# Effect of $\gamma$-Sterilization Process on PLGA Microspheres Loaded with Insulin-like Growth Factor – I (IGF-I)

C. CARRASCOSA, L. ESPEJO, S. TORRADO AND J. J. TORRADO*
*Departamento de Farmacia y Tecnología Farmacéutica*
*Facultad de Farmacia*
*Plaza Ramón y Cajal E-28040 Madrid, Spain*

**ABSTRACT:** The influence of $\gamma$-sterilization on the physicochemical properties of a controlled release formulation for the insulin-like growth factor-I (IGF-I) was investigated in this study. Recombinant human insulin-like growth factor-I (rhIGF-I) was efficiently entrapped in poly (D,L-lactide-co-glycolide) (PLGA) microspheres by water-in-oil-in-water (W/O/W) solvent evaporation technique. Microspheres were irradiated at a dose of 25 kGy and evaluated by means of scanning electron microscopy (SEM) and differential scanning calorimetry (DSC). The stability of the released protein was investigated by circular dichroism (CD) and sodium dodecyl sulfate polyacrilamide gel electrophoresis (SDS-PAGE). No difference was noticed in microsphere size and morphology before and after irradiation. Drug loading remains essentially the same after the sterilization process. However, rhIGF-I aggregation was detected by electrophoresis. In addition, subtle changes in DSC pattern were noticed for irradiated microspheres. *In vitro* drug release from irradiated microspheres was also affected, showing an increased burst effect. From this results it can be concluded that $\gamma$-sterilization process causes changes in the properties of rhIGF-I loaded microspheres.

**KEY WORDS:** poly(D,L-lactide-co-glycolide), microspheres, gamma irradiation, recombinant human insulin-like growth factor-I.

*Author to whom correspondence should be addressed. E-mail: torrado1@farm.ucm.es



0885-3282/03/02 0095–14 $10.00/0 DOI: 10.1177/088532803038026

## INTRODUCTION

**Parenteral drug delivery** systems based on biodegradable polyesters, such as the lactide and glycolide homo- and copolymers have been intensively investigated in the last decades as controlled release delivery systems for protein agents [1,2]. They have been proved to be useful for the controlled release of several protein and peptide drugs [3–5]. IGF-I is a naturally occurring protein which has many important biological effects [6]. The trophic, as well as neuromodulatory roles of the IGF-I in the brain have been studied over the last few years [7–10]. It is well known that brain concentration of IGF-I and its related molecules are involved in neurodegenerative processes in which IGF-I containing pathways are compromised, such as cerebellar ataxia, among others [11,12]. Like many other proteins, IGF-I has a short biological half-life [13]. Thus, the development of a controlled release system that allows protein delivery to be used for this neuroprotective indication is highly desirable. Entrapment in PLGA microparticles has been recently shown to be an attractive controlled delivery system for IGF-I, since it has been successfully achieved for other therapeutic indications [14,15]. These previous studies on microencapsulation of IGF-I have focused on protein stability and/or suitability for application issues. However, limited attention has been given to sterilization of these parenteral IGF-I controlled release systems. Since PLGA-based drug delivery systems are very sensitive to dry or moist heat, and ethylene oxide is not applicable due to its toxic residues, $\gamma$-irradiation currently remains the only accepted method for terminal sterilization of this type of substances. Potential new techniques, e.g. low-temperature plasma sterilization are still under evaluation [16].

A potential disadvantage of terminal $\gamma$-sterilization can be the radiolytic degradation of incorporated drug and polymer matrix [17]. It has been established that ionizing radiation induces crosslinking and/or chain scission and concomitant molecular weight loss of PLGA polymers in a dose-dependent manner [18]. Therefore, $\gamma$-irradiation accelerates polymer degradation rate and, thus, the release profile of microencapsulated drugs might be affected. In addition, the stability of the drug incorporated in the polymeric matrix has to be taken into account. The effects of $\gamma$-irradiation on the microparticulate systems made of PLGA can be also influenced by drug loading and are not easily predicted because of the different chemico-physical characteristics of the entrapped drug and its interactions with the polymer matrix [19,20].

In this work, the effects of $\gamma$-irradiation, at a dose of 25 kGy on the physicochemical properties and release rate of rhIGF-I incorporated in

PLGA microspheres were evaluated. A 25 kGy dose is accepted to be satisfactory for the purpose of sterilizing pharmaceutical products without providing any biological validation [21].

## MATERIALS AND METHODS

### Materials

rhIGF-I was provided by GroPep (Adelaide, Australia). PLGA 50 : 50, with an inherent viscosity of 0.8 dL/g was purchased from Boehringer Ingelheim (Resomer® RG 506), Polyvinyl alcohol (PVA) 15,000 was obtained from Fluka (Buchs, Switzerland) and Tween 20 was from Serva (Germany). Unless specified otherwise, all other reagents used were of analytical grade.

### Preparation of the Microspheres

Microencapsulation of rhIGF-I was performed by a W/O/W solvent evaporation technique. For this, 20 mg of lyophilized rhIGF-I were reconstituted in 0.4 mL of 10 mM sodium phosphate buffer (pH 6.0), containing 15 mg/mL of Tween 20. This aqueous solution was emulsified with 5 mL of methylene chloride (MC) containing 250 mg of PLGA at 13,500 rpm using an Ultraturrax® for 2 min to form the primary W/O emulsion. This W/O emulsion was then poured into 200 mL of a 2% (w/v) PVA/PBS solution (pH 7.4) and homogenized for 2 min at 13,500 rpm. The resulting W/O/W emulsion was stirred at 700 rpm for 4 h at room temperature to allow MC to evaporate. Microspheres were recovered after centrifugation at 6000 rpm for 20 min, washed three times with deionized water, freeze-dried, and stored at 4°C.

### γ-Irradiation of Placebo and IGF-I Loaded Microspheres

Placebo and loaded microspheres were irradiated by using $^{60}Co$ as irradiation source (Aragogamma, Barcelona, Spain). Irradiation was performed at room temperature. A dose of 25 kGy (2.5 Mrad) at a 3.33 kGy/h dose rate was applied.

### Characterization of Microspheres

Microparticle size and morphology before and after irradiation were evaluated. Microparticle size was determined by laser light

diffractometry (Galai® Cis-1). Samples of microspheres were suspended in deionized water, and analyzed with gentle stirring. The results are the average of three determinations. Morphology of the microspheres was analyzed by SEM (Jeol 6400 electron microscope, CAI, UCM, Madrid).

## Protein Loading Determination

Protein loading was determined by dissolving 10 mg of microspheres in 1 mL of 1 N NaOH, with shaking, at room temperature for 24 h as previously described [14]. The protein concentration was measured by UV absorption at 284 nm on a Beckman DU-7 spectrophotometer (Beckman, UK), using an extinction coefficient of 1.043 $(mg/mL)^{-1}\ cm^{-1}$ for rhIGF-I in 1 N NaOH. The loading level of rhIGF-I in the microspheres was expressed as % w/w of microparticles. The rhIGF-I loading efficiency was determined by expressing the amount of protein entrapped in the microparticles as a percentage of the total amount of protein used to make the rhIGF-I loaded microspheres.

## Differential Scanning Calorimetry (DSC)

Thermal analysis was performed on samples of loaded microspheres by using a DSC Mettler TA 4000 (Mettler, Switzerland). Approximately 4 mg samples were heated from 0 to 100°C in closed aluminium pans, at a heating rate of 10°C/min, under a constant flow of nitrogen.

## Protein Stability

Nonreducing SDS-PAGE and CD measurements were used to evaluate the integrity of rhIGF-I after microencapsulation and irradiation.

The presence of possible covalent aggregates was detected on Tris-Glycine nonreducing SDS-PAGE gels. Gels were run at a constant voltage, and stained with colloidal Coomassie blue.

CD spectroscopy was performed on a Jasco J-710 spectropolarimeter (Japan). Far-UV CD spectra were recorded at 25°C for protein solutions of ~100 μg/mL in PBS, pH 7.4. Scans were obtained from 260 to 200 nm at a step resolution of 1 nm, a scanning speed of 50 nm/min and with 50 millidegrees (mdeg) sensitivity, using 2 mm cuvettes. Each spectrum was the average of eight accumulated scans, from which the buffer spectrum was subtracted.

### *In vitro* Release Studies

*In vitro* release studies were carried out at $37 \pm 0.5^{\circ}C$, in PBS pH 7.4 (containing 0.02% (w/v) sodium azide to avoid microbial growth). Irradiated and nonirradiated drug loaded microspheres ($\sim 10$ mg) were suspended in 1 mL of release medium in 3 mL borosilicate vials and placed in a shaker bath. At predetermined time intervals, samples of the release medium were withdrawn, and replaced by fresh buffer. For sampling, microspheres and supernatant were separated by centrifugation at 3175 rpm for 10 min. rhIGF-I content in the withdrawn supernatants was analyzed by micro-BCA assay [22]. Experiments were performed in triplicate.

## RESULTS AND DISCUSSION

### Microspheres Morphology

As observed by SEM analysis, the microspheres possessed a regular spherical shape with a relatively rough surface and a few pores (Figure 1). No differences in microspheres morphology could be appreciated between irradiated and nonirradiated microspheres. As determined by laser light diffractometry, γ- irradiation caused a subtle, not significant, increase in particle size. The mean particle size of nonirradiated microspheres was $1.5 \pm 0.13\,\mu m$, whereas it was found to be $1.88 \pm 0.22\,\mu m$ for irradiated microspheres.

### Differential Scanning Calorimetry (DSC)

Figure 2 shows the DSC thermograms of rhIGF-I loaded microspheres, before and after irradiation. An endotherm-like peak in the region of approximately $50^{\circ}C$, characteristic of the PLGA copolymer, which represents glass transition, is maintained in the rhIGF-I loaded microspheres thermograms. In accordance with previously reported studies [20], a shift of the glass transition toward lower temperatures is observed as a consequence of γ-irradiation of PLGA polymers. Changes in the polymeric matrices occurring as a consequence of radiolytic events, such as chain scission, may be responsible for this shift of the glass transition [23]. The presence of rhIGF-I in the microspheres cannot be detected by this DSC analysis due to the lack of sensitivity of the apparatus.

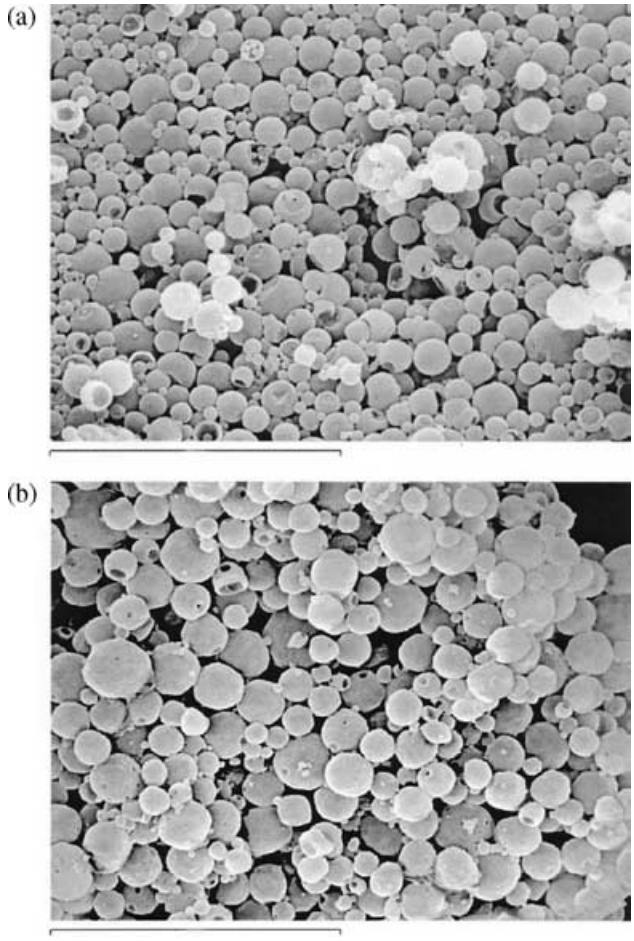


**Figure 1.** SEM of rhIGF-I loaded microspheres (bar = 30 μm). (a) Nonirradiated; (b) Irradiated.

## Drug Content

rhIGF-I was efficiently entrapped in PLGA microparticles by the W/O/W solvent evaporation technique. The actual load of protein in the microspheres was estimated to be 5.4% (w/w) for PLGA microspheres before irradiation, and 5.14% (w/w) for irradiated microspheres, which indicated an encapsulation efficiency of 74.3% and 70.7% respectively. It seemed that $\gamma$-irradiation did not have an effect in drug loading, since no significant difference was observed between irradiated and

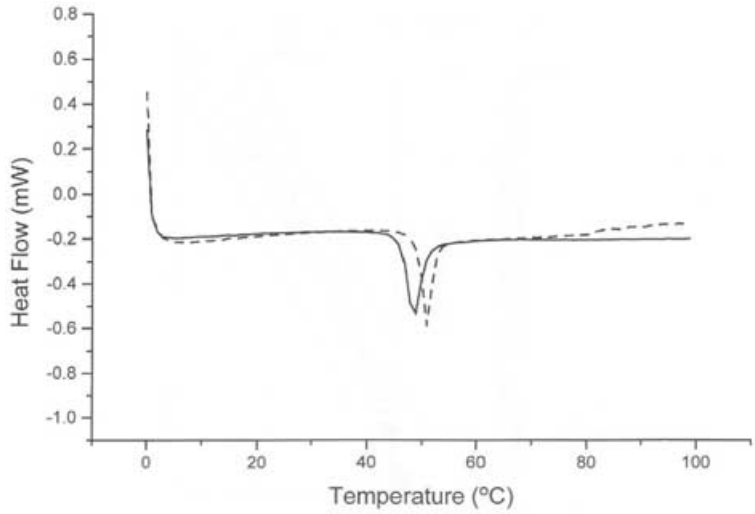


**Figure 2.** DSC thermograms of microspheres loaded with rhIGF-I before (- - - -) and after (——) γ-irradiation.

nonirradiated microspheres. Similar results have been reported previously in the literature [24].

## Protein Stability

Nonreducing SDS-PAGE analysis and CD measurements showed that rhIGF-I was released *in vitro* from irradiated as well as from nonirradiated microparticles in a predominantly intact form. Figure 3 shows a representative nonreducing SDS-PAGE gel of *in vitro* released rhIGF-I. As seen in this figure, identical bands were obtained for the native protein (7.6 kDa), and for the protein released from the nonsterilized microspheres, and no additional bands, indicating the presence of higher molecular weight species, were observed. However, in the sterilized samples, a weak additional band, of approximately 14 kDa is detected, indicating the aggregation (dimerization) of the peptide, probably as a result of the irradiation process. The formation of soluble dimers appears to be the most predominant form of covalent aggregation for rhIGF-I [25]. Fransson [25] suggested, among others, the implication of a radical-promoted pathway in rhIGF-I dimerization. Indeed, it is known that peptide and protein molecules irradiated in the solid state absorb radiation energy directly, producing various radicals [26]. These results strongly

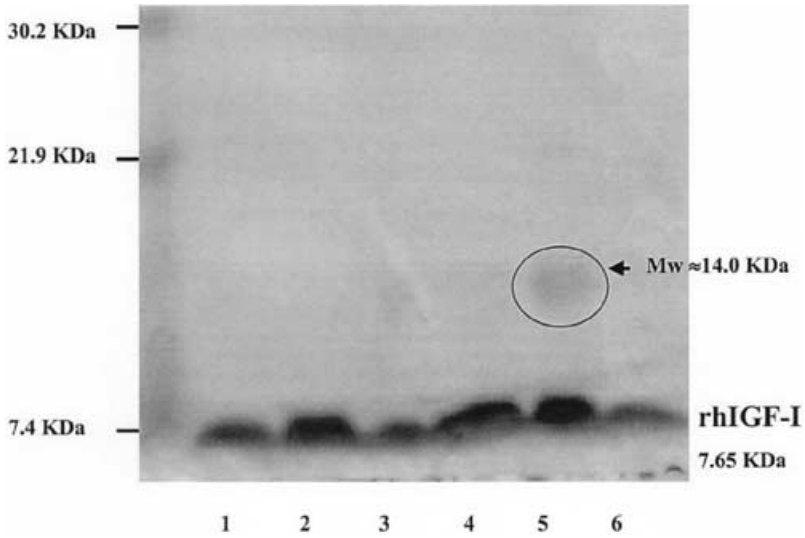


**Figure 3.** Nonreducing PAGE. (1) 10 μg raw material; (2) 10 μg microspheres, nonirradiated; (3) 10 μg microspheres, irradiated; (4) 20 μg microspheres, nonirradiated; (5) 20 μg microspheres, irradiated; (6) 10 μg raw material.

suggest possible $\gamma$-irradiation effects on rhIGF-I stability, and therefore, further HPLC analysis of these samples will be carried out to determine any degradation protein products due to $\gamma$-sterilization.

Far-UV CD measurements, which reflect the secondary structure of proteins, were performed to investigate the conformational stability of *in vitro* released rhIGF-I. Typical Far-UV CD scans of native rhIGF-I and rhIGF-I released from the microspheres *in vitro* are shown in Figure 4. This figure shows that the Far-UV spectra for the released protein did not deviate from the spectrum for the native rhIGF-I in PBS pH 7.4. As it can be seen in these spectra, rhIGF-I exhibits strong negative bands characteristic of this protein, which has been reported to have a 30% content of $\alpha$-helix structure [27,28]. The small differences in intensity, but not in spectral shape, observed for the irradiated sample strongly suggest that a small part of the protein could have been hydrolyzed by the $\gamma$-irradiation procedure. The hydrolyzed protein would still be measured by the BCA assay, but it would hardly contribute to the CD spectrum. Another explanation for this reduced intensity could be the presence of protein dimers, as revealed by SDS-PAGE.

These CD measurements showed that the rhIGF-I secondary structure has not been affected by microencapsulation, and that most of the protein

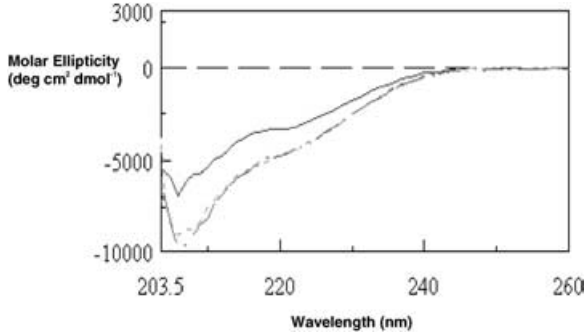


**Figure 4.** Far-UV CD spectrum of native and *in vitro* released rhIGF-I from PLGA microspheres before and after γ-irradiation. ( — — ) native rhIGF-I; (·····) rhIGF-I released from nonirradiated microspheres; (——) rhIGF-I released from irradiated microspheres.

retains its secondary structure after γ-sterilization. Moreover, rhIGF-I released from microspheres, following γ-irradiation, was detected in rat serum samples by ELISA (Diagnostic Systems Laboratories ref. 10-2800) (results not shown), confirming protein stability.

## *In vitro* Release

Figure 5(A) and (B) show the release profiles of rhIGF-I from irradiated, and nonirradiated microspheres over a period of 28 days. The release profiles exhibited an initial burst release effect, followed by a very slow release pattern.

Irradiated microspheres showed an initial burst release effect of $36.01 \pm 11.00\%$ whilst it was found to be $26.39 \pm 3.64\%$ for non-irradiated microparticles. These are relatively high burst release values, but are consistent with the type of polymer used in the present study. The initial burst is attributed to the immediate dissolution and release of the portion of the drug located on and near the surface of the microspheres. Factors affecting initial burst effect are protein loading and type of polymer. Previous studies [14,29], indicated that the end group of the polymer, (blocked or unblocked), could have a significant effect on the initial burst release of rhIGF-I from PLGA microspheres. Resomer® RG 506, used in the present investigation, presents a blocked-end form, containing hydrophobic alkyl end groups, which might be responsible for the observed large initial burst of rhIGF-I. The blocked PLGA tends to be more soluble in the organic solvents used in microsphere preparation, providing greater mobility for the protein

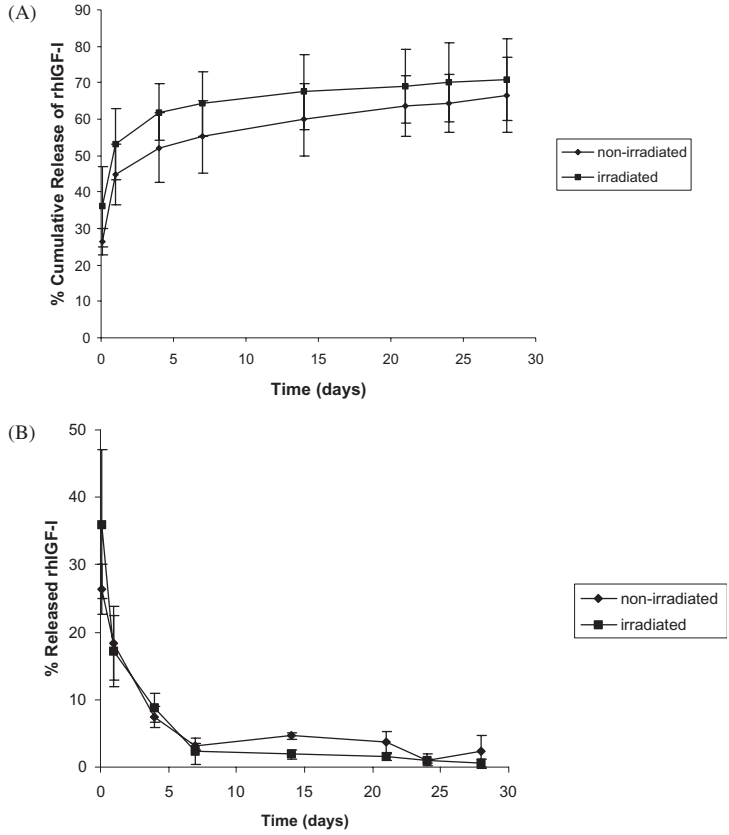


**Figure 5.** Cumulative (A) % and distributive (B) *in vitro* release of rhIGF-I from PLGA microspheres before and after $\gamma$-sterilization.

particles [30]. Protein particles may then migrate to the surface of the microspheres during production. This may cause a high initial burst of rhIGF-I from the microspheres followed by a slow release. In addition, the high initial burst effect could probably also be attributed to pores, which are initially present at the surface of the microspheres, as showed by Wang et al. [31].

Figure 5(B) shows that gamma sterilization leads to an increased ''burst'' effect, but does not significantly affect the release rate during the subsequent(s) release phase(s). The higher value for the sterilized microspheres can be explained by the chain cleavage effect of $\gamma$-irradiation on PLGA. With decreasing polymer molecular weight the mobility of the macromolecules increases and, thus, the free

volume available for diffusion and the diffusion coefficient of the drug increase [32].

The observed *in vitro* release profile for rhIGF-I is a typical finding and has been seen previously with many alternative proteins [1,33]. Release patterns of the irradiated and nonirradiated microspheres appeared to be similar, as can be seen in Figures 5(A) and (B). However, mainly as a consequence of the observed higher burst release effect, a higher amount of protein was released from γ-irradiated PLGA microspheres at the end of the study. For nonirradiated microspheres, the percentage of protein release over the 28 days period was $66.64 \pm 10.25\%$, whereas the corresponding value for the irradiated formulation was $70.74 \pm 11.24\%$. These results are in good agreement with the results previously reported by some authors [19,20] and show that *in vitro* release of rhIGF-I from PLGA microspheres is affected by γ-sterilization.

## DISCUSSION

These results strongly suggest that the physicochemical characteristics of rhIGF-I loaded PLGA microspheres are affected by the γ-sterilization process. Changes in the DSC patterns, as well as an increased *in vitro* burst release effect was noticed for the irradiated microspheres. Concerning protein stability, no changes on the protein conformation were observed by CD, although SDS-PAGE electrophoresis reveals the presence of aggregated forms of rhIGF-I. The investigation should be extended to other aspects of the system. Evaluation of physicochemical characteristics of the polymers such as molecular weight distribution, investigation of the effects of γ-irradiation on the formation on free radicals in polymer and drug, and further studies on protein stability by HPLC will be taken up in future works due to the therapeutic interest of this rhIGF-I controlled release formulation.

rhIGF-I loaded PLGA microspheres are a great therapeutic promise for chronic brain diseases where rhIGF-I has proven effective such as inherited cerebellar neurodegeneration [11]. *In vivo* additional studies carried out by our investigation group confirm the continuous rhIGF-I release from the microspheres, resulting in a prolonged behavioral recovery of mutant mice with inherited Purkinje cell degeneration (pcd) [34].

This study reveals the importance of evaluating the influence of γ-irradiation of every parenteral drug delivery PLGA-based system, since this sterilization method seems to modify the initial characteristics of this system, and its effects are not predictable.

## ACKNOWLEDGMENTS

This work has been supported by the Spanish Ministry of Health (FIS project PI020418).

## REFERENCES

1. Cohen, S., Yoshioka, T., Lucarelli, M., Hwang, L.H., and Langer, R. (1991). Controlled Delivery Systems for Proteins Based on Poly(lactic/glycolic acid) Microspheres, *Pharm. Res*, **8**: 713–720.
2. Baldwin, S.P. and Saltzman, W.M. (1998). Materials for Protein Delivery in Tissue Engineering, *Adv. Drug Deliv. Rev.*, **33**: 71–86.
3. Cleland, J.L., Duenas, E., Daugherty, A., Marian, M., Yang, J., Wilson, M., Celniker, A.C., Shahzamani, A., Quarmby, V., Chu, H. Mukku, V., Mac, A., Roussakis, M., Gillette, N., Boyd, B., Yeung, D., Brooks, D., Maa, Y-F., Hsu, C. and Jones, J.S. (1997). Recombinant Human Growth Hormone Poly(lactic-co-glycolic Acid) (PLGA) Microspheres Provide a Long Lasting Effect, *J. Control Release*, **49**: 193–205.
4. Okada, H. (1997). One- and Three-Month Release Injectable Microspheres of the LH-RH Superagonist Leuprorelin Acetate, *Adv. Drug Deliv. Rev.*, **28**: 43–70.
5. Menei, P., Daniel, V., Montero-Menei, C., Brouillard, M., Pouplard-Barthelaix, A. and Benoit, J.P. (1993). Biodegradation and Brain Tissue Reaction to Poly(D,L-Lactide-co-glicolide) Microspheres, *Biomaterials*, **14**: 470–478.
6. Zapf, J. and Froesch, E.R. (1986). Insulin-like Growth Factors Somatomedins; Structure, Secretion, Biological Action and Physiological Role, *Horm. Res.*, **24**: 121–130.
7. Torres-Aleman, I., Barrios, V. and Berciano, J. (1998). The Peripheral Insulin-like Growth Factor System in Amyotrophic Lateral Sclerosis and in Multiple Sclerosis, *Neurology*, **50**: 772–776.
8. Torres-Aleman, I. (1999). Insulin-like Growth Factors as Mediators of Functional Plasticity in the Adult Brain, *Horm. Metab. Res.*, **31**: 114–119.
9. Fernández, A.M., Gonzalez de la Vega, A.G., Planas, B. and Torres-Aleman, I. (1999). Neuroprotective Actions of Peripherally Administered Insulin-like Growth Factor I in the Injured Olivo-Cerebellar Pathway, *Eur. J. Neurosci.*, **11**: 2019–2030.
10. Doré, S., Kar, S., Zheng, W-H. and Quirion, R. (2000). Rediscovering Good Old Friend IGF-I in the New Millennium: Possible Usefulness in Alzheimer's Disease and Stroke, *Pharm. Acta. Helv.*, **74**: 273–280.
11. Torres-Aleman, I., Barrios, V., Lledo, A. and Berciano, J. (1996). The Insulin-like Growth Factor I System in Cerebellar Degeneration, *Ann. Neurol.*, **39**: 335–342.
12. Fernandez, A.M., De La Vega, A.G. and Torres-Aleman, I. (1998). Insulin-like Growth Factor I Restores Motor Coordination in a Rat Model of Cerebellar Ataxia, In: *Proc. Natl. Acad. Sci.*, USA., **95**: 1253–1258.

13. Guler, H.P. Zapf, J. and Froesch, E.R. (1989). Short Term Metabolic Effects of Recombinant Insulin-like Growth Factor in Healthy Adults, *N. Engl. J. Med.*, **317**: 137–140.
14. Lam, X.M., Duenas, E.T. Daugherty, A.L., Levin, N. and Cleland, J.L. (2000). Sustained Release of Recombinant Human Insulin-like Growth Factor-I for Treatment of Diabetes, *J. Control Release*, **67**: 281–292.
15. Singh, M., Shirley, B., Bajwa, K., Samara, E., Hora, M. and O'Hagan, D. (2001). Controlled Release of Recombinant Insulin-like Growth Factor from a Novel Formulation of Polylactide-co-glycolide Microparticles, *J. Control Release*, **70**: 21–28.
16. Mohr, D., Wolff, M. and Kissel, T. (1999). Gamma Irradiation for Terminal Sterilization of 17-β-Estradiol Loaded Poly-(D,L-lactide-co-glycolide) Microspheres, *J. Control Release*, **61**: 203–217.
17. Bruck, S.D. and Mueller, E.P. (1988). Radiation Sterilization of Polymeric Implant Materials, *J. Biomed. Mater Res.*, **22**: 133–144.
18. DeLuca, P.P., Kenley, R.A. and Hausberger, A.G. (1995). Gamma Irradiation Effects on Molecular Weight and *in vitro* Degradation of Poly (D,L-lactide-co-glycolide) Microparticles, *Pharm. Res.*, **12**: 233–242.
19. Volland, C., Wolff, M. and Kissel, T. (1994). The Influence of Terminal Gamma-sterilization on Captopril Containing Poly(D,L-lactide-co-glycolide) Microspheres, *J. Control Release*, **31**: 293–305.
20. Montanari, L., Cilurzo, F., Valvo, L., Faucitano, A., Buttafava, A., Groppo, A., Genta, I. and Conti, B. (2001). Gamma Irradiation Effects on Stability of Poly(lactide-co-glycolide) Microspheres Containing Clonazepam, *J. Control Release*, **75**: 317–330.
21. European Guideline 3AQ4a. (July 1992). The Use of Ionizing Radiation in the Manufacture of Medicinal Products, Official Publications of the European Communities, London.
22. Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J. and Klenk, D.C. (1985). Measurement of Protein using Bicinchoninic Acid, *Anal. Biochem.*, **150**: 76–85.
23. Hausberger, A.G., Kenley, R.A. and DeLuca, P. (1995). Gamma Irradiation Effects on Molecular Weight and *in vitro* Degradation of Poly(D,L-lactide-co-glicolide) Microparticles, *Pharm. Res.*, **12**: 851–856.
24. Bittner, B., Mäder, K., Kroll, C., Borchert, H-H. and Kissel, T. (1999). Tetracycline-HCl-loaded poly (D,L-lactide-co-glycolide) Microspheres Prepared by a Spray Drying Technique, Influence of γ-Irradiation on Radical Formation and Polymer Degradation, *J. Control Release*, **59**: 23–32.
25. Fransson, J.R. (1997). Oxidation of Human Insulin-like Growth Factor I in Formulation Studies. 3. Factorial Experiments of the Effects of Ferric Ions, EDTA, and Visible Light on Methionine Oxidation and Covalent Aggregation in Aqueous Solution, *J. Pharm. Sci.*, **86**: 1046–1050.
26. Rothen-Weinhold, A., Besseghir, K., Vuaridel, E., Sublet, E. and Oudry, N. (1999). Stability Studies of a Somatostatin Analogue in Biodegradable Implants, *Int. J. Pharm.*, **178**: 213–221.

27. Fransson, J., Hallén, D. and Florin-Robertsson, E. (1997). Solvent Effects on the Solubility of Human Insulin-like Growth Factor I, *Pharm. Res.*, **14**: 606–612.

28. Souillac, P.O., Middaugh, C.R. and Rytting, J.H. (2002) Investigation of Protein-carbohydrate Interactions in the Dried State. 2. Diffuse Reflectance FTIR Studies, *Int. J. Pharm.*, **235**: 207–218.

29. Tracy, M.A., Ward, D.L., Firouzabadian, L., Wang, Y., Cong, N., Quian, R. and Zhang Y. (1999). Factors Affecting the Degradation Rate of Poly(lactide-co-glycolide) Microspheres *in vivo* and *in vitro*, *Biomaterials*, **20**: 1057–1062.

30. Cleland, J.L., Lim, A., Barron, L., Duenas, E.T. and Powell, M.F. (1997). Development of a Single Shot Subunit Vaccine for HIV-1: Part 4. Optimizing Microencapsulation and Pulsatile Release of MN rgp 120 from Biodegradable Microspheres, *J. Control Release*, **47**: 135–150.

31. Wang, J., Wang, B.M. and Schwendeman, S.P. (2000). Characterization of the Initial Burst Drug Release from Poly(D,L-lactide-co-glycolide) Microspheres II. Alterations in Surface Permeability Implicating in Cessation of Burst Release, In: *Proceedings AAPS Annual Meeting*, Indianapolis, USA.

32. Faisant, N. Siepmann, J. and Benoit, J.P. (2002). PLGA-based Microparticles: Elucidation of Mechanisms and a New, Simple Mathematical Model Quantifying Drug Release, *Eur. J. Pharm. Sci.*, **15**: 355–366.

33. Crotts, G., Sah, H. and Park, TG. (1997). Adsorption Determines In-vitro Protein Release Rate from Biodegradable Microspheres: Quantitative Analysis of Surface Area during Degradation, *J. Control Release*, **47**: 101–111.

34. Carrascosa, C., Torres-Aleman, I., Lopez-Lopez, C., Carro, E., Espejo, L., Torrado, S. and Torrado, J.J. (2003) Microspheres Containing Insulin-like Growth Factor for Treatment of Chronic Neurodegeneration, *Biomaterials*, in press.

## BIOGRAPHIES

### C. Carrascosa and L. Espejo

C. Carrascosa and L. Espejo are Ph. D. students in the department of Pharmaceutical Technology.

### S. Torrado and J. J. Torrado

S. Torrado and J. J. Torrado are Professors of Pharmaceutical Technology who have published over 50 papers on the field of development of drug formulations. Their research is focused on the applications of new polymeric based controlled release formulations.