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PLGA–PEG microspheres of teverelix: influence of polymer type on microsphere characteristics and on teverelix in vitro release

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Abstract

Teverelix microspheres were produced by coacervation using a new type of poly(ester-carbonates) made of block copolymers of poly(lactic-glycolic acid) (PLGA) and poly(ethylene glycol) (PEG). Five different PLGA–PEG copolymers and one PLGA were used. The 'stability window' has been determined for all polymers. It varied depending on the molecular weight and the weight percentage of PEG. With increasing core loading (from 9.4 to 34.2%), the microparticle size increased from 10–50 to 5–1000 μm. The core loading did not have any influence on encapsulation yield, which remained above 80%. The influence of polymer type on microsphere characteristics was studied at two different core loadings: 9.4 and 28%. At a low core loading, the nature of the polymer had no influence on microsphere characteristics whereas at 28%, only PLGA–PEG copolymers gave acceptable microparticles in term of particle size. At 28%, the glass transition temperature (T_g) of loaded particles was 1–8 °C higher than the T_g of the corresponding polymer. Increasing the core loading increased teverelix release whereas polymer degradation was decreased. All microparticles made of PLGA–PEG copolymers showed a faster release of teverelix than PLGA-based microspheres, whatever the core loading. One PLGA–PEG was selected on the basis of in vitro release rate for further in vivo investigations.

Keywords: Microspheres; Coacervation; PLGA–PEG; PEG; In vitro release; GnRH antagonists

1. Introduction

Aliphatic polyesters like poly(lactide) (PLA), poly(glycolide) (PGA), and especially copolymers of lactide and glycolide poly(lactic-glycolic acid) (PLGA) probably constitute the most commonly used family of bioerodible polymers for drug delivery systems at present (Vert et al., 1981, 1991; Barrows, 1991; Szycher, 1991; Asano et al., 1989). High molecular weight PLGA copolymers are commonly synthesised by ring opening polymerisation of dilactide and diglycolide (Gilding and Reed, 1979). Ferruti et al. (1995a,b) and Penco et al. (1994, 1996a,b) described a novel chain-extension process, which, starting from PLGA and poly(ethylene glycol) (PEG) oligomers, makes poly(ester-carbonates). Many block copolymers of PLGA and PEG (Zhu et al., 1990) have already been described but these polymers are usually

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di or triblock copolymers while those synthesised in Ferruti's laboratory are multiblocks copolymers. The presence of PEG in poly(ester-carbonates) is of much interest because it increases the hydrophilicity of these polymers.

One major use of biodegradable polymers in the pharmaceutical field is the preparation of sustained release formulations of peptides and proteins. Indeed, because of their poor oral bioavailability, peptides and proteins are preferably administered through the parenteral route. For continuous administration, which is often the case with peptides, the alternative to daily injections is sustained release formulation.

Sustained release formulations are microspheres (Cleland, 1997; Jain et al., 2000), implants (Medlicott and Tucker, 1999) or gels (Jain et al., 1998). Three microsphere preparation techniques are commonly used (Jalid and Nixon, 1990; Lewis, 1990; Tice and Tabibi, 1991; Wu, 1995a,b): the first one is the single or double emulsion technique followed by solvent removal by evaporation or extraction, the second one is the spray drying method and the third one is the phase separation or coacervation method.

In this paper, we compare different types of PLGA–PEG using the coacervation technique with the LH–RH antagonist Antarelix^{TM} (teverelix^{INN}) (Boutignon et al., 1999; Erb et al., 1999). The aim was to determine the relation between particle characteristics (core loading, size), copolymer composition and peptide release.

2. Materials and methods

2.1. Materials

Silicone oil 47V500 (500 cSt) was purchased from Prolabo. Dichloromethane (DCM), acetonitrile, tetrahydrofuran (THF) and chloroform were obtained from Fisher. Heptane was purchased from Riedel-deHaen and erythrosine as a dye from Fluka. Trifluoracetic acid (TFA) was obtained from Aldrich. PLGA 50/50 D,L-lactide/glycolide copolymer DL436DA was purchased from Purac (Gorichem, The Netherlands). Teverelix was prepared by Degussa AG. PLGA–PEG were synthesised in the Department of Organic and Industrial Chemistry of the University of Milan, Italy, according to published methods (Ferruti et al., 1995a,b; Penco et al., 1994).

2.2. Methods

2.2.1. Preparation of block copolymers of PLGA and PEG

The synthesis of PLGA–PEG copolymers has been already described (Ferruti et al., 1995a,b; Penco et al., 1994). Briefly, PLGA oligomers were prepared by ring opening copolymerisation of dilactide and diglycolide monomers under nitrogen at 200 °C. PLGA–PEG segmented copolymers were obtained as follows: PEG-bis(chloroformate) was prepared from PEG and phosgene in the presence of *N*-ethyl-*N*,*N*-di-isopropyl amine (EDIPA) in chloroform. The resulting PEG-bis(chloroformate) was added to a solution of PLGA oligomers in chloroform in the presence of 4,4-dimethyl aminopyridine (DMP). After polymerisation, the resulting polycarbonate was purified by several precipitations in isopropanol and in ether. The general formula of the resulting product is shown in Fig. 1.

The polymers are denoted by PLGA–PEG (*A*/*B*) where *A* is the average molecular weight of PLGA and *B* is the molecular weight of PEG (Table 1).

2.2.1.1. Characteristics of block copolymers of PLGA and PEG. Viscosity was determined by using a Schott Geräte mod AVS 310 semi-automatic viscosimeter at 30 °C, in chloroform (0.1%, w/v). Gel permeation chromatography (GPC) was run on three Waters columns: Styragel HR 0.5–2 and 4, in THF at 1 ml min^{-1}. The system was constituted with a Waters 610 isocratic pump, a Waters 717 plus autosampler injector (injection volume: 100 μl). The controller was the Waters 600E and the refractometer was the Waters 410. The equipment was controlled by Millenium (Waters). Average molecular weights were evaluated using polystyrene standards. Differential

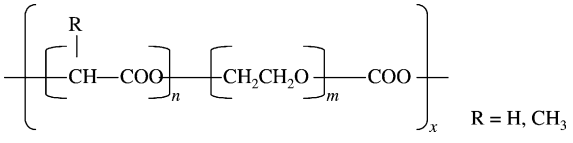

Fig. 1. General formula of block copolymers of PLGA and PEG.

Table 1
Characteristics of poly(lactic-glycolic acid) (PLGA)-based copolymers

Code: PLGA–PEG[a] (A^b/B^c)	Characteristics					
	$[\eta]^d$ (dl g^{-1})	M_n[e] ($\times 10^{-3}$ g mol^{-1})	M_w[e] ($\times 10^{-3}$ g mol^{-1})	D[f]	PEG content (%)	T_g[g] (°C)
PLGA–PEG (1800/194)	nd	37	22	6.0	10	26
PLGA–PEG (2300/600)	0.43	25	43	1.7	20	18
PLGA–PEG (1600/150)	0.50	36	64	1.8	9	32
PLGA–PEG (2000/194)	0.54	41	66	1.6	9	32
PLGA–PEG (2100/400)	0.84	58	94	1.6	16	38
PLGA[h]	0.45	24	42	1.8	0	42

nd: not determined.
[a] General formula: [–O–CO–(–CHR^1–O–CO–)x–R^2–]y, where R^1 is H or CH_3 and R^2 is PEG (number average molecular weight determined by end group titration of the starting PLGA oligomer).
[b] Molecular ratio between D,L-lactic acid and glycolic acid residues, was in all cases 1:1.
[c] B: average molecular weight of PEG.
[d] Intrinsic viscosity in chloroform at 30 °C.
[e] Average molecular weight calculated by gel permeation chromatography using a polystyrene calibration curve.
[f] D: polydispersity index: M_w/M_n.
[g] T_g: glass transition temperature determined by differential scanning calorimetry.
[h] PLGA 50/50 D,L-lactide/glycolide copolymer used as a reference, supplied by Purac.

scanning calorimetry (DSC) analyses were performed on a DSC7 Perkin-Elmer apparatus calibrated by Indium. The scanning rate was 5 °C min^{-1}.

2.2.2. Preparation of teverelix microspheres by coacervation

2.2.2.1. Phase diagrams and microscopic observations. The polymer (260 mg) was dissolved in 10 ml of DCM at room temperature in a covered beaker (Stassen et al., 1994). The solution was magnetically stirred at 250 rpm and 100 μl of concentrated aqueous solution of erythrosine was added; the stirring rate was risen to 500 rpm for a short time in order to disperse the aqueous solution, then the rate was stabilised at 250 rpm. Phase separation of the polymer solution was induced stepwise by addition of silicone oil (500 cSt, 1 ml increments) and followed by observation using optical microscopy on a Jenalab apparatus. The optimal composition of the medium induced encapsulation of the coloured aqueous droplets, as explained in the following sections.

2.2.2.2. Teverelix grinding. The peptide was manually ground by the use of a mortar and a pestle in order to obtain small particles. This step was necessary to obtain an homogeneous addition of the powder to the polymer solution.

2.2.2.3. Preparation of microspheres. This method was detailed elsewhere in the case of the use of polyesters (Jain et al., 1998; Stassen et al., 1994; Nihant et al., 1993, 1994, 1995; Thomasin et al., 1996). These techniques have been adapted to PLGA–PEG polycarbonates. The polymer (260 mg) was dissolved in 10 ml of DCM. This solution was poured into a covered beaker, the medium was thermostated at 5 °C by means of an ice bath. The solution was magnetically stirred at 300 rpm and the peptide powder was then added. After 15 min of stirring, the adequate volume of silicone oil was added to the dispersion with a polypropylene syringe. Then the phase separated system was transferred into a 250 ml reactor thermostated at 1 °C containing 200 ml of silicone oil. The medium was carefully stirred by means of a four-pitched blade propeller. After 20 min, the microparticles were filtered through a thermostated glass filter, washed with heptane and then dried overnight under vacuum at 5 °C. All microspheres were stored at low temperature (5 °C) in order to prevent the fusion of the particles, especially for particles with a glass transition temperature (T_g) below room temperature.

2.2.2.4. Characterisation of microspheres. Shape, surface and size of the microspheres were determined by microscopic observation using a micrometer

coverlid on a Jenalab apparatus. GPC was run on the same Waters apparatus as for the characterisation of the polycarbonates. Average molecular weights were evaluated using polystyrene standards. DSC analyses were performed on a DSC7 Perkin-Elmer apparatus calibrated by indium. The scanning rate was $5\,^{\circ}C\,min^{-1}$. Microspheres photographs were made on a camera adapted to the microscope apparatus.

2.2.2.5. Determination of teverelix content. Microspheres containing teverelix (15 mg) were dissolved in 2 ml of acetonitrile. After complete dissolution, 8 ml of water containing 0.1% (w/w) of TFA was added. The media was then centrifuged to separate the precipitated polymer. The supernatant was then analysed by a reverse phase HPLC using a C18 column (Merck). It was run on an Alliance apparatus (Waters), at $1\,ml\,min^{-1}$, under isocratic conditions with UV detection at 220 nm. The solvent used was a mixture of 71% of phosphate buffer (pH 2.5) and acetonitrile 29% (v/v). The peptide content was calculated using a teverelix standard curve.

2.2.3. In vitro release

In vitro release experiments were performed using a flow-through Erweka DFZ60 apparatus equilibrated at 37 °C.

Ten milligram teverelix samples were loaded in the presence of sea sand (matrix) in 20 ml flow-through cells. Then a $0.5\,ml\,min^{-1}$ flow of Ringer solvent (NaCl, 8.6 g; KCl, 0.30 g; $CaCl_2$, 0.25 g; NaN_3, 0.20 g; for 1 l) was applied. Teverelix release was monitored everyday by HPLC analysis using the same conditions as previously.

3. Results and discussion

3.1. Characteristics of teverelix

The LH–RH antagonist Antarelix^{TM} (teverelix^{INN}) is a decapeptide constituted of the following amino acids: Ac-DNal–DPhe(pCI)–DPal–Ser–Tyr–DHci–Leu–Lys(iPr)–Pro–DAla-NH_2 where Nal was the naphthylalanyl, pCI was the paracitrullyl and Hci, the homocitrullyl amino acid. Due to this amino acid constitution, teverelix is highly hydrophobic, the solubility in water being approximately $10\,mg\,ml^{-1}$.

The molecular weight of teverelix is $1459\,g\,mol^{-1}$.

3.2. Characteristics of block copolymers of PLGA and PEG

PLGA–PEG block copolycarbonates were synthesised as follows (Ferruti et al., 1995a,b; Penco et al., 1994): PEG oligomers, activated by phosgene, react with PLGA oligomers to conduct to block copolycarbonates of PLGA and PEG (Fig. 1). Several copolymers have been synthesised; their characteristics are described in Table 1. PLGA was used as a reference.

For all copolymers, the average molecular weights of starting PEG and PLGA were different. The resulting percentage of PEG varied from 0% for pure PLGA to 20% for PLGA–PEG (2300/600) and the T_g from 18 °C for polymer containing 20% of PEG to 38 °C for the polymer, containing 9% of PEG. PLGA–PEG copolymers showed only one glass transition temperature, showing that PEG and PLGA fragments form a homogeneous phase. For all PLGA PEG copolymers, the average molecular weight of the resulting polymer varied from 22,000 to $94{,}000\,g\,mol^{-1}$ and consequently viscosity varied from 0.43 to $0.84\,dl\,g^{-1}$. The PLGA, used as a reference, showed an average molecular weight of $42{,}000\,g\,mol^{-1}$ and a T_g of 42 °C.

3.3. Teverelix microspheres preparation

Teverelix microspheres were produced by a 'phase separation' or 'coacervation' method. In this technique, the reduction of polymer solubility following the addition of a polymer non-solvent leads to microparticles (Jain et al., 1998; Stassen et al., 1994). Three different solvents with particular properties are needed: the first solvent must dissolve the polymer; the second solvent must be miscible with the first solvent, but the polymer must not be soluble in it; and a third solvent which must have the same properties as the second solvent and which had to be miscible with it. Based on these rules, we chose DCM, silicone oil and silicone oil, respectively.

Typically, the peptide was dispersed in the polymer solution. The peptide was insoluble in any solvent used in this method. Silicone oil was added and it extracted gradually the DCM and left the medium separated into two different phases: the coacervate

(mostly constituted by the polymer) and the supernatant (containing most of silicone oil and DCM). Soft droplets were formed which engulfed the peptide. The soft droplets were then transferred into a large amount of silicone oil, which extracted residual DCM and gave the final hard microspheres.

3.3.1. Phase diagrams

The optimal quantity of silicone oil which had to be added to the media has to be precisely determined. It corresponds to a physico-chemical balance between the polymer, DCM and silicone oil, conducing to microsphere formation. This particular balance is called the 'stability window' (Ruiz et al., 1990).

Typically, the determination of the 'stability window' is made by the use of a dye, in place of the peptide, which allows observation of the phase separation by optical microscopy. Silicone oil was added dropwise to polymer solution and induced four successive steps (Stassen et al., 1994), which are described on phase diagrams (Fig. 2). The first step ("1" in Fig. 2) occurred when a relatively small amount of silicone oil was added, the media had the appearance of a pseudo-emulsion. The second step (2) occurred

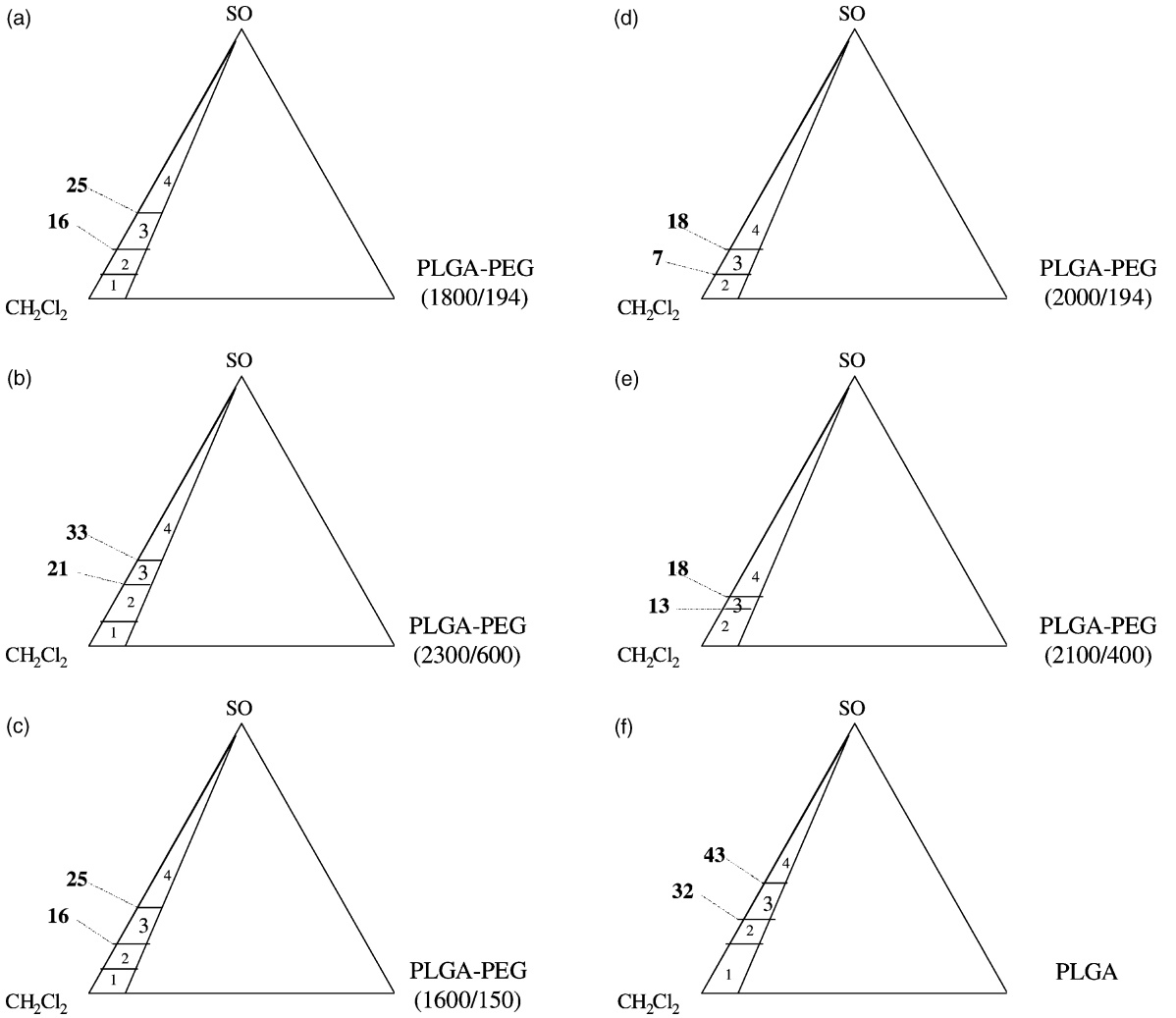

Fig. 2. Phase diagrams using (a) PLGA–PEG (1800/194), (b) PLGA–PEG (2300/600), (c) PLGA–PEG (1600/150), (d) PLGA–PEG (2000/194), (e) PLGA–PEG (2100/400) and (f) PLGA 50/50.

with increased amounts of silicone oil, which induced phase separation but did not correspond to a 'stability window' because the droplets, which appeared, were unstable and tended to coalesce and eventually fuse together. The third step (3) corresponded to the expected 'stability window' which meant that, with this particular amount of silicone oil, the preparation was stable. Droplets engulfed water-coloured droplets. The fourth and last step (4) was characterised by droplets, which engulfed many coloured droplets and tended to coalesce.

Fig. 2 shows the phase diagrams of the ternary CH_2Cl_2/PLGA–PEG/silicone oil system with all polymers described in Table 1. All experiments have been achieved with polymer concentrations smaller than 10%, as a weight percentage (Ruiz et al., 1990). The 'stability window' (3) corresponds to an interval of quantity of silicone oil added, expressed as a percentage of the total media weight. The highest amount of silicone oil needed to reach the 'stability window' was for PLGA; it appeared between 32 and 43% of silicone oil. All PLGA–PEG copolymers needed smaller amounts of silicone oil to reach the 'stability window'.

The effect of PLGA–PEG characteristics on the 'stability window' can be partially explained. First of all the influence of the molecular weight of the polymer was examined. PLGA–PEG (1800/194) and PLGA–PEG (2000/194) had very close characteristics except that their average molecular weights were 22,000 and 66,000 g mol^{-1}, respectively (Table 1). The amounts of silicone oil which had to be added in order to reach the 'stability window' were between 16 and 25% and between 7 and 18%, respectively. This is consistent with Ruiz et al. (1990) who noticed that for PLGAs, increasing amounts of silicone oil had to be added to the polymer solution to reach the 'stability window' when weight-average molecular weights decreased.

The molecular weight of the polymers was of course not the only parameter governing the amount of silicone oil to be added. PLGA–PEG (1600/150) and (2000/194) had almost the same molecular weight (Table 1) and yet the amounts of silicone oil were different. These two polymers are very close, concerning the initial molecular weight, but also the level of PEG, which is 9% for both (Table 1). In this case the only difference that could explain the different amounts of silicone oil is the composition of these two copolymers (Table 1).

Another parameter examined was the relative hydrophobicity. Stassen et al. (1994) studied the influence of hydrophobicity of PLGAs on the width of the 'stability window'. They compared PLGA 50/50, PLGA 75/25 and PLA and showed that the less hydrophobic the lactide-based polymer, the larger the area of the 'stability window'. In our study, the copolymer hydrophobicity mostly depends on PEG content, but no direct correlation was found between PEG content and the width of the 'stability window'. PLGA–PEG (2300/600) which had the higher amount of PEG (20%) among all PLGA–PEG showed a 'stability window' between 21 and 33% of silicone oil (Fig. 2). PLGA–PEG (2100/400) which contained 16% was between 13 and 18% of silicone oil whereas PLGA–PEG (1800/194) which had a smaller amount of PEG (10%) showed the larger 'stability window' of silicone oil: between 16 and 25%.

As in the case of PLGAs (Stassen et al., 1994), there is no simple correlation between copolymers composition and the amount of silicone oil to be added to reach the 'stability window'. Stassen et al. (1994) found that the stability of the system was more likely controlled by the coacervate/supernatant interfacial tension, which depends on the nature of the coating polymer. The nature of the polymer in our case may take into account all the characteristics of PLGA–PEG copolymers such as PEG content, average molecular weight of the resulting polymers and eventually average molecular weight of initial PLGA and PEG.

3.4. Teverelix microsphere characteristics

All PLGA–PEG copolymers were tested as coating material in microspheres of teverelix. Microparticles made of PLGA were prepared as a reference.

Encapsulation yields were calculated as the percentage of the analysed final core loading to the amount of teverelix added. Particle sizes were monitored by microscopic measurement and glass transition temperatures were determined by DSC. All characteristics are summarised in Table 2. Both influence of the initial core loading and the polymer type were tested on microsphere characteristics.

Table 2
Teverelix microspheres characteristics

Batch number	Polymer	Initial core loading (CL_i)	Final core loading (CL_f)	Encapsulation yield (%)	Particle size (μm)	T_g (°C)
54	PLGA	9.4	7.4	79	20–50	nd
40/42	PLGA–PEG (1800/194)	9.4	7.8	83	10–50	nd
66	PLGA–PEG (1800/194)	14.8	13.1	89	10–70	nd
69	PLGA–PEG (1800/194)	29.0	23.6	81	10–100	nd
29	PLGA–PEG (1800/194)	34.2	29.5	86	5–1000	nd
94	PLGA	28.5	23.5	82	100–200	44
76/80	PLGA–PEG (2300/600)	29.1	25.6	88	10–300	24
87	PLGA–PEG (1600/150)	28.2	25.1	89	10–300	38
93	PLGA–PEG (2100/400)	28.3	25.8	91	10–150	39
88	PLGA–PEG (2000/194)	28.3	27.9	99	10–200	40
108/111	PLGA–PEG (2000/194)	29.1	24.9	86	10–200	40

nd: not determined.

3.4.1. *Influence of the core loading*

The influence of the core loading on microspheres characteristics has been studied on one particular polymer, the PLGA–PEG (1800/194). Microspheres were prepared at four different initial core loadings: 9.4, 14.8, 29.0 and 34.2% (Table 2).

Whatever the initial core loading, all encapsulation yields were above 80%, whereas particle size depended strongly on the amount of peptide. It increased from 10–50 μm at 9.4% initial core loading to 5–1000 μm at 34.2%. At this particular core loading of 34.2%, size distribution of particles changed and was characterised by two types of particles: very thin walled microspheres of very low size (5–20 μm) agglomerated to the surface of big entities (1 mm); there were no particles in between these two types.

Increasing the amounts of peptide to be encapsulated, resulted in increasing particle size. We suggest that when the amount of peptide was too high, the elasticity of the polymer reaches a limit, the peptide pushes against the polymer wall, which became thinner and particle size increased. The particular value of 34.2% seemed to be a limit above which it was not possible to obtain small size distribution of microspheres. For further experiments, no initial core loading above 29% was used.

3.4.2. *Influence of the polymer type*

The influence of the polymer type on microspheres characteristics was studied at two different initial core loadings: 9.4 and 28% (Table 2).

At the low core loading (9.4%), two different polymers were tested: the PLGA–PEG (1800/194) and the PLGA (see the first two lines of Table 2). Microspheres had very close characteristics both in term of encapsulation yield and particles size. At the low core loading, the nature of the polymer did not seem to be a great factor influencing the microspheres characteristics.

At the high core loading (28%), six different polymers, including PLGA, have been used in microspheres preparation process (Table 2): PLGA–PEG (1800/194), (2300/600), (1600/150), (2100/400), (2000/194) and PLGA.

All microspheres prepared at that high initial core loading were characterised by high particle size. The size varied from 10 to 300 μm, whatever the polymer used. However, microparticles made of pure PLGA showed a thinner window of particle size, from 100 to 200 μm. Considering that only particles smaller than 150 μm were injectable, microspheres prepared from PLGA were not acceptable.

Microspheres morphology was also studied. All microspheres were spherical except microspheres made of PLGA. They were constituted of agglomerated non-spherical entities (not shown) whereas PLGA–PEG-based microparticles produced spherical microspheres (Fig. 3).

Encapsulation yields varied from 81 to 99% (Table 2). Differences observed between all batches produced may likely be due to both process and analytical variation.

DSC analyses of microspheres (Table 2) were compared to initial polymer T_g (Table 1), as Okada (1997)

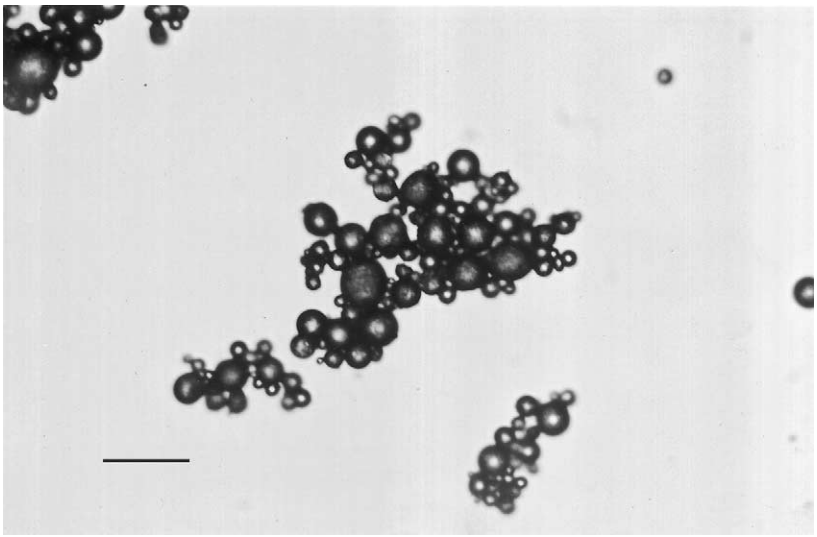

Fig. 3. Microscopic photograph of microspheres from batch no. 108/111. Bar represents 100 μm.

and Shameem et al. (1999) observed that T_g increased from 1 to 8 °C with the loading of peptide. This increase was ascribed to ionic interaction between the basic amino acids of the drug and the terminal carboxylic anions of the polymer (Okada, 1997). This interaction between the polymer and the peptide may increase the rigidity of the polymer macromolecule, that may increase the T_g. Interaction between the polymer and the peptide is consistent with the high hydrophobicity of the peptide, which create a high affinity between the peptide and the polymer matrix.

Microspheres whose T_g was higher than room temperature were of interest because this allowed storage at room temperature.

3.5. In vitro release of teverelix from microspheres

In vitro release of teverelix was tested with all microspheres batches. The influence of the final core loading and of the polymer type was studied. Teverelix release was monitored during 15 days, at 37 °C. The aim of in vitro release analysis was to choose between all PLGA–PEG polymers, the best candidate to administrate teverelix to animals.

3.5.1. Influence of the core loading

The influence of the core loading on teverelix release was studied on microparticles prepared from one particular polymer: the PLGA–PEG (1800/194). Three different core loadings were tested: 7.8, 13.1 and 23.6% (Table 2). The resulting release rates of teverelix are reported in Fig. 4.

Microspheres containing 23.6% of teverelix, released 45% of this amount in 15 days whereas microparticles containing 7.8% released only 13% in 8 days and released no more peptide after this time. Increasing the core loading of microspheres, both the

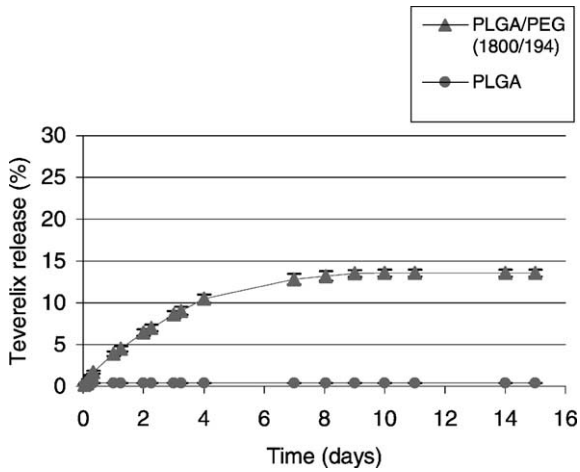

Fig. 4. Teverelix release rate of microspheres made of PLGA–PEG (1800/194) according to the final core loading.

total amount of released teverelix and the release rate increased.

After in vitro release, residual microparticles were analysed by GPC. The average molecular weight of the residual microspheres were 6500 g mol^{-1} at a core loading of 7.8%, 10,500 g mol^{-1} at 13.1% and 16,500 g mol^{-1} at 23.6%. The degradation degrees, which represented the percentage of degradation of the microspheres, were respectively 70, 52 and 25% for the corresponding core loadings, 7.8, 13.1 and 23.6%. The higher the core loading, the lower the polymer degradation.

In the case of microspheres made of PLGA, Witschi and Doelker (1998) proposed that the degradation rate of the polymer decreased slightly when increasing the core loading from 1 to 10%. The basic amino groups of the peptide were supposed to interact with the free carboxylic acid groups of the polymer, which reduced the autocatalytic effect of the carboxylic acid groups. In our case, the hypothesis of initial ionic interaction between amino groups of the peptide and carboxylic groups of the polymer is consistent with the T_g decrease of the loaded microspheres (see 'influence of polymer type', Chapter 3.3.2). It seemed that during degradation new carboxylic end groups were immediately involved in new ionic interactions, which reduced autocatalytic effect and consequently polymer degradation. This phenomenon was emphasised when the core loading is increased.

Surprisingly, in spite of slower polymer degradation when the core loading increased, peptide release was higher and quicker (Fig. 4). When the core loading increased, the amount of peptide not held in the matrix by ionic interactions may also increase, increasing amounts of peptide able to be released from the matrix.

3.5.2. *Influence of the polymer type*

Teverelix release was studied at two distinct core loadings: 7–8 and 25%.

At 7–8%, we compared the in vitro release of teverelix from two different productions made with the PLGA–PEG (1800/194) and with the PLGA. The results are shown in Fig. 5. Almost no release was observed from microparticles made of PLGA whereas microspheres made of PLGA–PEG released 13% in 8 days.

At a core loading of almost 25%, the influence of the polymer type was tested on in vitro teverelix release. Microspheres made with the six different copolymers were tested. Teverelix release results are presented in Fig. 6.

All microspheres made of copolymer of PLGA and PEG released more teverelix than PLGA microspheres. This may be due to the structural particularities of the new type of polymer family used in this study. These polymers were block copolymers made of PLGA fragments and PEG fragments.

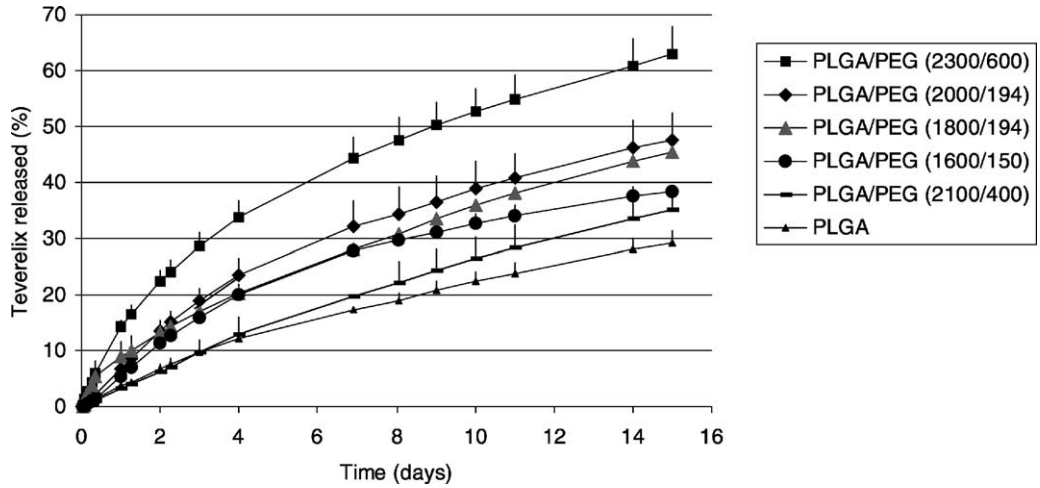

Fig. 5. Teverelix release rate at low core loading, according to the nature of the polymer.

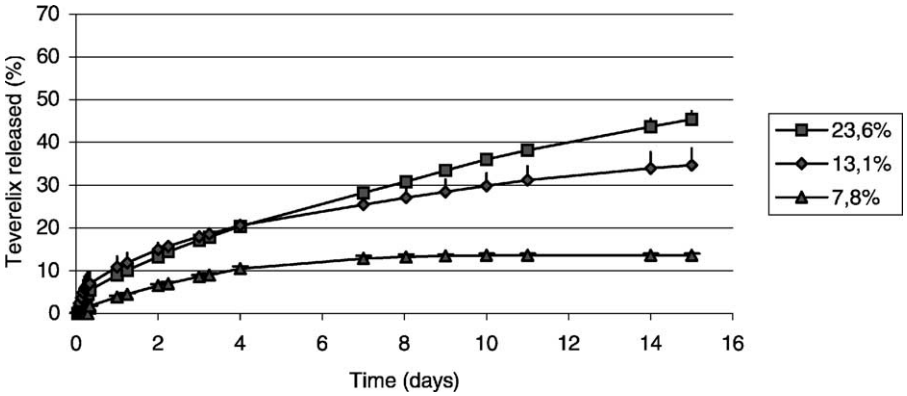

Fig. 6. Teverelix release rate as a function of the nature of the polymer.

PEG fragments were not hydrolysable but very hydrophilic. PEG fragments may act as 'water pump' inside the matrix of the microspheres, which may induce teverelix release. This hypothesis is consistent with the fact that teverelix release from microspheres seemed to be mostly governed by the PEG proportion inside the copolymer (Fig. 5). The formulation which released the higher and quicker amount of teverelix was the formulation which was made of the PLGA–PEG (2300/600). This copolymer contained the highest proportion of PEG: 20% (Table 1). All copolymers containing around 10% of PEG released teverelix almost in the same way: PLGA–PEG (2000/194), (1800/194) and (1600/150) (Fig. 6). Eventually, the PLGA, which obviously contained no PEG, released less teverelix compared to PLGA–PEG polymers. Surprisingly, the PLGA–PEG (2100/400) which contained 16% of PEG released less teverelix than all PLGA–PEG. This lowest release may be due to the high molecular weight of this polymer, which was 94,000 g mol^{-1}.

Changing the polymer used in microspheres production the release of teverelix changed, so teverelix release could be easily controlled by the type of copolymer.

The final goal of this study was to choose the best PLGA–PEG polymer in order to administrate teverelix to animals. The polymer which released the quickest and the highest amount of teverelix was the PLGA–PEG (2300/600) (Fig. 6) but unfortunately the T_g of the initial polymer was only 18 °C (Table 1) and the T_g of the loaded microspheres, 24 °C (Table 2). The T_g of the initial polymer does not allow room temperature processing and the T_g of the final particles does not allow room temperature storage and injection. This polymer was eventually not selected for further experiments. The copolymer following the PLGA–PEG (2300/600) concerning teverelix release was the PLGA–PEG (2000/194) (Fig. 6). It released 48% of teverelix in 15 days, initial polymer T_g reached 32 °C (Table 1) and final T_g, 40 °C (Table 2). This copolymer was chosen as a good candidate to formulate teverelix in order to make further in vivo study in dogs.

4. Conclusion

The GnRH antagonist teverelix has been encapsulated by coacervation using a wide variety of PLGA–PEG copolymers. It was demonstrated that this type of polymer has some advantages compared to PLGAs, based on particle size distribution as well as on in vitro release. Varying the percentage of PEG inside the polymer allow to modulate the release rate of peptide. This new family of bioerodible polymers may constitute a useful alternative to PLGAs for the encapsulation of peptide and proteins.

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