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Research paper

Biodegradable microparticles for sustained release of a new GnRH antagonist – part I: screening commercial PLGA and formulation technologies

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Abstract

The formulation of a new GnRH antagonist (degarelix) in biodegradable poly(DL-lactide-*co*-glycolide) (PLGA) microparticles was investigated for the development of a 3-month sustained release formulation to treat prostate cancer. The aim was to screen formulation technologies and distinct copolymers to produce microparticles (MP) of different types with good entrapment efficiency (>85%) and peptide purity (>95%) after gamma sterilization. Basically, three types of degarelix-loaded MP (4, 8 and 16% w/w nominal content) were produced with solvent and non-solvent technologies, namely double-emulsion solvent evaporation, spray-drying and two extrusion methods. Besides composition, commercial copolymers differing in residual monomer content and functional group at the carboxylic terminus (acid or ester) were characterized and employed. Peptide loading capacity and purity, as well as shape, size characteristics, and porosity of the produced microparticles were discussed in relation to technology and copolymer choice. Spray-drying and micro-extrusion were the two preferred formulation technologies because of higher entrapment efficiency and better preservation of peptide purity during production and γ-sterilization. The impact of formulation technologies on the MP characteristics overwhelmed the impact of copolymer selection. Nevertheless, one particular polymer was discarded since it was more susceptible towards radiolytic degradation. The resulting degarelix-MP will be tested in a biological assay for selection of the formulation based on performance.

Keywords: Peptide drug delivery; GnRH antagonist; Degarelix; Poly(lactic acid); Poly(lactic acid) copolymers; Microparticle; Microsphere; Extrusion; Prostate cancer

1. Introduction

Synthetic analogues of gonadotropin-releasing hormone (GnRH) are recognized as potent drugs for sex hormone-dependent diseases, most commonly for prostate cancer [1], but they are also indicated for endometriosis, uterine fibroids, in vitro fertilization, and precocious puberty [2–4]. In the 1980s, the therapeutic development of GnRH analogues, the so-called agonists and superagonists, which differ from natural GnRH in just one or two amino acids but which are much more stable and potent [5], led to the commercialization of several well known bioactive drug substances including leuprorelin, nafarelin, buserelin, goserelin, and triptorelin [2,3,6,7]. More recently, GnRH antagonists such as cetrorelix, ganirelix and abarelix, which are characterized by a more complex structure, where only 3–5 native amino acids of GnRH are preserved, were developed [2–4]. In initial trials, some antagonists caused severe adverse effects in humans because of high histamine release from mast cells. Only recently safer GnRH antagonists were developed which are close to clinical application for prostate cancer [2–4]. The advantage of the antagonists over the agonists/super-agonists is that they prevent the characteristic stimulatory

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phase of the pituitary-gonadal axis leading to an initial acute increase of hormonal secretion (surge) which may worsen disease symptoms (flare), e.g. in the case of prostate cancer [2–4].

Degarelix, a new antagonist of GnRH, has been recently synthesized and screened for duration of inhibition of luteinizing hormone (LH) release in a castrated rat assay [8]. In comparison to other antagonists (e.g. cetrorelix, Nal-Glu, ganirelix, abarelix and azaline B), this decapeptide exhibited a longer duration of action along with a lower potency at releasing histamine from isolated rat mast cells. Due to its improved physicochemical properties based on the urea/carbamoyl functionalities introduced in the substituents of amino acids at position 5 and 6, e.g. increased hydrophilicity and stability, degarelix is well adapted to clinical use [8].

Even though degarelix administered s.c. at 2 mg/kg was astonishingly able to suppress testosterone in rats over nearly 2 months [8], incorporation of degarelix in a bioresorbable polymeric carrier such as poly(DL-lactide-*co*-glycolide) was of interest for the development of a 3-month formulation for the prostate cancer indication. This approach presents the advantage to offer both flexibility for development (e.g. loading, copolymer, technology), and control over the system in terms of stability and release of the active. Moreover, such a formulation was successively developed for GnRH agonists [2,6,7], and would therefore be in line with existing clinical practice. In the present paper, we describe more specifically the production and characterization of degarelix microparticles taking advantage of the possibilities offered both by the technology and the polymer levels. The viability of the MP approach is dependent on good peptide entrapment efficiency and preservation of peptide purity. Therefore the objective of this work was to produce degarelix MP based on different copolymers but with entrapment efficiency higher than 85% and peptide purity higher than 95% after gamma sterilization.

2. Materials and methods

2.1. Materials

Degarelix (Fig. 1), molecular weight 1632 Da, was supplied by Ferring Pharmaceuticals A/S (Copenhagen, Denmark), and was used as received. Several sources of commercial poly(DL-lactide-*co*-glycolide) were used and compared: Medisorb 5050DL2A, 6535DL2A and 7525DL2A (hydrophilic series) from Alkermes Inc. (Cambridge, MA USA), Resomer RG502H (hydrophilic series), RG502 and RG752 (standard grade) from Boehringer Ingelheim Pharma KG (Ingelheim, Germany), and Phusiline PLA37.5GA25 from Phusis (Saint-Ismier, France). All PLGA were synthesized in the bulk in the presence of stannous octanoate as the catalyst, except the Phusiline PLGA catalysed with zinc lactate, and were used as received without additional purification. Polyvinyl alcohol, PVAL, was used as emulsifier (Mowiol 4-88, Hoechst). Solvents were all of analytical grade (Fluka).

2.2. Formulation technologies

Microspheres (MS) were obtained by double-emulsion solvent evaporation, w/o/w, in the presence of polyvinyl alcohol (PVAL). Typically, degarelix (50 mg) dissolved in water (1 ml) was first dispersed in a methylene chloride solution containing the polymer (500 mg/5 ml), at 20 500 rpm for 30 s with a mixer (Ultra-Turrax T25, Ika, Staufen, Germany). This primary emulsion was then added to the second aqueous phase (300 ml) containing 0.5% PVAL, and was stirred at 2000 rpm with a 35-mm paddle over 4 h at room temperature (secondary emulsion). MS were recovered by filtration and were rinsed four times with 65 ml water. Drying was carried out at reduced pressure (150 mbar) and at room temperature over 7 days. As an alternative method, degarelix-MS were also produced by spray-drying. An emulsion of the polymeric carrier (2 g in 20 ml methylene chloride) and degarelix (100 mg in 2 ml water) was atomized with the 0.7-mm nozzle of the spray-drying equipment (Büchi 190, Büchi, Flawil, Switzerland). Since no emulsifier was used, the emulsion was maintained during feeding at 2.5 ml/min with an Ultra Turrax (20 500 rpm). The settings for air flow, aspiration depression, and inlet temperature were 800 Nl/h, 40 mbar and 50 °C, respectively. The outlet temperature was in the 40–44 °C range. Microgranules (MG) were produced by milling a PLGA–degarelix intermediate produced by extrusion (cylindrical rods of c.a. 2 mm external diameter). Extrusion was either performed with a custom-made ram-extruder [9] or by micro-extrusion (1/4 inch Microtruder, Randcastle Extrusion systems Inc., Cedar Grove, NJ, USA). In the case of ram-extrusion, the polymer (1 g), previously dried at 40 °C under vacuum, was physically mixed with the peptide (100 mg) in a 3D Turbula mixer (WAB, Basel, Switzerland). The mixture was then directly fed to the barrel and after compression at 5 bars, the temperature was progressively increased until material came out of the die. In the case of micro-extrusion, the PLGA-degarelix mixture (5–10 g), prepared as previously, was fed to the heated barrel by rotation of a stainless steel LD 24 extrusion screw at 20 rpm. Extrusion was carried out through a 2-mm heated die.

2.3. Characterization

All selected PLGA were analysed for average molecular weight (M_w) and polydispersity (I) by size exclusion chromatography (SEC) (Waters 150C, styragel HR1, HR2 and HR4 columns at 30 °C, mobile phase tetrahydrofuran 1 ml/min, refractometric detection at 30 °C). Data were expressed with reference to a calibration curve obtained with polystyrene standards (Tosoh Corp., Japan). The DL-lactate/glycolate composition (L/G), the amount of residual monomers (lactide and glycolide) as well as the nature of

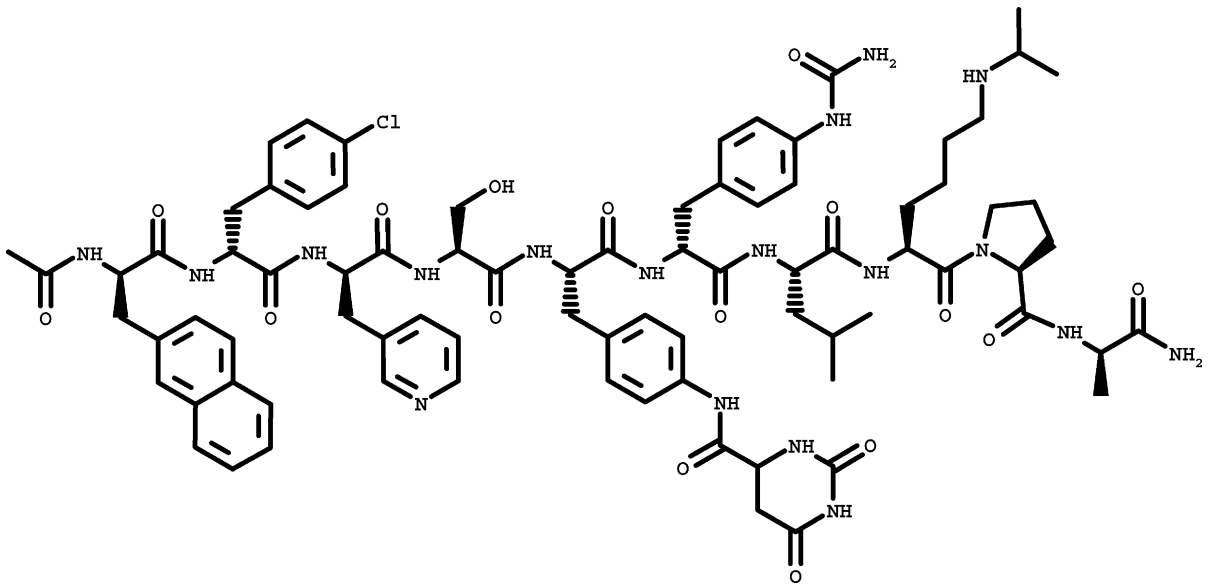

Fig. 1. Structure of Degarelix [Ac-DNal[1]-DCpa[2]-DPal[3]-Ser[4]-Aph(L-Hydroorotyl)[5]-DAph(Cbm)[6]-Leu[7]-ILys[8]-Pro[9]-DAla[10]-NH_2 (IUPAC rules are used for nomenclature except for the following abbreviations: Ac, acetyl; Aph, 4-aminophenylalanine; Cbm, carbamoyl; Cpa, 4-chlorophenylalanine; ILys, *N*-isopropyl lysine; Nal, 3-(2-naphthyl)-alanine; Pal, 3-(3-pyridyl)-alanine).

the chain ends were investigated by 400 MHz ^{1}H-NMR at 300 K, in DMSO-d_6. The following equations were used (where I_{a-f} stand for signal intensity as obtained by integration of a–f, Fig. 2): L/G composition, $G\% = 100 \times I_b/(2 \times I_a + I_b)$, $L\% = 100 - G\%$; residuals, lactide$\% = 100 \times I_c/I_{total}$, and glycolide$\% = 50 \times I_d/I_{total}$ with $I_{total} = I_f + 2 \times I_e + 0.5 \times I_b + I_a$. MP were analysed for peptide content (PC) and peptide purity (PP) on a Waters LC HPLC equipment, column YMC basic 5 μm 4.6 × 250 mm at 40 °C, mobile phase acetonitrile/water 36:64, 0.1% trifluoroacetic acid. Briefly, a given amount of MP (10 mg for 8% degarelix nominal content) was dissolved in 3.6 ml acetonitrile. Sonication was applied briefly and after 20 min, a 0.1% trifluoroacetic acid aqueous solution was added to completion of the 10-ml gauge flask. The mixture was vigorously shaken for peptide extraction and the polymer was separated by centrifugation (12 000 rpm, 10 min), at which point an aliquot of the supernatant solution was taken for analysis. Moisture content (Karl–Fischer titration, DL18 Mettler) and residual methylene chloride (Head-space sampler HP7694, gas chromatograph HP5890, column HP-innowax, oven temperature 80 °C, 4 extractions/vial, solvent *N*-methylpyrollidone) were also assessed. MP morphology was qualitatively investigated by scanning electron microscopy (SEM) (JSM-6400, Jeol Ltd., Japan) after gold sputtering. Mean volume diameter D[4,3] and characteristics of the size distribution (span, uniformity) were obtained from laser light scattering (Mastersizer S long bed, Malvern Instruments Ltd., Malvern, UK).

2.4. Gamma irradiation

MP sterilization with gamma rays was carried out using a ^{60}Co source, at 25 kGy (0.4–0.6 kGy/h), under dry ice and under air atmosphere at the FAW Swiss Federal Research Station (CH-8820 Wädenswil, Switzerland).

3. Results and discussion

3.1. Polymeric carriers

The performance of a peptide-containing bioresorbable long-acting formulation is dependent on three partners who closely interact: the bioresorbable polymer, the peptide, and the formulation technology. The polymer carrier was selected in the poly(DL-lactide-*co*-glycolide) family since it has proven its biocompatibility and degradability in several pharmaceutical applications [10], including commercialized peptide drug products like microparticles or implants, e.g. Decapeptyl [2], Lupron [6], Zoladex [7]. In this work, fast degrading copolymers with lactate/glycolate composition between 50:50 and 75:25 were preferred (Table 1). The choice was motivated by the propensity of the drug to maintain by itself a prolonged duration of action with a single bolus injection. PLGA copolymers of this selection, which are expected to degrade in several weeks, should therefore not hinder the release of the peptide, and provide additional sustained release to target the 3-month duration of action. Low molecular weight polymers also present the advantage that they can be extruded at a lower temperature thus limiting the risk of thermal peptide degradation.

Although PLGA are well known products, when combined and processed with peptide/protein drugs, there are still important difficulties in predicting the drug-release profile [11,12]. Therefore, particular attention was paid to PLGA characterization to better correlate with efficacy.

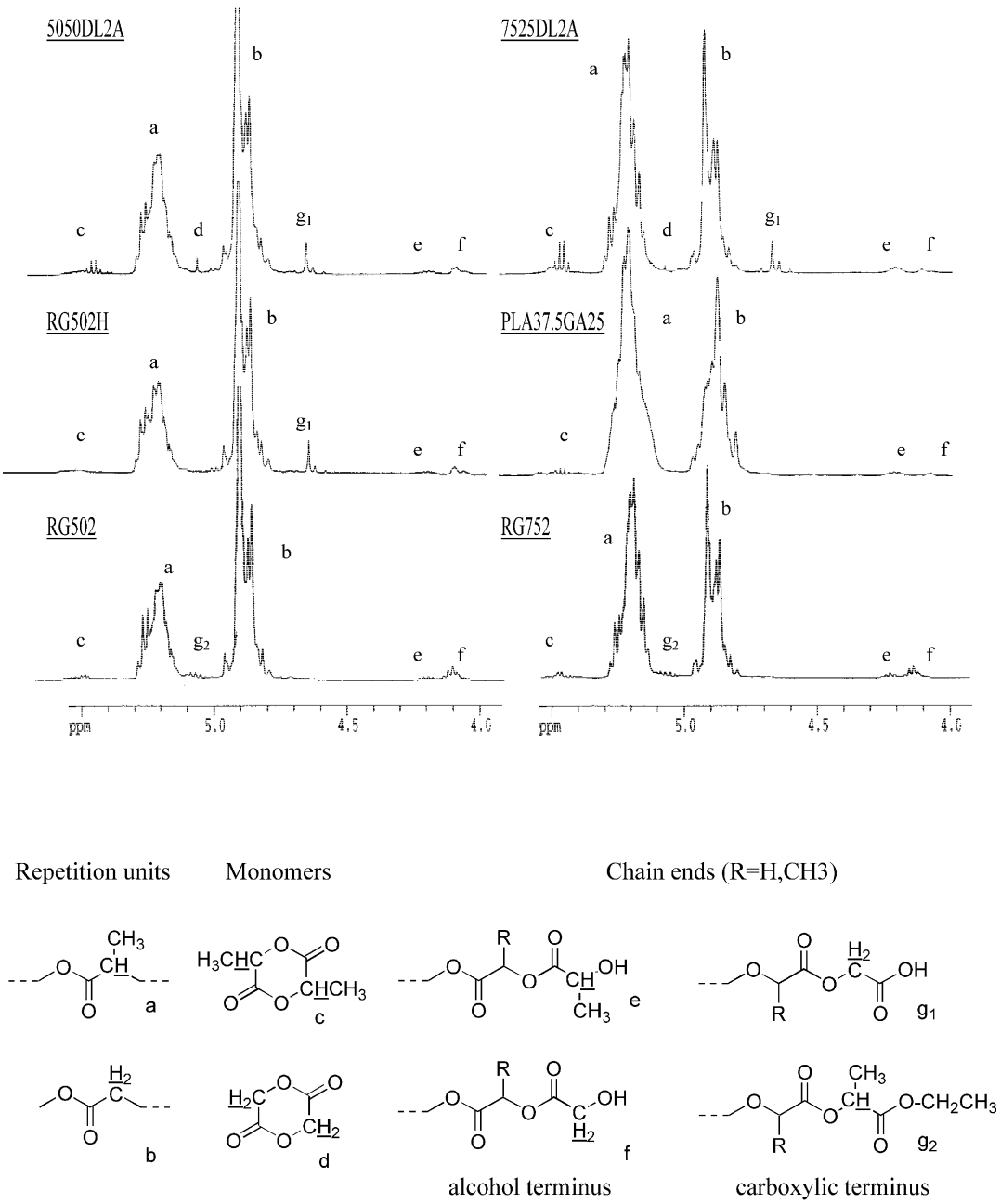

Fig. 2. ^{1}H-NMR spectra (400 MHz, DMSO-d6), lactic methine and glycolic methylene areas, of poly(DL-lactide-co-glycolide) from different commercial sources.

Several elements have already been provided on this issue [13,14], dealing with L/G composition, molecular weight and solubility, but important features like polymer purity or exact nature of chain ends were only poorly discussed with regard to possible influence on drug release. Lactate/glycolate compositions were consistent with stated values, in the limits of experimental errors (Table 1). Selected PLGA exhibited low and comparable molecular weight of ca. 10 kDa, the Phusiline copolymer having a higher M_w of ca. 20 kDa. Polydispersity indexes were all in the 2.2–2.5 range, which was considered as a relatively uniform molecular weight distribution. However, in the case of Phusiline PLA37.5GA25, polydispersity was found to be higher at 3.2 because of the presence of oligomers.

Discrepancies were found between manufacturers as far as residual amounts of monomers were concerned (Table 1 and Fig. 2). The comparison revealed higher amounts of residual monomers (lactide, c and glycolide, d in Fig. 2) for all Medisorb copolymers when compared to the Resomer examples, with Phusiline PLGA being intermediate. The literature has underlined the impact of

Table 1
Characteristics of selected bioresorbable polymeric carriers

PLGA[a]	Chain-end[b]	L/G composition		Molecular weight[c]	Residual monomers[b]	
		Stated	Found[b]	M_w (I)	Lactide	Glycolide
RG502H (Boehringer)	–OH –(G)COOH	50/50	50/50	8300 (2.5)	1.6	<LOD[d]
RG502 (Boehringer)	–OH $-COOCH_2CH_3$	50/50	51/49	12500 (2.2)	1.3	<LOD
RG752 (Boehringer)	–OH $-COOCH_2CH_3$	75/25	74/26	16200 (2.5)	1.1	<LOD
5050DL2A (Alkermes)	–OH –(G)COOH	50/50	54/46	9500 (2.2)	3.3	0.4
6535DL2A (Alkermes)	–OH –(G)COOH	65/35	66/34	10000 (2.4)	3.3	0.3
7525DL2A (Alkermes)	–OH –(G)COOH	75/25	74/26	10600 (2.4)	4.1	0.2
PLA37.5GA25 (Phusis)	–OH –COOH	75/25	76/24	18800 (3.2)	1.9	<LOD

[a] Commercial designations of PLGA according to the manufacturers.
[b] From ^{1}H-NMR.
[c] Average molecular weight in weight (M_w) and polydispersity (I) from SEC.
[d] LOD, limit of detection.

polymer impurities, particularly residual monomers, on microparticle characteristics, drug release and biocompatibility [15–17] (e.g. initial burst effect, surface porosity, polymer degradation and interaction with drugs). Therefore this feature may be of importance for MP performance.

Chain-ends were analysed focusing on the difference between hydrophilic and non-hydrophilic PLGA. Several authors have compared the degradation and/or release profiles from PLGA differing in hydrophilicity, typically by comparison between 'H' and 'non-H' polymer grades [9,18]. Differences in hydrophilic character are known to result from initiation. Chain-end modification on the carboxylic acid terminus takes place because the initiators (e.g. ethyl lactate and lauryl alcohol for hydrophobic, glycolic/lactic acids for hydrophilic) act as chain terminators to control the molecular weight of the copolymer [19]. However, to our knowledge, the nature of the polymer chain-ends was never compared in various commercial PLGA. Methine and methylene areas of ^{1}H-NMR spectra recorded in DMSO-d_6 are particularly informative (Fig. 2). Alcohol end groups appearing at ca. 4.1 ppm as $\sim C\underline{H}_2-OH$ (f), or at ca. 4.2 ppm as $\sim C\underline{H}(CH_3)-OH$ (e) were detected in all spectra. A more complex situation was found regarding the carboxyl end groups. In the case of standard (i.e. non-hydrophilic) Resomer copolymers (RG502, RG752), ethyl ester ends were detected as quadruplets g_2 ($\sim C\underline{H}(CH_3)-COOCH_2CH_3$, 5.1 ppm) and as a triplet ($\sim COOCH_2C\underline{H}_3$) at 1.2 ppm (not shown), esterification being a result of initiation by ethyl lactate. In comparison, for the hydrophilic PLGA (RG502H and Medisorb 5050DL2A, 6535DL2A (not shown), 7525DL2A), glycolic acid ends g_1 ($\sim C\underline{H}_2COOH$, 4.65 ppm) were clearly identified (two singlets with geminal coupling) as a result of glycolic acid initiation. Finally, for the Phusiline copolymer, carboxylic acid end signal ($\sim C\underline{H}(CH_3)-COOH$), expected at ca. 4.9 ppm [20], was not distinguished because of overlapping with the methylene signal arising from the glycolate repetition units (b), but the assignment was confirmed by the presence at ca. 1.3 ppm of the more intense methyl signal of the carboxylic ends ($\sim CH(C\underline{H}_3)-COOH$) (not shown). No additional initiator was identified in the case of the Phusiline copolymer directly obtained from zinc–lactate initiation [20]. The nature of the chemical groups at the polymer chain-ends is particularly important when dealing with polymer-drug interactions. For PLGA, it was shown that free carboxylic ends may interact with other functional groups of peptides or proteins thus delaying their release [6,11,21].

3.2. Microparticles

Numerous technologies are available for incorporating hydrophilic drug substances in a PLGA matrix [22–25]. Solvent-free formulation techniques in the melt (i.e. above the softening point of the PLGA polymer) require a relatively good thermostability of degarelix, up to 100–120 °C. On the other hand, techniques in solution require organic solvents and possibly surfactants, e.g. PVAL. For our development purposes, technologies permitting easy up-scaling were preferred. Therefore, solvent methods like double-emulsion solvent evaporation and spray-drying were both of interest since they are used for leuprorelin acetate (Enantone LP, Takeda) and bromocriptine mesylate (Parlodel LP, Novartis) biodegradable microspheres respectively. Extrusion found a commercial application in this field with the Zoladex PLGA implants containing goserelin acetate (AstraZeneca Pharmaceuticals)

[7], and has been recently investigated in other peptide formulation development work [26]. MP with various nominal degarelix contents (respectively approx. 4, 8 and 15 w/w%) were produced (Tables 2 and 3).

3.2.1. Solvent technologies

For double-emulsion microspheres (MS), encapsulation efficiency was in the 65–90% range (Table 2). Increasing the degarelix nominal content from 8 to 15% slightly favoured encapsulation (#8–10), a situation explained by a reduced loss, in proportion, of the water soluble peptide in the outer aqueous phase as suggested by Okada [6]. Despite the use of methylene chloride, the drug was not denaturated and peptide purity values were consistent at 98.5% in all cases. Typical MS average volume diameter D[4,3] was 100 ± 30 μm, with the exception of two batches exhibiting larger diameters (#3/8). MS were uniform (c.a. 0.5 uniformity) and size distribution was rather narrow (span < 2.7). Morphological investigations by SEM confirmed the spherical shape as well as the internal honeycomb porosity due to double emulsification (Fig. 3). Differences in the surface morphology were noticed with respect to polymer. The 50:50 copolymers exhibited a rough surface (#3/8) and a smoother surface was observed when increasing the L/G ratio. Surface porosity was clearly less observed with L/G 75:25 copolymers (#6–7/10) and higher molecular weight copolymers as expected from their higher glass transition temperatures.

The characteristics of MS produced by spray-drying were relatively different (Table 2, Fig. 4). Entrapment efficiency was generally improved when compared to double emulsion, values being beyond 85% in all cases. This situation, also reported in the literature when comparing both formulation methods [15], can be explained by the fact that the peptide can not diffuse out of the emulsion droplets, the interface being air in this case. In our case, increasing nominal peptide content increased EE (#16–19) whereas a negative effect was reported for the encapsulation of vapreotide with this technique [27]. Solvents and temperature had no deleterious effect on the peptide (PP = 98–99.5%). In comparison to double emulsion, spray-dried MS were smaller. Typical D[4,3] was of 50 ± 30 μm, the microspheres being less uniform in size and more broadly distributed than double emulsion MS because of some aggregation (Table 2). The surface was smooth and exempt of pores, whatever the copolymer and drug loading, but the internal structure could not be deduced from SEM since no broken spheres were present (Fig. 4). In the case of Phusiline copolymer (#19), some fibres were formed as mentioned in the literature with the use of higher molecular weight PLGA [28].

Table 2
Characterization of degarelix-loaded PLGA microspheres

Microspheres	PLGA	Degarelix[a]			Size[b]			Residuals[c]	
		PC (%)	EE (%)	PP (%)	D[4,3] (μm)	Span	Unif.	H_2O (%)	CH_2Cl_2 (ppm)
Double emulsion									
MS-1	RG502H	5.5	65.6	98.3	108	1.8	0.6	0.9	2900
MS-2	RG502	6.5	83.0	98.6	83	0.9	0.3	0.6	260
MS-3	5050DL2A	6.9	87.2	99.0	195	2.7	0.8	–	–
MS-4	6535DL2A	7.3	91.6	98.5	84	1.2	0.4	0.8	300
MS-5	RG752	5.9	74.0	98.2	116	1.5	0.5	0.8	350
MS-6	7525DL2A	6.9	84.9	98.9	135	1.5	0.5	1.2	300
MS-7	PLA37.5GA25	6.8	78.9	98.5	84	2.2	0.7	–	580
MS-8	RG502H	12.2	86.2	98.5	215	2.5	0.8	0.7	300
MS-9	6535DL2A	13.0	90.1	98.7	69	1.1	0.3	1.3	300
MS-10	PLA37.5GA25	13.7	84.3	98.6	123	1.1	0.3	0.8	3300
Spray-drying									
MS-11	RG502H	3.6	86.2	98.4	26	9.9	2.1	0.6	1300
MS-12	5050DL2A	3.8	90.9	98.7	63	9.2	2.8	1.4	–
MS-13	6535DL2A	3.5	86.6	98.7	74	14.4	3.7	1.1	300
MS-14	7525DL2A	3.7	87.7	99.0	–	–	–	1.4	300
MS-15	PLA37.5GA25	4.0	91.5	97.9	29	6.5	2.1	1.1	3400
MS-16	5050DL2A	7.3	95.6	99.0	151	19.0	5.8	1.5	-
MS-17	6535DL2A	7.8	100.0	98.9	70	2.7	1.0	1.3	350
MS-18	7525DL2A	7.2	90.8	99.5	24	9.0	3.2	1.3	–
MS-19	PLA37.5GA25	7.4	92.5	98.8	83	5.4	1.7	1.3	950

[a] Peptide content (PC) and purity (PP) from HPLC. Entrapment efficiency (EE) calculated from $100 \times PC/[m_{degarelix} \times CF]/[m_{PLGA} + m_{degarelix}]$ with CF = 0.87 (degarelix content in peptide powder) and m_{PLGA} and $m_{degarelix}$ the amounts of polymer and peptide employed.
[b] From laser light scattering.
[c] From Karl–Fischer titration and head-space gas chromatography.

Table 3
Characteristics of degarelix-loaded PLGA microgranules produced by extrusion

Microgranules	PLGA	$T_{Extrusion}$[a] (°C)	PLGA[b] (M_w − I) (Da)	Degarelix[c]		Size[d]			H_2O[e] (%)
				PC (%)	PP (%)	D[4,3] (μm)	Span	Unif.	
Ram-extrusion									
MG-1	RG502	73	12400 − 2.3	4.8	97.6	–	–	–	–
MG-2	RG502H	66	8500 − 2.1	6.0	96.9	–	–	–	–
MG-3	RG752	80	15300 − 2.2	6.8	97.6	257	2.2	0.7	–
Micro-extrusion									
MG-4	RG502	75	13300 − 1.5	2.8	99.6	182	1.8	0.6	0.9
MG-5	RG752	80	14700 − 2.6	1.4	98.3	–	–	–	–
MG-6	5050DL2A	75	10900 − 1.5	2.5	99.4	162	1.7	0.5	1.1
MG-7	6535DL2A	85	11500 − 1.5	3.6	99.2	152	2.0	0.6	1.3
MG-8	7525DL2A	85	12300 − 1.5	2.8	99.1	159	1.5	0.4	1.0
MG-9	PLA37.5GA25	85	19900 − 1.6	3.4	99.6	176	1.5	0.5	1.2

[a] Temperature of extrusion in the plastification zone.
[b] Average molecular weight in weight (M_w) and polydispersity (I) from SEC.
[c] Peptide content (PC) and peptide purity (PP) from HPLC.
[d] From laser light scattering.
[e] From Karl–Fischer titration.

From literature data, moisture is known to play a potentially important role both in drug stability and PLGA-microparticle degradation. The typical water content in the produced microspheres was ca. 1%, which is consistent with published values [11,29,30].

For the two formulation methods discussed so far, the use of an organic solvent is required to dissolve the polymeric carrier. The residual content in MP was assessed (Table 2). For most formulations, the level was compatible with the acceptance level, i.e. 500 ppm in pharmaceutical preparations [31]. Higher contents were however systematically found with the formulations based on the Phusiline 75:25 copolymer (#10/15/19), probably because this polymer was of higher molecular weight, and/or because the microspheres were pore-less. In this particular case, techniques of solvent removal like e.g. vacuum drying, temperature, nitrogen purge or aqueous washing ought to be investigated [32].

3.2.2. Extrusion

The comparative evaluation of custom-made ram-extrusion and bench-top screw-extrusion, or micro-extrusion on polymer and peptide integrity was undertaken. Even though the processing temperature was generally 5 °C higher in the case of micro- than for ram-extrusion, the less trivial

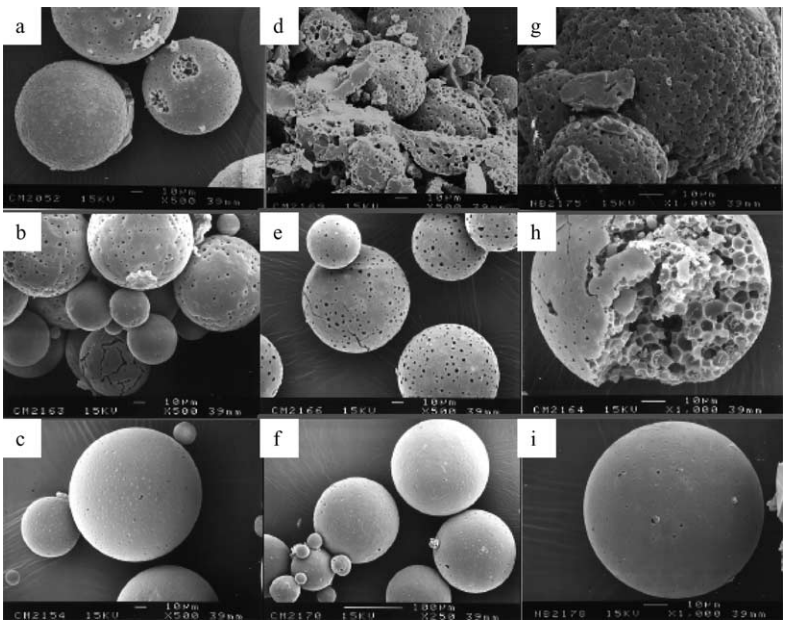

Fig. 3. Scanning electron microscopy pictures of double emulsion microspheres: MS-2 (a), MS-4 (b,h), MS-7 (c), MS-8 (d), MS-9 (e), MS-10 (f), MS-3 (g), MS-6 (i).

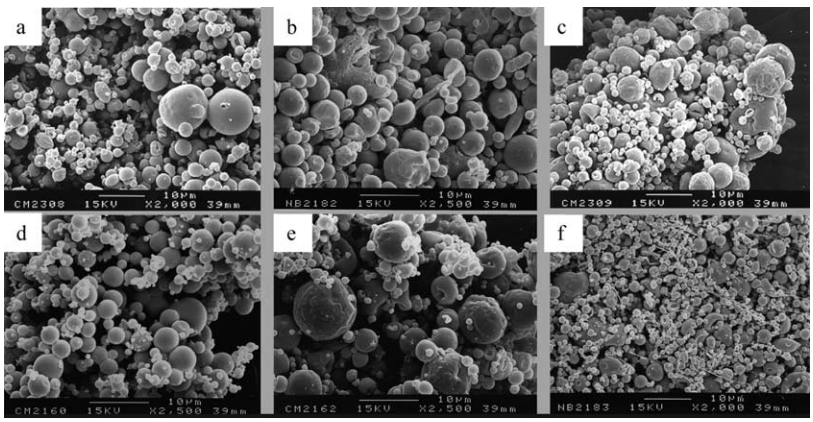

Fig. 4. Scanning electron microscopy pictures of spray-dried microspheres: MS-16 (a), MS-13 (b), MS-18 (c), MS-11 (d), MS-15 (e), MS-19 (f).

extrusion method exhibited several advantages (Table 3). For instance, because of the added mixing action of the screw, a better homogeneity of the peptide dispersion in the polymer was obvious visually. No difference was seen from SEM observations depending on the copolymer type (Fig. 5). Comparison of SEC data before and after extrusion (Tables 1 and 3) showed that the polymeric carrier was not degraded by the process. In the case of ram-extrusion, a 5% decrease in M_w was noticed with the higher molecular weight RG752 copolymer (#3), the values being stable for the other trials (#1–2). In the case of micro-extrusion (#4–9), M_w increased generally and the molecular weight distribution was narrower after extrusion ($I = 1.5$), which indicated that lower molecular weight chains were particularly subject to degradation. Similar results have been reported with the ram-extrusion of low molecular weight PLGA and a synthetic octapeptide [33] or with drug-free PLGA-block-poly(oxyethylene) copolymers [34]. In the case of ram-extrusion, the process affected degarelix integrity to a certain extent, since purity values were less than 98% for the three trials (#1–3). Micro-extrusion was less harmful, purity values being comparable to those obtained with solvent methods (Table 2). This may be explained by the improved peptide dispersion in the polymeric matrix resulting in a more effective shielding effect, and/or by a better control of the processing conditions between the custom-made and the small-scale industrial equipment. Particle size D[4,3] was found to be reproducible at 165 ± 15 μm (#4,6–9), uniformity and span values being similar to the double emulsion values. Water content was ca. 1% (Table 3) as for MS prepared from solvent methods.

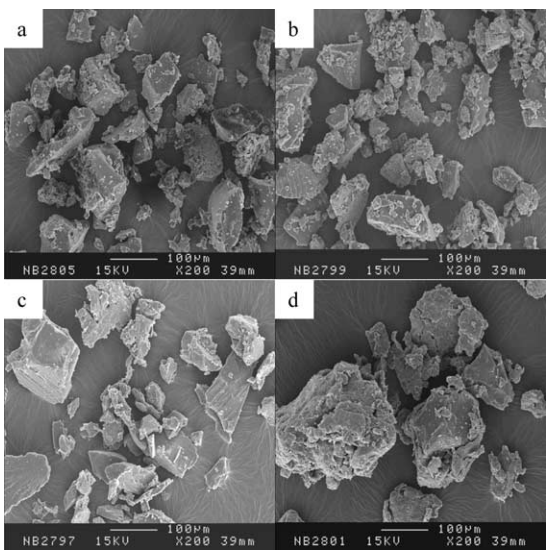

Fig. 5. Scanning electron microscopy pictures of micro-extrusion micro-granules: MG-6 (a), MG-8 (b), MG-4 (c), MG-9 (d).

3.3. Gamma sterilization

Gamma sterilization can be considered as a promising method for sterilization of biodegradable drug delivery systems provided the radiolytic degradation of the polymeric carrier and of the drug are limited. Several studies have clearly shown the mechanisms of free radical formation and stabilization are responsible for chain cleavage and reduction in average molecular weight [18,35]. Hence, at the recommended dose of 25 kGy, a 10–15% loss in M_w and an increase in polydispersity was documented for PLGA tetracycline-loaded microspheres [35], and in another study drug-free hydrophilic PLGA-based microspheres were shown to be more sensitive to radiolytic degradation (20%) than those based on capped PLGA of the same molecular weight and composition (Resomer RG503H vs. RG503) [18]. The data from our work (Table 4) are consistent with the expected loss in M_w, even if differences were noticed between polymers. For instance, the Phusiline copolymer (MS-7/10 and MG-9) appeared to be less resistant to radiolysis than Resomer and Medisorb copolymers. The observed difference may be attributable to the higher molecular weight and broader polydispersity of this particular polymer.

The susceptibility of degarelix to radiolysis was clearly evidenced as well, particularly for double emulsion (#2–10) MS. Purity remained, however, at an acceptable level for

Table 4
Effect of gamma-irradiation on PLGA and peptide degradation

Microparticles	Peptide purity[a] (%)	$M_w - I$[b] (Da)	M_w decrease[c] (%)
Degarelix (control)	97.5		
MS-2	96.0	7000 − 2.6	17.6
MS-3	98.1	–	–
MS-4	97.3	–	–
MS-6	96.5	–	–
MS-7	95.1	14100 − 3.7	25.0
MS-8	97.5	7400 − 2.4	10.8
MS-9	94.9	–	–
MS-10	95.9	13900 − 3.8	26.1
MS-11	96.8	8500 − 1.9	−2.4
MS-12	97.8	–	–
MS-13	97.2	–	–
MS-14	98.1	–	–
MS-15	98.3	–	–
MS-16	98.1	–	–
MS-17	98.1	–	–
MS-18	97.5	–	–
MG-4	98.5	10100 − 2.7	24.0
MG-6	99.2	8500 − 2.4	22.0
MG-7	98.6	8800 − 2.6	23.5
MG-8	98.0	9400 − 2.4	23.6
MG-9	98.2	13300 − 3.5	33.2

[a] From HPLC.
[b] Average molecular weight in weight M_w and polydispersity I from SEC.
[c] $100 \times (M_w - M_{w0})/M_{w0}$ with M_{w0} the starting molecular weight (Tables 2 and 3).

this stage of the development, i.e. beyond 95%. Optimization of the sterilization conditions e.g. under special atmospheres, or at a reduced dose, will certainly have to be considered in the future. Interestingly, MP produced by extrusion exhibited less degarelix degradation (MG-4–MG-9). Radiolysis protection induced by extrusion was previously reported for the polymeric carrier alone [9]. In addition, stabilization as a result of drug dispersion (molecularly solubilized vs. micro-domains) in a PLGA carrier was described [35] and could also apply to our observations. A gross dispersion as expected from extrusion may promote stability of the drug substance.

4. Conclusions

Degarelix-loaded PLGA microparticles with various peptide loadings were prepared by both solvent (double-emulsion solvent evaporation and spray-drying) and non-solvent methods (ram- and micro-extrusion). A better entrapment efficiency was featured by spray-drying in comparison to double emulsion and less peptide degradation was caused by micro-extrusion than by ram-extrusion. In addition to PLGA composition, residual monomer amount and nature of chain ends were shown to differ for polymers selected from distinct commercial sources. These properties are believed to be potentially of importance for the release of the active, particularly for the less pure Medisorb copolymers for instance. Basically three types of MP were prepared. Microparticles of type-1 refer to double-emulsion microspheres, 100 μm in average volume diameter, of important surface area because of internal and surface porosity. Spray-dried microspheres (type-2) are much smaller in size (50 μm), and are characterized by a poreless surface. No difference in MP characteristics was noticed with respect to PLGA, excepting fibre formation with the higher molecular weight Phusiline copolymer. Finally, type-3 particles are non-spherical, relatively large (165 μm) and compact microgranules produced by extrusion. Gamma sterilization at 25 kGy under dry ice caused some polymer degradation, particularly with the Phusiline PLGA. Degarelix radiolysis was limited, a better protection being offered to extruded microgranules in comparison to microspheres. Altogether, spray-drying and micro-extrusion were the preferred formulation technologies in combination with Resomer or Medisorb copolymers. However, different drug release profiles are expected from the different types of degarelix-MP, as well as variable potencies to suppress testosterone. Therefore, the formulations will also be tested for efficacy in an appropriate animal model.

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