

AN4001: Organic-phase encapsulation in protein-based nanocapsules studied by DLS

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Summary

Protein-based nanocapsules composed of human serum albumin and silk fibroin (*Bombyx mori*) were investigated for particle size using dynamic light scattering (DLS), hence proving ultrasound-induced crosslinking and the successful production of monodisperse HSA-SF nanocapsules.

Introduction

Protein-based nanocapsules are highly interesting for stimuli-responsive targeted drug delivery, as protein properties can directly influence particle stability and release behavior. In an ultrasound-assisted nanocapsules production approach, human serum albumin (HSA) and silk fibroin (SF) were combined in a two-phase system and nanoparticle self-assembly was induced.¹

Using [dynamic light scattering](#) and the [DynaPro[®] NanoStar[®] DLS/SLS instrument](#), the nanocapsules formulation and dispersion properties based on particle size were investigated.

Materials and Methods

HSA-SF nanocapsules were produced by sonication in a two-phase system consisting of phosphate buffer as aqueous phase and n-dodecane as organic phase. In order to investigate the impact of degradation, silk fibroin degradation was induced by boiling, which creates variations in polymer length.

Different ratios of HSA to SF were prepared, and two different degrees of SF degradation (i.e., different boiling times) tested for effect on the ultrasound-induced crosslinking.

Time-course DLS experiments were performed using a DynaPro NanoStar with disposable cuvettes and a sample

volume of 15 μ L. Each data point consisted of 20 acquisitions of 2 sec each, over a time period of 30 min. These experiments were performed in order to investigate the impact on size of the encapsulated organic phase in the HSA-SF nanocapsules. Data were acquired and analyzed with [DYNAMICS[®]](#) software.

Results and Discussion

Dispersion properties, such as dispersion stability, agglomeration and temperature stability based on particle size analysis were investigated.

The HSA-SF nanocapsules are core/shell particles, encapsulating the organic phase (n-dodecane) in the hydrophobic core. The organic-solvent core of the nanocapsules increases their buoyancy, leading to a visible upwards-directed motion of the larger nanocapsules. DLS experiments were done over 30 minutes allowing these large particles to form a separated phase at the surface of the aqueous buffer and then characterize the stable dispersion of the smaller nanocapsules.

The measured average hydrodynamic radius R_h of the nanocapsules showed high variations during the initial third of the measurement time (Figure 1). This change in apparent radius was clearly linked with the organic phase in the hydrophobic core which led to the visible upward movement of nanocapsules with larger diameters, as a higher amount of organic phase is entrapped in fractions with bigger particle sizes, reducing their average density to less than that of the aqueous phase. After about 10 minutes, the particle radius remained constant, indicating the formation of a stable dispersion after all the larger particles floated out of the optical measurement region.

As a result, R_h of the nanocapsules formulations were evaluated separately for two measurement periods of the

same measurement, e.g. the initial third and the remaining measurement time, as well as combined over the complete time period. The intensity-weighted size distribution of the stable dispersion is shown in Figure 2. An average R_h over the stable measurement time indicated particle sizes ranging from 339 to 762 nm, depending on the preparation conditions.

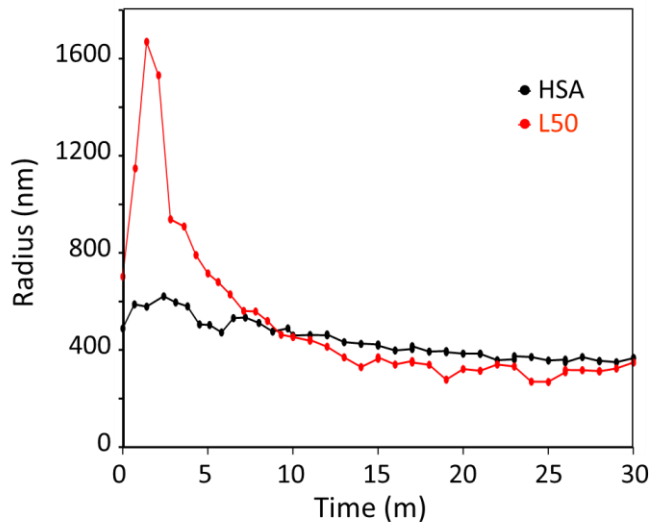


Figure 1. Hydrodynamic radius as a function of time for HSA (black) and HSA-SF (red) nanocapsules containing 50% of silk fibroin with low degradation degree (L50). The loss of larger particles over the course of ~ 10 minutes is due to their incorporation of low-density organic phase causing them to floating upwards and out of the measurement volume. Adapted from Ref. 1.

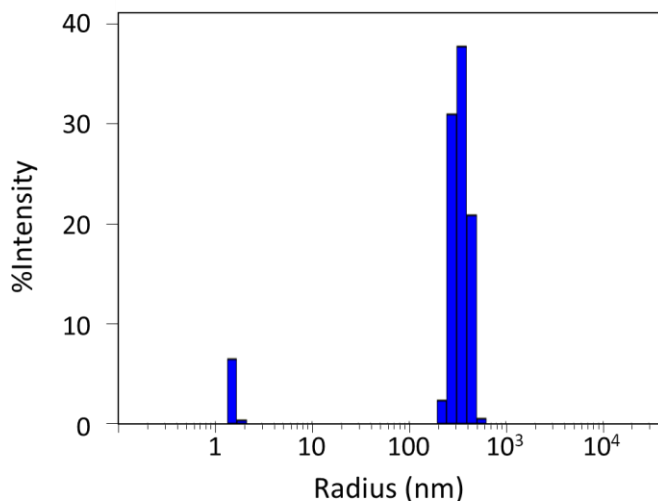


Figure 2. Intensity distribution of HSA-SF nanocapsule's (L50) mono-disperse phase, showing an average hydrodynamic radius of 346 nm (dominant peak) of the investigated nanocapsules.

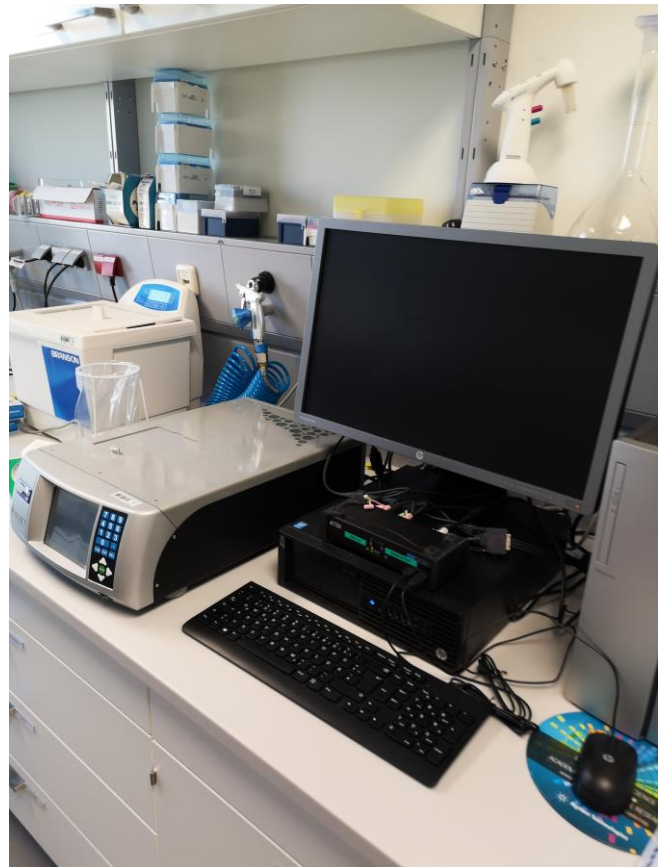


Figure 3. DynaPro Nanostar System

Conclusions

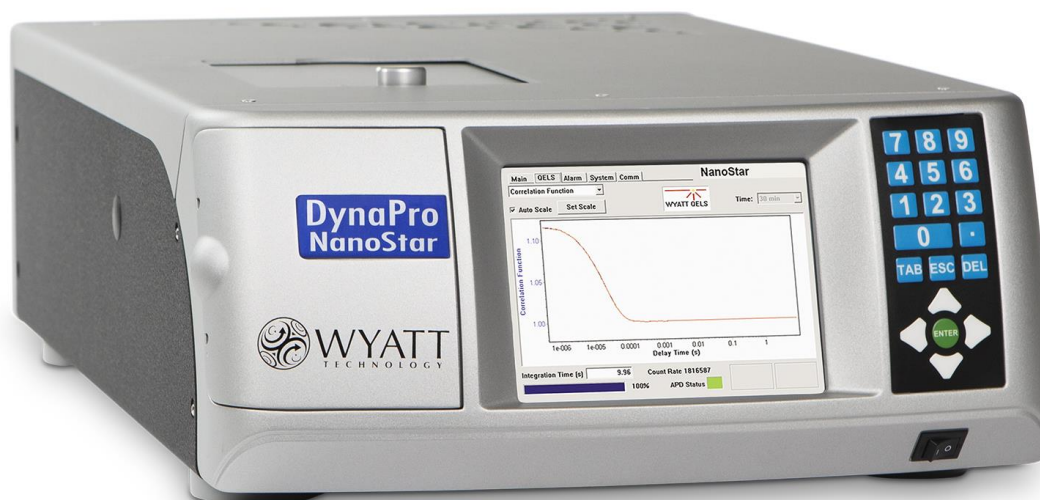
Based on the demonstrated results, successful HSA-SF nanocapsules production by ultrasound-assisted self-assembling was achieved, whereby a significant influence of the encapsulated organic phase on particle size and floating behavior was investigated by DLS. Though the larger nanocapsules moved to the surface, the remaining nanocapsule dispersion was stable and the radius measured by DLS stayed constant. This information on dispersion behavior served as basis for further experiments and led to the successful separation of a stable dispersion of nanocapsules with an average hydrodynamic radius of 346 nm.

Acknowledgements

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References

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