International Journal of Pharmaceutics 535 (2018) 428–437

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Penta-block copolymer microspheres: Impact of polymer characteristics and process parameters on protein release

Minh-Quan Le[a], Fabien Violet[a], Cédric Paniagua[b], Xavier Garric[b], Marie-Claire Venier-Julienne[a,*]

[a] MINT, UNIV Angers, INSERM 1066, CNRS 6021, Université Bretagne Loire, 4 rue Larrey, 49933 Angers Cedex 9, France
[b] Institut des Biomolecules Max Mousseron (IBMM), UMR5247, Université de Montpellier, CNRS, ENSCM, Faculté de Pharmacie, 15 Avenue Charles Flahault, 34093 Montpellier, France

ARTICLE INFO

Keywords:
Protein sustained release
Penta-block copolymer
Morphology
Microencapsulation

ABSTRACT

Here, we aimed to develop protein loaded microspheres (MSs) using penta-block PLGA-based copolymers to obtain sustained and complete protein release. We varied MS morphology and studied the control of protein release. Lysozyme was used as a model protein and MSs were prepared using the solid-in-oil-in-water emulsion solvent extraction method. We synthesized and studied various penta-block PLGA-based copolymers. Copolymer characteristics (LA/GA ratio and molecular weight of PLGA blocks) influenced MS morphology. MS porosity was influenced by process parameters (such as solvent type, polymer concentration, emulsifying speed), whereas the aqueous volume for extraction and stabilizer did not have a significant effect. MSs of the same size, but different morphologies, exhibited different protein release behavior, with porous structures being essential for the continuous and complete release of encapsulated protein. These findings suggest strategies to engineer the morphology of MSs produced from PLGA-based multi-block copolymers to achieve appropriate release rates for a protein delivery system.

1. Introduction

Numerous efforts have been made to develop protein delivery systems from poly(D, L-lactide-*co*-glycolide) (PLGA) microspheres (MSs) for a wide range of clinical applications (Ghorbani et al., 2016; Chiang et al., 2016; Gilert et al., 2016). PLGA is a biodegradable, biocompatible polymer that exhibits desirable degradation properties (Makadia and Siegel, 2011; Tian et al., 2012), whereas other biodegradable polymers, for example poly caprolactone has a very low degradation rate (from two to three years) (Nair and Laurencin, 2007; Asghari et al., 2017). However, protein instability and incomplete protein release from PLGA MSs has often been reported. This is mainly due to an acidified environment due to PLGA degradation and interactions between PLGA and encapsulated proteins (Weert et al., 2000; Wu and Jin, 2008; Cossé et al., 2016). Many solutions have been proposed to address these issues. Co-encapsulation with buffering salts or antacid agents have been effective in limiting acidification upon polymer degradation (Varde and Pack, 2004; Mundargi et al., 2008). The addition of hydrophilic or amphiphilic polymers (*e.g.* polyethylene glycol (PEG), poloxamer 188) have limited protein-PLGA interactions (Goppert and Muller, 2005; Tran et al., 2012). The copolymerization of amphiphilic/hydrophilic polymers and polyester PLA/PLGA to form multi-block copolymers has also been tested as an alternative (Ma, 2014). The presence of amphiphilic/hydrophilic segments increases the hydrophilicity of the entire polymer (Quesnel and Hildgen, 2005; Dorati et al., 2008; Ma, 2014). Studies on protein release from MSs prepared with PLGA-PEG-PLGA triblock copolymers have shown that PEG segments induce a rapid rate of water uptake and continuous protein release (Kissel et al., 2002; Tran et al., 2012). Feng et al. (2015) found that PLGA-mPEG diblock copolymers supported complete protein release (for an encapsulation rate of 4%) (Feng et al., 2015). Recently, MSs with a low protein encapsulation rate ($< 1\%$) prepared with PLGA-P188-PLGA penta-block copolymers showed sustained protein release, following a triphasic pattern, with the greatest release in the middle stage (Morille et al., 2016; Kandalam et al., 2017).

Varying the morphology and porosity of MSs to modulate the protein release profile is an alternative strategy (Klose et al., 2006; Mao et al., 2007; Vay et al., 2012). In general, MSs with a porous structure should have a faster release rate, regardless of the effect of other characteristics, such as MS size, encapsulation rate, and drug

Abbreviations: DCM, dichloromethane; DMF, dimethyl formamide; DSC, differential scanning calorimetry; EE, encapsulation efficiency; FDA, food and drug administration; FITC, fluorescein isothiocyanate; GA, glycolic acid; HCl, hydrochloric acid; LA, lactic acid; MS, microsphere; NMR, nuclear magnetic resonance; PEG, polyethylene glycol; PLGA, poly(D,L-lactic-*co*-glycolic acid); P188, poloxamer 188; SEC, size exclusion chromatography; SEM, scanning electron microscopy; S/O/W, solid-in-oil-in-water; TRIS, tris(hydroxymethyl)aminomethane

* Corresponding author at: Micro et Nanomédecines Translationnelles (MINT), INSERM U1066 – CNRS 6021, 4 rue Larrey, 49933 Angers Cedex 9, France.
E-mail address: marie-claire.venier@univ-angers.fr (M.-C. Venier-Julienne).

https://doi.org/10.1016/j.ijpharm.2017.11.033
Received 18 June 2017; Received in revised form 14 November 2017; Accepted 16 November 2017
Available online 20 November 2017
0378-5173/ © 2017 Published by Elsevier B.V.

distribution (Luan et al., 2006; Oh et al., 2011). Polymer characteristics and process parameters profoundly influence the porosity of the resulting MSs (Mousnier et al., 2014; Cocks et al., 2015; Zhang et al., 2016; Noviendri et al., 2016) and the presence of amphiphilic segments in multi-block PLGA-based copolymers supports pore-formation (Buske et al., 2012; Tran et al., 2012; Wei et al., 2016).

Here, our aim was to develop protein loaded MSs using PLGA-P188-PLGA penta-block copolymers to modulate MS porosity and obtain continuous protein release. Ideally, more than 80% of the encapsulated protein should be released by one month, with no significant burst during the first 24 h. We used lysozyme as a model protein with a low theoretical encapsulation rate (0.6% (w/w)). The MSs were prepared using the solid-in-oil-in-water (S/O/W) emulsion solvent extraction method, which is suitable for protein encapsulation and protein stabilization (Emami et al., 2009; Rawat et al., 2012; Marquette et al., 2014). We investigated the impact of the copolymer characteristics and process parameters on MS morphology and evaluated protein release from MSs of different porosity.

2. Materials and methods

2.1. Materials

Lysozyme (extracted from chicken egg white) and its substrate *Micrococcus lysodeikticus*, glycofurol, dimethylformamide (DMF), methylene chloride (DCM), acetone, and trizmabase (TRIS) were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Pluronic® F68 was obtained from BASF (Levallois-Perret, France). Polyvinyl alcohol (Mowiol® 4-88) was supplied by Kuraray Specialities Europe (Frankfurt, Germany).

2.2. Methods

2.2.1. Copolymer synthesis

Penta-block PLGA-based copolymers were synthesized from the combination of poloxamer 188 (P188), as the central segment, and PLGA segments at the extremities, forming ABA copolymers. P188 is a linear polymer of 150 poly(ethylene oxide) residues (PEO) and 30 poly (propylene oxide) (PPO) residues with a molecular weight of 8.4 kDa. The PLGA segments were modulated by varying the ratio of lactic acid/glycolic acid (LA/GA) and the molecular weight.

Copolymers were prepared by ring-opening polymerization of D,L-lactide and glycolide using poloxamer 188 as an initiator and stannous octoate [Sn(Oct)2] as a catalyst (Garric et al., 2008). Briefly, precise amounts of poloxamer 188, D,L-lactide, and glycolide were mixed before introduction into 100 mL round-bottom flasks containing the catalyst. The mixtures were heated to 140 °C and degassed by 15 vacuum-nitrogen purge cycles to remove moisture and oxygen, which inhibit polymerization. Flasks were then left at room temperature and sealed under dynamic vacuum at 10^{-3} mbar. Polymerization was carried out at 140 °C under constant agitation. After five days, the products were recovered by dissolving in methylene chloride and subsequently precipitated by adding a 10-fold volume of ethanol. Finally, the polymer was filtered, washed with cold ethanol, and dried at 45 °C under reduced pressure until obtaining a constant weight.

Two groups of polymers were synthesized to study the effect of the LA/GA ratio and PLGA segment weight. In one group, the LA/GA ratio was either 50/50 or 75/25 (molecular weight of 40 kDa). In the other group, the weight of the PLGA segments was 10, 30, or 40 kDa and the LA/GA ratio was fixed at 50/50.

2.2.2. Copolymer characterization

Copolymers were characterized by ^{1}H NMR and Size Exclusion Chromatography (SEC) (Waters, France). The molecular weight of the PLGA block was determined using the integration ratio of the resonance of -CH_2 PEG units at 3.6 ppm, -CH PLA units at 5.15 ppm, and -CH PGA units at 4.8 ppm in the ^{1}H NMR spectra of CDCl3. Copolymer dispersity (Đ) was determined by SEC using Waters Inc. equipment fitted with a 5 µm Pl gel mixed-C (60 cm) column as the stationary phase and a Waters 410 refractometric detector, with elution in DMF at 1 mL min^{-1}. Samples were dissolved in DMF at 10 mg/mL and filtered on a PTFE Millex®- FH (pore size 0.45 µm) filter (Millipore Corporation) prior to the injection of 20 µL polymer solution. The number average molecular weight (M_n) and the weight average molecular weight (M_w) were expressed depending on the calibration against poly(styrene) standards. Differential scanning calorimetry (DSC) measurements were also carried out under nitrogen on a Perkin-Elmer DSC 6000 thermal analyzer. Samples were subjected to a first heating scan from −50 °C to 200 °C (10 °C min^{-1}), followed by cooling (10 °C min^{-1}), and a second heating scan from −50 °C to 200 °C (10 °C min^{-1}). The glass transition temperature (Tg) was measured during the second heating ramp.

2.2.3. MS preparation

Penta-block PLGA-based MSs were prepared as described previously (Tran et al., 2012). The theoretical protein loading was 0.6% (w/w). Briefly, lysozyme (LYS) and P188 (at a LYS/P188 ratio of 1/10 (w/w)) were dissolved in water. Subsequently, 3.12 g of glycofurol was introduced into the solution to form a suspension. After incubation at 4 °C for 30 min, the precipitated protein was recovered by centrifugation (10,000 g, 4 °C, 30 min). The precipitated product was re-dispersed in 2 mL dichloromethane/acetone (DCM/Ace) 3/1 (v/v) containing 150 mg penta-block copolymer. The suspension was emulsified in 90 mL PVA at 4% (w/v) at 1 °C and mechanically stirred at 550 rpm for 1 min. After the addition of a determined volume of deionized water, the emulsion was stirred for 10 min more, added to 500 mL deionized water, and stirred continuously for 20 min to extract the solvent. Finally, the MSs were filtered on a 5-µm filter (HVLP type, Millipore SA, Guyancourt, France), washed, freeze-dried, and stored at −20 °C.

The impact of process parameters on MS morphology was investigated by varying the nature of the organic solvent (DCM or a mixture of DCM/Ace (3:1, v/v)), copolymer concentration (50 mg/mL or 100 mg/mL), volume of additional water for extraction (40 mL or 160 mL), PVA content (2, 4, or 6% (w/v)), and emulsifying rate (550 rpm or 1000 rpm). The other parameters were kept constant.

2.2.4. MS characterization

2.2.4.1. MS morphology and mean size. MS mean size was measured using a Coulter® Multisizer (Coultronics, Margency, France). The MSs were dispersed in isotonic saline solution prior to analysis. Three independent measurements were performed for each MS batch and at least three different batches were examined.

MS surface morphology was assessed by scanning electron microscopy – SEM (JSM 6310F, JEOL, Paris, France). Freeze-dried MSs were mounted onto metal stubs using double-sided adhesive tape and vacuum-coated with a film of carbon using a MED 020 (Bal-Tec, Balzers, Lichtenstein) before being investigated. MS internal morphology was also examined by SEM. First, an appropriate amount of MSs was dispersed into a 1 mL Tissue-Tek® dish (Sakura Finetek, USA) and frozen at −20 °C for 1 h. The block was cut into 20-µm-thick slices at −15 °C maximum with a micro-cutting device (Leica Microsystem, Nanterre, France). The slices were rinsed three times with cold water (1 °C) before being freeze-dried. The resulting samples were then analyzed by SEM as described above.

2.2.4.2. Lysozyme-FITC distribution within MSs. Lysozyme-FITC was prepared as described previously by Bezemer et al. (2000). MSs were loaded with lysozyme-FITC using the same protocol as for lysozyme encapsulation. A laser-scanning confocal imaging system (Leica TCS SP8, Germany) was used to observe lysozyme-FITC distribution within the MSs. Freeze-dried MSs were dispersed on a glass slide and fluorescence images of cross sections were taken by optical sectioning. All images were obtained at a single resolution.

Download English Version:

https://daneshyari.com/en/article/8520655

Download Persian Version:

https://daneshyari.com/article/8520655

Daneshyari.com