum dose of microspheres. The best results were obtained when gelatin, as the stabiliser agent, was incorporated in the preparation of microspheres. The release constant of aciclovir from these microspheres was 1.13 μg/mg per day microspheres for 49 days, a period of time longer than 14 days obtained by Conti et al. (1997). It was estimated that an amount of 0.74 mg of microspheres would be enough for the treatment of herpes simplex and Epsteins–Barr infections, and 7.4 mg for varicella zoster infections (Martínez-Sancho et al., 2003).

On this basis, when microspheres are prepared for the administration of a drug in a practically isolated zone as the vitreous, release must allow to reach therapeutical levels with the minimum dose. Moreover, the size of microspheres has to be adequate for its injection through a suitable needle. Thus, the objective of the current study was to optimise a microspheres formulation for intravitreal administration in order to minimise as much as possible the dose of microspheres to be administered with a suitable particle size for its injection through a 27G needle in a single dose. For this purpose, a central rotatable composite 2^2 + star experimental design was applied where two variables, aciclovir and gelatin, were studied. Aciclovir was chosen because it influences on the incorporated amount into the microspheres and the amount of microspheres to be administered, and gelatin because improved the release rate of aciclovir from PLGA microspheres when added to the external phase of the emulsion. The amount of aciclovir included in formulations ranged from 40 to 120 mg and gelatin from 9.5 to 150.5 mg, according to the experimental design. The studied responses, in all formulations, were the yield of production, encapsulation efficiency, initial burst release, aciclovir released from 1 to 14 days and at the end of the assay (73 days).

2. Materials and methods

2.1. Experimental design

A two-factor, five-level central rotatable composite design 2^2 + star (Cochran and Cox, 1957) was used for the optimisation procedure. This design is suitable for the exploration of quadratic response surfaces and constructs a second order polynomial model, thus helping in optimising a process using a small number of experimental runs. The design consists of two replicated centre points and the set of points lying at the

Table 1
Variables and level of variation 2^2 + star design

Level of variation	Aciclovir (mg)	Gelatin (mg)
Independent variables		
−1.414	40	9.5
−1	51.6	30
0	80	80
1	108.4	130
1.414	120	150.5
Dependent variables		
Y_1: yield of production (%)		
Y_2: encapsulation efficiency (%)		
Y_3: initial burst release (%)		
Y_4: cumulative amount of aciclovir released from 1 to 14 days (μg/mg microspheres)		
Y_5: cumulative amount of aciclovir released at the end of the assay (μg/mg microspheres)		

midpoints of each edge of the multidimensional cube that define the region of interest. A rotatable design puts the star points on a circle around the centre of the design, giving equal predictive power in all directions. The studied factors were aciclovir and gelatin. The different formulations of the design consisted of all possible combinations of the factors at all levels and were conducted in a fully randomised order. The independent factors and measured responses are listed in Table 1. The replicate design number was 2 with a total of 30 experiments.

2.2. Materials

Aciclovir (acicloguanosine, 9[2-(hydroxyethoxy) methyl]-guanine) was supplied by Reig Farma, S.A. (Madrid, Spain). PLGA 50:50 (Resomer® RG502, inherent viscosity 0.2 dL/g, weight-average molecular weight M_w 15,000 Da) was purchased from Boehringer Ingelheim Chemicals Division (Ingelheim, Germany). Polyvinyl alcohol (PVA) 72,000 M_w and gelatin (type A, 100–120 bloom) were obtained from Fluka Chemie AG (Germany) and Merck (Spain), respectively.

Dichloromethane (CH_2Cl_2) and sodium hydroxide solution, analytical grade, were provided by Merck (Barcelona, Spain).

2.3. Preparation of microspheres

Microspheres were obtained by the O/W emulsion solvent evaporation technique (Beck et al., 1979; Martínez-Sancho et al., 2003).

Briefly, 400 mg of PLGA were dissolved in 1 ml CH_2Cl_2 by vortex mixing. The appropriate amount of aciclovir according to the experimental design (40–120 mg, with respect to a drug:polymer ratio from 1:10 to 3:10) was suspended in the organic solution with a vortex mixer (IKA Labortechnik, Germany). The aqueous phase consisted of a 0.1% PVA solution, including the respective amounts of gelatin (9.5–150.5 mg). The solution was prepared by dispersing gelatin in approximately 10 ml of cold 0.1% PVA, allowing the gelatin particles to swell, and afterwards heating the dispersion to 50 °C under magnetic stirring. Then, this gelatin solution was added to 0.1% PVA to complete volume (100 ml). The inner phase was slowly poured into the aqueous phase, and the solvent evaporation step was performed by continuous stirring for 3 h under room temperature. After evaporation of methylene chloride, microspheres were vacuum-filtered through a 5 μm filter, washed three times with water and freeze-dried. All formulations were kept in a dessicator until use.

2.4. Morphological characterisation

Microspheres morphology was evaluated by scanning electron microscopy (SEM, Jeol, JSM-6400, Tokyo, Japan). The dried samples were gold sputter-coated before observation by SEM at 20 kV.

Granulometric analysis of each batch of microspheres was performed with a Galai Cis-1 computerised inspection system (Galai Production Ltd., Israel) in the 0.5–150 μm range.

2.5. Differential scanning calorimetry (DSC)

Thermal analysis was performed with a Mettler 820 DSC analyser (Mettler Toledo, Switzerland). Samples (5–10 mg) were heated from 25 to 300 °C at a heating rate 10°/min in nitrogen atmosphere (flow rate 40 ml/min).

2.6. Injectability

Aciclovir microspheres (10 mg) were suspended in 1 ml of saline solution. Maximum force needed to inject this suspension using a 2 ml syringe attached to a 27G needle was determined in an Instron 4501 instrument (Instron Corporation, Canton, MA).

2.7. Encapsulation efficiency

Encapsulated aciclovir was determined by dissolving the freeze-dried microspheres (10 mg) in 1 ml of CH_2Cl_2 and the drug was extracted with 9 ml of a 10^{-4} M sodium hydroxide solution by vortex mixing for 3 min, three times. The mixtures were centrifuged at 6000 × g for 5 min and the extracted aqueous solutions were filtered through a 0.45-μm syringe filter (Tracer, Spain). The aciclovir content in the PLGA microspheres was determined spectrophotometrically at 254 nm (DU-6, Beckman, OH). The components of the microspheres did not interfere with aciclovir at this wavelength. The total amount of aciclovir was calculated from the aliquots of each extract.

2.8. In vitro release studies

In vitro drug release profiles were obtained by incubating the microspheres (10 mg) in 3 ml (sink conditions) of isotonic phosphate buffer saline (PBS) pH 7.4 under continuous shaking (100 strokes per minute) in a water bath (NE-5, Clifton, UK) at 37 °C. At regular time intervals, the PBS was removed, filtered through a 0.45 μm filter and aciclovir was quantified spectrophotometrically at 251 nm (microspheres components did not interfered with aciclovir). The same volume of fresh PBS was replaced to continue the release study. All the experiences were performed in duplicate from each batch.

2.9. Statistical analysis

The experimental data were analysed by the response surface regression procedure with a lack of fit option to fit the second-order polynomial equation:

$$y_u = \beta_0 + \beta_1 x_{1u} + \beta_2 x_{2u} + \beta_{11} x_{1u}^2 + \beta_{22} x_{2u}^2 + \beta_{12} x_{1u} x_{2u} + e_u$$

where y_u is the measured response, β_0, β_1 and β_2 are the regression coefficients, x_{1u}, x_{2u} are the studied factors and e is the error term.

The statistical analysis of the obtained results has been done by the corresponding analysis of variances of the selected experimental design, in order to determine the regression significance, its adjust to the model and the significance of the coefficients of the polynomial terms. This statistical study has been done by the Statgraphics Plus 4.0® (John Wiley and sons, New York).

The agreement between the predicted and the experimental values was determined by bias. Bias was calculated using the equation:

$$\left[\frac{\text{predicted value} - \text{experimental value}}{\text{predicted value}}\right] \times 100$$

3. Results and discussion

The objective of the present study was to optimise a formulation of biodegradable microspheres loaded with aciclovir to improve the release rate of the drug. The studied variables were aciclovir and gelatin. Aciclovir was chosen in order to minimise the amount of microspheres to be administered, and, based on previous studies carried out by the authors, gelatin was selected from several additives (fatty and non-fatty) because it provided an improvement of the in vitro release rate of aciclovir from PLGA microspheres.

In this work, morphologically, SEM revealed that all microspheres obtained from the experimental design resulted in spherical shapes and possessed a smooth surface (Fig. 1). The particle size is an important microsphere property, as it can influence the biopharmaceutical properties of the particle preparations. The particle size of microspheres was not significantly influenced by the amount of aciclovir and gelatin. The mean particle size of microspheres prepared from the formulations was 42.36 ± 15.56 μm, being considered suitable for intravitreal administration through a 27G needle (inner diameter 0.19 mm).

DSC measurements showed the same thermal behaviour in all microspheres. The PLGA T_g value (44.67 °C) and the aciclovir melting endotherm (242 °C) remained located practically at the same temperatures for all the microspheres formulations.

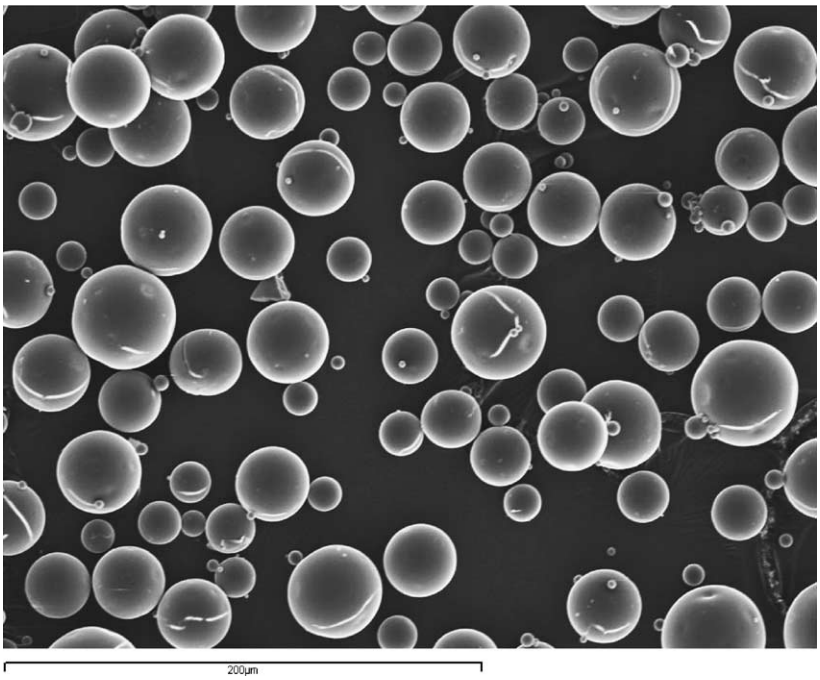

Fig. 1. SEM photograph showing the spherical shapes and smooth surfaces of microspheres.

The thermograms also showed a broad endotherm in the range 75–125 °C corresponding to a loss of residual water and a small endothermic peak around 175 °C probably due to the fusion of a morphous form of aciclovir. As an example, the thermogram of the formulation prepared with 80 mg of aciclovir and 80 mg of gelatin (0,0) of the experimental design, is shown in Fig. 2.

The results obtained for the measured responses of the experimental design and two replicates (mean values ± S.D.) are listed in Table 2.

The yields of microparticles (Y_1) were up to 50% (most of formulations had yields of more than 60%), which reflects a good efficiency of the preparation method. This response was significantly affected by the amount of aciclovir employed in the microspheres preparation ($P < 0.05$). The yields of microspheres lower to 60% corresponded to those formulations prepared with amounts of aciclovir related to a drug:polymer ratio higher than 2.7:10, which is logical because these formulations have a high solute ratio promoting the precipitation of

Table 2
Observed experimental data for the design and two replicates (mean values ± S.D.) of the responses for the design 2^2 + star

Formulation	Y_1	Y_2	Y_3	Y_4	Y_5
1 (−1,−1)	66.18 ± 1.46	49.02 ± 1.79	3.79 ± 0.85	3.38 ± 0.28	54.96 ± 1.42
2 (1,−1)	60.03 ± 3.59	17.61 ± 1.50	3.44 ± 0.16	3.28 ± 0.10	38.06 ± 1.11
3 (−1,1)	63.58 ± 2.51	60.30 ± 1.12	2.17 ± 0.60	4.62 ± 0.62	69.56 ± 0.87
4 (1,1)	56.88 ± 4.15	23.88 ± 0.45	8.69 ± 0.02	6.53 ± 0.54	51.50 ± 0.81
5 (−1.4,0)	67.36 ± 1.84	70.83 ± 1.71	1.28 ± 1.32	13.56 ± 1.61	64.23 ± 1.45
6 (1.4,0)	58.98 ± 0.95	18.24 ± 1.15	9.82 ± 1.26	11.46 ± 1.25	41.66 ± 2.89
7 (0,−1.4)	63.10 ± 2.69	44.32 ± 2.62	7.07 ± 3.20	6.48 ± 0.28	73.00 ± 3.81
8 (0,1.4)	62.57 ± 2.22	47.04 ± 1.59	7.44 ± 0.98	13.24 ± 0.80	79.40 ± 2.82
9 (0,0)	71.94 ± 3.47	71.40 ± 2.97	4.56 ± 0.44	32.72 ± 1.65	117.63 ± 4.31
10 (0,0)	68.34 ± 3.60	70.13 ± 2.66	6.45 ± 1.00	26.96 ± 3.11	118.83 ± 4.63

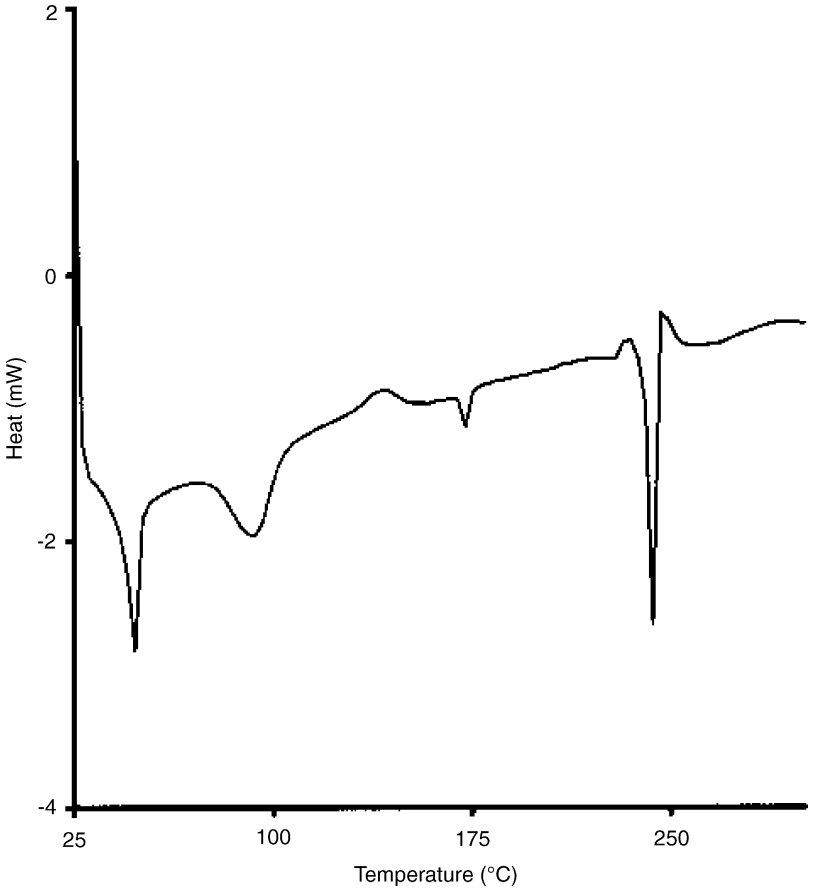

Fig. 2. DSC thermogram of aciclovir PLGA microspheres formulation (0,0).

the polymer which leads to a low yield of production.

The values for encapsulation efficiency of aciclovir in PLGA microspheres (Y_2) ranged between $17.62 \pm 1.51\%$ and $70.77 \pm 2.62\%$, and were significantly influenced ($P < 0.05$) by the two studied factors (aciclovir and gelatin). Very low encapsulation efficiency values were observed for those formulations which incorporated an aciclovir:polymer ratio higher than 2:10, this was probably due to the fact that during the microspheres preparation process a suspension is formed (aciclovir is not soluble in methylene chloride) whose stability decreases when a high proportion of drug was incorporated, decreasing the percentage of aciclovir incorporated into the microspheres. The obtained values for this parameter (Y_2) were fitted to the selected model ($R = 0.96$), according to the following equation: $Y_2 = 70.76 - 17.77X_1 + 2.67X_2 - 14.96X_1^2 - 14.39X_2^2 - 1.25X_1X_2$, being the coefficient term which reflects the interaction between the variables (X_1 and X_2), not significantly different from zero. Optimum formulation for this response corresponded to (−0.60,0.12).

Optimum microspheres formulation was selected considering not only the yield of production and encapsulation efficiency, but also the drug release characteristics including the initial burst. Tzafriri (2000) assumed that the total drug release is supplied by two uncoupled pools, one pool of fast drug diffusion (responsible from the burst), and another pool of slow diffusion drug controlled by polymer degradation. The relative dominance between diffusion and erosion plays a major role in the release kinetics. In particular, the velocity of erosion, the effective dif-

fusion coefficient of the drug molecule in the wetted polymer, the average pore length, and the initial pore diameter are sensitive parameters, whereas the porosity and the effective diffusion coefficient of the drug in the solvent-filled pores are seen to have little influence, if any, on the release kinetics (Lemaire et al., 2003).

The initial burst release is sometimes attributed to the rapid release by diffusion of dissolved drug initially deposited inside the pores. The most commonly supported hypothesis for the explanation of the burst is that some drug particles could have migrated at the surface during the drying of microspheres (Cohen et al., 1991). In this work, the percentage of initial burst release (Y_3) for all formulations was low, ranging between $1.28 \pm 1.32\%$ and $9.82 \pm 1.26\%$ of formulations (−1.4,0) and (1.4,0), respectively. There was a significant effect of the variable aciclovir ($P < 0.05$), with an increase of the initial burst release when increasing the incorporated amount of aciclovir. Despite there can be found differences, the initial burst releases were always low.

The aciclovir release profiles of all formulations in pH 7.4 PBS are shown in Figs. 3 and 4.

As can be observed in formulations 1, 2, 3, 4, 7 and 8 aciclovir was released from 50:50 PLGA microspheres slowly within 1–14 days. Afterwards, aciclovir was continuously released for approximately 70 days. This behaviour was concordant with the release kinetic of drugs from PLGA, where, in general, a two-phase release can be observed, firstly a slow release (diffusion phase) followed by a faster release (bulk erosion). But aciclovir release for the first 14 days from formulations 5, 6, and 9 was not as slowly as observed from day 14 onwards, probably due to the optimum amount of gelatin incorporated in the preparation process of these microspheres (80 mg). According to Lemaire et al. (2003) release profiles from porous biodegradable matrices depends not only on the interaction of polymer erosion and drug diffusion, but also on the structure of the porous microenvironment. In our case, incorporation of gelatin, in the appropriate amount, in the outer phase of the emulsion would lead to a change in the physicochemical characteristics of this phase which

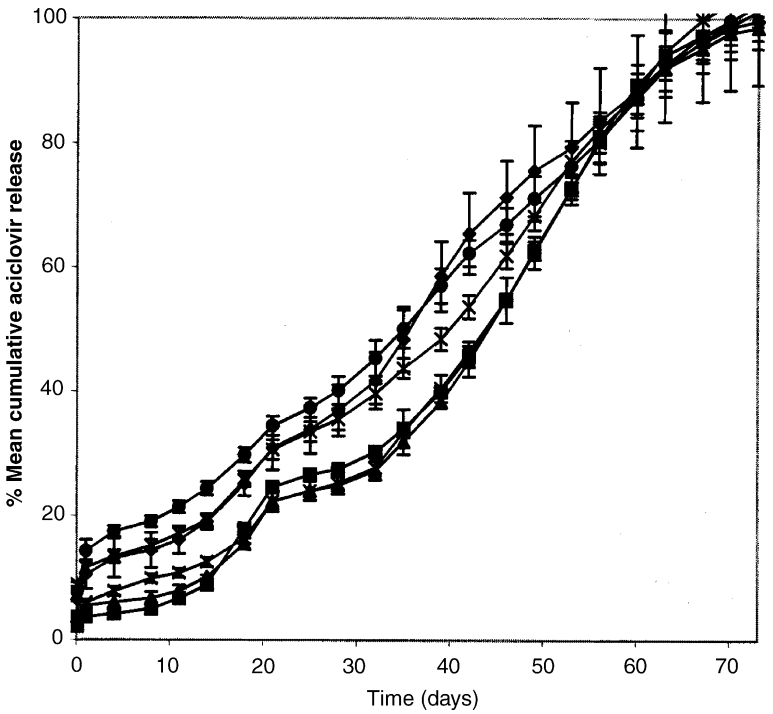

Fig. 3. Mean cumulative release (%) profile of formulations 1 (▲), 2 (×), 3 (■), 4 (+), 7 (◆) and 8 (●), and their replicates from 10 mg microspheres in 3 ml PBS.

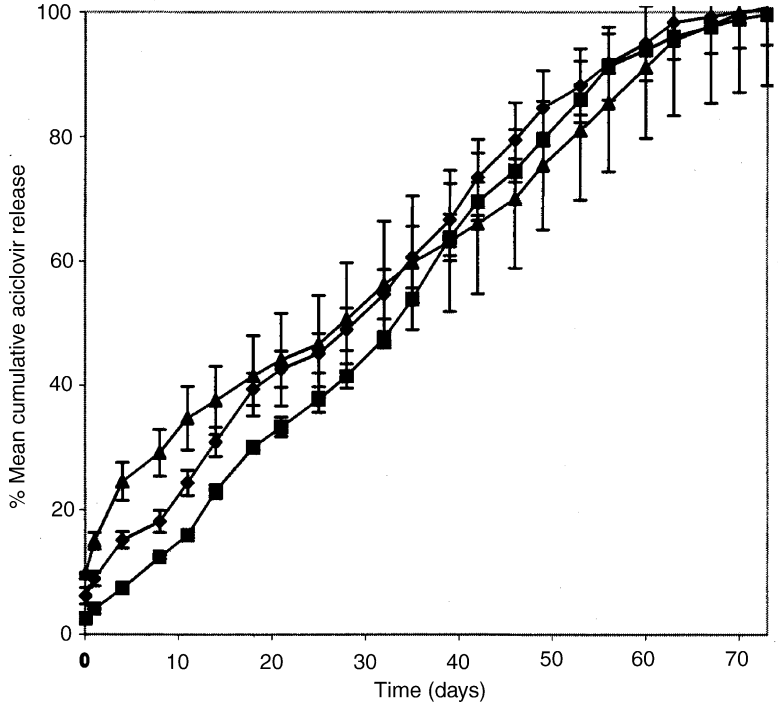

Fig. 4. Mean cumulative release (%) profile of formulations 5 (■), 6 (▲), 9–10 (◆), and their replicates from 10 mg microspheres in 3 ml PBS.

can influence on the evaporation rate of the polymer and the microparticle structure can be affected. In the microspheres preparation process, a porous structure is generated and the drug released depends on the pore size. The drug molecules in the microspheres are either trapped within the polymer, or deposited inside the pores. During the release phase, a drug molecule located inside a pore naturally diffuses towards one of the endpoints of the pore, and eventually reaches the outside. A drug molecule located within the network of micropores first diffuses toward the closest pore. At the same time, the internal surface of the pores erodes slowly by its contact with the solvent, thereby bringing parcels of the polymer and additional material to the outside. When the suitable amount of gelatin is used in the microspheres preparation, the pore generation is improved, the drug diffusion is increased, thus accelerating the release of aciclovir. A SEM photograph illustrating the internal porous structure of the studied microspheres is shown in Fig. 5.

These facts take place particularly during the first 14 days of the present release assay. The cumulative amount of aciclovir released for 14 days is very important to reach therapeutical levels with the minimum dose of microspheres because if the drug release were too low the drug concentration in the vitreous would not be enough. That was the reason why it has also been studied this response, the amount of aciclovir released during this period of time. The optimum value for this response (Y_4) corresponded to formulation (0,0.06), closes to formulation (0,0). The experimental values showed a significant effect of the gelatin variable ($P < 0.05$). The different release rate of formulations was probably due to gelatin, because when it is incorporated in the appropriate amount in the microspheres preparation, it accelerates the diffusion/dissolution process in the first stages of the release.

Aciclovir and gelatin significantly influenced cumulative amount of aciclovir/mg microspheres released at the end of the assay ($P < 0.05$). The range of responses was 38.06 ± 1.11 μg/mg microspheres in formulation (1,−1) (minimum) to 118.4 ± 4.25 μg/mg microspheres in formulation (0,0) (maximum). This response was adequately fitted to the selected model

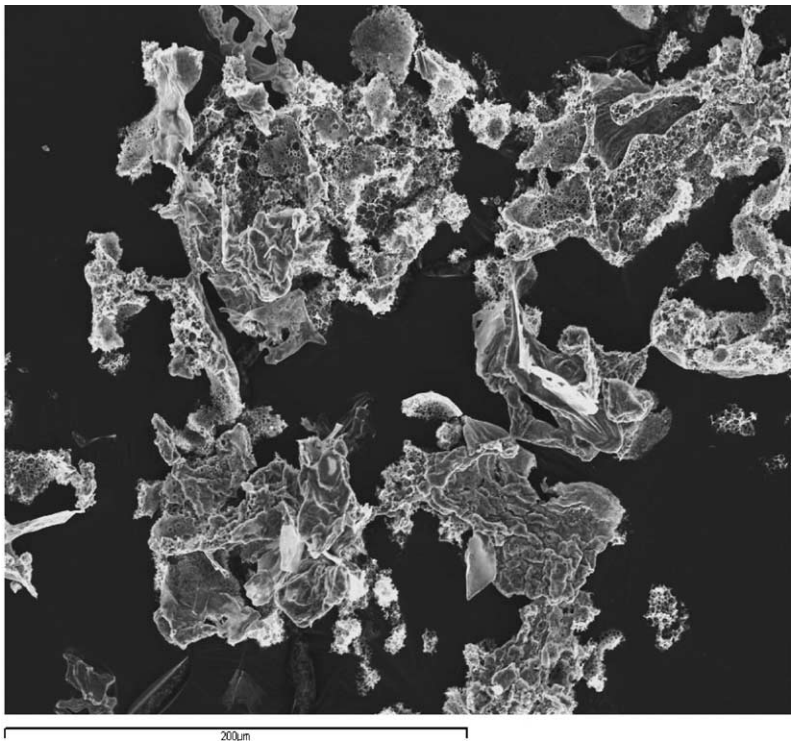

Fig. 5. SEM photograph illustrating the internal porous structure of the microspheres in the 73rd day of the release assay.

according to the equation $Y_5 = 118.4 - 8.36X_1 + 4.63X_2 - 35.48X_1^2 - 23.86X_2^2 - 0.29X_1X_2$. The regression coefficient obtained for Y_5 was 0.95. In order to assess the reliability of the equation that describes the influence of the factors on the microspheres characteristics, in Table 3 there is a comparison between the experimental and predicted values (mean values ± S.D.) of the response Y_5. It can be seen an agreement between the predicted and the experimental values, since low values of bias were found. For this reason it can be concluded that this equation describes adequately the influence of the selected independent variables on the response under study.

Table 3
Observed and fitted values (mean values±S.D.) for the Y_5 response of the experimental design and two replicates

Formulation	Observed value	Fitted value	Bias (%)
1 (−1,−1)	54.96 ± 1.42	62.48 ± 0.37	12.02 ± 1.98
2 (1,−1)	38.06 ± 1.11	46.34 ± 0.37	17.83 ± 2.86
3 (−1,1)	69.56 ± 0.87	72.33 ± 0.36	3.81 ± 1.41
4 (1,1)	51.50 ± 0.81	55.03 ± 0.37	6.41 ± 1.50
5 (−1.4,0)	64.23 ± 1.45	59.24 ± 0.37	8.41 ± 1.80
6 (1.4,0)	41.66 ± 2.89	35.60 ± 0.37	13.85 ± 5.58
7 (0,−1.4)	73.00 ± 3.81	64.11 ± 0.37	13.85 ± 5.58
8 (0,1.4)	79.40 ± 2.82	77.23 ± 0.37	3.73 ± 2.76
9 (0,0)	117.63 ± 4.31	118.40 ± 0.37	3.14 ± 1.17
10 (0,0)	119.16 ± 4.98	118.40 ± 0.37	3.16 ± 0.93

According to the applied model, the optimum value for this response (Y_5) corresponded to formulation (−0.1,0.09), which was practically (0,0) of our experimental design, prepared with 80 mg of aciclovir and 80 mg of gelatin, and this was located in the optimum zone of the response surface (Fig. 6).

This formulation did not correspond to that which showed the best encapsulation efficiency (−0.60,0.12), but it released the highest amount of drug at the end of the assay, which was the total amount incorporated in the microspheres. This formulation also presented a high yield of production (70.14 ± 3.72%) and encapsulation efficiency (70.77 ± 2.62%) being considered as the optimum one. The release kinetic of this formulation from 1 to 14 days was similar than from 14 days onwards

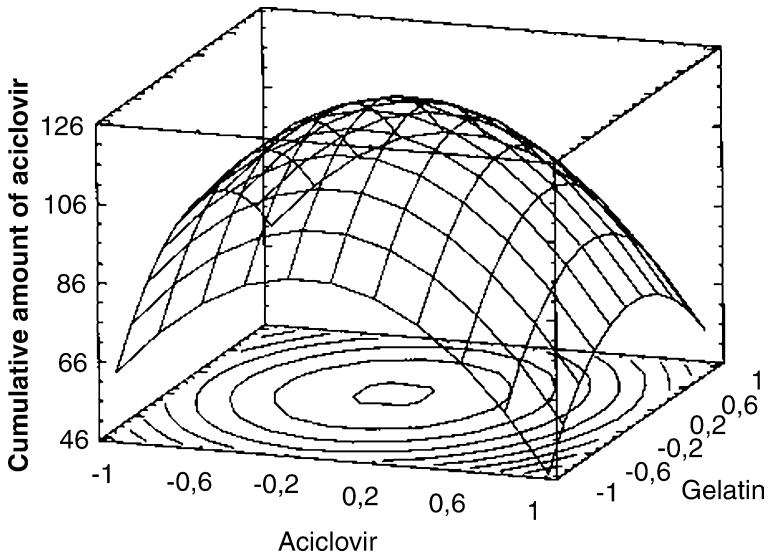

Fig. 6. Surface plot showing the effect of aciclovir and gelatin on the response Y_5.

and can be adjusted to a linear release curve. This behaviour can be observed when the quantity of drug released by diffusion equals the quantity of drug transferred by erosion. Thus, experimental values were adjusted to a zero-order kinetic from 1 to 63 days ($r > 0.99$) in all cases ($n = 6$), being the mean release constant $1.73 \pm 0.08\,\mu$g/day per mg microspheres. Several authors (Conti et al., 1997) have prepared aciclovir microspheres by the spray drying technique with PLA and PLGA. These authors found that the slower release rate was promoted by PLA (M_w 28,000). These microspheres released a 50% of the encapsulated drug in 8 days (end of the assay). In our case, the selected formulation showed a slow release rate, being the 50% of the incorporated drug released in 30 days. A prolonged release the drug is important for the treatment of ocular pathologies caused by herpes simplex and varicella zoster viruses, which consists of two phases: an induction phase and a maintenance phase that implies long-term treatments for several weeks to prevent reinfections. In our case, administration of a single dose of the selected formulation would be enough to maintain drug levels during a period of time necessary for this kind of treatments. Due to the high loading and its adequate release properties, the selected formulation (2:2:10 aciclovir:gelatin:polymer ratio) could offer a good alternative to successive injections, avoiding the risk of cataracts, retinal detachment, haemorrhages and endophthalmitis. Taking into account the mean release constant ($1.73 \pm 0.08\,\mu$g/day per mg microspheres), and knowing the vitreous distribution volume in rabbits (1.5 ml), the vitreous elimination half-life of the drug (2.98 h), and the in vitro sensitivity of herpes simplex virus, types 1 and 2, to aciclovir (up to $0.1\,\mu$g/ml) and of varicella zoster virus (up to $1\,\mu$g/ml) (Brigden and Whiteman, 1985), it could be estimated that 0.48 and 4.8 mg of microspheres, respectively, could deliver an adequate amount of the drug in a single injection into the vitreous of an animal model which is in the therapeutical level for 63 days. These doses of microspheres resulted a 40% lesser than those obtained in previous studies of the authors, which demonstrated that the addition of several substances (fatty and non-fatty additives) improved the release rate of aciclovir from PLGA microspheres, with the best results when gelatin was added to the external phase of the emulsion. This formulation corresponded to (−1.4,0) of the design.

The redispersion characteristics of the selected formulation in saline solution without viscous agent were good and particles showed no aggregation. In accordance with practical experience, these microspheres showed an adequate injectability (12.6 N) over 10 s for a 27G needle, the minimum diameter used for intravitreal administration (Herrero-Vanrell and Refojo,

2001). Furthermore, the administration of this pharmaceutical dosage form through a 27G needle does not require surgical incision or retirement (biodegradable carriers disappear from the site of action once the drug has been released from it).

Microspheres for intraocular administration have been under evaluation for ophthalmic drug delivery purposes for the two past decades, as well as biodegradable polymers for the sustained delivery of drugs. These polymers do not require removal after drug release and are completely eliminated from the body after degradation in non-toxic products. Moreover, poly(α-hydroxy acids) as polylactide, polyglycolide or their copolymers are known to be perfectly biocompatible (Visscher et al., 1985). Although the intraocular administration of particulate systems carries the risk of blurred vision and a foreign body reaction, these adverse effects gradually decrease with time and disappear when the polymer is hydrolysed in its monomers (lactic and glycolic acids). However, when injected in the eye, care must be taken to inject the microspheres in such a way that they do not interfere with the visual pathway. The rate of degradation of microspheres in vivo depends on the presence of drug, the amount of injected microparticles, the nature and molecular weight of the polymer, and the surface area of particles. In our case, the use of an amorphous 50:50 PLGA, the low molecular weight of this polymer and the small size of the microspheres may affect the rate of degradation of microspheres making it faster. Furthermore, the high payloading obtained promotes the generation of pores in the inner matrix enhancing the breakdown of the particles at the first stages of the release assay. These facts make the microspheres reported in this work potentially useful for intravitreal injection.

4. Conclusions

The application of a two-factor five-level central rotatable composite 2^2+star design resulted a useful tool for the characterisation and optimisation of aciclovir PLGA microspheres prepared by a O/W emulsion solvent technique. The multiple regression analysis of the obtained results led to polynomial equations that describe adequately the influence of the selected variables (aciclovir and gelatin) at different levels on the responses under study in the present work. According to the studied factors, the selected optimum formulation was that prepared with 80 mg of aciclovir and 80 mg of gelatin corresponding to (0,0) of the experimental design. This formulation released aciclovir at a constant rate for 63 days allowing the treatment of ocular pathologies caused by herpes simplex and varicella zoster viruses by intravitreal administration of a single dose of 0.48 and 4.8 mg of microspheres, respectively, with a 40% reduction of the dose of microspheres to be administered with respect to previous studies.

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