Poster

Insulin-loaded poly(D,L-lactide-*co*-glycolide) micro- and nanoparticles obtained by Flow Focusing technology

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Introduction

The subcutaneous injection of insulin typically results in an inappropriate control of glycaemia, and poor treatment compliance. Despite oral administration could be much advantageous, many difficulties must be faced, e.g., the protection of the biomolecule against self-aggregation, and enzymatic degradation. Intense research is undergoing to develop oral delivery systems for the enhancement of the efficacy and safety of insulin [1, 2]. In this work, we describe the preparation of insulin-loaded poly(D,L-lactide-*co*-glycolide) (PLGA) particles using two methods: the flow focusing (FF) technology, and the traditional solvent evaporation method (SEV). It is compared the effect of both methodologies on the: *i*) geometry and physicochemical properties of the particles; *ii*) chemical structure of insulin inside PLGA; and *iii*) insulin loading and release properties.

Materials and Methods

Preparation of the insulin-loaded PLGA particles: a water-in-oil emulsion was prepared by mixing a 10 % (v/v) acetic solution containing insulin (10 mg/mL) with 1 mL of a 1 % (w/v) solution of PLGA in ethyl acetate. When PLGA particles were prepared by SEV [3], this emulsion was added to a 0.3 % (w/v) PVA solution and homogenized. Then, PLGA particles were spontaneously formed when the double emulsion was diluted with 20 mL of a 2 % (w/v) PVA solution. Following the FF procedure [4], the previously prepared emulsion was used as focused fluid in a simple flow focusing nozzle [Avant 2 (D = 50 μm), Ingeniatrics Tecnologías S.L., Spain]. Distilled water was used as focusing fluid. PLGA particles were formed into a 0.3 % (w/v) PVA solution.

Characterization methods: the mean particle size and particle size distributions were measured at room temperature by laser scattering (Partica LA-950V2, Horiba). Insulin loading was determined by reverse phase-high performance liquid chromatography (RP-HPLC) (Hitachi LaChrom® (D-7000) Series HPLC system). Insulin content was expressed in terms of insulin entrapment efficiency (EE, %) and insulin loading (%) [5]. The zeta potential (ζ)-pH trend and ζ-ionic strength dependence were investigated in order to characterize surfaces of particles [4]. Fluorescence spectroscopy (maximum excitation λ = 311 nm) was carried out to establish if the tertiary structure of insulin is kept unmodified after encapsulation process and, additionally, to qualitatively check the efficacy of the protein loading [6]. Insulin release from PLGA was performed *in vitro* at 37.0 ± 0.5 ºC by following the dialysis bag method, and using PBS (pH 7.4 ± 0.1) as the release medium [5].

Results and Discussion

SEV method allows obtaining smaller particles than the FF technology: 0.49 ± 0.32 μm and 1.26 ± 0.12 μm, respectively. On the opposite, a more narrow size distribution was defined by FF (figure 1). The morphology and surface of the particles were not affected by the preparation method. Regarding to formulations electrokinetic, similar results were obtained for non-loaded and insulin-loaded PLGA, independently of the formulation method (data not shown for brevity). This point out that the protein was not surface adsorbed and, thus, a very efficient entrapment have led to insulin-loaded PLGA particles which, from an electrokinetic point of view, are indistinguishable from non-loaded PLGA.

Greater insulin EE (%) and loading (%) were obtained by following the FF technology: 98.95 ± 0.21 (SEV: 61.27 ± 0.64) and 4.74 ± 0.01 % (SEV: 2.92 ± 0.04 %), respectively. These high values for both methods could be due to a stronger electrostatic interaction between the positively charged insulin ($-NH_2$ groups protonated) and the negative polymer. In addition, as PLGA particles are obtained in just one step by FF, the possibility of drug loss during the synthesis is negligible [3]. Insulin absorption into PLGA was also qualitatively checked by fluorescence spectroscopy (figure 2a): the characteristic band of insulin is present in the spectrum of the protein-loaded PLGA particles. Interestingly, a significant reduction in the intensity of the insulin band is observed when the protein-loaded PLGA particles are prepared by SEV. This is the consequence of the lower protein content obtained by SEV.

Insulin release follows a biphasic profile (figure 2b) due to diffusion-cum-degradation mediated processes. During the rapid early phase, protein release could occur by the loss of the surface-associated insulin and by protein diffusion from the core. During the slower phase, the release may result from polymer degradation, from insulin diffusion through the polymeric core, or both [3]. At this point, we should also take into account that particle size is an important parameter that could affect the polymer degradation: an increase in particle size reduces the surface area/volume ratio of the polymer, leading to decreased buffer penetration into PLGA and slower protein release. This may define the slower insulin release from PLGA obtained by FF [3].

Conclusions

We have analyzed two formulation procedures, the classic SEVM and the novel FF technique, for the preparation of spherical PLGA particles loaded with insulin. Compared to SEVM, it was found that FF allowed obtaining microparticles with a clear more narrow size distribution. Importantly, the microparticles

obtained by FF showed a higher loading and a much slower insulin release. This demonstrating the potential use of FF for the engineering of PLGA micromedicines based on proteins (or peptides).

Acknowledgements

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References

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Figure 1. Scanning electron microphotographs of insulin-loaded PLGA particles formulated by SEV (a), and by FF (b). (c) Size histograms of insulin-loaded PLGA particles prepared by both methods.

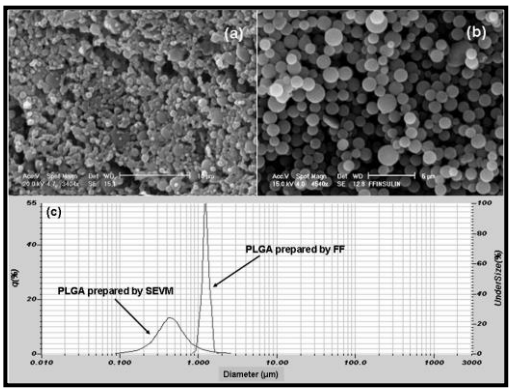

Figure 2. (a) Fluorescence spectra of insulin, and insulin-loaded particles obtained by SEV, and FF. (b) Release of insulin from PLGA prepared by SEV (■) or FF (□), as a function of the incubation time in PBS.