Stabilization of TiO_2 Nanoparticles in Cell Culture Media

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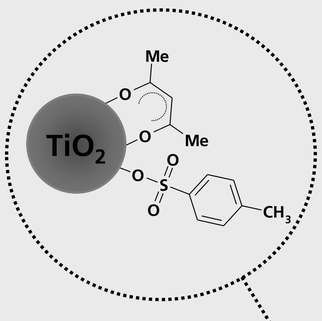
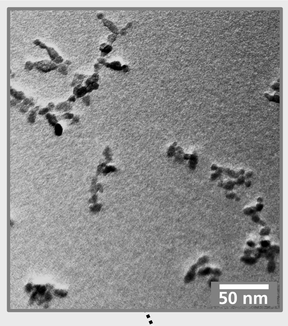
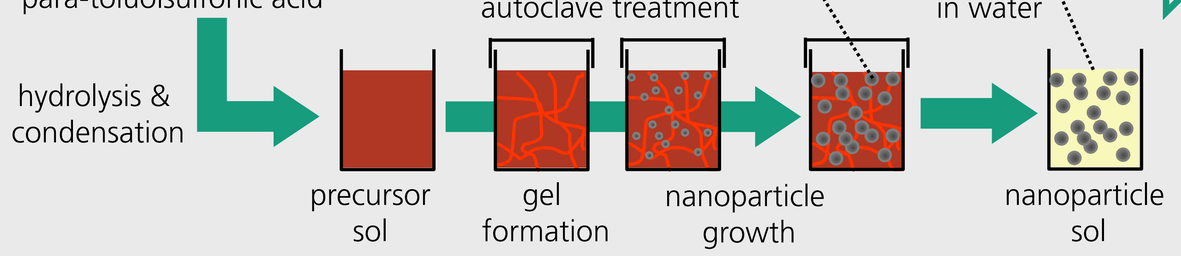
Titanium dioxide nanoparticles are of great interest for tumor therapy due to their photocatalytic activity. Stable TiO_2 nanoparticle dispersions are a crucial requirement for reliable cell culture experiments concerning toxicity studies or tumor therapy experiments [1, 2]. As most nanoparticles do not show colloidal stability in cell culture media due to its pH and/or salt content, suitable surfactants have to be found. The effectivity of a surfactant depends on the nanoparticle type, its surface properties and the chosen cell culture media. Therefore, there is no general solution for stabilizing nanoparticles and it has to be found the most adequate surfactant in every single case [1, 3, 4]. Consequently, this study intended to stabilize self-synthetized TiO_2 nanoparticles in three different cell culture media (Dulbecco's Modified Eagle Medium (DMEM), Roswell Park Memorial Institute Medium (RPMI) and Bronchial Epithelial Cell Growth Medium (BEGM)). For the stabilization, various commercial products were utilized that had already been cell culture tested, like BSA (bovine serum albumine), FBS (foetal bovine serum) or tween80. Furthermore some surfactants were examined that had not yet been used for this purpose, e.g. PCE (Polycarboxylate ether) or Brij30. The most promising stabilizers FBS and PCE for RPMI are presented here.

TiO_2 Nanoparticles

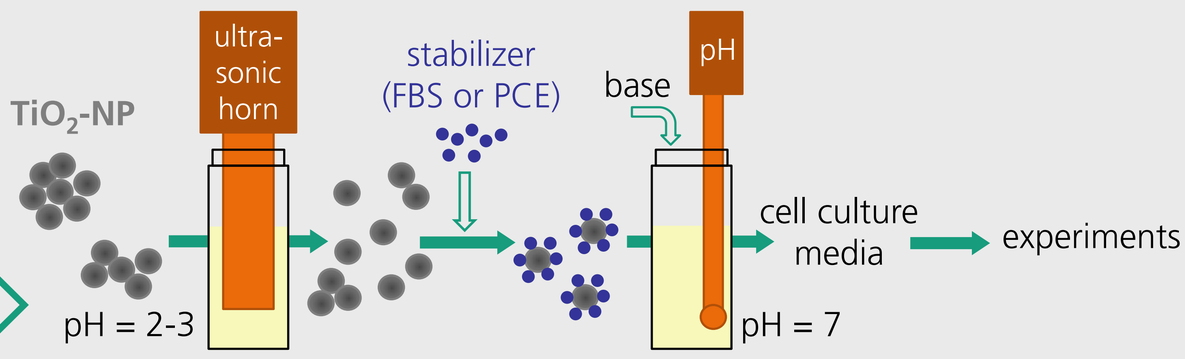
- Hydrothermal synthesis
- Controllable, monodisperse particle and crystallite size: 8 nm
- Anatase phase
- Organic surface moieties



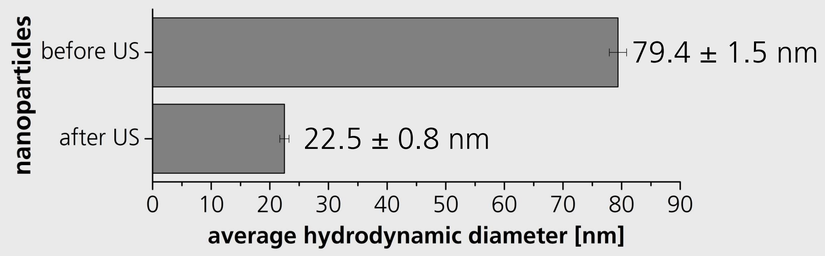




Stabilization Protocol



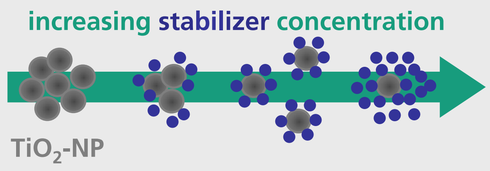
Dynamic light scattering measurement before and after ultrasonic (US) treatment



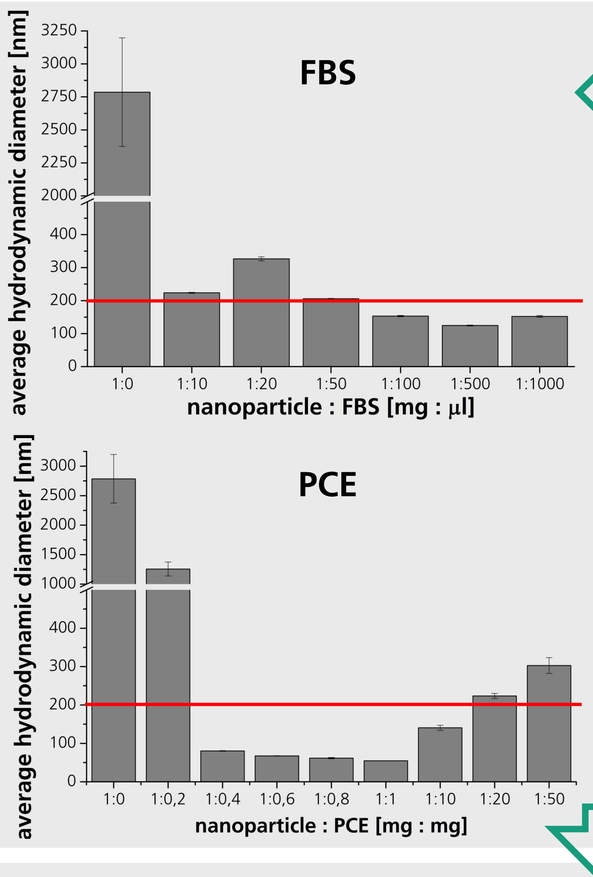
By this stabilization protocol the optimal dispersion and stabilization of NPs in cell culture media can be achieved.

Agglomerate Size

- Dynamic light scattering measurement of nanoparticles with different FBS or PCE concentrations in RPMI
- Stabilized following the developed protocol

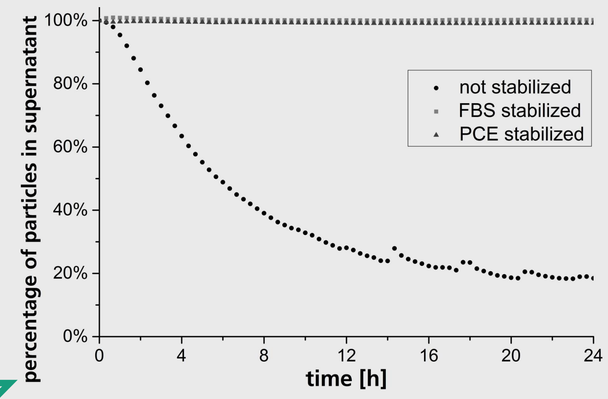


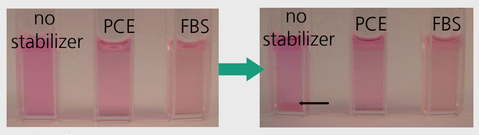
By variation of the stabilizer Concentration, the optimal agglomerate size can be obtained.



Sedimentation

- Absorption measurement at 490 nm, 24 h (every 20 min), at room temperature
- 0.5 mg/ml particles in RPMI
- Particles not stabilized, PCE stabilized (NP : PCE = 1 mg : 400 µg), FBS stabilized (NP : FBS = 1 mg : 100 µl)





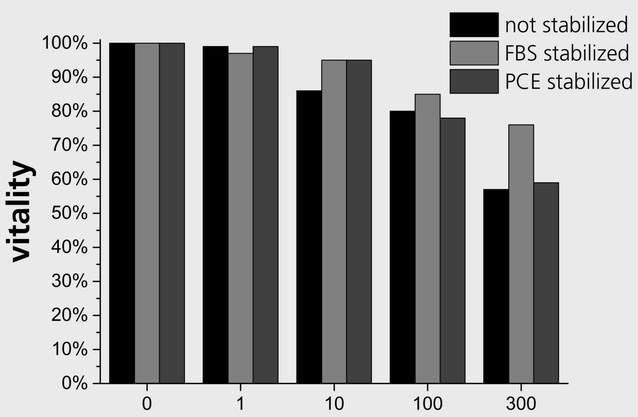
Sedimentation after 0 h

Sedimentation after 24 h

The stabilized NP do not sediment in cell culture media during 24 h.

Cytotoxicity of stabilized NPs

- MTT assay
  (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
- FaDu human epithelial cell line
  (from a squamous cell carcinoma of the hypopharynx)
- in RPMI, 24 h incubation, 37 °C, 5 % CO_2
- Particles not stabilized, PCE stabilized (NP : PCE = 1 mg : 400 µg), FBS stabilized (NP : FBS = 1 mg : 100 µl)



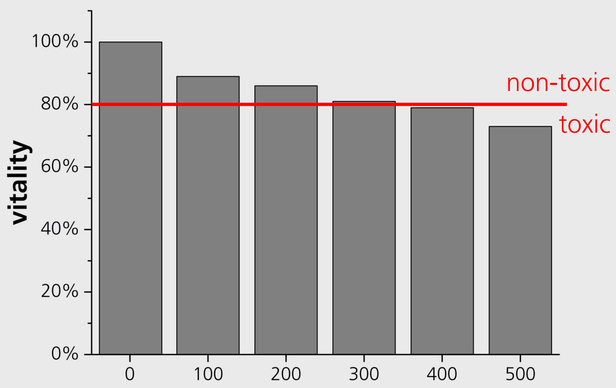
No negative effects of the stabilizers on the cytotoxicity of the NP can be detected.

Cytotoxicity of PCE

- MTT assay
- FaDu human epithelial cell line
- in RPMI, 24 h incubation, 37 °C, 5 % CO_2

Concentration for stabilization:
NP : PCE = 1 mg : 400 µg

maximum NP concentration: 300 µg/ml
=> maximum PCE concentration: 120 µg/ml



PCE is non-toxic in the concentration range utilized.

Summary

- Stabilization of NP in cell culture media with the help of FBS or PCE:
- No sedimentation over 24 h
- Agglomerate size smaller than 200 nm in diameter
- No negative effects of stabilizers on cell toxicity of the NP

Outlook

- Examination of NP uptake by cells via TEM
- In vitro test for tumor treatment via photocatalytic activation of the TiO_2 NP

Acknowledgement

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Literature

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