Standard Operation Procedure (SOP)

for the Dispersion of Polydisperse Polymers Particles

Date

08.06.2021

Version

1.0, English

Authors

Amelie Vogel[1], Wendel Wohlleben[2], Kerstin Hund-Rinke[3], Martin Wiemann[4], Andrea Haase[1]

1 German Federal Institute for Risk Assessment (BfR), Berlin, Germany

2 BASF SE, Ludwigshafen, Germany

3 Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany

4 IBR R&D gGmbH, Institute for Lung Health, Muenster, Germany

Content

1 Introduction

Polymer particles (PP) often have a low buoyant density and, therefore, may be cumbersome to handle when used for *in vitro* cell culture experiments under submersed conditions. This standard operating procedure (SOP) was established within the BMBF-funded project InnoMat.Life (www.innomatlife.de) to ease the aqueous dispersion of polydisperse PP such as for instance polypropylene, polyethylene, polyamide, polyurethane particles.

The method described here aims to reduce the floatation of PP materials in aqueous solutions. Also, the attachment of PP particles to the non-polar pipette tip may pose considerable inaccuracy to the transfer of PP dispersions by pipetting. Both difficulties with PP material handling are overcome by pre-wetting the PPs with 96 % ethanol and immersing them in 0.05% w/v BSA-water in the presence of a very low and, therefore, cell compatible concentration of surfactant (Tween® 40).

2 Equipment and Reagents

2.1 Reagents

Bovine serum albumine (BSA), fraction V, purity 98 %, endotoxin-free and cell culture tested

96 % ethanol

MilliQ water

Tween® 40

PP to be tested

2.2 Equipment

Pipettes: 5 ml, 1000 µl, 100 µl

Pipette tips 5 ml, 1000 µl, 100 µl

Calibrated microbalance

Refrigerator (4°C)

100 ml glass container for dissolution of BSA

100 ml glass container for sterile-filtered 1 % w/v stock BSA-water solution

100 ml container for 0,05 % w/v BSA-dispersion medium

10 ml glass vials for weighting in the polydisperse powders

Plastic syringes and sterile filters (0.1 and 0.2 µ pore width)

Magnetic stirrer

Magnetic stir bar

Scalpel

Vortexer

3 Experimental Protocol

3.1 Preparing the dispersion fluid

Dissolve 1 g of BSA fraction V in 100 ml MilliQ water in a glass beaker. Sterilize the solution by passing through a low protein binding protein filter with a 0.1 µm pore size, to receive a sterile 1 % w/v BSA stock solution. The sterile solution can be stored at 4°C.

Prepare a 1% (w/w) stock solution of Tween® 40. For this weigh in 500 mg Tween® 40 and fill up with water to achieve 50 g in total. To facilitate pipetting of the viscous Tween® 40, the pipette tip may be cut off with a scalpel. Mix carefully. Tween® 40 might flocculate, therefore, filter the solution with a 0.2 µm pore size. The received 1% (w/w) stock solution of Tween® 40 can be stored at room temperature.

Prepare the dispersion fluid by mixing 2 ml of the BSA stock solution, 20 µl of the Tween® 40 stock solution and 38 ml sterile MilliQ water in a 100 ml sterile glass vial. Prepare the dispersion fluid freshly for each experiment.

3.2 Dispersion of Polymer Particles

Weight in 15 mg ± 1 % of the polymer into a 15 ml glass vial. Pre-wet the powder with 30 µl ethanol. Add the BSA/ Tween® 40 dispersion fluid in two steps: Pipette 970 µl into the glass vials and mix well. To receive a 2.5 mg/ml polymer dispersion, add 5 ml of the BSA/ Tween® 40 dispersion fluid and vortex. If agglomerates are still visible, the dispersion may be put into a sonication bath for at least 5 min. Effects of Tween® 40 on particle dispersion and pipetting the PP suspension are shown in Figure 1.

4 Final notes and handling the PP suspension:

As particles tend to settle, vortex the vials immediately before use.

Mixing 1 volume of the particle suspension with at least 19 volumes of cell culture medium yields an upper particle concentration of 125 µg/mL. This is an acceptable upper concentration limit for cell culture experiments. The moderate lowering of osmolarity should be well-tolerated by most cells. Alternatively, the lowered osmolarity may be compensated by first mixing an equal amount of polymer dispersion (2.5 mg/ml) with an equal amount of double concentrated medium to obtain a 1.25 mg/ml polymer dispersion in cell culture medium, prepare the final working dilutions from this.

The final concentration of Tween® 40 was shown to be non-toxic to CaCo-2 cells within the InnoMat.Life project. However, for all other cell types this needs to be verified prior to the experiments.

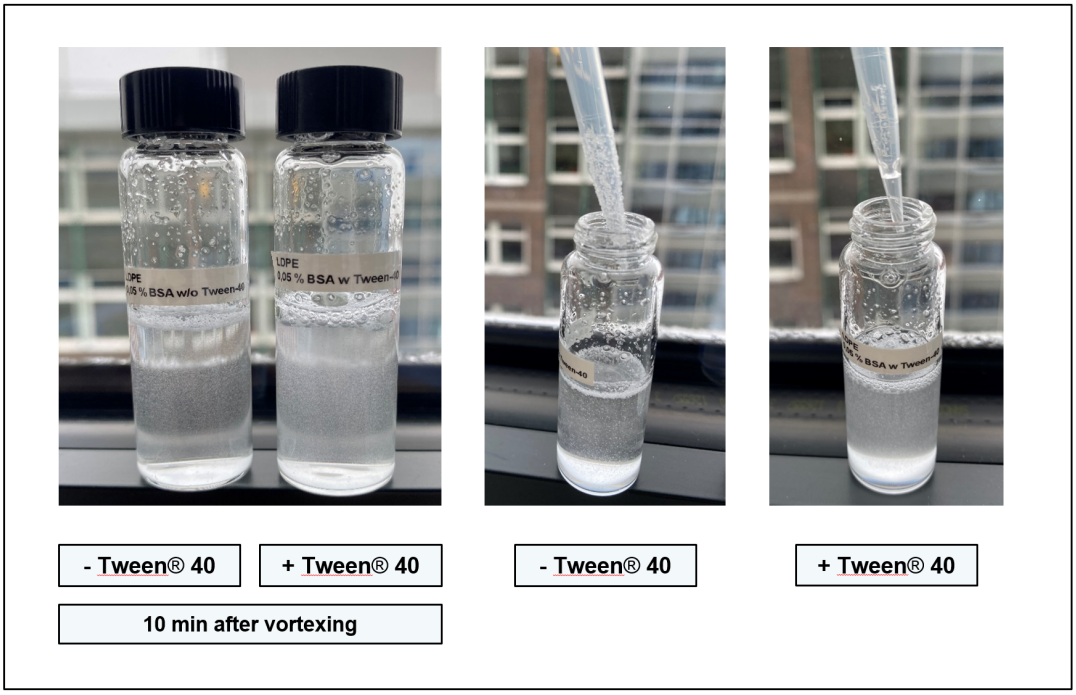

Figure 1. Improvement of the polymer dispersion by Tween® 40. By reducing the surface tension, less particles are floating and they appear to be more stable in the dispersion (left picture). Secondly, pipetting was improved since particles no longer stick on the outer pipette tip.