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A mathematical model for interpreting in vitro rhGH release from laminar implants

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Abstract

Recombinant human growth hormone (rhGH), used mainly for the treatment of growth hormone deficiency in children, requires daily subcutaneous injections. The use of controlled release formulations with appropriate rhGH release kinetics reduces the frequency of medication, improving patient compliance and quality of life. Biodegradable implants are a valid alternative, offering the feasibility of a regular release rate after administering a single dose, though it exists the slight disadvantage of a very minor surgical operation. Three laminar implant formulations (F_1, F_2 and F_3) were produced by different manufacture procedures using solvent-casting techniques with the same copoly(D,L-lactic) glycolic acid (PLGA) polymer (Mw = 48 kDa). A correlation in vitro between polymer matrix degradation and drug release rate from these formulations was found and a mathematical model was developed to interpret this. This model was applied to each formulation. The obtained results where explained in terms of manufacture parameters with the aim of elucidate whether drug release only occurs by diffusion or erosion, or by a combination of both mechanisms. Controlling the manufacture method and the resultant changes in polymer structure facilitates a suitable rhGH release profile for different rhGH deficiency treatments.

Keywords: PLGA; rhGH; Polymeric laminar implant; Solvent-casting; Drug release

1. Introduction

Recombinant human growth hormone (rhGH) is used to treat short stature in children due to growth hormone deficiency (GHD), human immunodeficiency virus-associated wasting, Turner's syndrome and growth failure due to chronic renal insufficiency; and it has also been approved for treatment of adults with GHD. In addition, because of its anabolic effects, there is clinical evidence that rhGH may be useful in treating trauma, clinical malnutrition, and osteoporosis and in wound healing (Takada et al., 2003a). Currently, rhGH requires daily injections over a period of several years, causing the patient discomfort with risk of dosing errors and noncompliance. Reduced frequency of medication would greatly improve patient compliance and increase patient convenience in terms of quality of life. Controlled-release systems based on biodegradable polymers have been studied so as to reveal the influence of different polymer variables, such as monomers ratio, molecular weight (Mw) and preparation method, on the release rate. Takada et al. prepared biodegradable microcapsules of rhGH, resulting in a controllable sustained release profile by selecting the proper copoly(D,L-lactic)glycolic acid (PLGA) (Takada et al., 2003b). After this study, the same authors achieved a pharmacological action equivalent to daily rhGH injections using the correct ratio of lactic/glycolic (L/G) and Mw of the polymer (PLGA) to obtain microcapsules with 2-week or 1-month sustained-release profiles (Takada et al., 2003a). A different rhGH release profile was observed from PLA (semi-crystalline) and PLGA (amorphous) microspheres with different pore wall morphology. Release from PLA microspheres was complete after approximately 20% of initial burst but PLGA microspheres showed a similar burst level followed by much slower, incomplete release after the same one month period (Kim and Park, 2004). A laminar implant formulation provided a sustained release of rhGH for at least 2 weeks and a greater efficacy than the frequent administration of the conventional injectable (García et al., 2002). The main advantage of this formulation is the versatility of its design which permits adjusting the formulation variables to optimise the release rate

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although, as a minor drawback, its administration requires a very small surgical operation.

Laminar implants can be classified into two main types; monolithic and reservoir devices. In the first type, the drug is dispersed in a polymer matrix. In the second type, the therapeutic agent forms a core surrounded by a polymer barrier. Combinations of both devices can also be prepared. Drugs are released from these systems by diffusion through the polymer barrier, by its erosion, or by a combination of both mechanisms (Jain et al., 1998). On the other hand, drug release profiles from bulk-eroding PLGA matrices are complex because the polymer phase properties change continuously during degradation, resulting in drastic changes in drug diffusivity and permeability (Kunou et al., 2000). Shah et al. produced solvent-cast films in which the onset and rate of their more rapid drug release correlate directly with the kinetics of polymer Mw loss. In this case, the erosion occurs by a bulk rather than a surface process, depending on the rate of hydrolytic scission of the polyester chain and the time required to reduce the initial polymer Mw to an oligomeric range (Shah et al., 1992). The formulation variables to be taken into account in the laminar implants manufactured by solvent-casting methods are: polymer and solvent characteristics, solvent evaporation method (Zilberman et al., 2001), mould material and, finally, the different implant preparation procedures. The PLGA polymers form easy-to-handle transparent laminar implants. Dichloromethane (DCM) used as solvent, after slow reduced-pressure evaporation at 5 °C, produces homogeneous films without holes or trapped air. Whereas teflon, aluminium and polyvinyl chloride (PVC) are the best materials to optimize separation between the film and the mould, without tearing (Dorta et al., 2002; Santoveña et al., 2004; Santoveña et al., in press). Implant preparation varies from the simplest method of drug and polymer dispersion (Dorta et al., 2002; Santoveña et al., 2004), a mixture of drug solution and polymeric dispersion (Bodmeier and Chen, 1989; Shah et al., 1992; Gould et al., 1994) to a combined method of solvent-casting and compression techniques (Hashizoe et al., 1994) using combined multilayer discs with a central drug monolithic layer, or multilayer discs with a central lyophilized drug reservoir (Santoveña et al., in press). Wang et al. manufactured two polymeric devices with a central hole, one passing through the polymer matrix and the other on only one side, which permit simple drug diffusion by first order and zero order kinetics, respectively (Wang et al., 1996). Release from polymeric implants can be delayed by immersion in a polymer solution or by contact with an organic solvent that dissolves the polymer but not the drug (Shah et al., 1992; Bodmeier and Chen, 1989).

Our aim in the present study was to compare the rhGH release profiles from different types of laminar implants and their relationships with the changes in polymer Mw in the formulation, employing a mathematical model that relates hormone release rate with the polymer matrix degradation.

2. Materials and methods

Lyophilized rhGH was provided by Novo Nordisk as Norditropin® vials. Poly(D,L-lactic-co-glycolic acid) copolymer was obtained in our laboratory by ring-opening polymerization of its dimers. All other reagents were those of analytical grade.

2.1. PLGA synthesis

The biodegradable PLGA was synthesised by ring-opening polymerisation of initial monomers D,L-lactide (Aldrich) and glycolide (Boehringer, Ingelheim) following the method described by Gilding and Reed (1979) (Gilding and Reed, 1979). D,L-Lactide (LA) was recrystallized in ethyl acetate (Merck) at room temperature until the racemic mixture melting point was attained (124–126 °C), and the glycolide (GA) was used directly (m.p. 88–90 °C). Stannous octoate (0.1%, w/w) and lauryl alcohol (0.01%, w/w) (both Sigma) were used as catalyst and chain transfer agent, respectively. This mixture (45 g) of 75 mol% LA and 25 mol% GA was loaded into three 30 mm inner diameter glass ampoules. These ampoules were then immersed in an oil-bath maintained at 140 °C for 1 h to obtain the solid copolymer, which was extracted in chloroform (Merck) followed by precipitation with methanol (Merck). The polymerization yield was 98%.

2.2. Characterization of copolymers

The weight-average molecular weight (Mw) and number-average molecular weight (Mn) of the raw polymer and implants were determined by gel permeation chromatography (GPC) relative to polystyrene standards (Tokyo Soda Ltd.) with molecular weights 2800–700,000 Da, using Waters equipment (Milford, MA, USA). Tetrahydrofuran (THF) was used as the mobile phase, at a flow rate of 0.9 ml/min.

The copolymer composition was determined by nuclear magnetic resonance of proton (^{1}H NMR) with a Bruker AMX-400 spectrometer using deutered chloroform ($CDCl_3$) (Aldrich) as a solvent. The relative proportions of lactic–glycolic (LA–GA) and glycolic–glycolic (GA–GA) acid bonds were assessed by nuclear magnetic resonance of carbon 13 (^{13}C NMR) at 100.61 MHz using dimethyl-sulphoxide-d6 (DMSO-d_6) (Sigma) as a solvent (Dorta et al., 1993).

The Mn, Mw and polydispersivity of PLGA were found to be 22,000, 40,000 Da and 1.81, respectively. The ratio of LA/GA was 66:34 and of LA–GA/GA–GA was 1:2.

2.3. Preparation of rhGH-PLGA laminar implants

Three different formulations of PLGA laminar implants (F_1, F_2 and F_3) containing freeze-dried rhGH were prepared using a solvent-casting technique (Santoveña et al., in press).

2.3.1. Formulation F_1

An aqueous solution of Norditropin® containing 0.078% (w/v) rhGH was poured into 40 mm diameter teflon moulds and freeze-dried in a Labconco Lyphlock 6 by a freezing step of 30 min at −45 °C, a primary drying step of 24 h at −10 °C; and finally, a secondary drying step of 10 h at 5 °C. A 20% (w/v) PLGA solution in dichloromethane (DCM) was then poured onto the lyophilized cake and the DCM allowed to evaporate

slowly at 4 °C for 48 h. The resulting rhGH polymer-film was then vacuum-dried in a desiccator at room temperature for 12 h so as to remove any residual solvent, then cut into 6 mm diameter discs and 420 ± 29 μm (n = 10) thick (Mitutoyo digital micrometer). Theoretical declared rhGH content was 85 μg.

2.3.2. Formulation F_2

The Norditropin® solution (rhGH: 0.078%, w/v) was dispersed into the PLGA solution (20%, w/v) and mixed (Vortex) prior to casting in teflon moulds at 4 °C for 48 h. The resulting rhGH polymer-film was then vacuum-dried for 12 h in a desiccator at room temperature to remove the residual DCM, then cut into 6 mm discs. The thickness of these films was 350 ± 20 μm (n = 10), the theoretical declared rhGH content being equal to the F_1 formulation.

2.3.3. Formulation F_3

A combined method of both solvent-casting and compression techniques was used to prepare formulation F_3. Firstly, two drug-free PLGA-films were prepared by casting 3 ml of polymer solution on two teflon moulds, then we let the DCM to be evaporated slowly at 4 °C for long enough to remove any residual solvent. Afterwards, 2.5 ml of an aqueous solution of Norditropin® (rhGH: 0.078%, w/v), was cast over each PLGA-film. These polymer-films containing hormone solution were freeze-dried (Labconco Lyphlock 6) by the same cycle used for F_1, then placed as a sandwich, i.e. a drug-core completely encased by polymer. This system was compressed at 3 metric tons for 1 min at room temperature using a hydraulic manual press (Perkin-Elmer) and then cut into smaller discs, 5 mm in diameter and thickness 180 ± 11 μm (n = 10). Each disc was again encased individually by two drug-free PLGA-films, each 7 mm in diameter. The resulting double-cast discs were compressed at 1 metric ton for 4 min to obtain a final thickness of 266 ± 9 μm (n = 10). Finally, a central hole was made right through them using a 0.5 mm diameter punch. The theoretical declared rhGH content in this formulation was 60 μg.

2.4. Stability studies

The stability of rhGH in DCM and THF was tested. A known amount of hormone was suspended in a known volume of solvent and the rhGH concentration determined by size exclusion chromatography (SEC-HPLC) at predetermined intervals for 24 h. The SEC-HPLC system was used with a Shodex column and Waters system. Samples were eluted at 1.0 ml/min, with 25 mM sodium phosphate, 300 mM sodium chloride, pH 7.0. The absorbance at 214 nm was measured and the total protein in each sample determined using the ratio of peak areas and known standard concentrations. The chromatographic method was validated according to the International Conference on Harmonization (ICH) guidelines (International Conference of Harmonization, 1996). Likewise, the stability of a standard solution was tested for several days in both mobile phase at room temperature and in release medium (isotonic phosphate buffer (Sörensen) pH 7.4) at 37 °C.

2.5. Drug content determination

The rhGH content in the laminar implants was determined by dissolving each disc in THF. Once the polymer was dissolved, the dispersion was centrifuged at 4000 rpm for 10 min (Econospin Sorvall Instruments) the supernatant removed and residue vacuum-dried. This precipitate was dissolved in phosphate buffer and the hormone quantified measured by SEC-HPLC.

2.6. In vitro release studies

To determine the release kinetics, the pre-weighed loaded discs were maintained in individual vials containing 2 ml of isotonic phosphate buffer (Sörensen) pH 7.4 in a water bath (Stuart Scientific) at 37 °C for 15 days. The release medium was periodically removed and replaced by equal volumes of fresh buffer, taken into account in the calculations of the cumulative drug release analysed by SEC-HPLC as described above. The drug content in each implant in each in vitro release study was measured by dissolving each disc in THF and assaying in triplicate as described above for drug content.

2.7. PLGA degradation

The average molecular weights were determined by GPC as above, and the advance of the in vivo degradation process was expressed by the degradation index (DI) (Glynn et al., 1976), which represents the number of cleavages and bonds initially present (Dorta et al., 1993), using the calculation:

$$\mathrm{DI} = \frac{\mathrm{Mn}^0}{\mathrm{Mn}^t} - 1$$

Mn^0 is the initial Mn, and Mn^t that at time t.

3. Results and discussion

The rhGH maintained its integrity during the contact time (24 h) with the organic solvents (DCM and THF). However, there was a significant reduction (20%) in the hormone concentration after 48 h in both mobile phase and phosphate buffer. For this reason, the release medium was changed every 24 h, and the sample removal and analysis were not delayed more than 24 h.

The tested formulations conformed to the uniformity requirements for the dosage units in accordance with USP XXIII ed., the coefficients of variation (CV) of formulations F_1, F_2 and F_3 being 4.34 ± 4.63, 3.17 ± 3.29 and 1.9 ± 2.11% (n = 10), respectively. Therefore, it can be concluded that the procedure used to incorporate the hormone allows its homogeneous distribution in the film.

The in vitro rhGH release from F_1, F_2 and F_3 formulations and the DI values of the polymeric film immersed for two weeks in phosphate buffer at pH 7.4 are shown in Fig. 1A, B and C, respectively. As can be seen, the polymer degraded slowly from the first day of immersion up to 15 days, with a final DI of 2.5. In a previous study (Santoveña et al., 2004), we noted that when this type of PLGA formulations was tested in the same medium

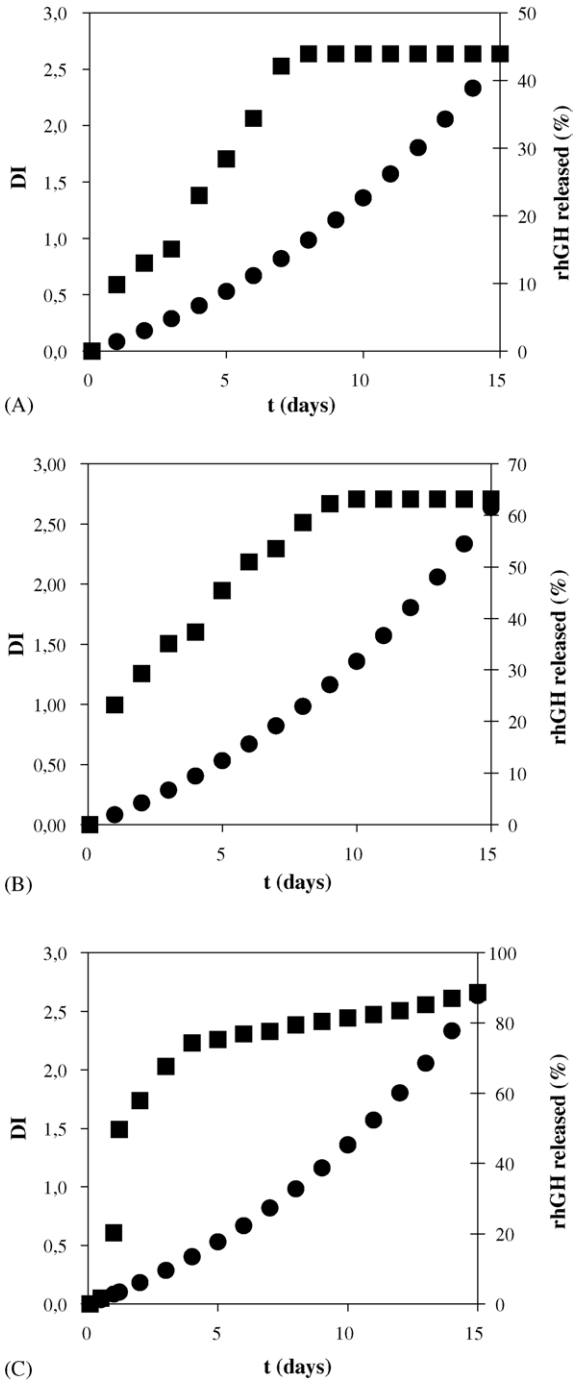

Fig. 1. rhGH in vitro release (■) and polymer implant DI values for F_1 (A), F_2 (B) and F_3 formulation (C) (●) during a 15 days period in phosphate buffer.

and time period their weight dropped slightly but continuously whereas the consistency and rigidity increased. This is a consequence of their low affinity with the aqueous medium, due to changes in conformation that minimize contact with solvent molecules (Katayama et al., 1984). In vitro, the rhGH hormone was gradually released from F_1 and F_2 devices for 8 and 10 days, respectively, followed by a plateau (Fig. 1A and B), but after 15 days only ~40% was released by F_1 formulation and ~60% by F_2. However, the release from laminar implant F_3 showed a biphasic profile (Fig. 1C), consisting of an initial high release rate (77% of rhGH dose released over the first 6 days) followed by a much slower release phase between 7 and 15 days.

Since the decrease in polymer Mw is a consequence of its degradation, this process implies changes in the internal structure of the PLGA matrix which apparently increase permeability to the drug with time, modifying the relative contribution of diffusion-erosion to the release rate (Heller, 1980).

If we assume the release rate (dX/dt) depends on the percentage of drug remaining inside the polymer matrix (X), and polymer degradation causes changes in the structure of the PLGA matrix, the next equation may be applied:

$$\frac{-\mathrm{d}X}{\mathrm{d}t} = kX \tag{1}$$

being k a parameter related to the polymer Mw as follows:

$$k = b_0 + b_1\frac{1}{\mathrm{Mw}} \tag{2}$$

where b_0 is the relative initial release rate and b_1 provides information about the contribution of polymer degradation to the release rate, showing the dependency of polymer permeability on its Mw. Then, after replacement Eq. (2) in (1), we obtain this equation:

$$\frac{\mathrm{d}X}{\mathrm{d}t} = -\left(b_0 + b_1\left(\frac{1}{\mathrm{Mw}}\right)\right)X \tag{3}$$

or the same,

$$\frac{-\mathrm{d}LnX}{\mathrm{d}t} = b_0 + b_1\frac{1}{\mathrm{Mw}} \tag{4}$$

Taking into account this last equation (Santoveña et al., 2004), there are eight possible cases of relative release rate evolution (see Fig. 2 and Table 1) depending on whether b_0 and b_1 values are positive or negative, or are equal or not to zero.

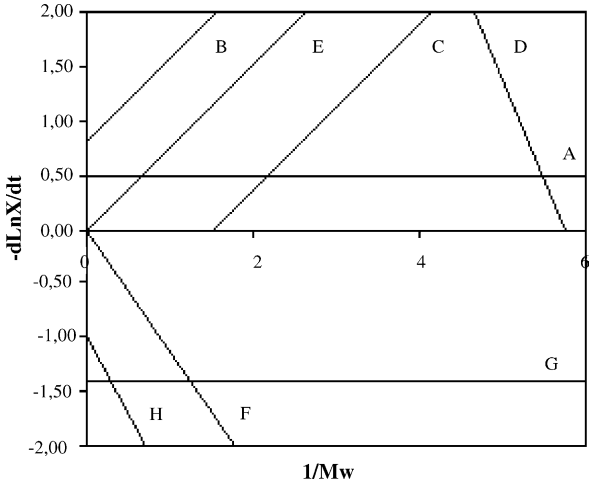

Fig. 2. Possible cases of relative release rate evolution versus the inverse of polymer Mw according to Eq. (2). The b_0 (days^{-1}) and b_1 (Da) values used to generate the plots are; case A: $b_0 = 0.5$ and $b_1 = 0.0$; case B: $b_0 = 0.8$ and $b_1 = 0.8$; case C: $b_0 = -1.1$ and $b_1 = 0.7$; case D: $b_0 = 11.6$ and $b_1 = -2$; case E: $b_0 = 0.0$ and $b_1 = 0.8$; case F: $b_0 = 0.0$ and $b_1 = -0.8$; case G: $b_0 = -1.4$ and $b_1 = 0.0$; case H: $b_0 = -1.0$ and $b_1 = -1.7$.

Table 1
Possible cases of relative release-rate evolution in the model

Case	b_0	b_1	Release rate evolution
A	>0	=0	Lumped release (Cussler, 1984)
B	>0	>0	Continuous release rate controlled by polymer degradation after an initial release rate
C	<0	>0	Release rate initially controlled by a certain polymer DI value (Santoveña et al., 2004)
D	>0	<0	Higher initial rate decreasing with time
E	=0	>0	Increasing release rate without initial rate
F	=0	<0	Decreasing release rate without initial rate
G	<0	=0	Negative lumped release
H	<0	<0	Negative initial rate followed by a release rate decreasing with time

Fig. 3 shows the relationship between the time-derivative of the logarithm of the amount of peptide released: $-\mathrm{d}LnX/\mathrm{d}t$ and the inverse of polymer Mw: 1/Mw from formulations F_1, F_2 and F_3. As can be seen in Fig. 3A, a linear relationship is achieved ($-dLnX/dt = -0.049 + 0.03 \times (1/\mathrm{Mw})$) with a correlation coefficient (r) equal to 0.829 for the first 7 days of release from formulation F_1. Up to a particular value of polymer DI (~0.2) is reached, the hormone starts being released from the implant at a rate that rose as polymer Mw fell, as in case C of the model where the initial release rate is controlled by a certain polymer DI value (Shah et al., 1992; Vogelhuber et al., 2001). When the Mw decreased after 8 days, the release rate dropped dramatically, with no association with polymer Mw degradation. For laminar implant F_2, Fig. 3B, there was a lumped release (Cussler, 1984) ($-\mathrm{d}LnX/\mathrm{d}t = 0.082$ days^{-1}; $r = 0.075$) like in case A of the model, for 7 days of incubation where the release rate stayed the same, independently of the inverse Mw value (Huang and Brazel, 2001), followed by an increase when a particular value (~1.0) of polymer DI was reached, to immediately decrease on the 9th day. Another relationship between the parameters of the model was found for this formulation between days 8 and 11, ($-\mathrm{d}LnX/\mathrm{d}t = 0.46 - 0.07 \times (1/\mathrm{Mw})$; $r = 0.966$), a first order release like case D of the model. This consists of a higher initial rate decreasing with the drop in polymer Mw value (Huang and Brazel, 2001). For formulation F_3 (Fig. 3C), a linear relationship ($-\mathrm{d}LnX/\mathrm{d}t = 1.7 - 0.39 \times (1/\mathrm{Mw})$; $r = 0.991$) is seen between the time-derivative of the logarithm of the remaining amount of rhGH and the inverse of polymer Mw, but with $b_1 < 0$ as in case D of the model, due to the lower release rate in the first 6 days of immersion in phosphate buffer. After this, no relationship between the parameters of the model was found until day 15 of release.

The different rhGH release from these types of laminar implants occurs as a consequence of their diverse manufacture procedures and matrix degradation. Because the lyophilized rhGH cake is inside the polymer film used to prepare formulation F_1, the hormone release rate from this device depends on polymer Mw variations since its diffusion from the core depends on the laminar implant degradation (Dorta et al., 2002; Santoveña et al., 2004) until the eighth day, in which the high degree of erosion allows the hormone to flow independently through the film matrix. In contrast, in the F_2 formulation, the hormone was dis-

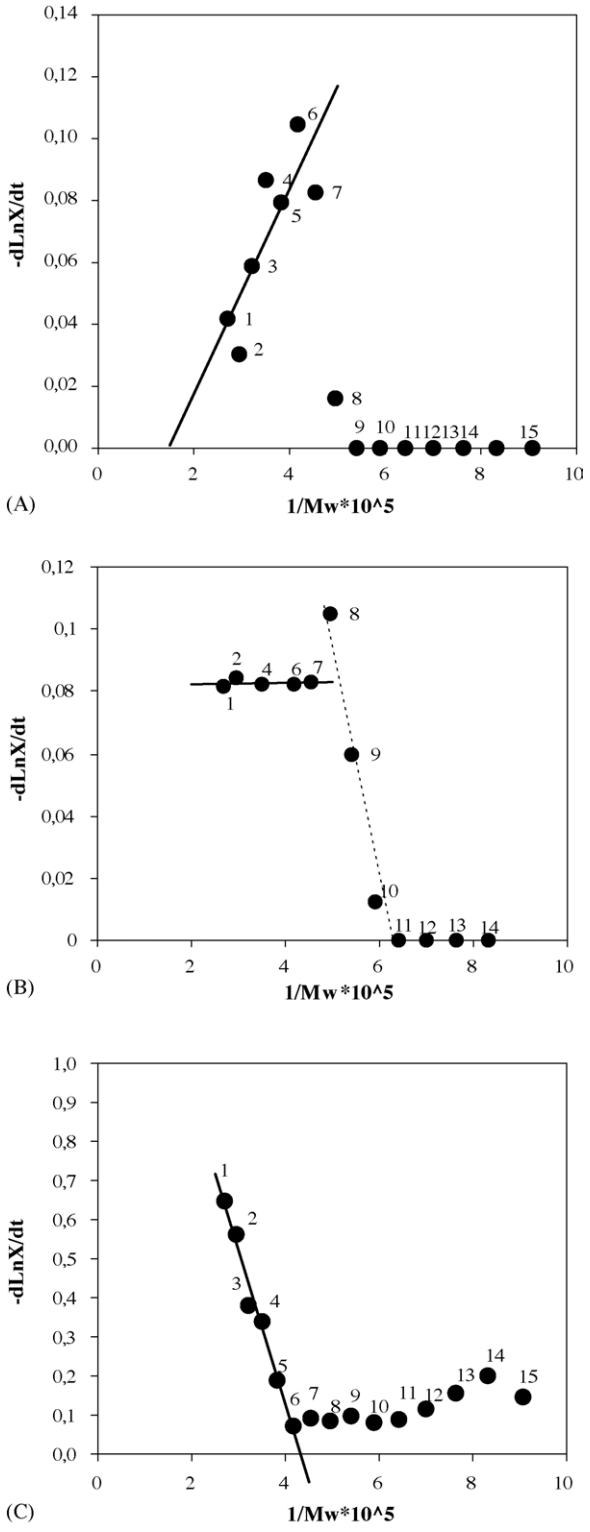

Fig. 3. Relationship between the time-derivative of the logarithm of the rhGH amount released and the inverse of polymer Mw, in formulations F_1 (A), F_2 (B) and F_3 (C). The numbers correspond to release days.

persed into the PLGA solution to prepare the film, and it should be consequently distributed as much over the surface as inside the laminar matrix (Zilberman et al., 2001; Huang and Brazel, 2001), so the hormone flows out earlier from the surface constantly for 7 days, independently of polymer degradation. The rhGH inside the polymeric structure is therefore released at an independent matrix degradation rate until the seventh day when the drug starts to flow out at a higher rate due to a combination of erosion of the PLGA matrix and diffusion of the drug through the degraded laminar implant structure. For formulation F_3, a linear relationship with a negative slope is found. This probably happens because in the first day in phosphate buffer, the hormone flows out at a higher rate from its lyophilized cake inside the implant through the central hole. On the contrary, during the final days (Wang et al., 1996) the release mechanism was not only simple diffusion but a combination of erosion and diffusion.

These different drug release profiles from different laminar polymer implants provide information about how therapeutic agent release kinetics can be affected by implant manufacture methods. Up to now, this had been obtained by the incorporation of different excipients to delay or accelerate the drug release beyond the normal dissolution characteristics of the solid drug particles in the dosage form (Cardinal, 2000; Kou, 2000). The development of this mathematical model for the manufacture of biodegradable film implants allows us to predict the shape of the drug release curve, to eliminate extensive trial and error experimentation, and to optimize laminar implant preparation conditions (Lemaire et al., 2003; Altinkaya et al., 2004).

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