Towards a comprehension of Zinc Oxide Nanoparticles behavior in inorganic and biological fluids

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TNH Lab

MOTIVATION

ZnO nanoparticles (NPs) for their unique characteristics represent potential diagnostic and therapeutic tools in cancer therapies.
Little is known about their toxicity mechanisms and stability in the biological context.
Investigations of ZnO NPs aggregation and biodegradation behavior in solvents and biological media are needed to produce dispersed and stable nanoparticles for clinical applications.

SYNTHESIS AND CHARACTERIZATION

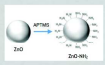
ZnO NPs synthesis: SOLVO-THERMAL

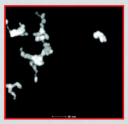
Sol 1: Zinc Acetate di-hydrate 818.2 mg (3,73mmol)
Methanol 42 mL
H_2O b.d. 318 µL
Sol 2: NaOH 288,8 mg (7,22 mmol)
H_2O b.d. 23 mL

- Dropwise addition of Sol 2 in Sol 1 for 15 min at 60°C under stirring
- Stir at 60°C for 5 hours
- Centrifugation at 5000 rpm for 10 min
- Wash with EtOH twice
- Final dispersion in EtOH

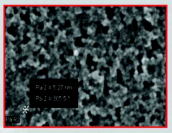
Chemical functionalization with aminopropyl groups

- 100 mg (1,22mmol) ZnO NPs in MeOH in a glass round flask
- 21,9 mg (0,122mmol) of aminopropyltrimethoxysilane (APTMS), 10 mol% of ZnO molar amount
- Reflux the solution for 6 h under nitrogen atmosphere
- Wash functionalized NPs ($ZnO-NH_2$) to remove unbound molecules
- Redisperse in clean EtOH





Transmission Electron Microscopy (TEM) of unfunctionalized ZnO NPs

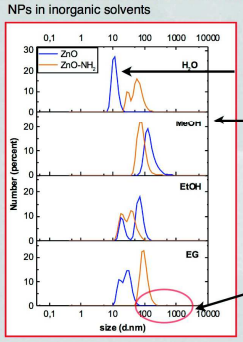


Field Emission Scanning Electron Microscopy (FESEM) of unfunctionalized ZnO NPs

COLLOIDAL STABILITY STUDY

Which is the best

DLS measurements of ZnO and $ZnO-NH_2$ NPs in inorganic solvents

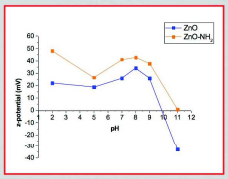


H_2O b.d. is the best solvent for ZnO NPs

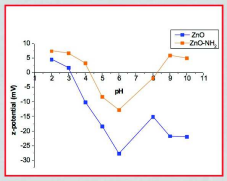
Due to the hydrophobic amine group, $ZnO-NH_2$ NPs are more stable in MeOH

EXTREME AGGREGATION! ZnO NP is around 7 nm!!

The influence of pH



ZnO and $ZnO-NH_2$ NPs are positive at **physiological pH** in NaOH 1M and HCl 1M titrated water solution



However in buffered solution*

…this is the behavior!!!

*citric acid, hydrochloric acid, sodium hydroxide, hydrogen carbonate, potassium hydrogen phtalate, potassium di-hydrogenphosphate and boric acid

THE BIOLOGICAL CONTEXT: LONG TERM BIODEGRADATION ANALYSIS

Structural characterization

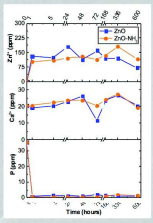
25 days biodegradation assay

- 2mg/ml ZnO and ZnO-NH2 NPs
- in Simulated Body Fluid (SBF) and cEMEM (complete Minimal Essential Eagle's Medium) culture medium
- Stirred at 37°C
- pH measurements at each time point*

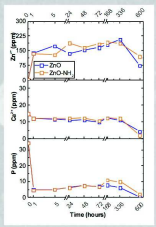
Dissolution analysis

ICP/MS measurements of dissolved Zn^{2+}, Ca^{2+} and PO_4^{2-}

SBF



cEMEM



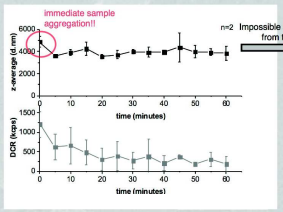
*pH variations are not significant

A dimensional point of view

72 hours DLS measurements

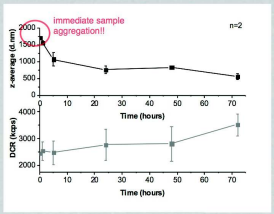
- 500µg/ml ZnO Nps
- in Simulated Body Fluid (SBF) and cEMEM (complete Minimal Essential Eagle's Medium) culture medium
- stirred at 37°C

ZnO NPs in SBF: aggregation and sedimentation



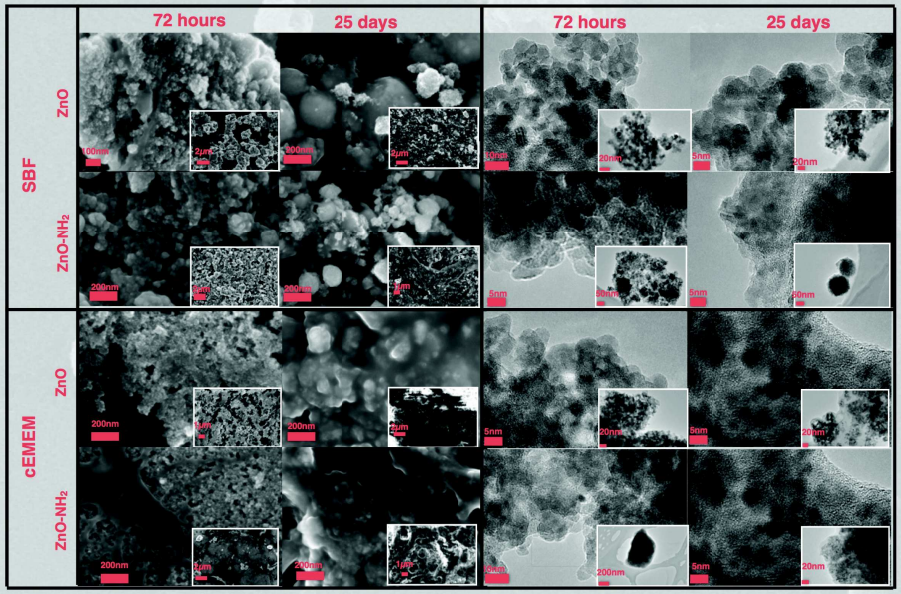
Huge ZnO-NPs aggregation!!

ZnO NPs in cEMEM: only aggregation



DCR= derived count rate (theoretical count rate obtained with 100% laser power)
z-average= intensity weighted mean hydrodynamic size of the ensemble collection of particles

Field Emission Scanning Electron Microscopy (FESEM) — **Transmission Electron Microscopy (TEM)**



EDX RESULTS: Main components in SBF sample: Zn, O, N, P, C
Main components detected in cEMEM sample: Zn, O, N, C, Ca, Si, Cl, P, S, Mg, K

CONCLUSIONS AND FUTURE OUTLOOK

- ZnO NPs strongly aggregate in any fluid, reaching the maximum in biological media like EMEM and in SBF.
- Functionalized ZnO NPs seem to aggregate more than unfunctionalized and to prefer non-aqueous solvents.
- The z-potential of both ZnO and $ZnO-NH_2$ is strongly influenced by fluid composition and shift to negative values in salted solution.
- The huge aggregation in biological media persists until 25 days, accompanied by little dissolution in the first hour that does not affect the ZnO NPs crystalline structure.
- The strong aggregation behavior of ZnO NPs in biological fluid suggests that these NPs must be protected by the biological microenvironment in order to obtain stable and mono disperse solutions for clinical applications.

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