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Development of prilling process for biodegradable microspheres through experimental designs

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ABSTRACT

The prilling process proposes a microparticle formulation easily transferable to the pharmaceutical production, leading to monodispersed and highly controllable microspheres. PLGA microspheres were used for carrying an encapsulated protein and adhered stem cells on its surface, proposing a tool for regeneration therapy against injured tissue. This work focused on the development of the production of PLGA microspheres by the prilling process without toxic solvent. The required production quality needed a complete optimization of the process. Seventeen parameters were studied through experimental designs and led to an acceptable production. The key parameters and mechanisms of formation were highlighted.

1. Introduction

The prilling or "laminar jet break-up technology" is a process used for the production of uniform particles from 10 to 10,000 micrometers. Liquid droplets are produced by extrusion of a flux through a vibrating nozzle; then the droplets are solidified by different ways. The prilling presents many advantages comparing to other formulation techniques, as an one-step process, a highly control of the narrow-size distribution of the prills (Bocanegra et al., 2005; Séquier et al., 2014), the possibility to create core–shell particles (Berkland et al., 2004), and the easy transfer of the manufacture of particles in sterile chamber (Brandenberger et al., 1999) with the required Good Manufacturing Practices.

The prilling technique was developed from the first theoretical approach by Joseph Plateau and Lord Rayleigh at the end of the 19th century (Rayleigh, 1878). It is essentially used in the agronomic and ceramic industries. Fertilizers are produced with ammonium nitrate, calcium nitrate, urea (Carson and Ozores-Hampton, 2012) . . . These droplets are dried during the fall of over 50 m in environmental controlled towers, for the production of 2 mm prills.

One of the most other used materials is sodium alginate for the easily production and controllability of biocompatible prills for a drug delivery system. In this case alginate is solidified after a few centimeters fall into ethanol or aqueous calcium chloride solution. These prills are used and developed in phytosanitary (Fravel, 1996, 1995; Lumsden, 1992; MacKenzie et al., 2000) and pharmaceutical (Auriemma et al., 2013, 2011a,b; Del Gaudio et al., 2005, 2013) fields. For example, Del Gaudio et al. (2014) presents alginate core–shell particles (produced from a double-nozzle) encapsulating piroxicam (PRX). The *in vitro* studies show a complete release of PRX before the 7th day.

Other materials are used to develop a drug-loaded system for a sustained release over one month. Poly(lactic-*co*-glycolic) acids or PLGA is a FDA-approved polymer commonly used as biocompatible and biodegradable microspheres. Microparticles are mainly produced by emulsion—solvent extraction (Jain, 2000; O'Donnell and McGinity, 1997; Vila et al., 2002). In few cases PLGA microparticles are prepared by break-up and extrusion processes (Widmer et al., 1998). All of these techniques need a previous PLGA organic solution introduced by different ways into a non-solvent of

Abbreviations: DMSO, dimethyl sulfoxide; Eth, ethanol; FDA, food and drug administration; HCl, hydrochloric acid; PEG, polyethylene glycol; PLGA, poly(D,L-lactic-*co*-glycolic acids); PG, propylene glycol; PRX, piroxicam; SEM, scanning electron microscopy; SD, standard deviation; W, water.

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the PLGA for solidification. Different extrusion techniques lead to objects with diameters in the order of magnitude of 1 mm (Desai et al., 2008; Ghalanbor et al., 2013; Vesna Milacic and Schwendeman, 2014). The only one jet break-up technique used in this case is the flow focusing, giving microsphere diameters about 2–3 μm or more with increasing the size distribution (Gañán-Calvo and Gordillo, 2001; Schneider et al., 2011).

Moreover, PLGA was commonly used as drug delivery system. PLGA microspheres allow many advantages as scaffolds for regeneration therapy (Huang et al., 2014) supporting adhered stem cells and for the encapsulation, the conservation and the sustained release of protein (Morille et al., 2013; Tran et al., 2012a). Proteins are considered as highly breakable products.

In order to create spherical scaffolds for the encapsulation of therapeutic proteins and for the survival of adhered stem cells (Menei et al., 2005; Tatard et al., 2005; Tran et al., 2012a,), the prilling would be an alternative to produce narrow-size microspheres with an intermediate diameter of 50–100 μm. According to a potential manufacturing scale-up, some parameters have to be highlighted. Halogenated solvents appropriated to PLGA, are toxics. Glycofurol, acetone and ethyl acetate are acceptable solvents as class 3according to the European Pharmacopoeia. For the protein microencapsulation, a nanoprecipitation step is performed to improve the protein stability (Giteau et al., 2008). The protein is nanoprecipitated in glycofurol, pelleted by centrifugation then resuspended in the PLGA solution. Nevertheless, the centrifugation step should be avoided for the pharmaceutical manufacturing in sterile chambers. So the development of a centrifugation-free process makes easier the scale-up. This is possible if the protein is directly nanoprecipitated in the PLGA solution, given that glycofurol is also a PLGA solvent (Aubert-Pouëssel et al., 2004; Tran et al., 2012a,b). All these requirements need a radical process adjustment.

The aim of this study is to develop protein loaded PLGA microspheres by the prilling process. Experimental designs were chosen to consider all the process parameters simultaneously.

2. Materials and methods

2.1. Materials

Lysozyme (chicken egg white) and its substrate *Micrococcus lysodeikticus*, glycofurol (tetraglycol or α-[(tetrahydro-2-furanyl) methyl]-ω-hydroxy-poly (oxy-1,2-ethanediyl)), hydrochloric acid (HCl), acetone, ethanol, dimethyl sulfoxide (DMSO), Pluronic® F68 (Poloxamer P188), were obtained from Sigma–Aldrich (Saint Quentin Fallavier, France). Uncapped PLGA 37.5/25 (M_W 24 kDa, PI 1.8) was obtained from Evonik Corporation (Birmingham, UK).

2.2. Methods

2.2.1. Characterization of the extraction medium

The surface tension was measured as described in Hirsjärvi et al. (2012) using a drop tensiometer device (Tracker Teclis, Longessaigne, France). The surface tension was evaluated for the interface of a rising air bubble (5 μL, controlled all along the experiment), in 3 mL of the studied solvent combinations, using the Laplace equation.

The dynamic viscosity was measured as described by Moysan et al. (2014) using a Kinexus® rheometer (Malvern Instruments, S. A., United Kingdom), with a cone plate geometry (diameter 40 mm, angle 2°) at room temperature.

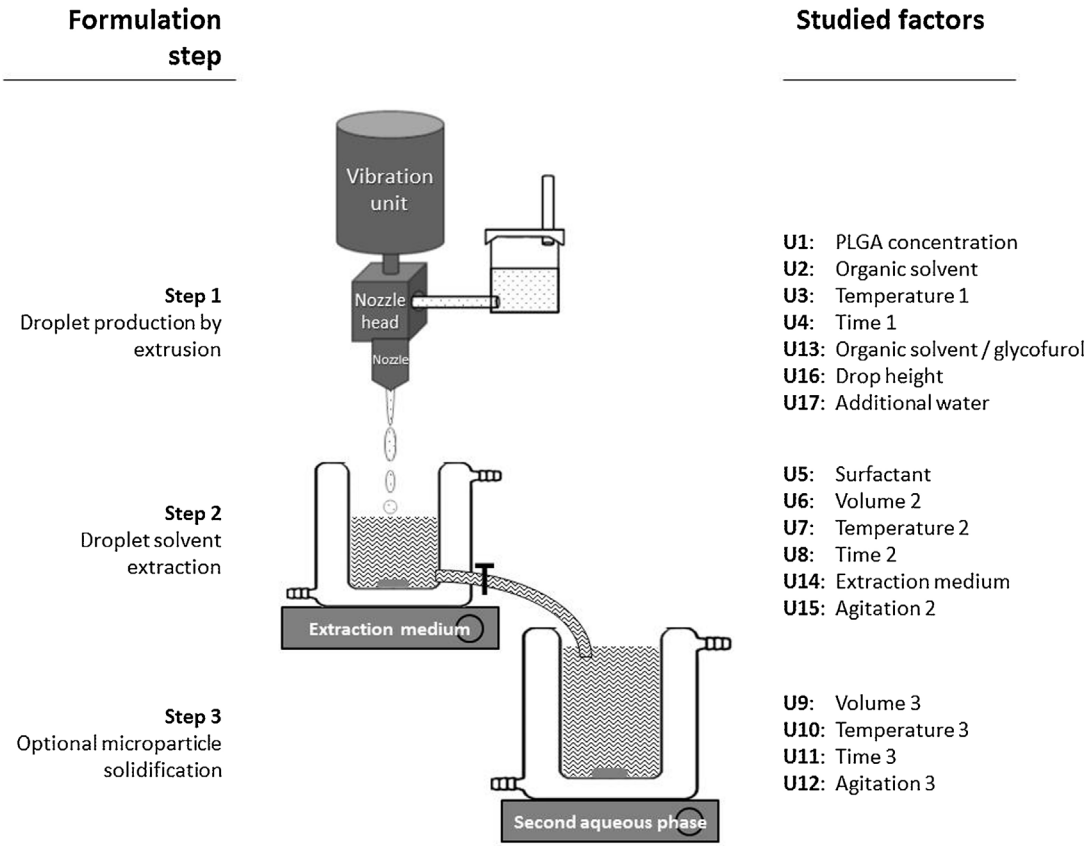

Fig. 1. Schema of the prilling process and input variables.