

Electronic Supplementary Material to

## „Focused Ultrasound as a Scalable and Contact-Free Method to Manufacture Protein-Loaded PLGA Nanoparticles”

By S. Schiller<sup>1,2</sup>, A. Hanefeld<sup>2</sup>, M. Schneider<sup>1</sup>, and C.-M. Lehr<sup>1,3</sup>

<sup>1</sup>Department of Pharmacy, Biopharmaceutics & Pharmaceutical Technology, Saarland University, 66123 Saarbrücken, Germany

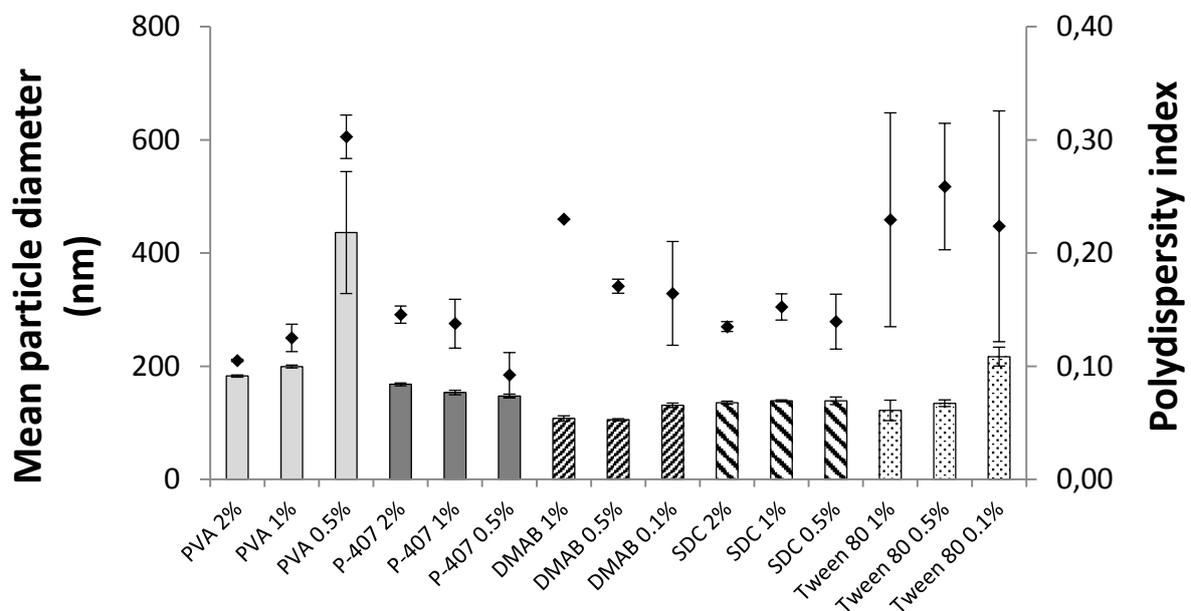
<sup>2</sup>Research Pharmaceuticals & Drug Product Development, Merck Serono, 64293 Darmstadt, Germany

<sup>3</sup> Department of Drug Delivery (DDEL), Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Center for Infection Research (HZI), Saarland University, 66123 Saarbrücken, Germany

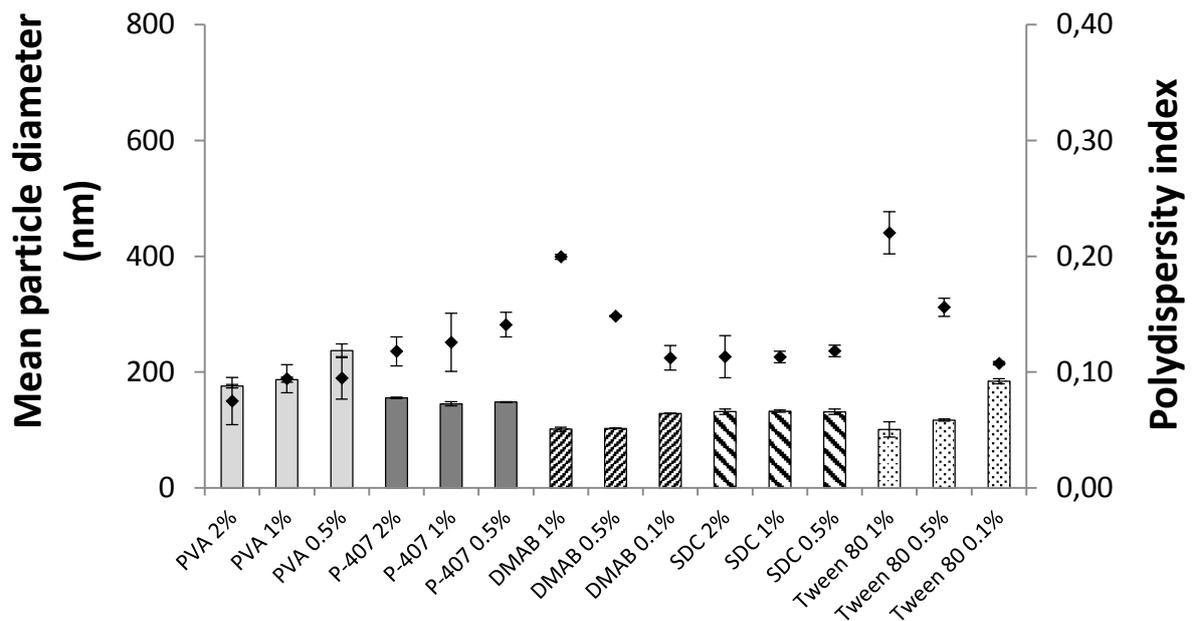
### Stabilizer screening for the preparation of PLGA nanoparticles

The following stabilizers were evaluated: polyvinyl alcohol (PVA; Mowiol® 4-88,  $M_w \sim 31,000$ ) and dimethyldidodecylammonium bromide (DMAB) were obtained from Sigma Aldrich (St. Louis, USA).

Sodium deoxycholate (SDC) and polysorbate 80 (Tween® 80) were obtained from Merck KGaA (Darmstadt, Germany). Poloxamer 188 (P-188) and poloxamer 407 (P-407) were kindly provided by BASF (Ludwigshafen, Germany).



**Fig. S1** Mean particle size (bars) and polydispersity index (♦) of formulations manufactured with different stabilizers. The size of three independent batches was determined by DLS, and the standard deviation depicted as error bars. P-188 was omitted due to emulsion instability and subsequent film formation.



**Fig. S2** Mean particle size (bars) and polydispersity index (♦) of formulations manufactured with different stabilizers after removal of aggregates by centrifugation (15 min at 1,000 x g). The size of three independent batches was determined by DLS, and the standard deviation depicted as error bars. P-188 was omitted due to emulsion instability and subsequent film formation.

Nanoparticles were prepared by double emulsion solvent evaporation as described in the methods section. 0.4 mL of an aqueous solution of 3.75 mg/mL ovalbumin, 2.5 mL of 12 mg/mL PLGA in ethyl acetate, and 5 mL of the respective aqueous stabilizer solution were subsequently homogenized by focused ultrasound in a glass tube. The first homogenization was done with 50% duty factor, 1000 cycles per burst, and 200 W peak incident power for 60 s. The second homogenization was done using the same settings for 5 min. After solvent evaporation, the mean particle size was determined by dynamic light scattering before (Figure S1) and after (Figure S2) removal of aggregates by centrifugation (15 min at 1,000 x g) as described in the methods section. The  $\zeta$ -potential was additionally determined by laser Doppler anemometry (Zetasizer Nano ZS, Malvern Instruments, Worcestershire, UK) when using charged stabilizers: 1 mL of freshly prepared nanoparticle suspension was pelletized by centrifugation (5 min at 21,000 x g), the supernatant removed, the nanoparticles redispersed in 1 mL of 1 mM phosphate buffer pH 7.4, and transferred to a folded capillary cell

(DTS1061). Each sample was measured three times with automatic measurement duration and voltage selection, data recorded using Zetasizer Software v7.01 and analyzed using “General Purpose mode”.

The different stabilizers were evaluated for their ability to prevent droplet coalescence and nanoparticle agglomeration during solvent evaporation and after particle formation, respectively. The absence of large aggregates is indicated by minimal differences of mean particle size and polydispersity index between the three independent runs for each stabilizer concentration before removal of aggregates (Figure S1), as well as minimal differences before and after centrifugation (Figures S1 and S2). A good stabilization for both the emulsion and the particle suspension could be observed for PVA at 1 and 2%, P-407 at 0.5, 1 and 2%, DMAB at 0.1%, and SDC at 0.5, 1 and 2%. Visible agglomeration occurred when using PVA at 0.5%, DMAB at 0.5 and 1%, and Tween® 80 at 0.1, 0.5 and 1%. Interestingly, while a minimum concentration of 1% for the nonionic, steric stabilizer PVA was required, formulations using the cationic, non-steric stabilizer DMAB only remained stable at DMAB concentrations of not more than 0.1%. The heavy precipitation observed for DMAB concentrations of 0.5 and 1% may be due to higher charge interactions and crosslinking between positively charged particles, free stabilizer and the negatively charged free ovalbumin. At 0.1% DMAB seems to be feasible for the preparation of small, positively charged PLGA nanoparticles ( $\zeta$ -potential of  $35.0 \pm 1.0$  mV). Nanoparticles prepared using 0.5% P-407 were slightly negatively charged ( $-30.4 \pm 2.1$  mV), while the anionic stabilizer SDC yielded a stronger negative surface charge ( $-51.7 \pm 4.1$  mV).

For formulations containing P-188 at 0.5, 1 and 2%, particle size measurement was omitted because of the formation of a continuous film at the surface of the emulsion. The poor emulsification efficiency of P-188 in contrast to P-407 may be explained by the very high hydrophilicity of P-188 (HLB > 24), while P-407 (HLB between 18-23) is more lipophilic due to its more than doubled poly(oxypropylene) middle block.