P-012 **Insulin Encapsulation into polymeric micelles**

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INTRODUCTION AND OBJECTIVES

In the last years, therapeutic peptides and proteins emerged as promising drugs in the treatment of various diseases such diabetes or cancer, presenting growing share in the global market. Despite their high therapeutic potential, its administration in the active conformation especially through non-invasive routes, has been shown to be an enormous challenge for the pharmaceutical industry (Andrade F, 2011).

Some of the problems such low mucosal permeability and physicochemical instability could be overcome using nano-based drug delivery systems, including micelles, that protect the peptides and proteins from degradation, and enhance their transepithelial transport and cellular uptake (Soppimath K, 2001).

Micelles are spherical nanosized systems composed by block copolymers of hydrophilic and hydrophobic polymers or by lipids-graft-hydrophilic polymers. Like liposomes, polymeric micelles allow the encapsulate drugs with different polarities, however, they are more stable then liposomes (Torchilin V, 2007).

Poloxamers are nonionic triblock copolymers composed by a central block of relatively hydrophobic polyoxypropylene surrounded on both sides by the blocks of relatively hydrophilic polyoxyethylene conforming to the general formula $HO(C_2H_4O)_a\ (C_3H_6O)_b\ (C_2H_4O)_a$. Soluplus® is a polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer with amphiphilic properties that, like poloxamers, present the capacity to self-assembly into micelles at concentrations above the critical micelle concentration (Kabanov AV, 2002).

The main objective of this work was to produce insulin-loaded polymeric micelles and assess the effect of type of polymer and type of agitation during production in the characteristics of micelles.

MATERIALS AND METHODS

Materials

Poloxamer 338 was purchased from Sigma-Aldrich (Portugal). Poloxamer 188, poloxamer 407 and Soluplus® were kindly provided by BASF (Portugal). Lyophilized human biosynthetic insulin was a gift from Lilly (Portugal). The other reagents used were chloroform from analytical grade, acetonitrile and trifluoroacetic acid from HPLC grade and deionized water (Milli-Q®).

Micelles preparation

Insulin-loaded micelles were prepared using the hydration of polymeric film technique. The polymers (30mg) were dissolved in 3ml of chloroform that was removed under vacuum with consequent obtainment of a polymeric film. Insulin aqueous solution (1mg) was added to this film to get 10 mg/ml polymer concentration, followed by extensive vortexing or sonication. Non-incorporated insulin was separated by filtration of micelle suspension through a 0.2 µm filter and quantified using the HPLC method developed and validated previously (Sarmento B, 2006).

Micelles characterization

Particle size was measured by dynamic light scattering (DLS) at 25 °C with a detection angle of 90° and zeta potential by phase analysis light scattering (PALS) using a 90Plus Particle Size Analyzer supplemented with a ZetaPALS (Brookhaven Instruments Corporation) (n=3).

Association efficiency (AE%) as the amount of insulin associated with the micelles was calculated measuring the free insulin in filtrate after micelles filtration by HPLC using the following equation:

$$AE\% = \frac{\text{total amount of insulin} - \text{free insulin in filtrate}}{\text{total amount of insulin}} \times 100$$

RESULTS AND DISCUSSION

The characteristics of insulin-loaded micelles are resumed in the tables 1 and 2:

Table 1: Characterization of micelles in terms of mean diameter and polydispersity

		Mean Diameter (nm)		Polydispersity	
Agitation		vortexing	sonication	vortexing	sonication
Polymer	Pol188	241.07 ± 186.50	257.63 ± 177.83	0.23 ± 0.37	0.03 ± 0.05
	Pol 338	416.60 ± 63.99	278.80 ± 145.27	0.25 ± 0.21	0.07 ± 0.12
	Pol 407	305.27 ± 91.26	254.73 ± 142.68	0.19 ± 0.25	0.10 ± 0.05
	Solup	88.3 ± 5.51	87.2 ± 14.26	0.22 ± 0.12	0.25 ± 0.06

Pol-Poloxamer, Solup-Soluplus®, n=3, mean±SD

Micelles produced in this work present a mean diameter between 80 and 420 nm and small polydispersity. It is possible verify that, compared to vortexing, sonication appears to produce particles of poloxamer with lower

mean diameters and less polydispersity, with the exception of micelles produced with poloxamer 188. It also appears that the increasing in the size of micelles is proportional to the increasing of the molecular weight of poloxamer used (188<407<338) (Table 3). In all cases, micelles produced by poloxamer have a charge close to neutrality, which suggest the presence of polyoxyethylene on their surface (Brus C, 2004).

Table 2: Characterization of micelles in terms of zeta potential and association efficiency

		Zeta Potential (mV)		AE%	
Agitation		vortexing	sonication	vortexing	sonication
Polymer	Pol 188	3.43 ± 2.86	1.46 ± 0.73	22.04 ± 2.72	17.76 ± 4.07
	Pol 338	-0.98 ± 5.63	1.06 ± 4.11	23.65 ± 0.48	21.12 ± 2.16
	Pol 407	3.68 ± 7.06	-1.00 ± 7.95	26.69 ± 4.47	18.93 ± 2.11
	Solup	-10.57 ± 1.74	-9.71 ± 1.56	29.47 ± 4.35	22.12 ± 15.92

AE-Association Efficiency (% of initial), Pol-Poloxamer, Solup-Soluplus®, n=3, mean±SD

In the case of Soluplus®-based micelles, both mean diameter and polydispersity were similar between vortexing and sonication. In addition, the slightly negative charge of Soluplus®-based micelles suggests the possible presence of insulin on their surface. Interestingly, although Soluplus® have higher molecular weight than poloxamers (Table 3), it produces particles with low mean diameter.

The association efficiency was about 20-30% of the initial amount of insulin and present similar values between the different types of polymer, although the micelles produced by sonication appear to produce micelles with slightly lower association efficiency. This could be due to the lower mean diameter of particles obtained by sonication.

Table 3: Molecular Weight of the different polymers

Type of polymer	Average molecular weight (g/mol)
Poloxamer 188	7680 – 9510
Poloxamer 338	12700 – 17400
Poloxamer 407	9840 – 14600
Soluplus®	90000 - 140000

CONCLUSIONS

In this work we study the effect of type of polymer and agitation during production in the characteristics of insulin-loaded micelles. Although the appearance of the slightly differences between the different micelles, they are not significant ($p>0.05$). Further studies are needed with higher number of batches to analyze the effect of other parameters like polymer:insulin ratio, agitation time or the solution used during the film hydration.

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