Monoclonal antibody encapsulation in polymer microspheres using the solid-in-oil-in-water method with ethyl acetate as organic solvent.

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INTRODUCTION AND OBJECTIVE

The parenteral route is the most common method for the delivery of proteins but is associated to rapid clearance of the drug from the bloodstream and shortened half-lives in vivo, necessitating frequent injections of drug. These drawbacks prompted the development of sustained-release systems designed to prolong the effects of therapeutic proteins in vivo by entrapment of these drugs in a carrier system such as biodegradable polymer-based formulation that could control their release over 1-3 months. Therefore, the aim of this study was to develop and evaluate the encapsulation of antibody such as a full length antibody (mAb) in poly-lactic-co-glycolic-acid (PLGA) microspheres.

MATERIALS AND METHODS

Materials
mAb was obtained from UCB-Pharma; PLGA was purchased from Boehringer Ingelheim (PLGA 50/50, Resomer RG 504, Germany). PBS (Phosphate Buffer Saline) system, Eth Ac, polysorbate 20, polyvinyl alcohol (PVA, 87-90% hydrolyzed), polyols such as Mannitol, sugars such as Sucrose and Trehalose and amino acids such as L-Glutamic acid and L-Histidine were supplied by Sigma Aldrich (Germany). Dichloromethane (DCM), ethyl acetate (Eth Ac) and propan-2-ol were purchased from Merck (Germany).

Freeze drying - Formulation screening for spray-drying process
The formulation screening was carried out to select appropriate buffer and excipients for further spray-drying and encapsulation processes. This study was performed using the freeze-drying as the drying process due to material consumption considerations. A conservative cycle was applied on the pilot Freeze Dryer EPSILON 2- 6D (Martin Christ, Germany) using the following process parameters: freezing at -40°C, annealing at -25°C, sublimation at -30°C and second drying at 20°C

Spray-drying - Preparation of solidified antibody particles
Antibody particles were prepared by spray-drying using a Mini Spray Dryer B-190 (Büchi, Switzerland) (Schule 2008). The inlet temperature (Tin) and the liquid flow rate were set at 130°C and 3 mL/min, respectively. The aspiration was fixed at 25m^3/h and the atomization flow rate at 800 L/h.

S/O/W encapsulation method - Preparation of PLGA microspheres
Spray-dried antibody particles were dispersed into Eth. Ac. solution containing PLGA at 10% w/v concentration using high speed homogenization. This suspension was added rapidly to the aqueous solution containing a stabilizer, such as PVA, using high speed homogenization and maintained under magnetic stirring during solvent extraction. The final microspheres were filtered and dried at room temperature. (Wang 2004)

Particle size evaluation by laser diffraction and scanning electron microscopy (SEM)
The particle size distribution was measured by dispersion using a Mastersizer Hydro 2000 S (Malvern Instruments, UK). Surface morphology and internal porosity were evaluated by SEM.

Drug loading and encapsulation efficiency evaluation (EE%)by size exclusion chromatography (SEC)
20mg of PLGA microspheres were immersed in 750µL DCM for 2h at room temperature to dissolve the polymer. The mAb which was insoluble in DCM was further extracted with PBS buffer (4x750µL). The aqueous phases were collected after phase separation by centrifugation and analyzed by SEC with detection at 280 nm.

In vitro release
The in-vitro release profiles of the mAb from the PLGA microspheres were evaluated by incubation in PBS buffer at 37°C under agitation. The percentage of released mAb was determined by SEC analysis up to 10 weeks.

Activity evaluation by ELISA test and Bioassay
The quantification of mAb was determined by binding to appropriate factor immobilised on 96 well plate. The relative potency of the mAb was measured by cytotoxicity neutralization assay.

RESULTS AND DISCUSSION

Freeze-drying – Formulation screening
A screening design was carried out to evaluate the effects of formulation factors, such as the buffer, the type and the concentration of the bulking agent and the stabilizer, on the stability of the mAb during the freeze-drying used as drying process model. Based on this study, a stable formulation composed as sucrose

and glutamate buffer was selected for further spray-drying evaluation.

Spray-drying - Preparation of solidified antibody particles

Using optimized spray-drying process parameters and formulation selected from the freeze-drying study, the antibody particles were produced at a 10mL scale with an observed yield higher than 60% and characterized by a median diameter of 5µm. No increase in aggregates was detected by SEC (Fig. 1) and no loss in activity was shown using ELISA and Bioassay.

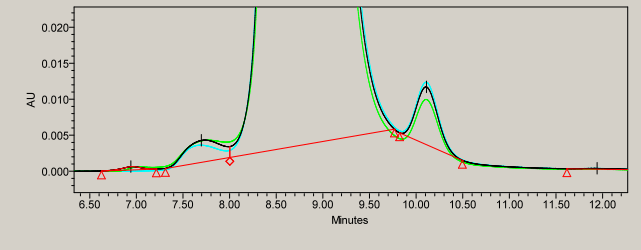

Figure 1: SEC chromatograms comparison (in blue) before drying;(in green) after spray-drying; (in black) after freeze drying)

Characterization of PLGA microspheres prepared by S/O/W encapsulation method

The PLGA microspheres were produced using process parameters previously optimized by DoE. Different theoretical drug load from 9 to 16%w/w were tested.

Table 1. Characteristics of PLGA microspheres

Run	Theo drug load	EE	Monomer loss (T0)	Burst effect = 24h % released	d(0.5)
1	9%	79%	0.9%	26%	33µm
2	11%	80%	1.2%	18%	41µm
3	16%	71%	0.0%	19%	40µm

The loss of monomer takes into account the increase of aggregates and fragment calculated after extraction (T0) and through the in vitro release testing. An increase of fragmentation and a monomer peak displacement linked with a loss of activity around 10% was observed after 4 weeks incubation which could be linked with the acidic PLGA degradation.
As illustrated in the Fig. 2, the release profiles were linear over the first 7 weeks after the initial burst stage.

As observed in Fig. 3 and 4, the SEM showed low surface porosity and internal pores size up to more than 10µm which did not affect the release.

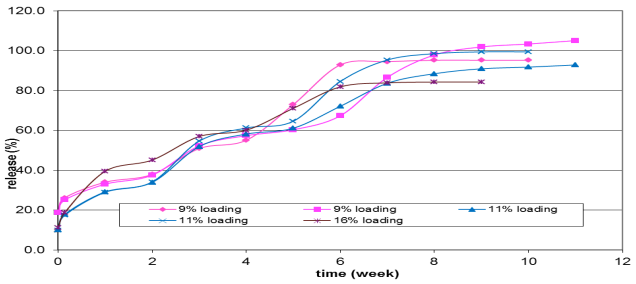

Figure 2: In vitro release profiles – mAB:PLGA microspheres

In addition, agglomeration tendency of microspheres was highlighted which could be limited using a freeze-drying as terminal process to reduce residual moisture.

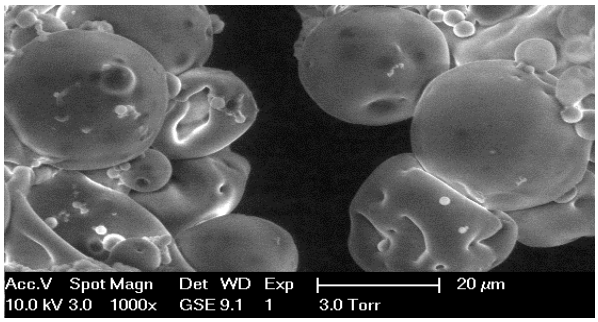

Figure 3: SEM – surface of PLGA microspheres

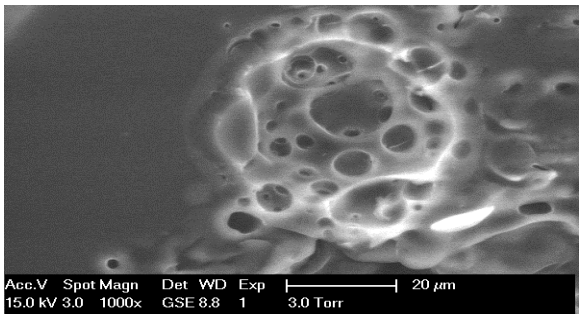

Figure 4: SEM – internal porosity of microspheres

CONCLUSIONS

The s/o/w method using ethyl acetate as the organic solvent and antibody particles produced by spray-drying was considered to be appropriate for preparing microspheres with up to 11% (w/v) loading (run 3) without degradation and with a constant release with limited burst effect. However, the stability during in vitro release should be improved using efficient buffers or other polymer. In addition, the microspheres agglomeration should be limited adding the drying process as terminal process.

REFERENCES

- S. Schule et al. (2008) *Stabilization of IgG1 in spray-dried powders for inhalation*. Eur. J. Pharm. Biopharm., 69, 793-807
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