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Sustained Release Micro-Encapsulates to Administer Highly Water-Soluble Peptides for Retinal Diseases

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Ocular Delivery of Peptides

Retinal disorders such as choroidal neo-vascularization (CNV), diabetic retinopathy (DR) and age-related macular degeneration (ARMD), etc. can cause irreversible loss of vision due to damage to the optic nerve. Therapeutics currently administered (Lucentis, etc.) require frequent injections to the posterior segment of the eye, which increase the probability of infection and retinal detachment. One way to eliminate frequent injection regimens is to deliver these molecules via sustained release microspheres that can injected into the vitreous. Another advantage of encapsulating drugs is the protection of biologics from rapid *in-vivo* degradation.

Development objectives for these delivery systems include achievement of high encapsulation efficiencies of intact drug, retention of biological potency of the encapsulated drug, sustained release profile over at least 3 months and injectability via a 27G needle. One major issue encountered during the encapsulation process is the loss of the water-soluble drug, resulting in very low encapsulation efficiencies and typically, high "burst" release.

We have encapsulated a highly water-soluble peptide as a model compound in PLGA using our proprietary methodology, with high encapsulation efficiencies, percent "burst“ <1% and release profiles that can be modulated, based on polymer matrix composition and internal microstructure.

Concepts described in this work are part of a development program to develop a proprietary platform delivery system, EySite™. Small molecules, proteins and peptides can be delivered either singly or in combination formats.

Objectives

- *Develop encapsulation methodology with flexibility to achieve optimum size for long-term release of biologics*
- *Achieve optimum size for injectability.*
- *Develop encapsulation methods to minimize “burst”.*
- *Achieve in vitro sustained release of a water-soluble model peptide, MW 4200 Daltons.*

Methods

Preparation of Micro-encapsulates: PLGA microencapsulates were prepared by a proprietary process, to achieve a narrow size distribution, optimum size and injectability. A model peptide of MW 4200 Da was encapsulated.

Characterization:

- Particle Size Distribution (PSD): Samples were suspended, 20mg/mL in a carboxymethyl cellulose-based diluent, diluted with an equivalent volume of water. 500uL of the suspension was dispersed in 10 mL of a dispersal medium (diluent diluted 30-fold in distilled water). Particle size was measured on a Horiba LA-950V Laser Diffraction Particle Size Analyzer.
- Imaging: Dry encapsulates were characterized by cryo-scanning electron microscopy.
- Peptide content (mg/g): 20 mg of the microencapsulate was dissolved in 1mL of acetonitrile, and 4mL of 30mM acetate buffer, pH 4.4, added drop-wise while mixing. 1mL of the slurry was centrifuged (5min; 6000 RPM), and the supernatant was removed for HPLC analysis. HPLC analysis was performed on a RP C18 column.
- *In-Vitro* Burst (%): 30 mg of the micro-encapsulates were reconstituted in PBS, pH 7.4 and rotated at 37°C, assayed after 1 hour.
- *Accelerated In-Vitro* Release: Samples were suspended 60 mg/mL in acetate buffer (100mM; pH 4.2) and placed in cellulose ester dialysis tubes (Spectrum; Spectra/Por; MWCO: 1000 kD; 5mL vol.). The tubes were suspended in 45mL and incubated at 50°C. Time point samples were obtained after 1 hour and daily over the course of fifteen days.
- *Injectability* of the microspheres suspended in the reconstituting fluid were tested both through 23G and 27G needles.

Results

Figure 2 shows the solid internal microstructure and pore-free surfaces of microspheres produced using the optimized proprietary process below. Peptide is encapsulated in nano-sized pores.

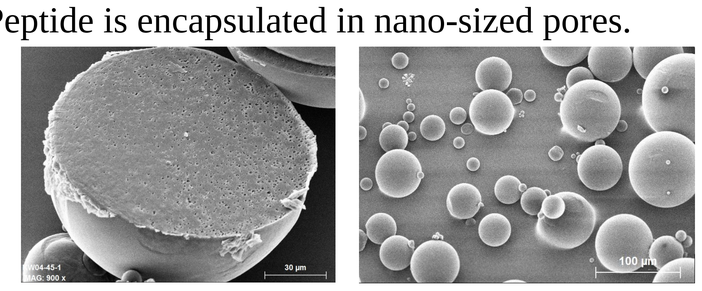
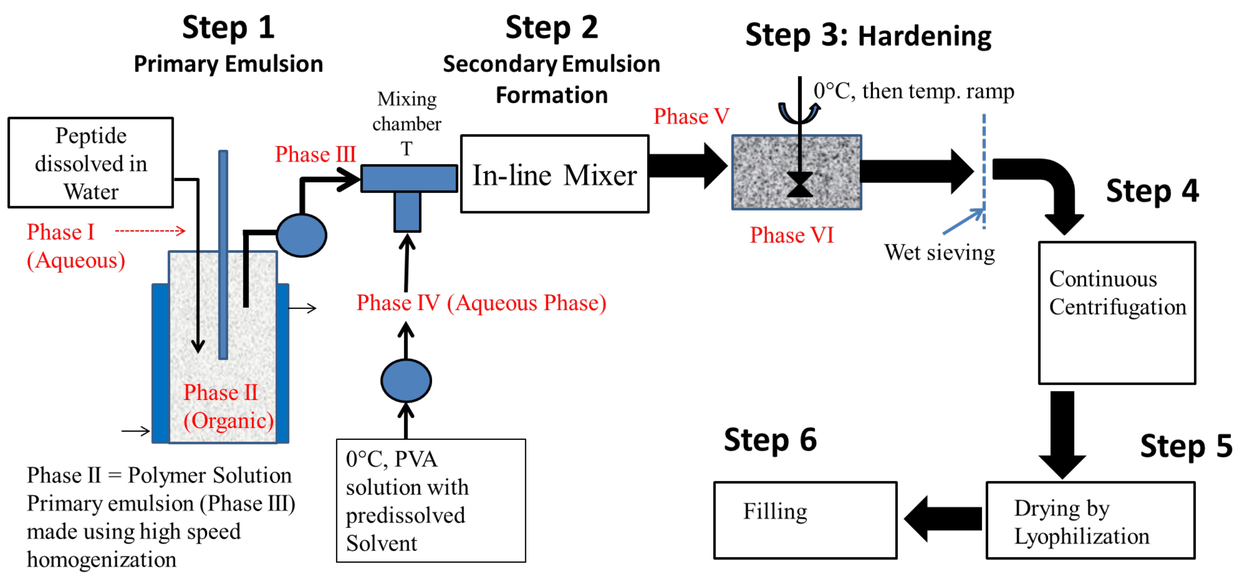


Figure 2: SEM of Peptide Micro-encapsulates

Figure 1: Schematic of Microsphere Process for Large Scale Batches



Results

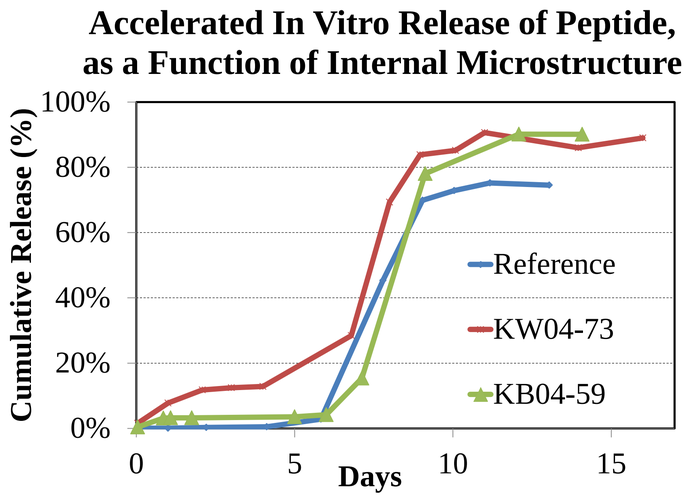


Fig. 3: Accelerated In Vitro Release, 50°C 12g Batch Scale, RG504H

Table 1: Peptide Microsphere Characteristics

Characteristic	Batch KW04-73	Batch KB04-59
PSD (D50, µm)	51.7 µm	61.4 µm
In vitro burst release (37°C)	0.46%	0.12%
Peptide content	44.7 mg/g	43.6 mg/g

INJECTABILITY

- Proprietary process can flexibly produce microspheres with D50 < 20 µm, required to inject through a 27G or smaller needle (Fig. 4).
- Microspheres with D50 of 3-15 µm were injectable through both 23G and 27 G needles.

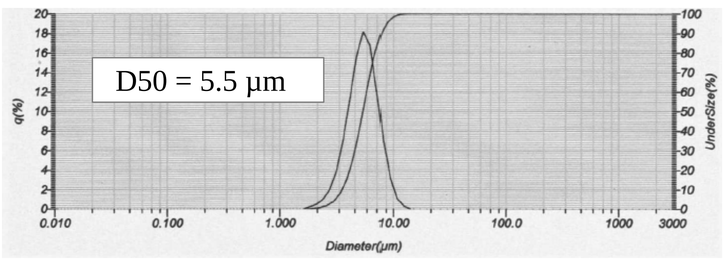


Figure 4

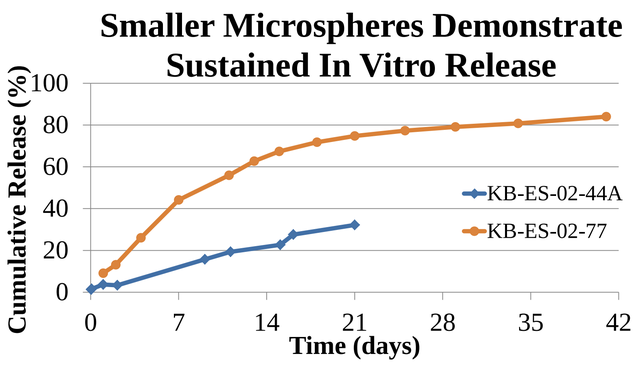


Figure 5: In Vitro Release of a Corticosteroid, with (02-77; d50 = 7.2 µm) and without (02-44A; d50 = 3.1 µm) a release modifier.

Conclusions and Next Steps

- A process for peptide-loaded microspheres with high encapsulation and low burst, due to “solid” internal microstructure, has been developed.
- The process has built-in flexibility and scalability.
- These advantages will be used to modulate the size for injectability and to modulate release using longer-duration polymers.

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