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Note

Technological considerations related to the up-scaling of protein microencapsulation by spray-drying

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Abstract

Research and development of therapeutics and vaccines based on biodegradable polymers are intensive and one of the most promising fields in controlled drug delivery. However, new applications necessitate successful technology transfer and industrial scale-ups. In an endeavour to produce clinical samples of a single-administration tetanus vaccine based on poly(lactide-co-glycolide) microspheres, we report on technological parameters that are of importance in the up-scaling of the spray-drying process. The results show that an up-scaling of the encapsulation of protein vaccines or drug by spray-drying is feasible, but that additives, the type of polymer solvent, the polymer concentration, the w/o ratio and the product collection method influence process and product quality.

Keywords: Biodegradable polymer; Emulsion stability; Microspheres; Spray-drying; Up-scaling

1. Introduction

Biodegradable and biocompatible polymers increasingly find their way into biomedical applications, such as in the prolonged delivery of peptide or protein-type therapeutics and vaccines using microspheres (MS) fabricated from poly(lactide-co-glycolide) or poly(lactide) (PLGA and PLA). MS have become a realistic alternative to multiple-dosing therapy, and a number of products are already available on the market. Starting with hormone therapeutics in the 1980s (e.g. Decapeptyl® Retard), the most recent product is Nutropin® Depot, a human growth hormone released during 1 month from PLGA MS.

The process of microencapsulation of water soluble peptides and proteins, generally requires the use of a w/o emulsion from which the particles are normally prepared by various techniques, such as co-acervation (phase separation), solvent evaporation from a w/o/w system and spray-drying. It has been demonstrated that these processes can be detrimental to the biological activity of proteins [1–3]. Therefore, it might be advantageous to reduce process time or to use alternative processes, such as the freeze-spray-drying, which is conducted at temperatures below 0°C, and obviates the need for water surfactants [4]. Herein, we report on critical, technological steps in the up-scaling of regular spray-drying for encapsulation of water-soluble proteins into PLGA MS. It can be viewed as a preliminary work for a possible technology transfer and a more engineer-guided up-scaling of the spray-drying process.

2. Materials and methods

2.1. Microsphere preparation

Solutions of protein in water and of 14 kDa (M_w) PLGA 50:50 (unless otherwise stated, Resomer® PLGA RG502H from Boehringer Ingelheim, Ingelheim, Germany) in ethyl formate or dichloromethane were emulsified by ultrasonication (2×20 s, 1–4°C, 20 kHz and output 40 on Vibracell®, Sonics & Materials, Danbury, CT) before spray-drying (Büchi 191, Flawil, Switzerland) [5]. Air flow (550 Nl/min), aspiration (−40 mbar) and inlet temperature (45°C) were kept constant, whereas the feeding rate of the w/o emulsion to the spray-dryer, the concentrations of polymer, protein and additives were varied (see Table 1). For development-scale batches (5–10 g), the emulsion was stirred during the process. For research-scale batches, no further mixing was performed. The proteins encapsulated were either human serum albumin (HSA, from Swiss Red

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Table 1
The stability of w/o emulsions prepared with aqueous solutions of albumin (HSA) or trehalose and PLGA solutions in dichloromethane (DCM) or ethyl formate (EF) on a research scale (0.5 g polymer)[a]

Test parameter	Experimental condition	Stability range (min)
w/o ratio	0.05	20
	0.1	30
	0.15	60
Polymer concentration	5% PLGA	30
	10% PLGA	90
Temperature (°C)	4	30
	20	20
Organic solvent	EF	10
	EF + 5% HSA	30
	DCM	30
	DCM + 5% HSA	>120
Additive concentration	5 or 10% HSA	>120
	5 or 10 % trehalose	>120
	5% HSA + 10% trehalose	>120

[a] Unless otherwise specified, the emulsions were prepared at 4°C with 5% PLGA in DCM; w/o volume ratio was 0.1.

Cross, Berne, Switzerland) or tetanus toxoid (TT lot 166 from Berna, Berne, Switzerland) and the co-encapsulated additives were HSA plus trehalose (Fluka, Buchs, Switzerland). The MS were harvested from the glass equipment either by washing-out procedures using either a 0.1% aqueous solution of poloxamer 188 (Synperonic® PE/F 68, ICI chemicals, Middlesbrough, UK) or poly(vinyl alcohol) 15000 (Fluka), heptane or by dry sampling. For both wet procedures, the particles were collected on a regenerated cellulose filter (0.2 μm pore size), washed once with 250 ml of water, flushed with pressurized air at 4 bar for 10 min; then, the filter cake was washed with 50 ml of hexane or heptane and flushed again with pressurized air as described. All formulations were finally dried overnight at room temperature and 25 mbar pressure.

2.2. Assessment of w/o stability

The stability of the w/o emulsions used in the spray-drying was studied as a function of various parameters, as described in Table 1. Unless otherwise specified, the emulsions were prepared by sonication (30 s) at 4°C of a w/o mixture (v/v of ratio 0.1) containing 5% PLGA 50:50 in dichloromethane or ethyl formate. A total of 10 ml were used for each experiment. The content of tetanus toxoid, HSA and trehalose are relative to the total dry weight of polymer and additives. Finally, the light transmission of each emulsion was measured at 700 nm using a spectrophotometer (Uvikon® 931, Kontron Instr., Milano, Italy). For simplicity, the relative change in transmission over time was translated into a arbitrary score value, reflecting the stability of the emulsion. The response was determined as relative change in light transmission of the emulsion, and emulsions were qualified as stable, when light transmission changes were below 10%.

2.3. Product characterization

MS size and morphology were examined by visible light microscopy and laser light diffractometry (Mastersizer® X, Malvern Instr., Worcestershire, UK). Injectability of the MS through needles of different sizes (0.5–0.9 mm of inner diameter) was tested after dispersing the particles in distilled water, 0.5% polysorbate 20 in 67 mM PBS of pH 7.4, or in 2% aqueous soya lecithin of pH 6.0 (Epikuron® 145 V, Lucas Meyer, Hamburg, Germany). Protein loading and release were studied as described [1]. Briefly, HSA content and tetanus toxoid antigenicity in the MS was determined by dissolving the particles (10–20 mg) in dichloromethane, recovering the undissolved protein on a 0.2 μm regenerated cellulose filter and eluting the protein in an isotonic buffer (67 mM PBS (pH 7.4) 0.02% sodium azide). HSA content was then assayed by fluorimetry, whereas tetanus toxoid was analyzed by ELISA. Burst release of protein from 20 mg MS was determined in 4 ml PBS in rotating borosilicate vials. Samples were assayed by fluorimetry after 24 h incubation at 37°C and separation of the particles and supernatant by centrifugation at 3500 rev./min for 10 min. Protein load and release experiments were performed in triplicate.

3. Results and discussion

3.1. Assessment of stability

Table 1 shows the results of emulsion (w/o) stability as a function of various physico–chemical and technological parameters. The most important feature from these experiments performed on a research scale (0.5 g polymer) was that the addition of 5 or 10% of trehalose or HSA to the aqueous phase of the emulsion resulted in dispersions which were stable for more than 2 h; the percentage of additives are relative to the total dry weight of polymer and additives. According to the classification system used, the dispersions without additives were defined as unstable, with a change in light transmission of more than 10%. Furthermore, the emulsions were more stable at higher w/o volume ratios (0.05, 0.1 ad 0.15) and polymer concentrations (5 and 10%). Finally, the emulsions were slightly less stable at 20 than at 4°C and when using ethyl formate rather than dichloromethane as polymer solvent. Dichloromethane produced white emulsions of lower light transmission than the colloidal appearance with ethyl formate. This indicates that ethyl formate allowed smaller water droplets than did dichloromethane.

In a supplementary experiment, the stability of an emulsion that contained tetanus toxoid was studied, with respect to polymer type and amount of additive. Table 2 shows that a small amount of tetanus toxoid alone (0.25% relative to total dry weight) was insufficient to produce stable emulsions. Emulsions containing tetanus toxoid without stabiliz-

Table 2
The stability of w/o emulsions prepared with an aqueous solution of tetanus toxoid (0.25% relative to total dry weight of product) and stabilizing additives and polymer solutions in dichloromethane (10% PLA) or ethyl formate (8.5% PLGA 50:50)

Polymer type	Tetanus toxoid concentration (%)	Additive (%)[a]	Emulsion stability
PLA[b]	0.25	0	Strong flocculation
		1.25	Slow flocculation
		5	Stable
PLGA 50:50	0.25	0	Strong flocculation
		1.25	Stable
		5	Stable

[a] 1:1 (w/w) mixtures of HSA and trehalose.
[b] PLA: Resomer® PLA R202, 14 kDa (M_w).

ing additives very rapidly showed flocculations. This was in contrast to the slower process of phase separation so characteristic for the toxoid-free emulsions. Whereas samples prepared with PLGA 50:50 using ethyl formate were stable over 5 min, those prepared with PLA in dichloromethane immediately collapsed upon ultrasonication. The differences in stability between PLGA- and PLA-based emulsion are likely linked to differences in hydrophobicity and in polymer endgroups. The terminal ester normally present in these PLGA and PLA qualities is capped for PLGA, giving rise to a free carboxylic terminal group which is dissociated at a pH of 7. Also, TT is negatively charged at this pH, PLGA and TT can be mutually repulsive, a phenomenon that may prevent associative aggregation. By adding HSA and trehalose, the emulsion stability improved substantially and sufficiently for practical purposes of spray-drying. One possible explanation is that amphiphilic compounds such as HSA and tetanus toxoid can reduce the interfacial tension between aqueous and organic phase. This should reduce coalescence of the disperse phase. Furthermore, additives which increase overall viscosity of the emulsion or which reduce the density difference between the dispersed and the continuous phases are potential emulsion stabilizers, e.g. when DCM ($\rho = 1.32$) is used as polymer ($\rho = 1.28$) solvent, sugars and proteins increase the density of the aqueous phase.

3.2. The effect of various technological parameters on the microsphere quality

The rate at which the emulsion (w/o) was spray-dried, the polymer concentration utilized (7.5–20%) and the method of product collection were the variables investigated with respect to MS properties, such as product yield, particle size

Table 3
Effect of various technological parameters of spray-drying on the quality of the PLGA 50:50 MS ($n = 1$)[a]

Parameter and level	Product yield (%)[b]	Particle size (μm)[c]	Loading efficiency (%)[d]	Burst release (%)[d]
Emulsion feed (ml/min)				
0.9	31	6.3	32	65
2.2	57	4.1	25	58 ± 9
4.6	59	3.9	18	69
Polymer concentration (%, w/v)				
7.5	57	5	19	55
10	57	4.1	25	58 ± 9
12.5	55	5.5	22	54
15	51	4.9	26	63
20	38	5.1	26	64
Product collection				
Poloxamer 188	57	4.1	25	58 ± 9
PVA	45	6.2	24	56
Heptane	40	4.9	61	91 ± 5
Dry sampling	59	5.4	67	89
Up-scaling using metal and glass cyclones[e]				
5 g/metal	69	3.9	29	51 ± 6
10 g/metal	67	6.1	25	48
5 g/glass	58	5.3	25	52
10 g/glass	56	5.1	22	55 ± 7

[a] Unless otherwise stated, an emulsion of 900 mg PLGA 50:50 in dichloromethane (10%) and 50 mg each of trehalose and HSA in water was spray-dried at a feed rate of 2.2 ml/min. The w/o ratio was 5%.
[b] Product was collected from both the cyclone and collection flask.
[c] Volume-weighted mean particle size as measured by laser-light diffractometry.
[d] Means of measurements from one MS batch. Relative standard deviations were less than 4% for all determinations unless otherwise specified ($n = 3$).
[e] Product was collected by wet sampling using poloxamer 188/water.

and morphology, and protein loading and in vitro release (Table 3).

When the emulsion was spray-dried at lower rates, product yield was lower, because more product deposited in the spraying chamber. Moreover, the lower feed increased the loading efficiency, whereas the fraction of HSA released after 24 h incubation in PBS and the mean particle size was not affected by this variable. Wan et al. [6] found that the spray rate (7–15 ml/min) influenced the MS product. Higher rates produced lower yields and more aggregates. We did not observe such effects probably because at these low rates (0.9–4.6 ml/min), atomization is complete and ensures the manufacture of individuals particles which deposit in cyclone or collection flask. Finally, the spray rate has also shown a strong influence on the outlet temperature [6] which might be critical for the solvent-evaporation efficiency and, consequently, for aggregation of MS or denaturation of protein load due to residual water or organic solvent. At the relatively low spray rates applied in this investigation, no such influence was observed.

Polymer concentration exerted no consistent effect on the parameters tested, with the exception of the low yield (38%) obtained at the highest polymer concentration (Table 3). The latter effect was again related to the increased amount of polymer deposit in the spraying chamber. It has been described that a polymer concentration of 0.5–5% did not notably affect the production yield and the particle size [7].

The experimental rationale for increasing spray rate and polymer concentration was to assure the formation of stable w/o emulsion due to a shorter process time (Table 1) and due to slower sedimentation of water droplets in a more viscous organic phase (Stoke's law). Furthermore, this should have a positive effect on the physicochemical and biological stability of encapsulated compounds such as therapeutic and vaccine proteins.

Loading efficiencies in the preparations described so far were unusually low and the burst releases after 24 h very high. Therefore, additional experiments were performed to study whether this was an effect of the type of polymer solvent (dichloromethane vs. ethyl formate), the co-encapsulated trehalose, the type of polymer (end-group uncapped, RG502H vs. capped PLGA 50:50, RG502), the way the emulsion was fed into the spray-dryer (peristaltic pumping in PTFE tubes vs. gravitational feeding by a byrette), or the method by which the product was collected (wet vs. dry collection). For illustration, the use of ethyl formate as polymer solvent or the absence of trehalose as additive very interestingly both increased the loading efficiency of HSA from 25 to 38 and 32%, respectively, as compared with a formulation with dichloromethane and trehalose, and the burst release decreased by 5–15% from 58%; in both cases, the product yield was also slightly higher. We have previously shown that the type of polymer solvent radically influences the spray-drying process and the product quality [5,8]. Product collection by non-aqueous methods resulted, as expected, in higher HSA loading and burst release (Table 3). When the MS were rinsed out by aqueous poly(vinyl alcohol) solution or by heptane, the product yield was low possibly due to these liquids' inadequate wetting properties. For the other variables tested, none or only minor influences on the product quality were observed.

During spray-drying of PLGA/PLA systems, much product can deposit in the cyclone dependent on materials and equipment settings used. The Büchi 191 spray-dryer is equipped with a glass cyclone in which the product is only accessible from a small opening (25 mm) at the bottom. To simplify the harvesting of MS, a metal cyclone with a detachable top cover was fabricated. Hence, the product could be collected through a wider opening (82 mm). This cyclone was compared with the conventional glass cyclone in a 10-fold up-scaling of the spray-drying process, and the product was recovered by wet sampling in 0.1% poloxamer 188. A higher product yield was obtained with the metal cyclone (Table 3). Particle size and morphology, and protein loading and release were not affected by the cyclone type. Generally, we obtained higher product yield for both cyclone types than previously reported spray-dryings of polyesters or cellulose polymers using the same apparatus [6,7,9].

Finally, a research-scale batch (1 g product) and a development-scale batch (10 g product), both containing tetanus toxoid were compared (Table 4). Most importantly, the prolonged process time and consequently prolonged contact time between the toxoid and the emulsion (w/o) did not considerably impair the ELISA-antigenicity of encapsulated antigen. The loading efficiencies in the research-scale and the development-scale batches were 35.8 and 31.9, respectively, and the inter-dose variation of the two batches were comparable. Similarly, the percentage of antigen released during 24 h was not significantly higher for the development-scale than for the research-scale batches.

These results show that an up-scaling of the encapsulation

Table 4
Process and product properties of PLGA 50:50 (RG502H) MS with tetanus toxoid and HSA[a]

Attribute	Research scale (1 g)	Development scale (10 g)
Process time (min)	3	18
Yield (%)	63	69
Particle size (μm)[b]	2.1/5.1/14.2	1.5/3.9/21.0
Antigen loading (%)[c]	35.8 ± 5.7	31.9 ± 4.2
Antigen burst release (%)[c]	29.8 ± 2.1	36.5 ± 3.6

[a] Emulsions (w/o) with 10% PLGA 50:50 in ethyl formate were spray-dried using a w/o ratio of 0.067. Nominal loadings of toxoid, HSA and trehalose, relative to total dry weight of product, were 3 Lf/mg, 50 and 100 μg/mg, respectively. The product was collected by aqueous washing with poloxamer 188 and recovered by filtration.

[b] Volume-weighted undersizes $d(0.1)$, $d(0.5)$ and $d(0.9)$, as measured by laser light diffractometry.

[c] Tetanus-toxoid amounts in Lf (flocculating units) were measured by ELISA ($n = 3$).

of proteins (vaccines) by spray-drying is feasible. Approximately 300–400 human doses at 10 Lf of a MS-based tetanus vaccine (clinical trials are presently under planning and preparation in our laboratory) can be produced with the described technology.

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