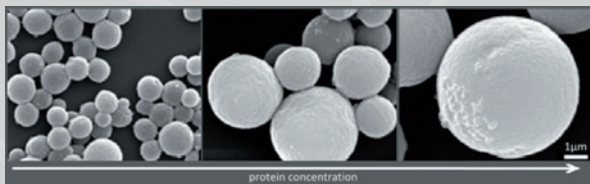


AMSilk®-Silkbeads – general description

Microparticles are one of the manifold morphologies spider silk proteins can adopt. These particles are formed by precipitating aqueous silk protein solutions with kosmotropic salts such as potassium phosphate or ammonium sulfate. The resulting particles are, like natural spider silk fibers, stable against chemicals and protein denaturants such as urea or guanidinium chloride.

The surface of the particles is smooth and they are tightly packed with no obvious substructure as seen with Cryo-SEM studies on freeze fractioned spheres. The main secondary structure element is β -sheet, the most rigid secondary structure a protein can possess. This is a prerequisite for the extraordinary chemical stability. The diameter of the Spidersilk Beads is variable and depends on the initial protein concentration and the chosen kosmotropic salt ranging from nanometers up to several microns.



The mechanism of Spidersilk Beads formation is described in “An engineered spider silk protein forms microspheres” by Slotta et al. (Angew. Chem. Int. Ed.; 2008; 47; 4592-4594), further information can be found in “Processing conditions for the formation of spider silk microspheres” by Lammel et al. (ChemSus-Chem; 2008; 1; 5; 413-416).

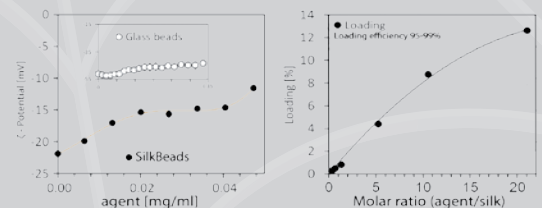
Controlled drug delivery with Spidersilk RX

Spidersilk RX is not immunogenic and causes no inflammatory reactions in the body. This makes it an excellent tool for pharmaceutical applications.

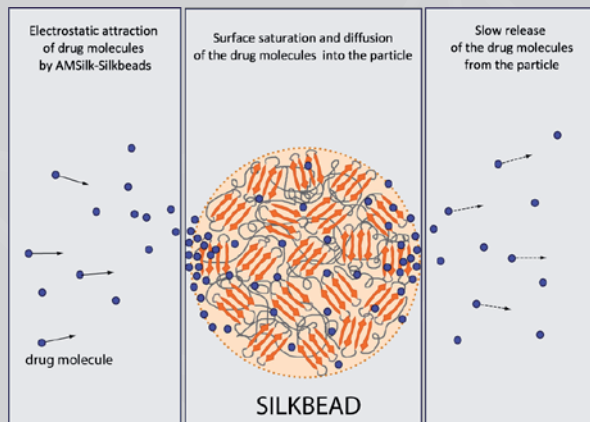
Spidersilk Beads are negatively charged at pH 7 and can thus form complexes with positively charged molecules via electrostatic interactions. To test the loading efficiency, Spidersilk Beads loaded with drug molecules were compared

to glass beads (dense matrix, no diffusion into the bead). The zeta-potential of glass beads is with a value of -50 mV much higher than that of Spidersilk Beads (approximately -22 mV). This should theoretically cause a higher loading efficiency with positively charged molecules.

In an experimental setup, methyl violet was used as model substrate. Measuring the change in zeta-potential while loading the Spidersilk Beads with methyl violet showed, that the loading follows a triphasic process. First, the drug molecules attach to the Spidersilk Beads by electrostatic forces. The small molecular drugs saturate the surface and start to diffuse into the particle. The drugs are retained in the particle matrix by hydrophobic and electrostatic interactions. Incubation in



a release medium is necessary to disturb the interaction between silk and drug molecules. Forced by a gradient driven process, the drug diffuses back to the surface. There it is slowly released into the environment. For more detail



see Lammel et al. (Biomaterials; 2011; 32; 8; 2233-2240).

AMSilk's **Spidersilk Beads** have many advantages compared to conventional drug delivery systems. **Spidersilk Beads** are produced and loaded with drug molecules in an aqueous environment and under ambient conditions. Conventional drug delivery systems often use toxic production. Therefore application restrictions may occur or extensive purification strategies have to be pursued.

AMSilk's **Spidersilk Beads** contain mainly hydrophobic and negatively charged protein molecules. Due to their amphiphilic properties they can also easily interact with hydrophilic drug molecules.

AMSilk's **Spidersilk RX** has several advantages as a drug delivery platform:

- Adjustable particle size with a minimal diameter distribution (as low as +/- 3 %)
- Good to high drug loading efficiency
- High loading capacity
- Monodisperse building blocks
- No harsh preparatory conditions required (no elevated temperature, no organic solvents, no vigorous agitation, no surfactants)
- Switched to insoluble with most solvents after formation of beads from aqueous solvents; stable

- Stable, yet biocompatible and degradable carrier (temperature over 100°C; high pH and mechanical stability)
- No aromatics and therefore very low absorption in the UV/VIS range; no quenching; no interference with analytical methods
- Non immunogenic
- Uniform particle homology
- No animal source material required

AMSilk's **Spidersilk Beads** have an appropriate diameter for phagocytosis by macrophages or cellular uptake in carcinoma cells.

Data presented resulted from a collaboration with the groups of Prof. Dr. Winter at the LMU Munich and Prof. Dr. Scheibel at the University of Bayreuth, Germany.

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