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Improving release completeness from PLGA-based implants for the acid-labile model protein ovalbumin

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ABSTRACT

The objectives of this study were to assess the feasibility of hot melt extrusion (HME) for the preparation of PLGA-based ovalbumin-loaded implants as well as to characterize and improve protein release from the implants. Ovalbumin (OVA) was stable during extrusion, which was attributed to a protective effect of the biodegradable matrix. OVA release was characterized by a low burst, a slow release up to day 21, which plateaued thereafter resulting in incomplete release for all evaluated protein loadings. Release incompleteness was accompanied by the formation of an insoluble residual mass. Further characterization of this mass indicated that it consisted of non-covalent protein aggregates and polymer, where ovalbumin was ionically bound as the pH inside the degrading matrix decreased below the pI of the protein. Although higher protein release was obtained with the inclusion of weak bases because of their neutralizing effect, OVA aggregation and release incompleteness were not fully avoided. With the use of shellac, a well-known enteric and biocompatible polymer, as protective excipient, a distinct late release phase occurred and release completeness was increased to more than 75% cumulative release. Shellac apparently protected the protein against the acidic microclimate due to its low solubility at low pH. Protected OVA was thus released once the pH increased due to a declining PLGA-oligomer formation. The result was a triphasic release profile consisting of an initial burst, a slow diffusion phase over about 7 weeks, and an erosion-controlled dissolution phase over the next 3 weeks. An acid-labile protein like OVA was thus feasibly protected from interactions with PLGA and its degradation products, resulting in a controlled delivery of more than 85% of the original payload.

1. Introduction

The development of delivery systems for peptides and proteins is still a lively research area due to the growing number of therapeutic macromolecular candidates (Teekamp et al., 2015; Vaishya et al., 2014). The parenteral route is preferred for their administration, because peptides and, especially, proteins possess a complex structure that results in physical and chemical stability issues and poor membrane permeability (Ibraheem et al., 2014).

The development of controlled release systems has arisen from the need to avoid rapid metabolization and thus prolong protein circulation in vivo (Teekamp et al., 2015). Poly(lactide-co-glycolide) (PLGA) is the most common biodegradable polymer, being used in different FDA approved products (Ghalanbor et al., 2012). PLGA-based formulations possess a challenge for proteins due to the complex denaturation and aggregation mechanisms potentially induced by manufacturing processes and the polymer surface (i.e. hydrophobicity), its degradation mechanism as well as its acidic degradation products (Giteau et al., 2008; Houchin and Topp, 2008; Teekamp et al., 2015).

Different manufacturing routes have been studied for the preparation of PLGA-based drug delivery systems. Preparation of micro- and nanoparticles is one of the most investigated approaches, due to the ease of administration (i.e. sc, im, iv). However, micro- and nanoencapsulation usually involves the use of organic solvents and the creation of new interfaces that can compromise stability (Ghalanbor et al., 2013; Teekamp et al., 2015).

Hot melt extrusion (HME) is a solvent-free manufacturing process facilitating incorporation of – the more stable – dry protein (Ghalanbor et al., 2010; Stanković et al., 2015a). It allows for high drug loadings within polymeric matrices, and it is easier to scale up. However, the use of high temperatures and shear forces characteristic of this process might impact protein integrity (Ghalanbor et al., 2010; Repka et al., 2012; Stanković et al., 2015a).

OVA is a globular glycoprotein with a molecular weight of 45 kDa and an isoelectric point (pI) of 4.9. It is more hydrophobic than other proteins such as lysozyme or BSA (Ianeselli and Zhang, 2010), and is

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Table 1
Reagents used and their expected effect on residual mass of implants after release.

Reagent; concentration	Expected effects	References
THF; 100%	PLGA Solvent	Ghalanbor et al. (2012)
Ethanol; 100%	Shellac solvent	Limmatvapirat et al. (2007)
SDS; 2%	Dissociation of non-covalent bonds and protein adsorbed to PLGA by hydrophobic interaction; solubilization of aggregates	Park et al. (1998), Stanković et al. (2015b) and Wang (1999)
GnHCl; 6 M	Dissociation of non-covalent bonds	Park et al. (1998) and Wang (1999)
ACN: H_2O 50:50	Disruption of protein–protein contacts formed by hydrogen bonding formation; denaturation of protein	Gekko et al. (1998) and Wang (1999)
NaOH; 1 M	Alkaline hydrolysis of esters; disruption of strong ionic interactions	Ghalanbor et al. (2012)
DTT; 0.01 M	Cleaving disulfide bridges and thioester bonds at an intra- and intermolecular level	Fenton and Fahey (1986), Ghalanbor et al. (2012), Stanković et al. (2015b) and Wang (1999)
Hydroxylamine; 0.2 M	Hydrolyzing thioesters but not disulfide bridges	Fenton and Fahey (1986), Ghalanbor et al. (2012), Stanković et al. (2015b) and Wang (1999)

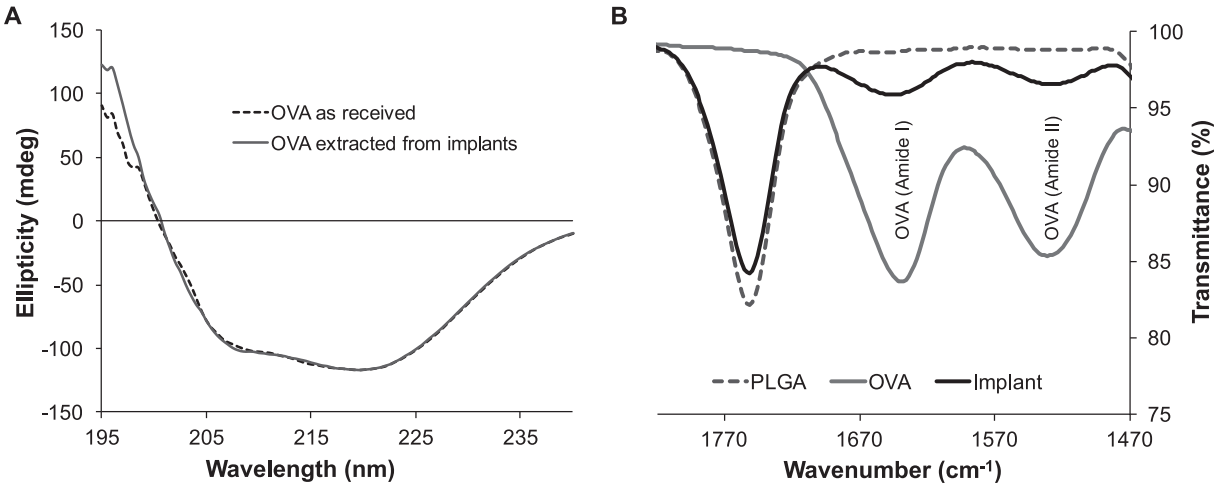

Fig. 1. CD spectra (A) and FTIR spectra (B) of OVA standard and 10% OVA-loaded PLGA based implants.

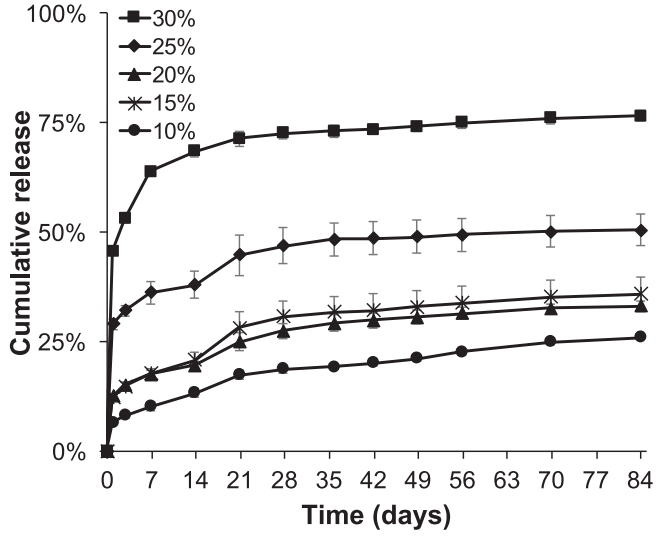

Fig. 2. Release profile of PLGA-based implants loaded with different concentrations of OVA.

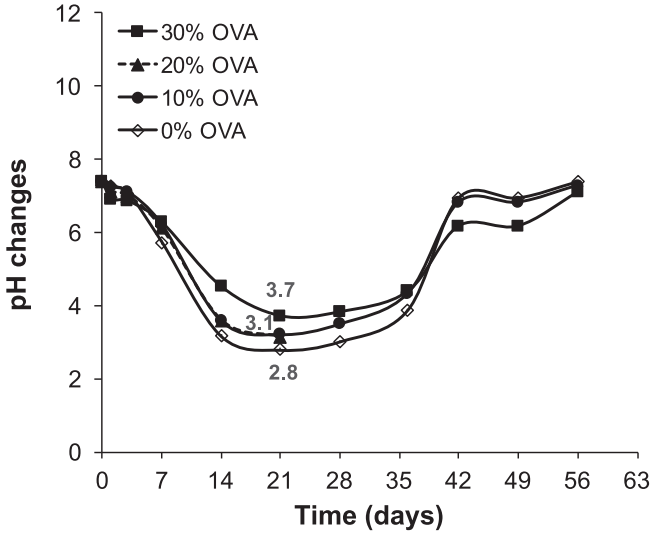

Fig. 3. pH changes of dissolution medium during OVA release from implants with different protein loadings.

very sensitive to environmental conditions (i.e. high temperatures, pH < pI), leading to protein aggregation and detrimental interactions with other molecules (Hu and Du, 2000; Huntington et al., 1995; Ianeselli and Zhang, 2010). Studies on other globular proteins have shown that incomplete release from PLGA-based implants was related to thioester formation of free cysteine groups of the protein with acidic degradation products of PLGA (Ghalanbor et al., 2013, 2012, 2010). Protein release by diffusion, and thus release prior to the formation of acidic polymer degradation products, could enhance the release completeness. This, however, limits the release duration substantially and leads to polymer residues after completing protein delivery, which could result in undesirable accumulations upon repetitive administrations. Different methods can be used to achieve continuous protein release (Schwendeman et al., 2014). For example, co-excipients have