European Journal of Pharmaceutics and Biopharmaceutics 86 (2014) 393–403

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Encapsulation of immunoglobulin G by solid-in-oil-in-water: Effect of process parameters on microsphere properties ☆

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ARTICLE INFO

Article history:
Received 27 June 2013
Accepted in revised form 22 October 2013
Available online 31 October 2013

Keywords:
Microspheres
Solid-in-oil-in-water
Antibody
PLGA
Ethyl acetate
Design of experiment

ABSTRACT

Antibodies (Abs) are prone to a variety of physical and chemical degradation pathways, which require the development of stable formulations and specific delivery strategies. In this study, injectable biodegradable and biocompatible polymeric particles were employed for controlled-release dosage forms and the encapsulation of antibodies into polylactide-co-glycolide (PLGA) based microspheres was explored. In order to avoid stability issues which are commonly described when water-in-oil (w/o) emulsion is used, a solid-in-oil-in-water (s/o/w) method was developed and optimized. The solid phase was made of IgG microparticles and the s/o/w process was evaluated as an encapsulation method using a model Ab molecule (polyclonal bovine immunoglobulin G (IgG)). The methylene chloride (MC) commonly used for an encapsulation process was replaced by ethyl acetate (EtAc), which was considered as a more suitable organic solvent in terms of both environmental and human safety. The effects of several processes and formulation factors were evaluated on IgG:PLGA microsphere properties such as: particle size distribution, drug loading, IgG stability, and encapsulation efficiency (EE%). Several formulations and processing parameters were also statistically identified as critical to get reproducible process (e.g. the PLGA concentration, the volume of the external phase, the emulsification rate, and the quantity of IgG microparticles). The optimized encapsulation method has shown a drug loading of up to 6% (w/w) and an encapsulation efficiency of up to 60% (w/w) while preserving the integrity of the encapsulated antibody. The produced microspheres were characterized by a d(0.9) lower than 110 μm and showed burst effect lower than 50% (w/w).

1. Introduction

The number of therapeutic monoclonal antibodies (MAbs) which are currently in development has increased dramatically over the past few years. There are more than 34 therapeutic MAbs approved or under review in the European Union and United States [1]. Their pharmacological activity is highly specific and commonly leads to minimal side effects. MAbs may be also conjugated to other therapeutic compounds or radioisotopes in order to increase the efficacy of a drug to a target site, thus reducing its potential systemic side effects, or for specific diagnostic purposes, respectively. Nowadays, less immunogenic human MAbs are available but they are still prone to a variety of physical and chemical degradation pathways, although MAbs, seem to be more stable than other proteins [2,3].

Antibodies are characterized by relatively high molecular weight (150,000 Da) but engineered antibody fragments are much smaller (50,000 Da) than intact full-length MAb and are characterized by different systemic distributions and plasmatic clearances [4].

Injectable biodegradable and biocompatible polymeric particles could be used both to protect MAbs from *in vivo* degradation and to control their release after administration. In the last two decades, synthetic biodegradable polymers have been increasingly used in drug delivery as they are more stable than natural polymers. Thermoplastic aliphatic poly(esters), such as poly-lactide (PLA), poly-glycolide (PGA), and especially PLGA, have generated tremendous

Abbreviations: (M)Ab(s), (monoclonal) antibody (antibodies); EE%, encapsulation efficiency; EtAc, ethyl acetate; IgG, immunoglobulin G; MC, methylene chloride; PGA, poly-glycolide; PLA, poly-lactide; PLGA, polylactide-co-glycolide; PVA, polyvinyl alcohol; PVP, polyvinylpyrrolidone; SEC, size exclusion chromatography; SEM, scanning electron microscopy; s/o/w, solid-in-oil-in-water; SD, spray-dried; T_{in}, inlet temperature; T_{out}, outlet temperature; w/o, water-in-oil.

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0939-6411/$ - see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.ejpb.2013.10.013

interest due to their excellent biocompatibility and biodegradability. Various polymeric drug delivery systems such as microspheres, microcapsules, nanoparticles, pellets, implants, and films have been produced using these polymers for the delivery of a variety of drugs. They have also been approved by the US Food and Drug Administration (FDA) and European Medical Agency (EMA) for human drug delivery [5].

PLGA and its various derivatives have been extensively used in recent development of nano/microparticles containing therapeutic material in controlled-release applications due to their inherent advantages over conventional drug delivery systems. However, PLGA seems to present a negative effect on protein stability during both preparation and storage, mainly due to the acid-catalyzed nature of its degradation. In addition, processing conditions used in the manufacture of PLGA drug delivery vehicles present detrimental effects on protein secondary structures [6].

Protein adsorption and denaturation at the water/solvent interfaces is one of the major issues that lead to a decrease in the protein bioactivity occurring during the microencapsulation process. In order to avoid the denaturation of the encapsulated protein which mainly occurs during the formation of the water-in-oil (w/o) emulsion, a solid-in-oil-in-water (s/o/w) method has been developed. It is thought that the solid state proteins maintain their bioactivity by drastically reducing conformational mobility [7]. In the s/o/w method, solid protein particles are dispersed in the polymer solution to generate the primary suspension. Generating micronized protein particles is one of the major issues in the s/o/w method. This may be achieved with micronization methods including freeze-drying, spray-drying, and spray-freeze-drying [8].

The organic solvent methylene chloride (MC) is commonly used in the double emulsion technique (w/o/w). In this study, MC (a class 2 solvent) was replaced by ethyl acetate (EtAc) (a class 3 solvent) [9], which is considered to be more suitable for both environmental and human safety [10].

High protein loading and high encapsulation efficiency (EE%) should be considered in such development due to the high cost of therapeutic proteins. Moreover, the mean diameter of the microparticles produced must allow for suitable syringeability of the resulting suspension. Usually, 22–25 gauge needles (inner diameters of 394–241 μm) are used for intravenous infusion as well as intramuscular and subcutaneous injections. Therefore, microparticles characterized by a mean diameter lower than 250 μm, more preferably lower than 125 μm, are considered to be suitable for this purpose. The particle size distribution should also be evaluated as the release of the encapsulated protein will depend on the total surface area [11].

This study aims to validate the concept of encapsulating antibodies into a stabilizing formulation of biodegradable PLGA microspheres with a high efficiency of encapsulation. Polyclonal bovine IgG was used as a model antibody as well as MAb fragments (for which the value of applying controlled-release formulation is clear). In addition to the IgG stability and the release profile of the MAb, the effects of the volumetric mean diameter (D[4.3]) and the drug loading were evaluated. Internal and external porosities were observed by scanning electron microscopy (SEM). The IgG distribution within the microspheres was evaluated using a fluorescence labeled IgG. A Design of Experiment was conducted to determine the most critical parameters in the s/o/w encapsulation process.

2. Materials and methods

2.1. Materials

IgG was used as a model molecule and was purchased as a lyophilized powder from Equitech (Kerrville, USA). PLGA (Resomer® RG504), supplied by Boehringer Ingelheim (Ingelheim, Germany), was used as the biodegradable polymer. EtAc (Sigma Aldrich, Diegem, Belgium) was used as the organic solvent during the s/o/w encapsulation process. MC (Merck, Darmstadt, Germany) was used to dissolve the PLGA during the encapsulation efficiency evaluation. Polyvinyl alcohol (PVA) – 87–90% hydrolyzed – and Polyvinylpyrrolidone (PVP) (Sigma–Aldrich, Diegem, Belgium) were used as surfactants. Mannitol and L-histidine (Sigma Aldrich, Diegem, Belgium) were used to stabilize the IgG during the spray-drying process. PBS pH 7.2 (Sigma Aldrich, Diegem, Belgium) was used to buffer the IgG solution. The microspheres were recovered using nylon filters with a porosity of 0.2 μm (Millipore, Billerica, USA). Amicon 15–30 K membranes (Millipore, Billerica, USA) were used to perform the diafiltration.

2.2. Methods

2.2.1. Preparation of IgG microparticles using a spray-drying process

Aqueous IgG solutions, with an IgG concentration of 25 mg/mL in 30% (w/w) mannitol in a 20 mM histidine buffer pH 6.0, were spray-dried using a Mini Spray-dryer B-190 assembly with 0.7 mm spray nozzle (Büchi Labortechnik, Flawil, Switzerland) [12]. The inlet temperature (T_{in}) and the liquid flow rate were set at 130 °C and 3 mL/min, respectively. The drying air flow rate was fixed at 30 m^3/h and the atomization flow rate at 800 L/h. The resulting outlet temperature (T_{out}) was 80 °C.

2.2.2. Encapsulation of IgG microparticles in PLGA microspheres using a s/o/w emulsion process

The IgG was encapsulated using a s/o/w emulsion evaporation/extraction method [13,14] and a brief description is given below.

PLGA was dissolved in a fixed volume of 5 mL EtAc under magnetic stirring at a concentration range of 1–15% (w/v) at room temperature. The solid-in-oil (s/o) dispersion was formed by adding 30–150 mg of IgG spray-dried powder (SD IgG) into the PLGA organic solution using a T25 digital Ultra-Turrax® high-performance disperser equipped with the S25N – 8G dispersing tool (IKA®, Staufen, Germany) set at 13,500 rpm. This suspension was added to 30–100 mL of an aqueous external phase containing 0.1–2.0% (w/v) of a surfactant such as PVA or PVP and maintained under agitation using the Ultra-Turrax® stirrer at 3400–13,500 rpm as emulsification rate. Finally, the s/o/w emulsion was added into an additional volume of water (100–400 mL extraction phase) to produce the final s/o/w emulsion. The resultant emulsion was maintained under magnetic stirring at for 30 min to allow both the extraction and the evaporation of the EtAc. As the polymer was insoluble in water, microspheres containing encapsulated IgG were produced due to the rapid extraction of the EtAc in the aqueous phase resulting in a fast solidification of the polymer. The microspheres were recovered by filtration, washed several times with Milli Q water and left under vacuum for 48 h at room temperature [15].

2.2.3. Particle size and morphology evaluation

Both the particle size of the IgG:PLGA microspheres and the spray-dried (SD) IgG microparticles were evaluated in triplicate using a Malvern Mastersizer Hydro 2000 S (Malvern Instruments, Malvern, UK). The particle size of the SD IgG microparticles was evaluated by laser diffraction after dispersion in isopropanol (refractive index = 1.38). A refractive index of 1.52 was used for the SD IgG microparticles. PLGA microspheres were analyzed in water as the dispersion medium, using refractive indexes of 1.33 and 1.55 for water and PLGA, respectively. The volumetric mean diameter D[4.3] was used to evaluate the particle size of the produced microparticles.