Safe-by-design strategies based on the use of proper biocompatible surface modifiers for the ink & paint sector

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Helsinki, April 15, 2015

Outline

- Properties of surface modifiers used in inks and pigments.
- Selection of surface modifiers and surface modifiers with biological origin
- Strategies for surface modifications
- Silver nanoparticles (AgNPs)
- Zinc oxide (ZnO NPs)
- Quantum Dots
- Conclusions

Surface Modifiers

- **Proven Biocompatibility:** There are a number of studies demonstrating the biocompatibility and low toxicity of several molecules and molecular structures such as biopolymers in the literature . The ligands, biopolymers or biocompatible polymers are targeted while selecting and designing the surface modifiers.
- **Compatibility with NMs' properties:** The influence on the physical chemical properties of NMs upon attachment of surface modifiers onto the NM surface is important. Since the aim of the project to reduce the toxicity of NMs used in inks and pigments, the ligand or polymeric structure attached to the NM should not interfere with their physicochemical properties (at least with their most interested properties such as colour).
- **Stability in Formulation:** Dispersibility in the ink or pigment formulation and stability of the coating on the NM surface are taken into account.
- **Chemical Suitability:** The ligand that is planned to chemically attach to the NM surface should be suitable for chemistry. For example; carbohydrates have several hydroxyl groups for further chemistry.
- **Cost and Applicability:** The selected ligand should also be a commodity and should not increase the cost of the material excessively.
- **Processability:** the ligand shall not hinder the successful application of the nanoparticles, e.g. in case the ink is to be used for printing electro-conducting elements, the ligand must be removable at acceptable conditions.

Selected Biomacromolecules

- Carbohydrates
- Oligonucleotides and peptides
- Biocompatible polymers (as a first layer before further modification)
- Derivatized biomacromolecules

Modifiers should fulfill the requirements

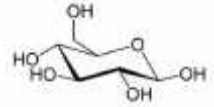
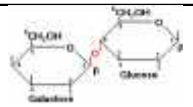
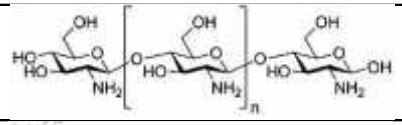
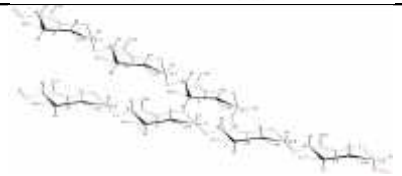
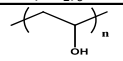
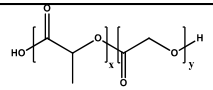
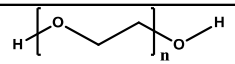
Application Concerns

- Proven biocompability
- Stability in formulation
- Chemical suitability
- Low cost
- Applicability

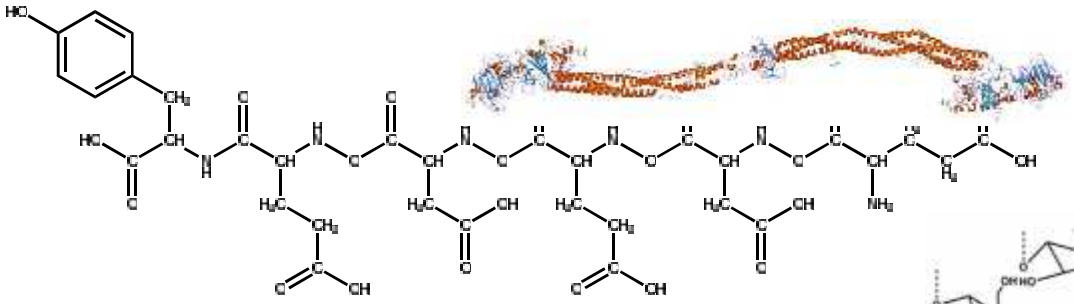
Toxicity Concerns

- Dissolution
- Size
- Surface Chemistry/Chemical nature
- Aggregation

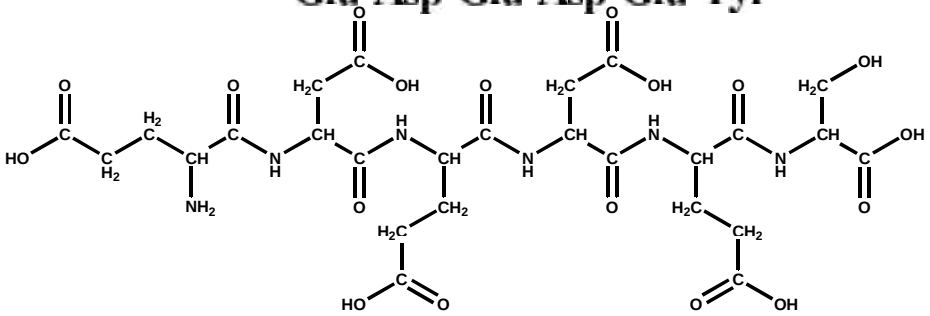
List of Surface Selected Modifiers

Ligand/Polymer	Structure
Glucose	
Lactose	
Chitosan	
Starch (Amlose and Amylopectin)	
Peptides	**For Metal Oxides:** Glu-Asp-Glu-Asp-Glu-Tyr Glu-Asp-Glu-Asp-Glu-Ser
	For QDs and AgNPs: Cys-Cys-Glu-Asp-Glu-Asp Cys-Glu-Asp-Glu-Glu-Asp
Peptidoglycans	Lactose-Ser-Ser-Lactose
Oligonucleotides	**For Metal Oxides:** AAAAAAAAAA GGGGGGGGGG TTTTTTTTTT CCCCCCCCCC
	For QDs and AgNPs: $SH(CH_2)_6$-TTTTTTTTT (From 5' to 3') $SH(CH_2)_6$- AAAAAAAAAA (From 5' to 3') $SH(CH_2)_6$- GGGGGGGGGG (From 5' to 3') $SH(CH_2)_6$-CCCCCCCCCC (From 5' to 3')
Polyvinyl alcohol	
Poly (D,L-lactide-co-glycolide)	
Polyethylene glycol	

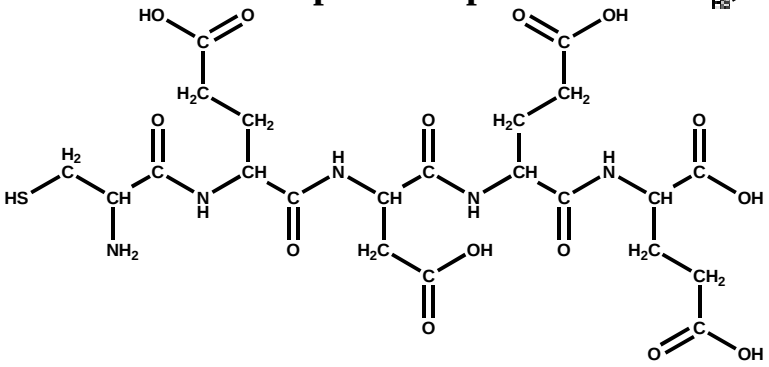
Chemical Structures of Selected Surface Modifiers



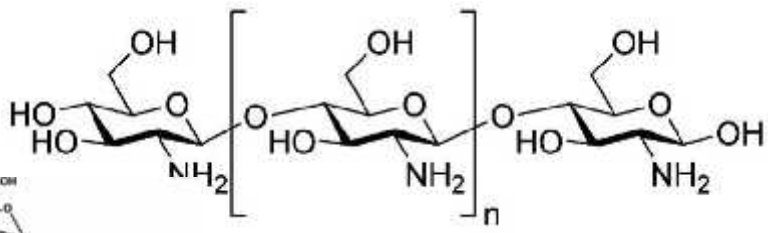
Glu-Asp-Glu-Asp-Glu-Tyr

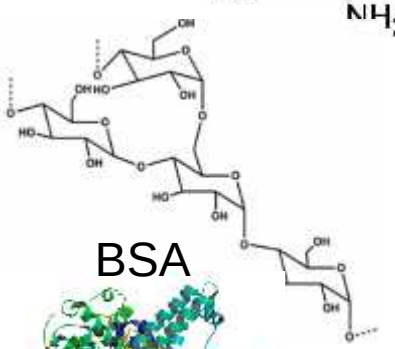


Glu-Asp-Glu-Asp-Glu-Ser

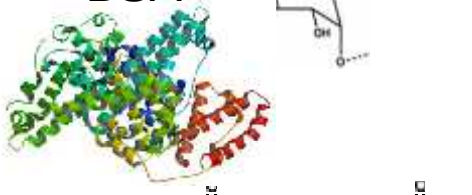


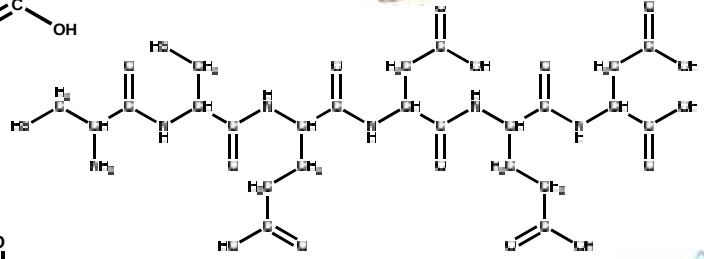
Cys-Glu-Asp-Glu-Glu-Asp



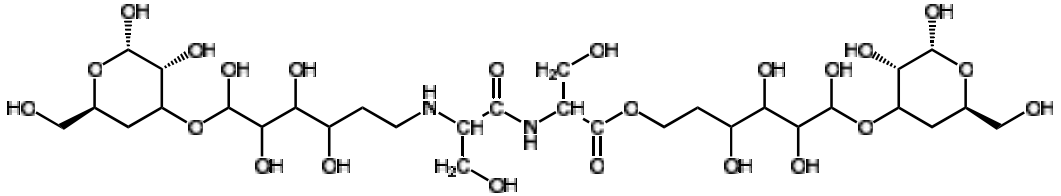


BSA





Cys-Cys-Glu-Asp-Glu-Asp



Lactose-Ser-Ser-Lactose

Some points to consider

- Surface properties of NMs
  - Defined by the synthesis procedure
  - Impurities remained on the NM surface
  - Agglomeration (a major problem)

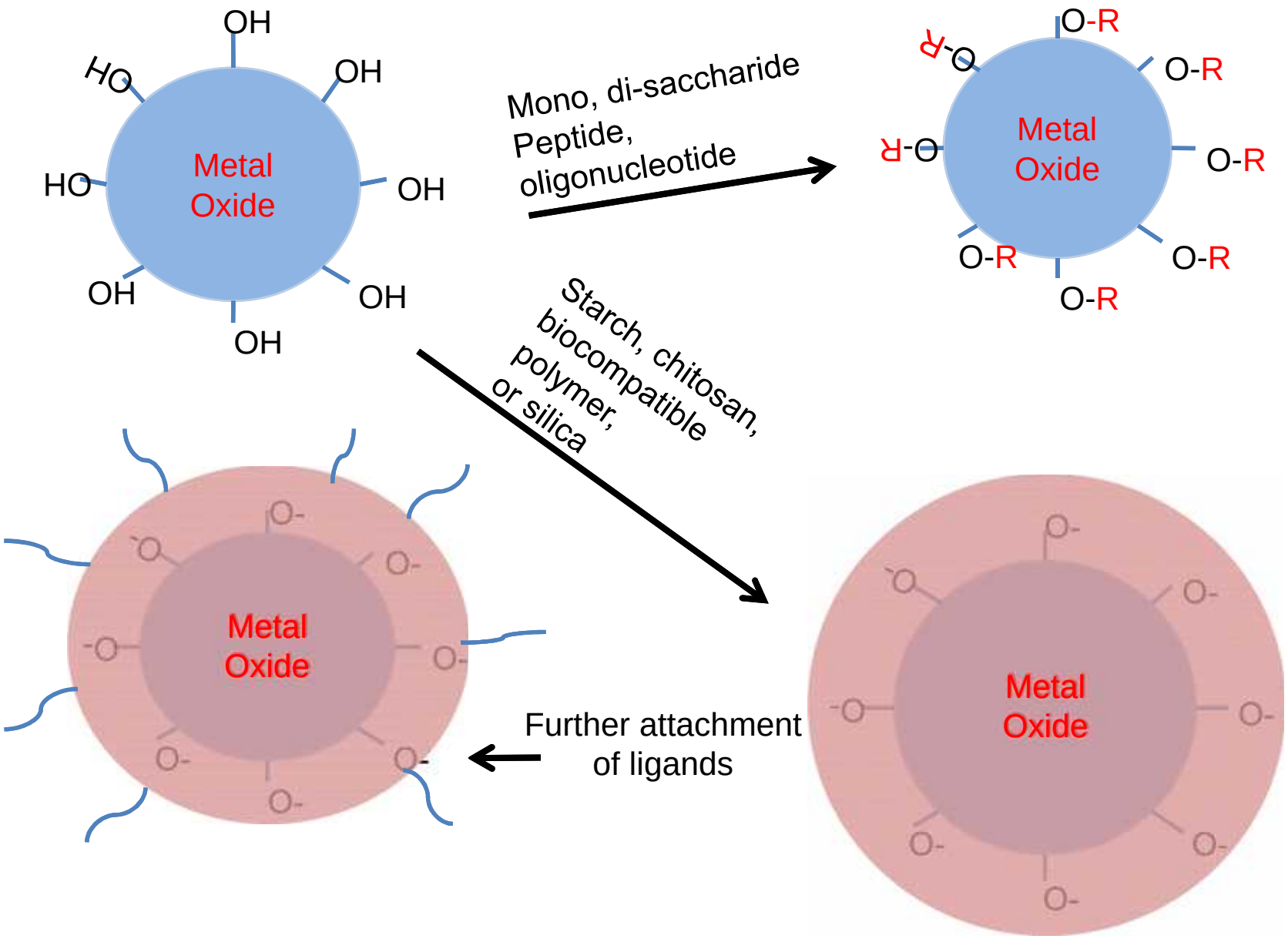
- Possible causes of toxicity: size, shape, dissolution and chemical nature

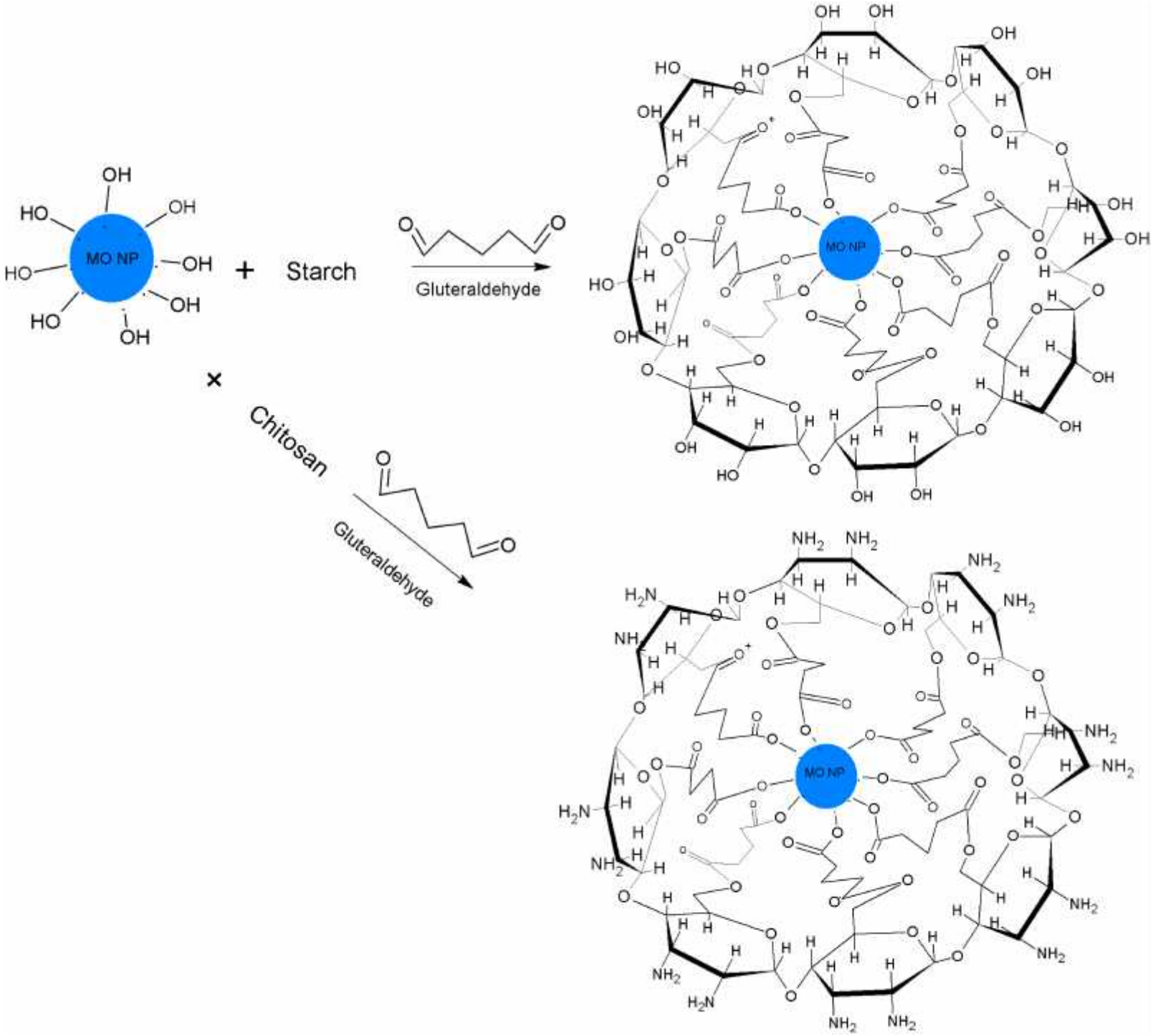
Two Main Routes For Modification

- The functionalization of the **metal oxide** NMs through the hydroxyl groups via bio-ligand possessing hydroxyl group will be **directly cross-linked** to the NP surface or **a silica shell on to the surface** of the NPs will be formed through **the cross-linking chemistry**. Further chemistry can be pursued through the functional groups **on the surface of the silica shell for the attachment of bio-ligands** such as monosaccharides, peptides, oligonucleotides and proteins.

- The surface chemistry of **QDs** and **AgNPs** are quite different from metal oxide NMs, well-known thiol-noble metal **(-S-AgNP)** and thiol-QD **(-S-ZnS/CdSe)** formation is utilized for the chemical attachment of the bio-ligands and polymeric structures to the NM surfaces. In a similar fashion, either **thiol-modified bio-ligands** can be directly attached to the NP surface or a shell structure completely surrounding the NM surface can be formed.

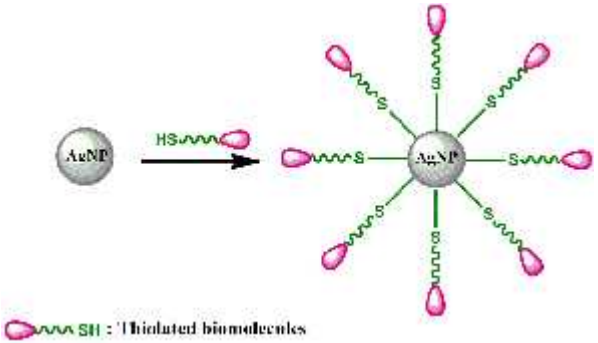
Two Main Routes For Modification Metal Oxide NPs



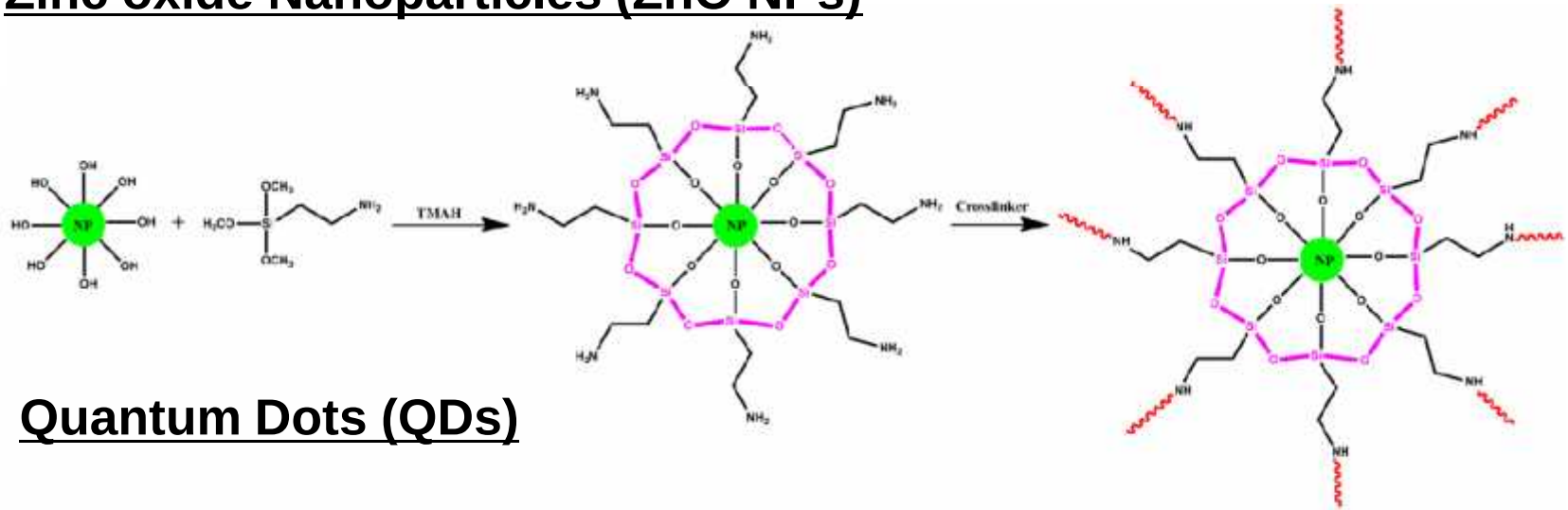

MO NP
+
Starch
Gluteraldehyde
×
Chitosan
Gluteraldehyde
MO NP
MO NP

Surface Modification Strategies

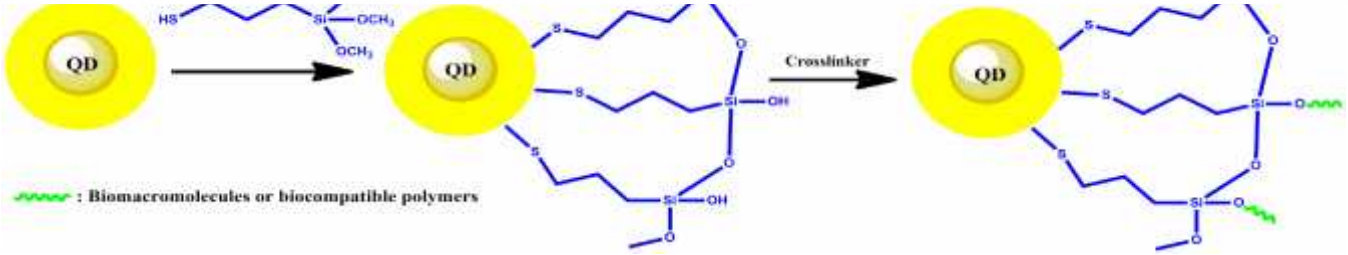
Silver Nanoparticles (AgNPs)



Zinc oxide Nanoparticles (ZnO NPs)



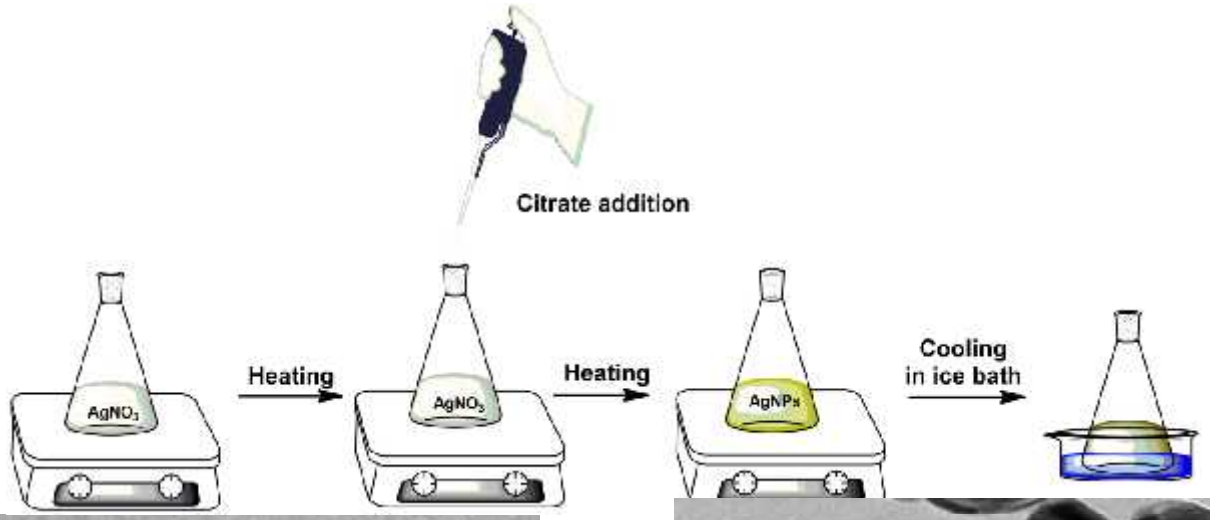
Quantum Dots (QDs)

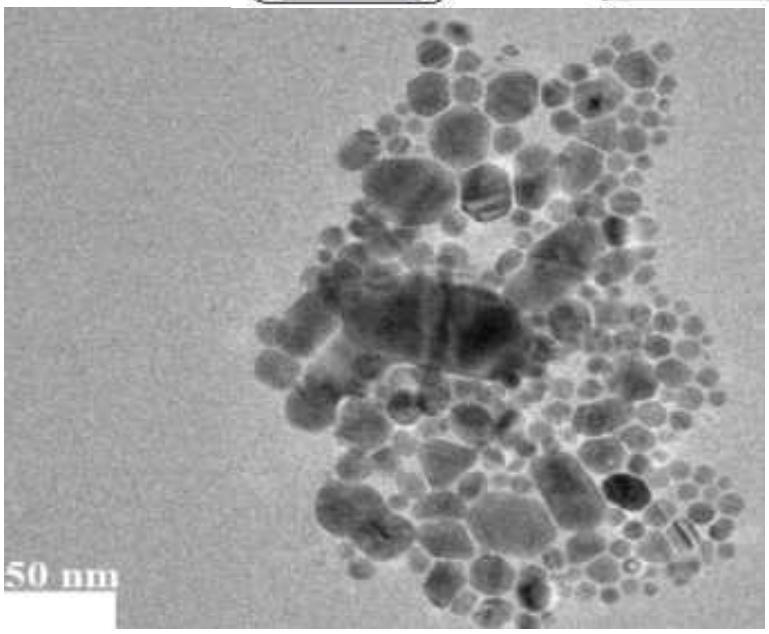


Nanomaterials aimed to modify in Nanomicex Project

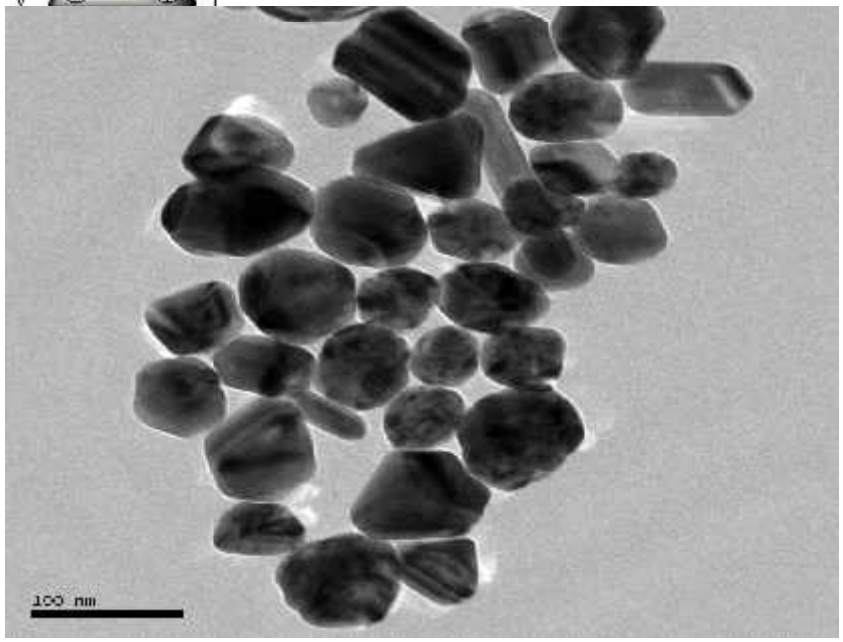
- **AgNPs:** Used to prepare conductive inks
- **ZnO NPs:** For adsorption in UV region or antibacterial properties
- **CdSe-ZnS QDs:** For their fluorescence properties

Silver Nanoparticles (AgNPs)
Lee - Meisel Method



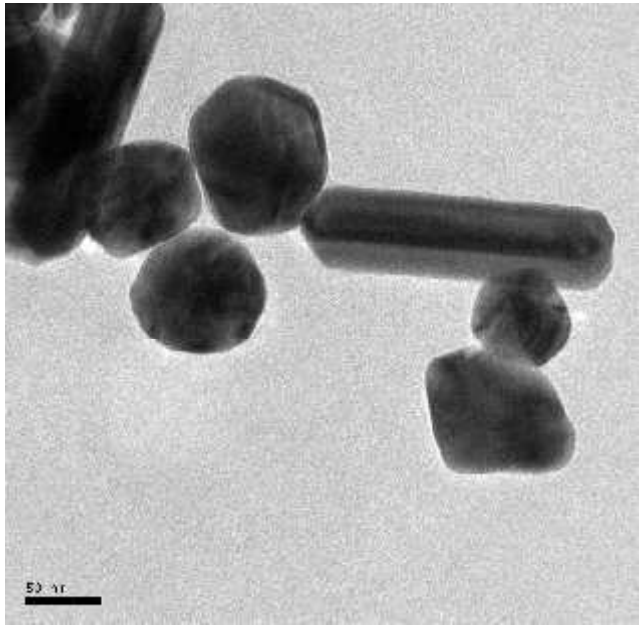


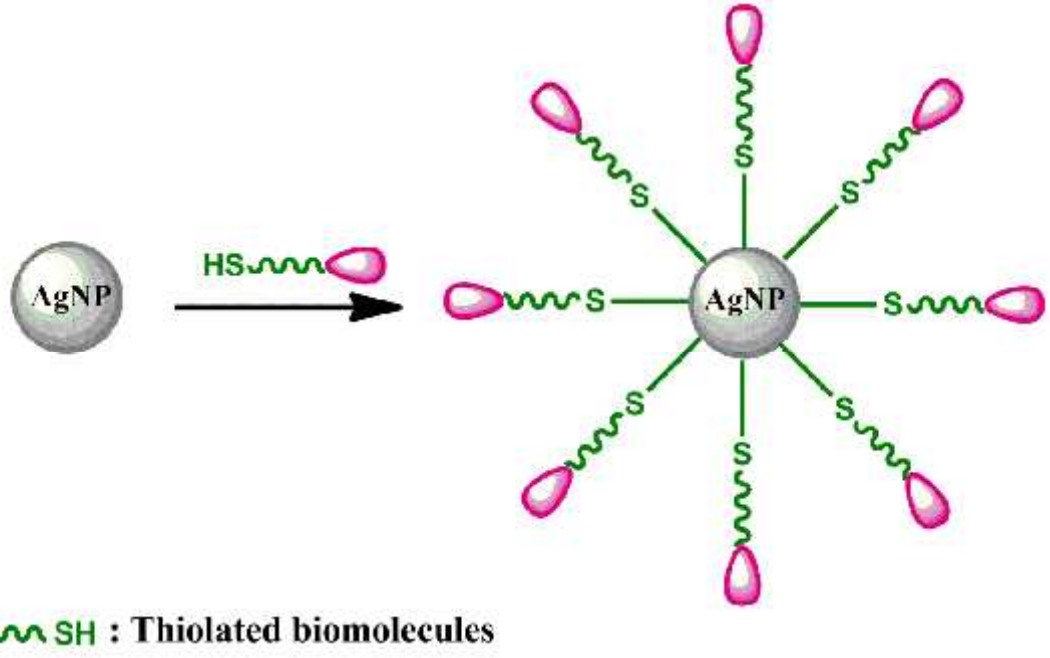
Provided by Plasma Chem



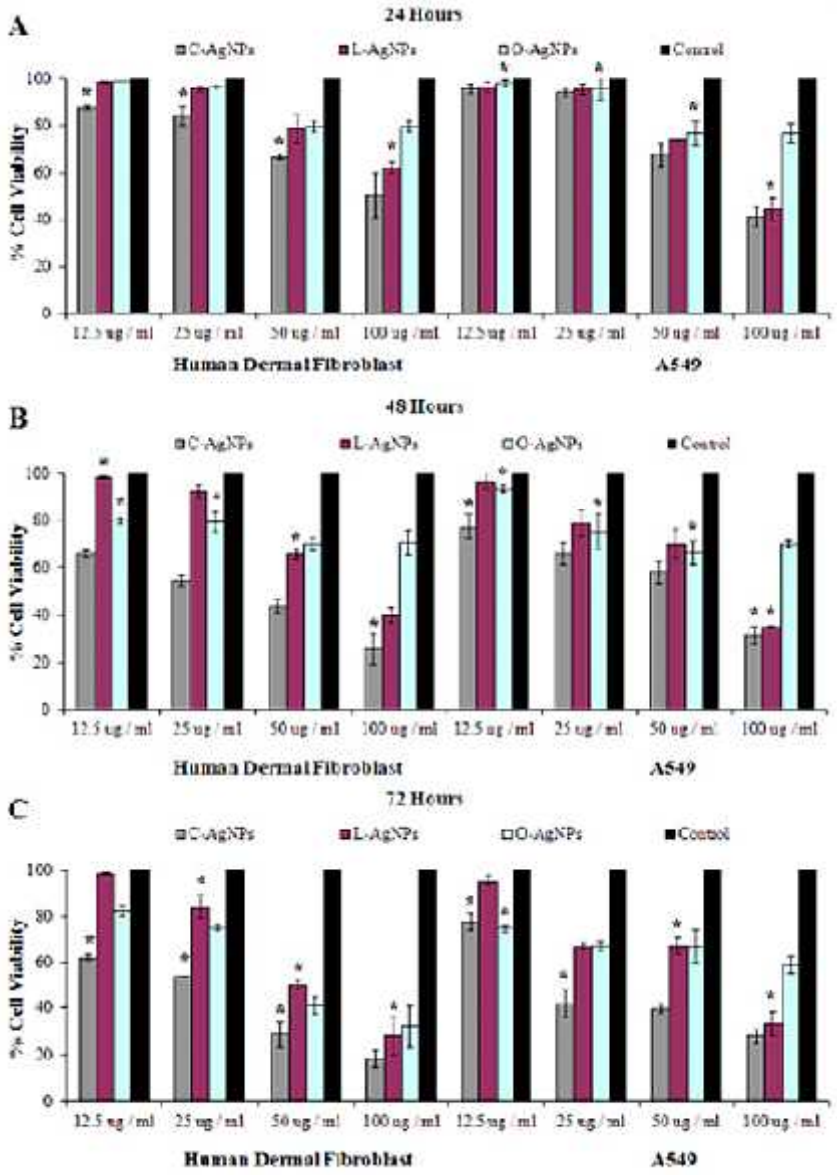
Synthesized in our lab with citrate reduction

Modification of AgNPs with thiolated carbohydrates and biomolecules





Influence of modification after dialysis of AgNPs on cytotoxicity

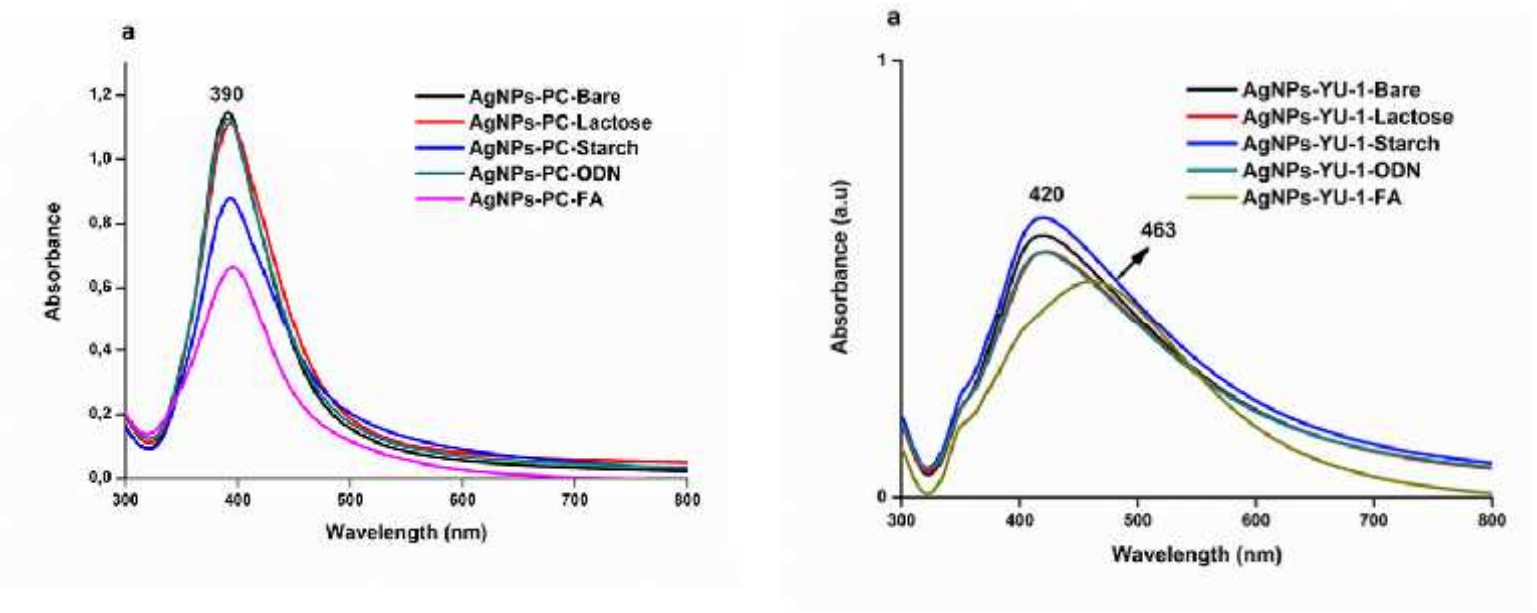


Evaluation of the cytotoxicity of the AgNPs. Percent viability of HDF and A549 cells (A)–(C) during a three-day incubation period in the presence of 12.5, 25.0, 50.0 and 100.0 ug/ml AgNP concentrations. The control (negative) column represents the untreated cells.

Modification with Lactose (L) and oligonucleotide (O) decreases the cytotoxicity on both cell types.

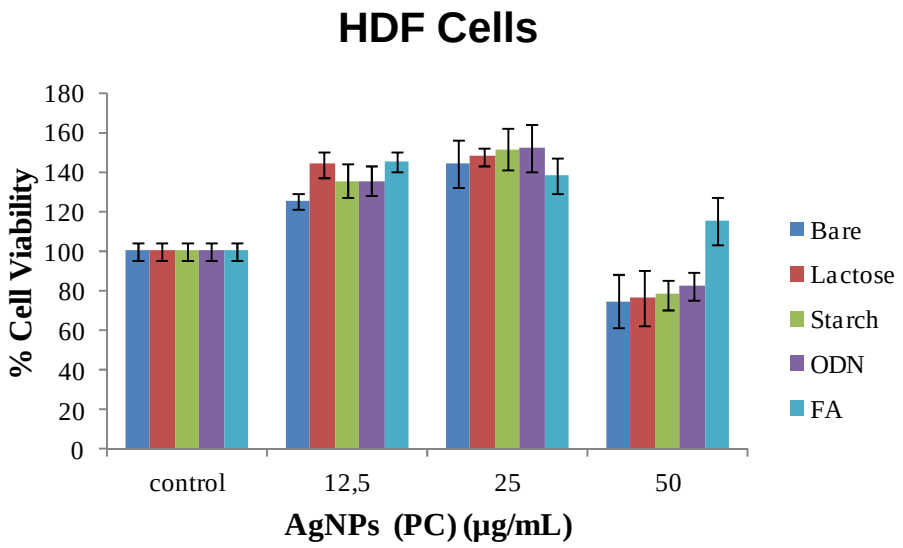
However, a concentration based toxicity is observed.

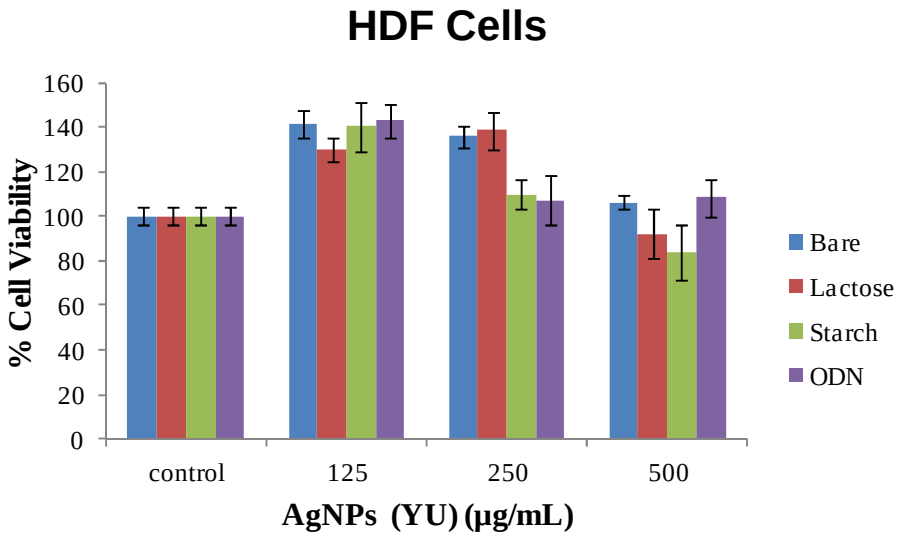
Characterization of AgNPs with thiolated carbohydrates and biomolecules

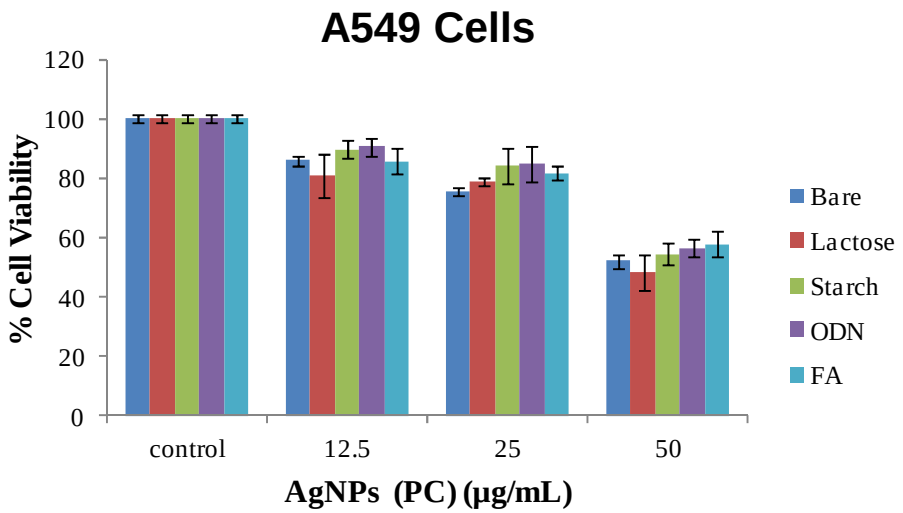


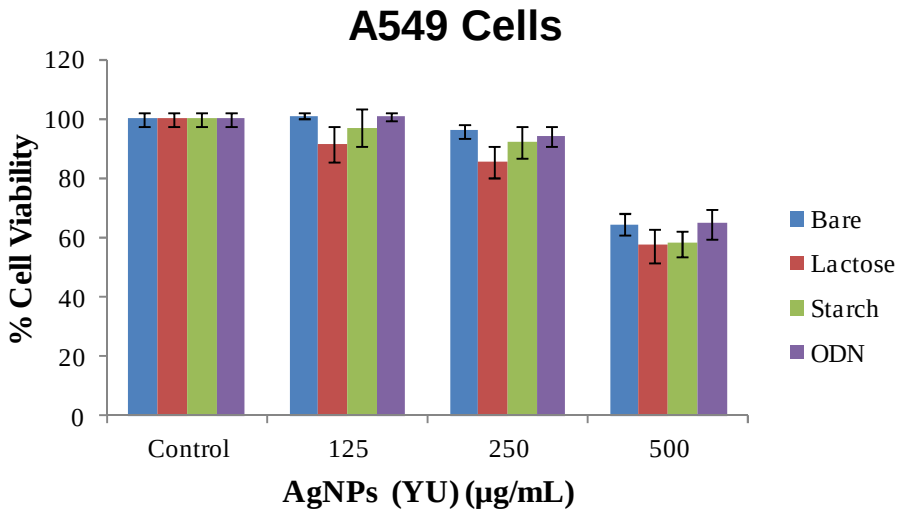
UV-Vis (a) and DLS (b) spectra of lactose, starch, oligonucleotide (ODN), and folic acid (FA) modified AgNPs-YU (Yeditepe University) and AgNPs-PC (Plasma Chem)

Cytotoxicity of modified AgNPs





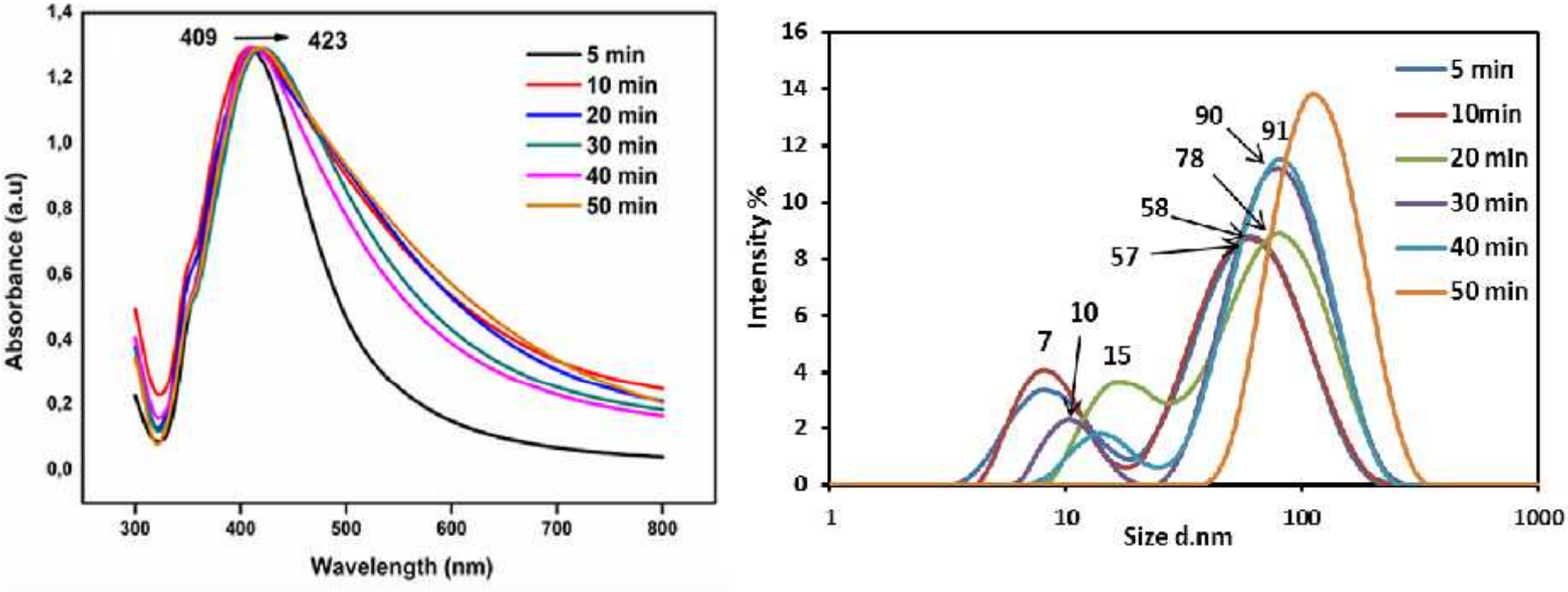




What is the source of toxicity in a AgNP colloidal suspension?

- Is it AgNPs?
- We are investigated weather we can prepare a AgNP suspension with low toxicity by minimizing the effects from the synthesis procedure.
- This procedure can still be considered surface modification (not a ligand) but reducing agent.

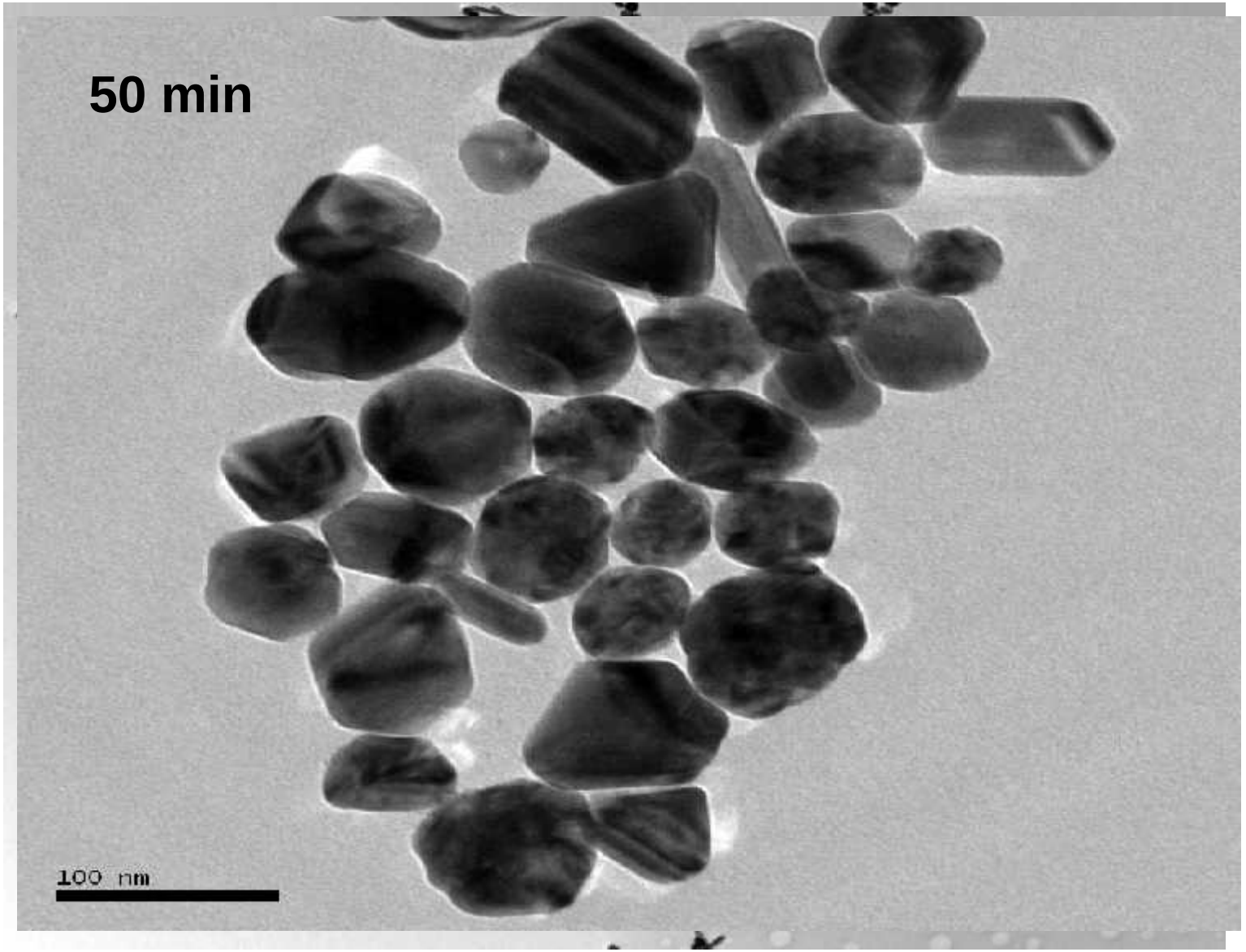
AgNPs synthesized at varying reaction time



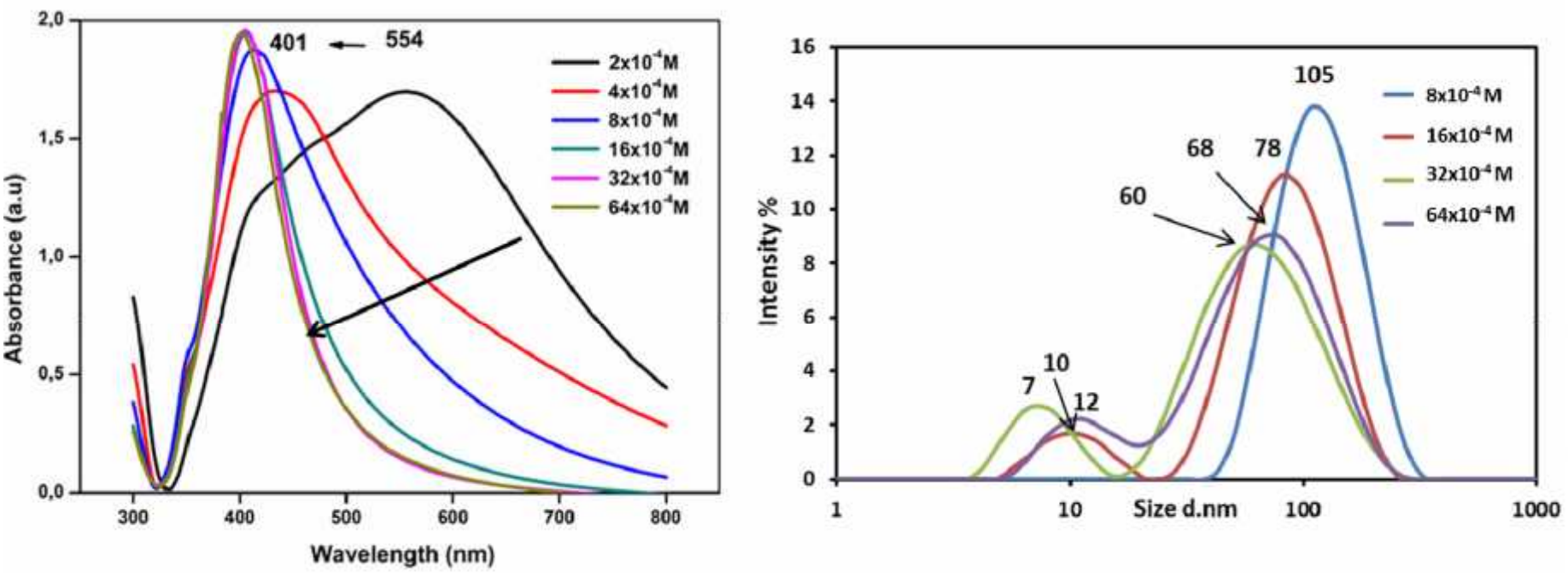
UV-Vis a and DLS spectra b of AgNPs prepared with increasing reaction time from 5 min to 50 min.

Accepted, Nanotechnology (IF 3.7)

Characterization of AgNPs synthesized via varying reaction time

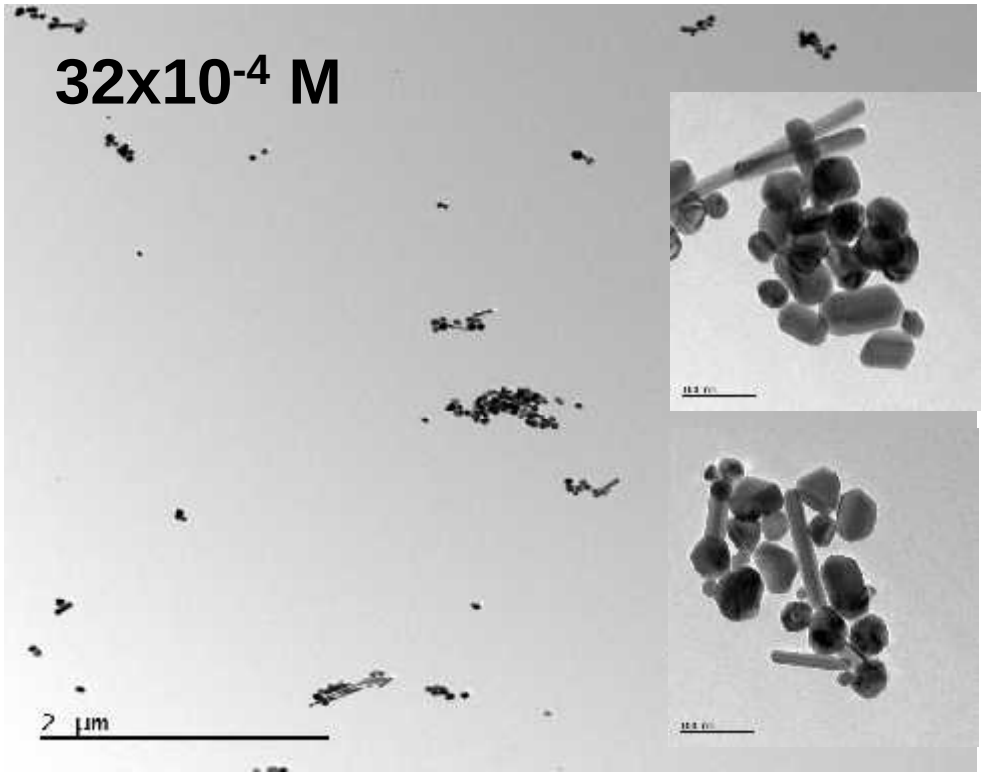


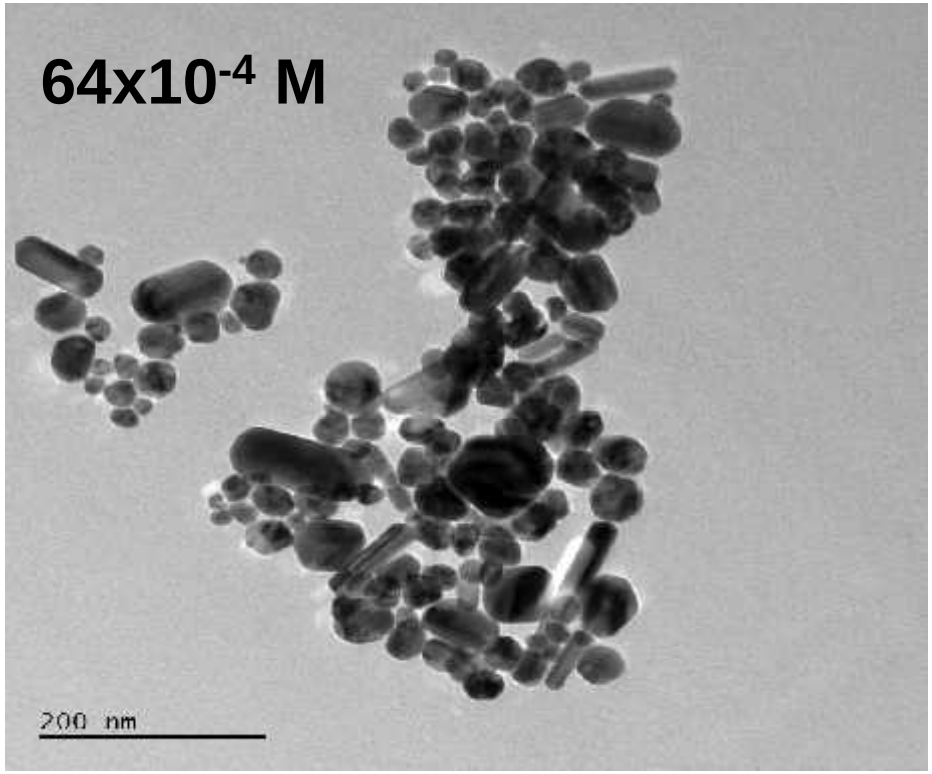
AgNPs synthesized at varying citrate concentration



UV-Vis a and DLS spectra b of AgNPs prepared at different citrate concentrations.

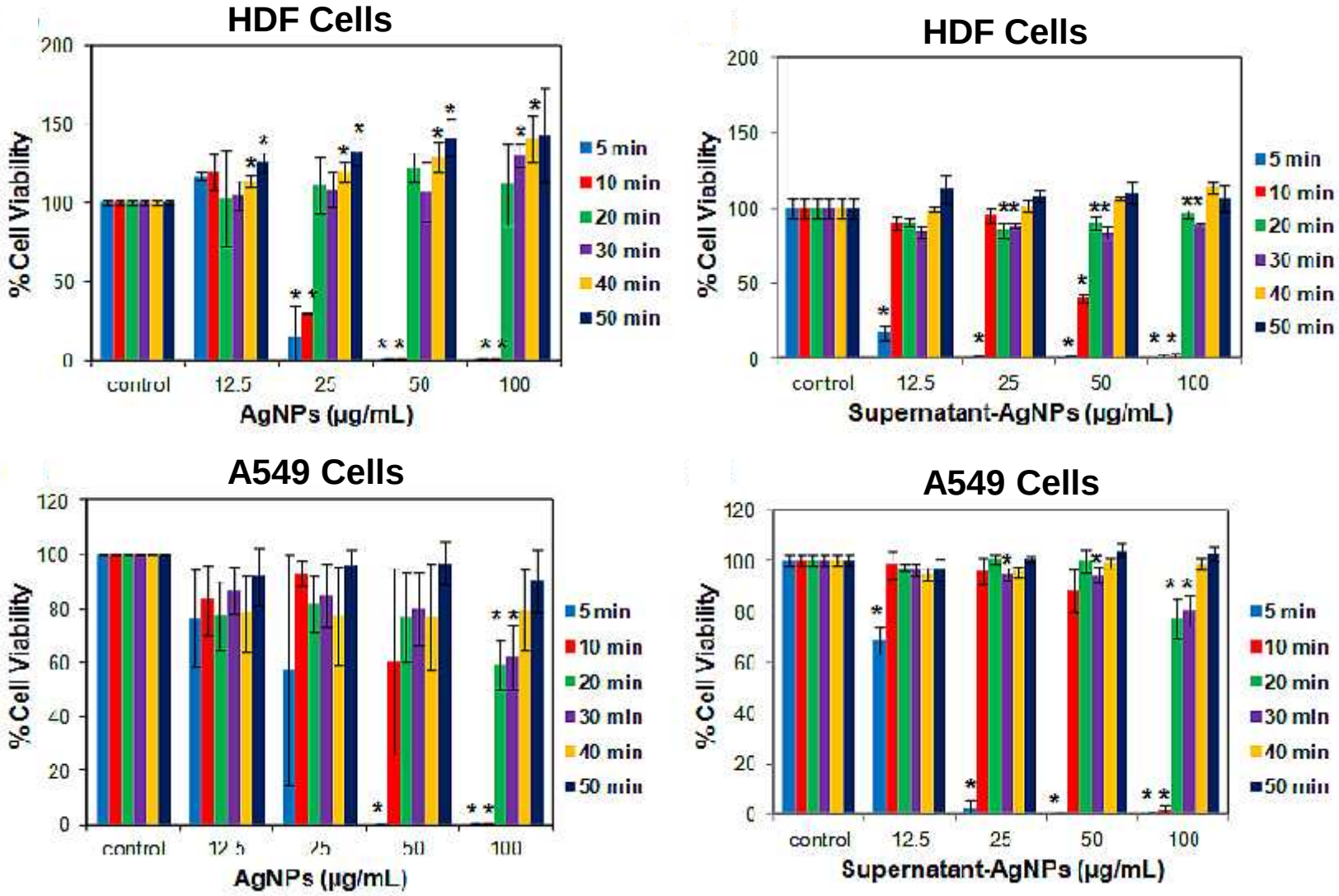
Characterization of AgNPs synthesized via varying citrate concentration





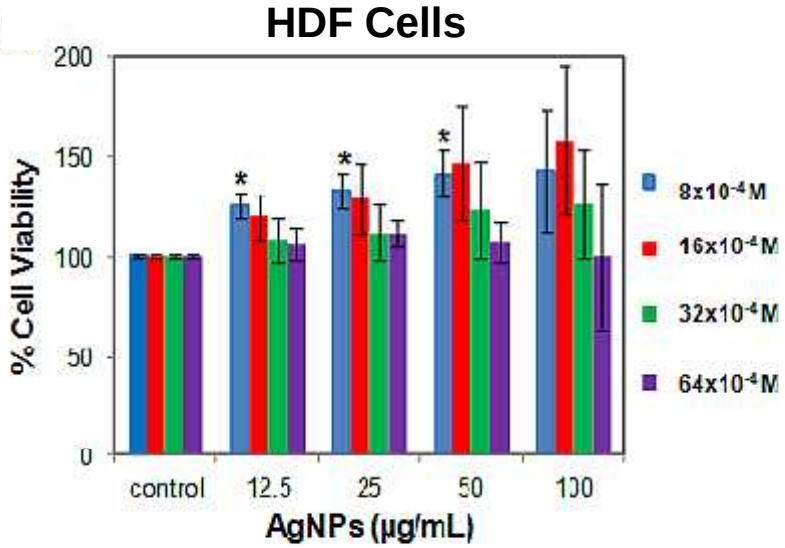
Accepted, Nanotechnology (IF 3.7)

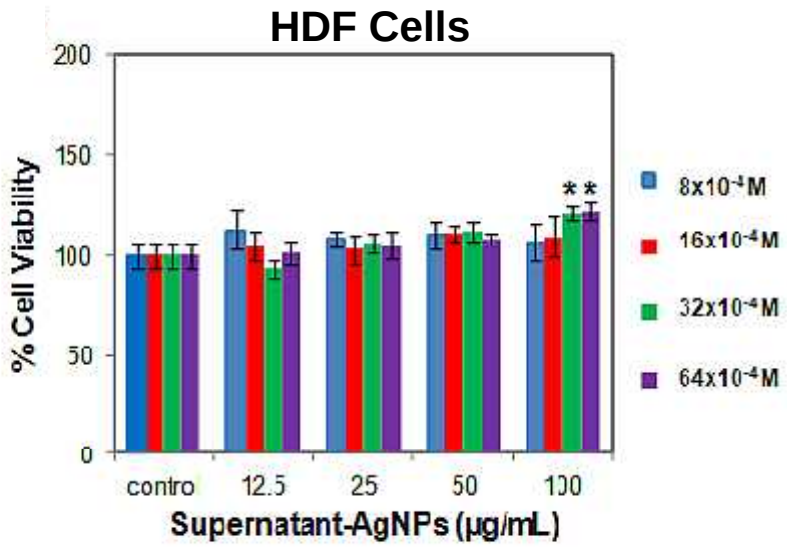
Cytotoxicity of AgNPs synthesized by varying reaction time and their supernatants

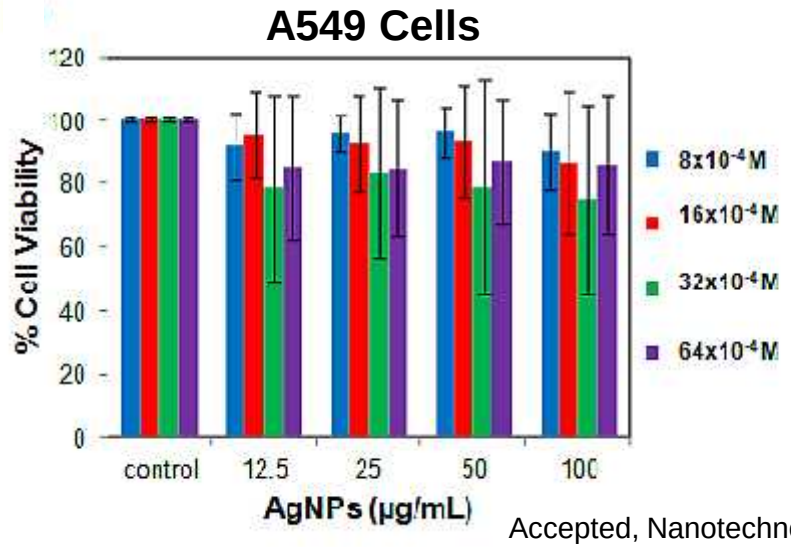


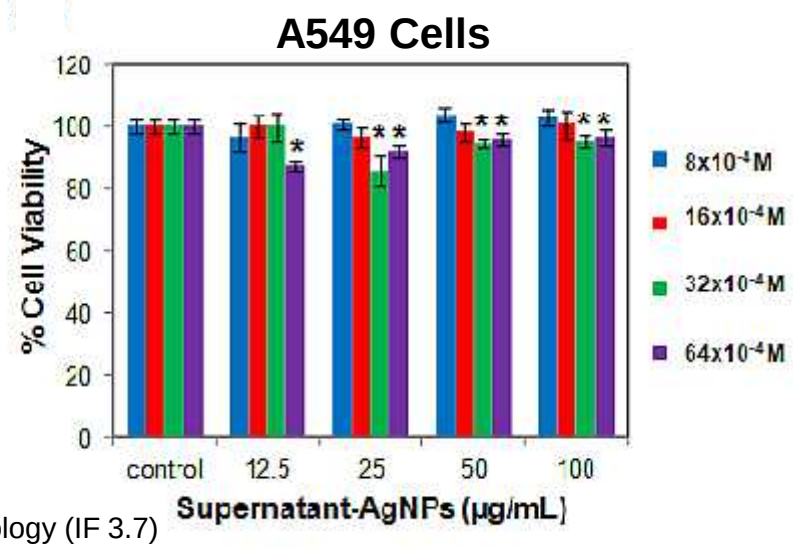
Accepted, Nanotechnology (IF 3.7)

Cytotoxicity of AgNPs synthesized by varying citrate concentration and their supernatants









Accepted, Nanotechnology (IF 3.7)

CONCLUSIONS AND RESULTS FOR AgNPs

- The main reason for cytotoxicity of AgNPs is free Ag^{+} either left in the suspension or released through dissolution, and very small sized AgNPs. Even the surface modification works for larger particles in the batch, the smaller particles continue to be toxic through their sizes (because they are very small, only a few nm).

- A strategy “safety by design” is suggested by considering nature of the target application. For example, change of ink formulation to a hydrophilic one is suggested.

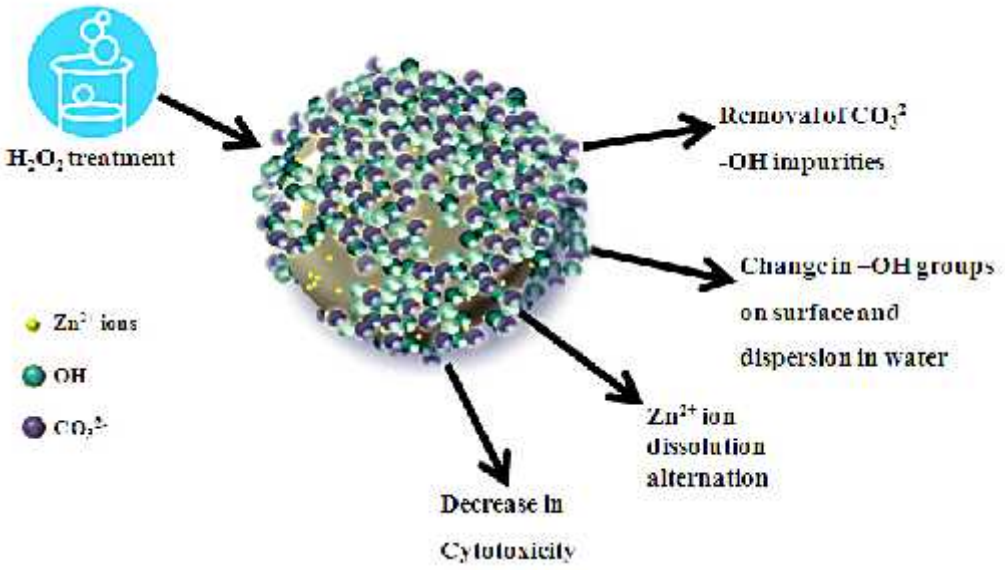
- The need for hydrophobic surface chemistry for AgNPs for their use in ink formulations is another problem since they cannot be reliably tested.

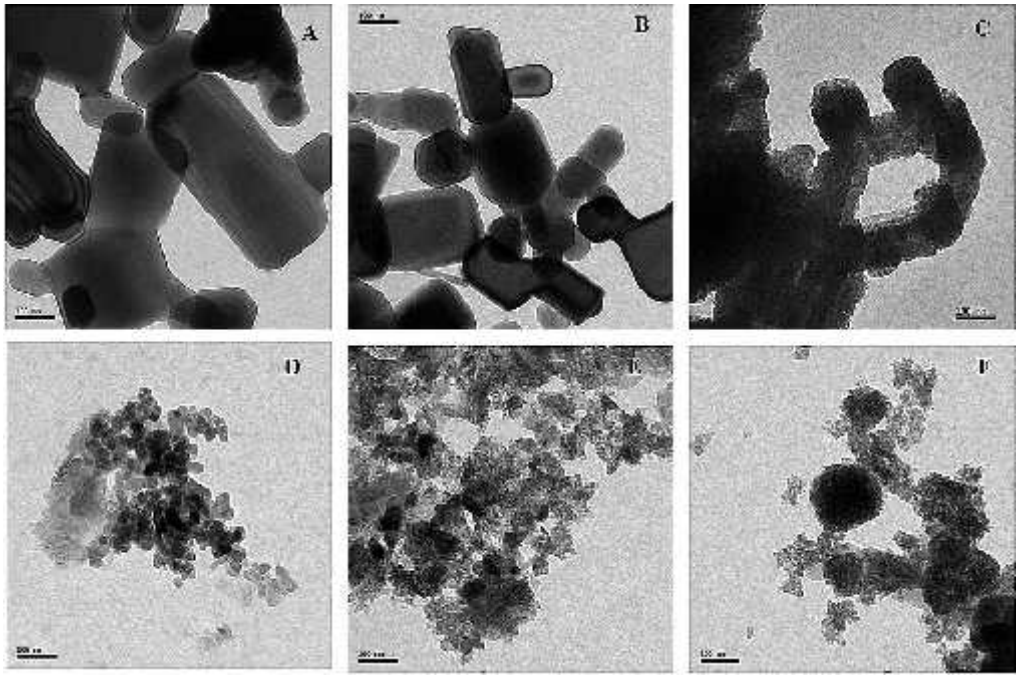
ZnO NPs

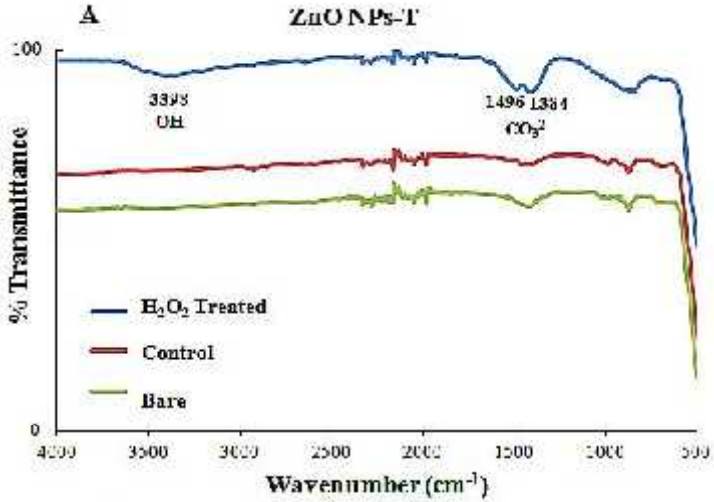
- Decrease cell-NPs contact (referring to size)
- Decrease ion release (stop dissolution)

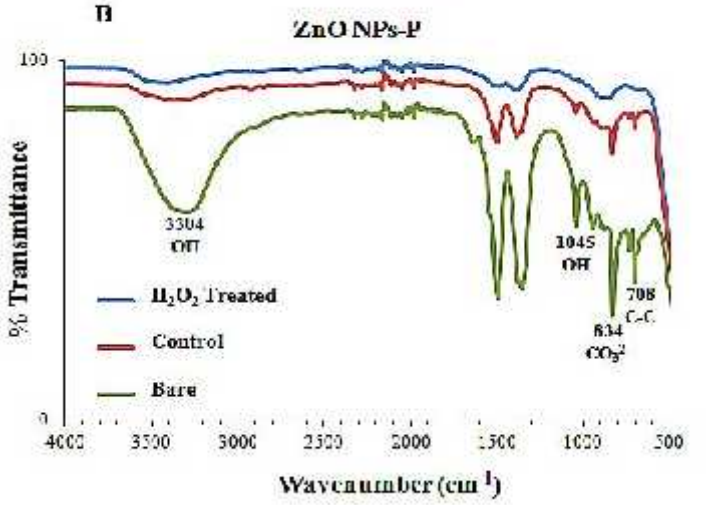
- Problems: Agglomeration and difference in surface chemistry from source to source

Zinc Oxide NPs (ZnO NPs)



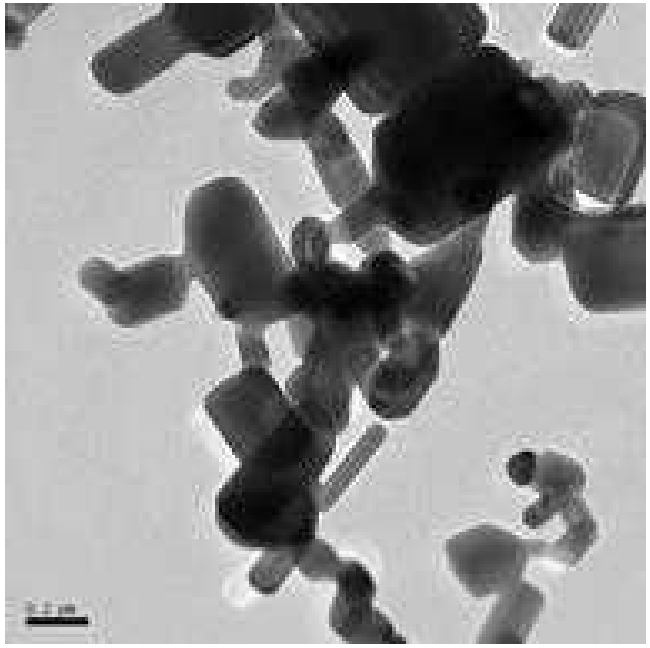






M. Altunbek, A. Baysal, M. Çulha / Colloids and Surfaces B: Biointerfaces 121 (2014) 106–113 (IF 4.2)

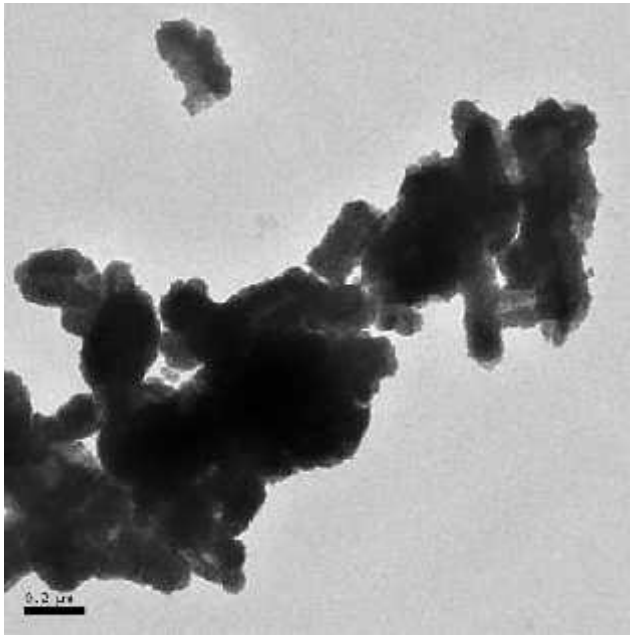
Characterization of hydroxylated ZnO NPs



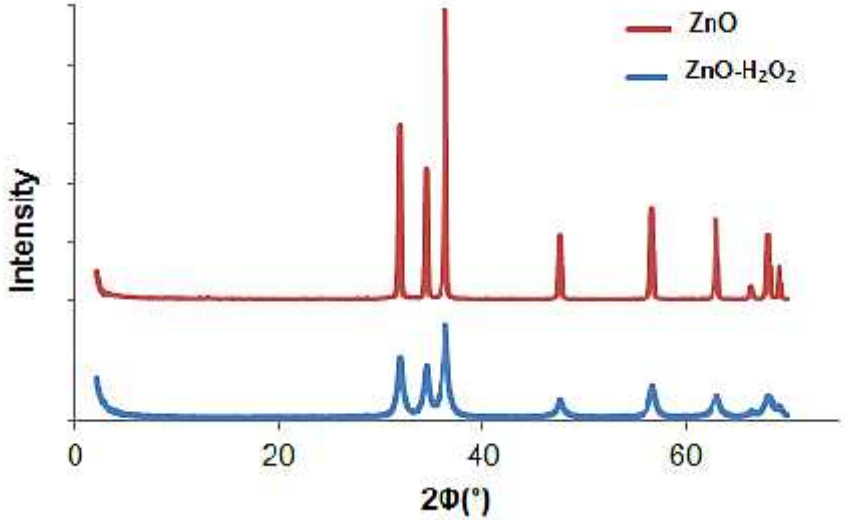
Bare ZnO NPs

Hydroxylation

H_2O_2 treatment

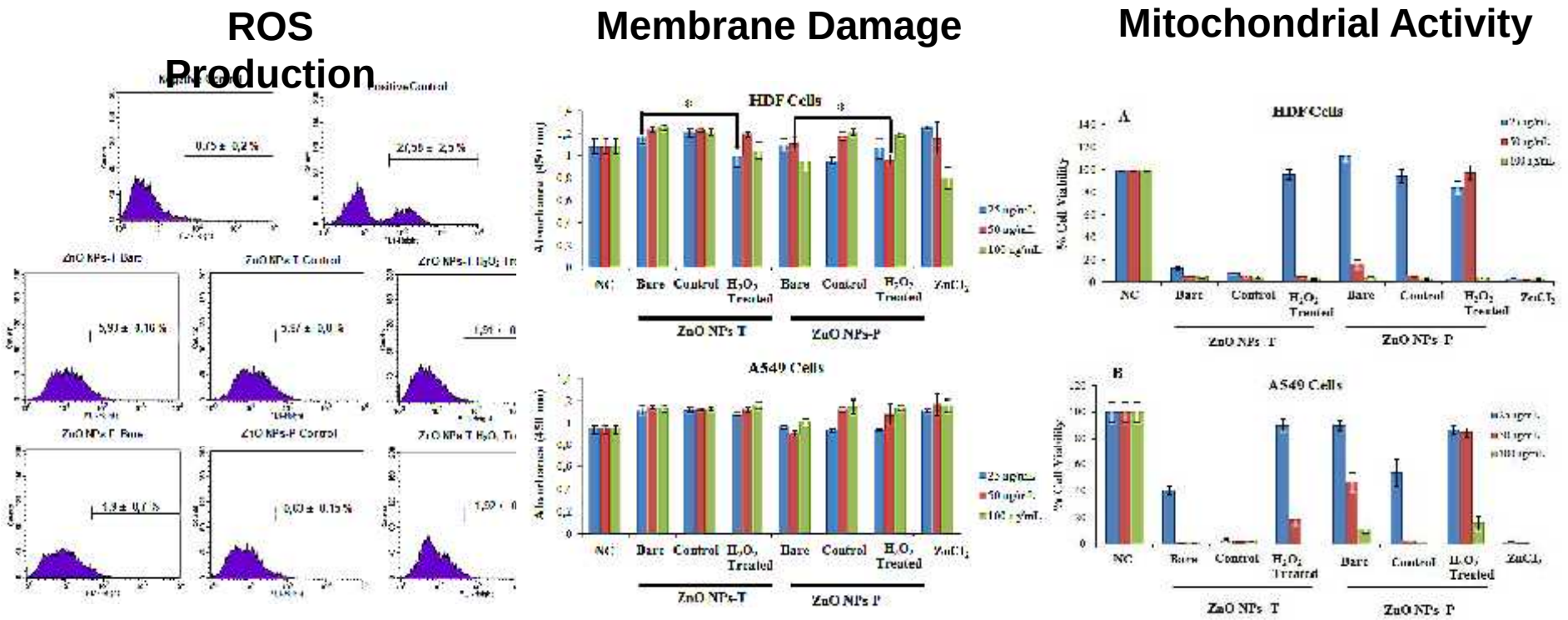


Hydroxylated ZnO NPs



XRD spectra of ZnO NPs before and after H_2O_2 treatment

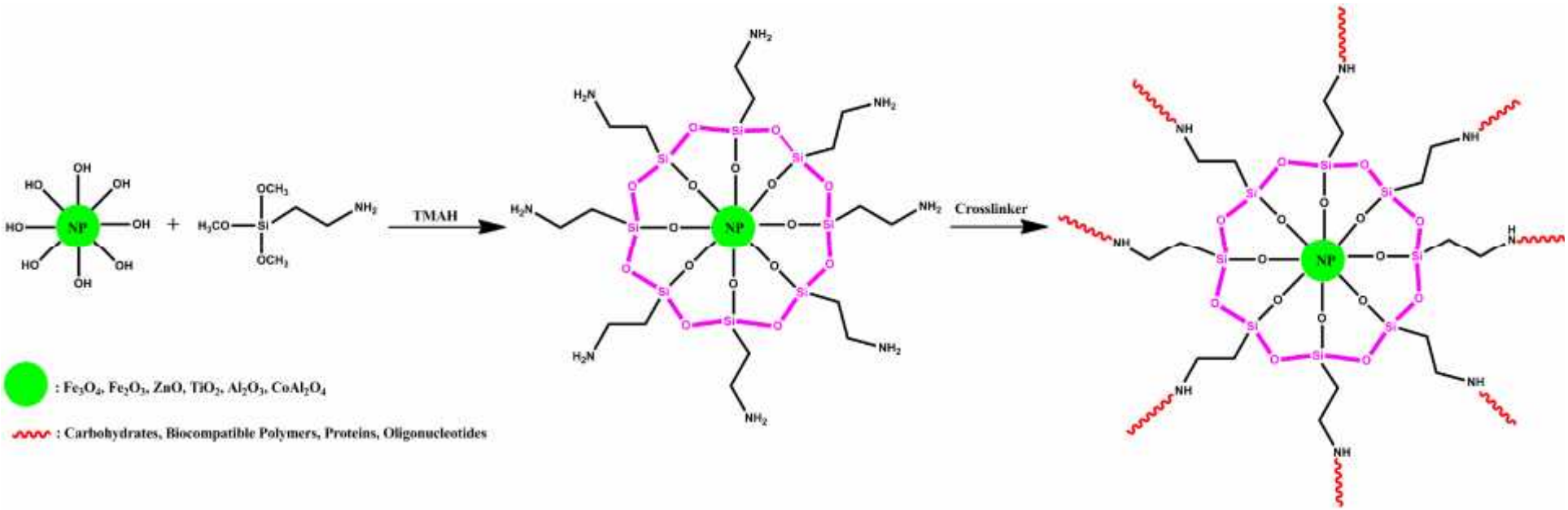
Effect of Surface Properties on Cytotoxicity



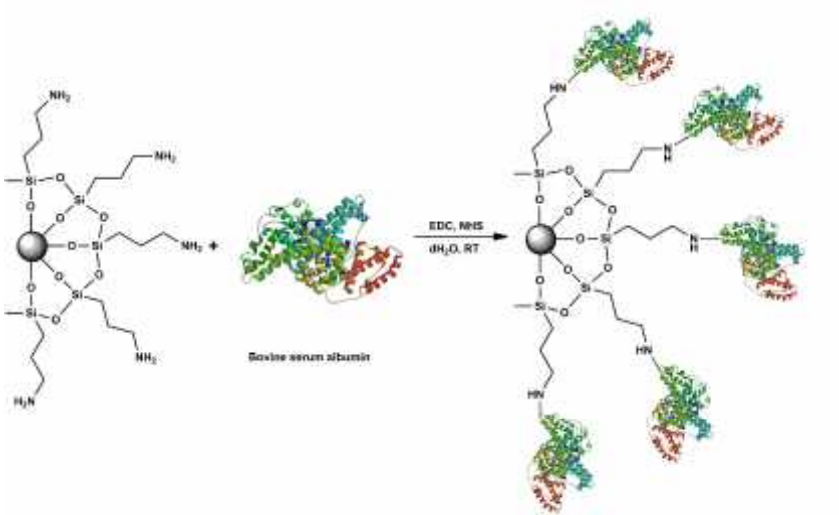
ZnO NPs cytotoxicity is associated with the production of intracellular reactive oxygen species (ROS). The oxidative stress results in damage in the cellular components such as lipids, proteins and DNA. The fatty acid oxidation results in cell death due to destruction of plasma and organelle membranes. The increased OH group density on the NP surface upon H_2O_2 treatment of ZnO NPs–T, decreased level of ROS production in a half ratio compared to the bare form due to the decrease of the cell-NPs interaction. The membrane integrity destruction as a result of the ROS production upon ZnO NPs exposure was measured lactate dehydrogenase (LDH) release to the extracellular media. The significant decrease in LDH release after exposure of the H_2O_2 treated ZnO NPs–T and ZnO NP–P at 25 µg/mL and 50 µg/mL concentration levels, respectively, was the evidence of the decrease in the cytotoxicity of ZnO NPs.

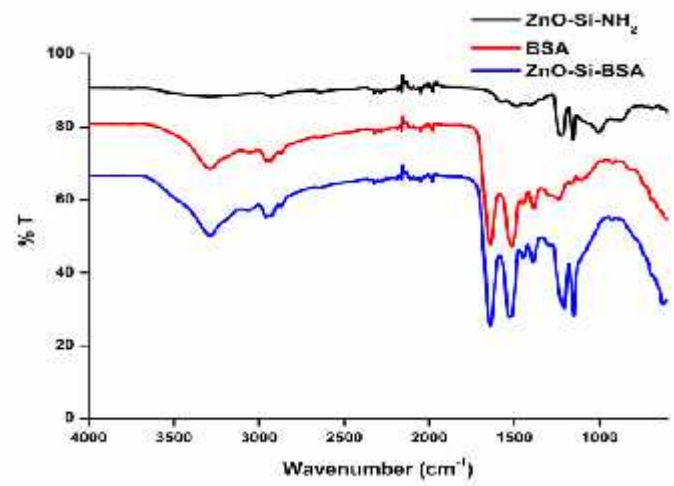
M. Altunbek, A. Baysal, M. Çulha / Colloids and Surfaces B: Biointerfaces 121 (2014) 106–113

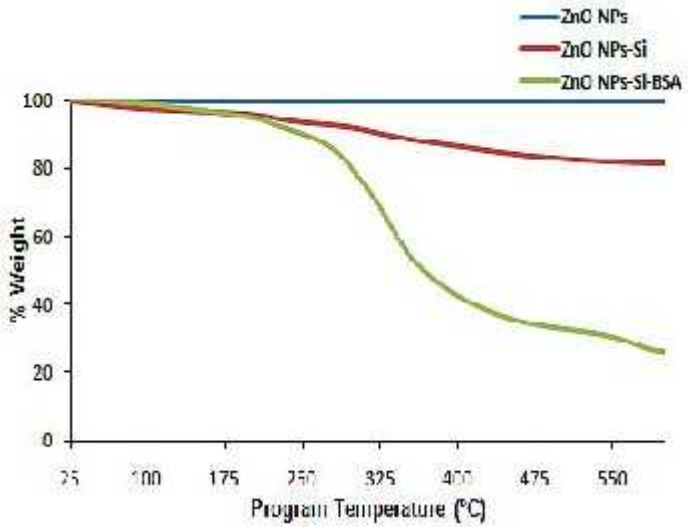
Coating with Silica (3-Aminopropyl)-triethoxylsilane (APTES)



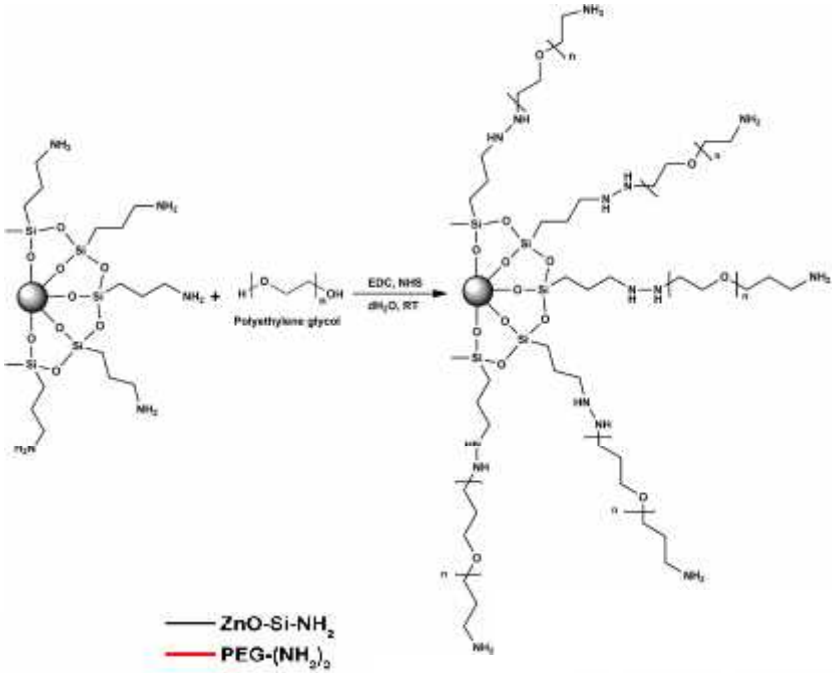
Bovine serum albumin (BSA) attachment onto silica coated ZnO NPs

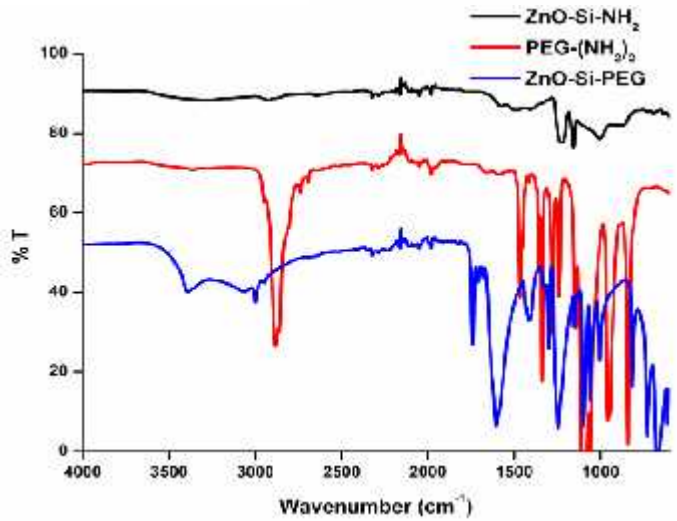


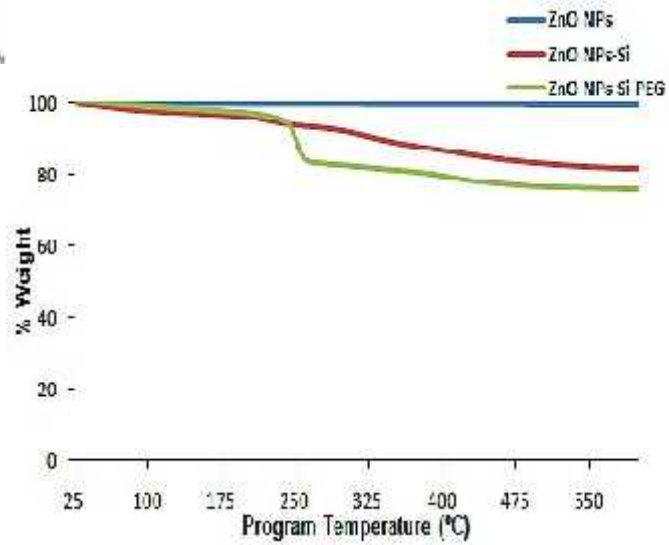




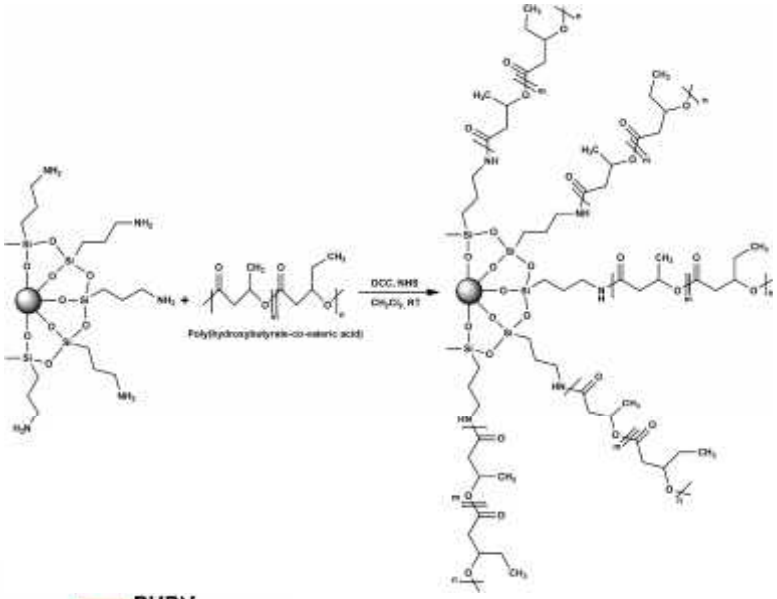
Amine terminated poly(ethylene glycol) (PEG-$NH_2)_2$ attachment onto silica coated ZnO NPs

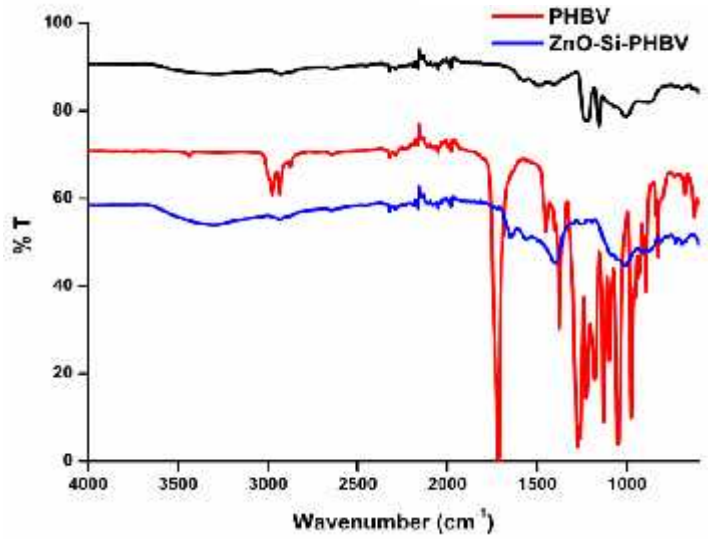


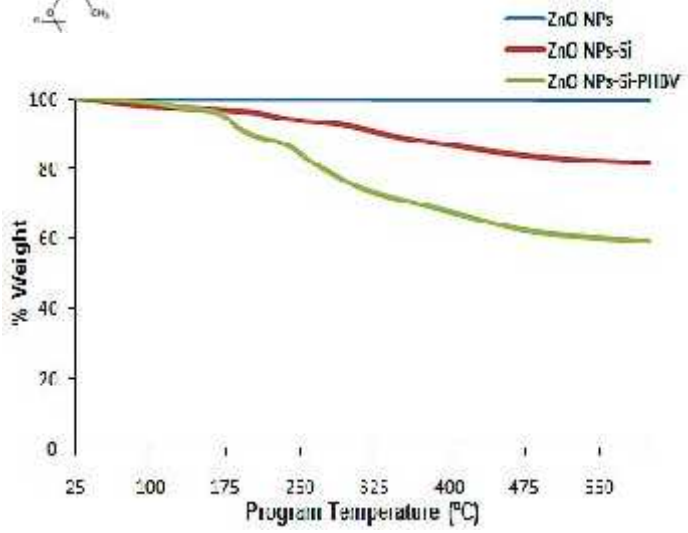




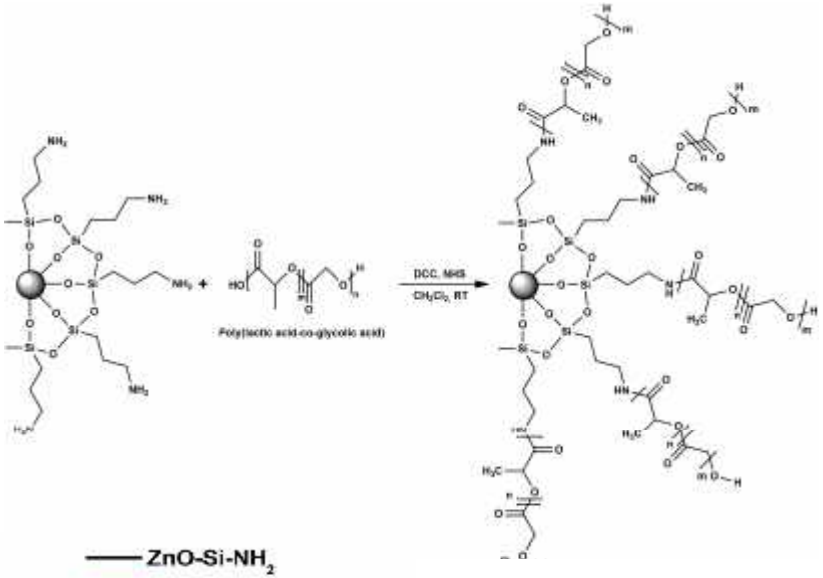
Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) attachment onto silica coated ZnO NPs

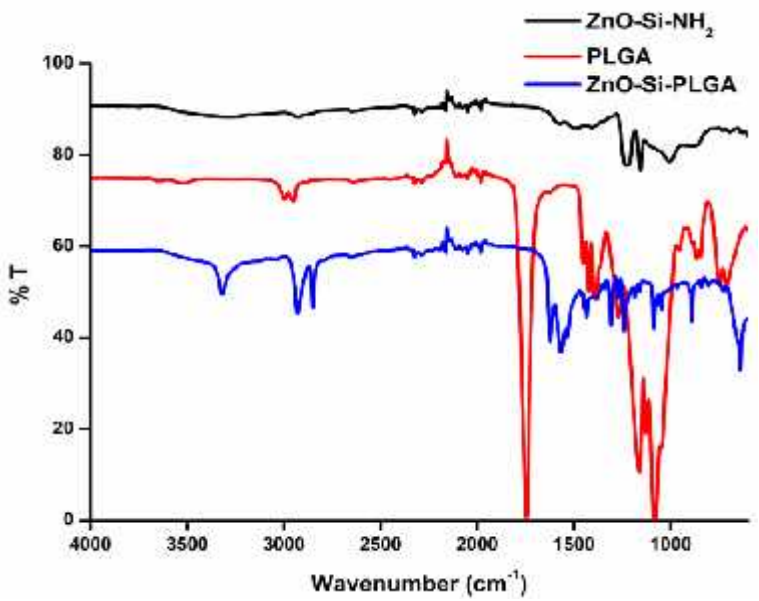


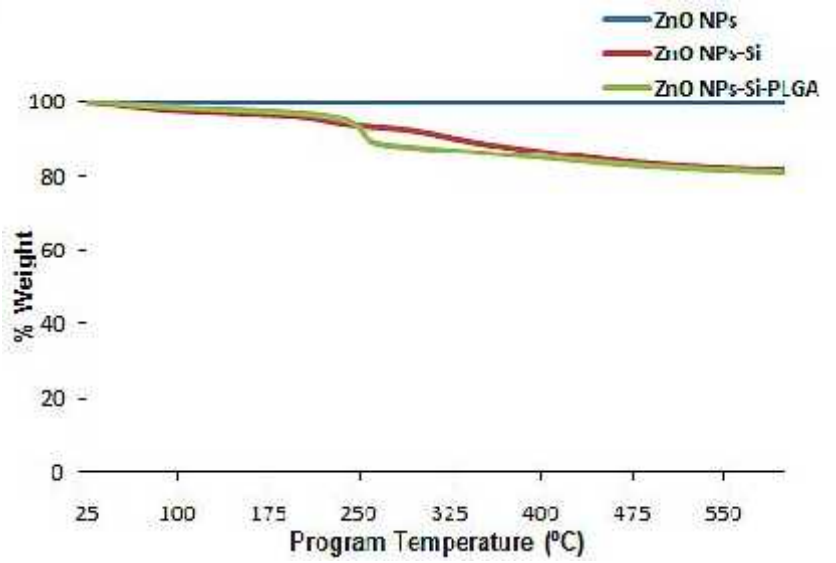




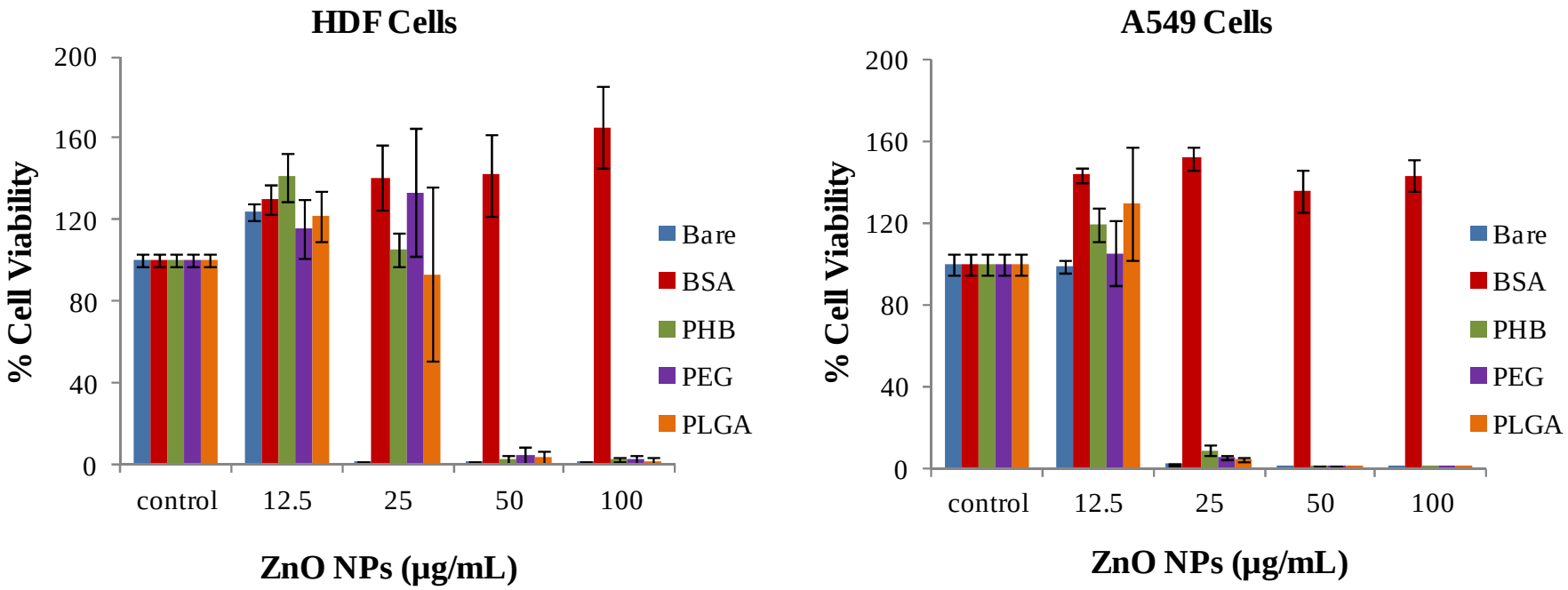
Poly(lactide-co-glycolide) (PLGA) attachment onto silica coated ZnO NPs







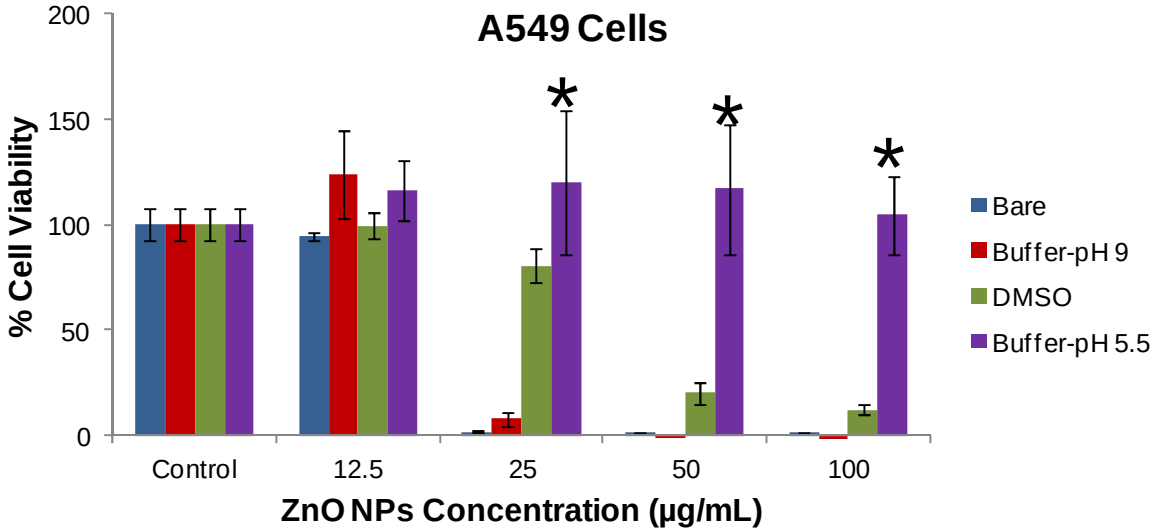
Cytotoxicity of Modified ZnO NPs



Dissolution Problems with ZnO NPs

Determination of Zn content of BSA modified ZnO NPs with ICP-MS

	Initial amount (mg)	Determined amount by ICP-MS (mg)
Buffer-pH 9	56	36.4
DMSO	56	5.6
Buffer-pH 5.5	56	9.3



Cytotoxicty of ZnO NPs prepared at different conditions

Toxicology Research

PAPER

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Cite this: *Toxicol. Res.*, 2015, **4**, 159

Label-free monitoring of the nanoparticle surface modification effects on cellular uptake, trafficking and toxicity†

D. Bartczak, M.-O. Baradez, H. Goenaga-Infante and D. Marshall*

Changing the surface functionality of nanoparticles (NP) through th
on approach to reduce their potential toxici
ange. NP's mechanism of action and beha
lenge. It is often exacerbated by necessary
imiting provided information, yet is require
techniques. In this study we have adopted
ity performed in biological matrix *in situ* to
ically changes their toxicity profiles, due to
NP capping with proteins alters their cellu
al endosomes acidification is an important
supported by other commonly used techn
their bioactivity, which could be used to in

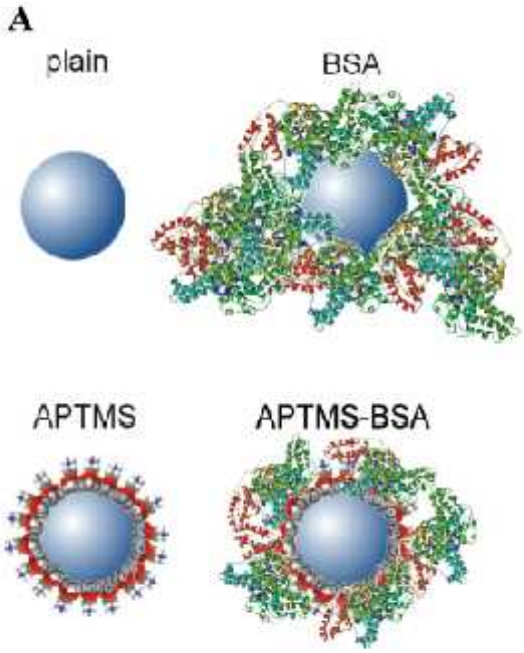


Fig. 1 Schematic representation of plain and capped ZnO NP (A) and es

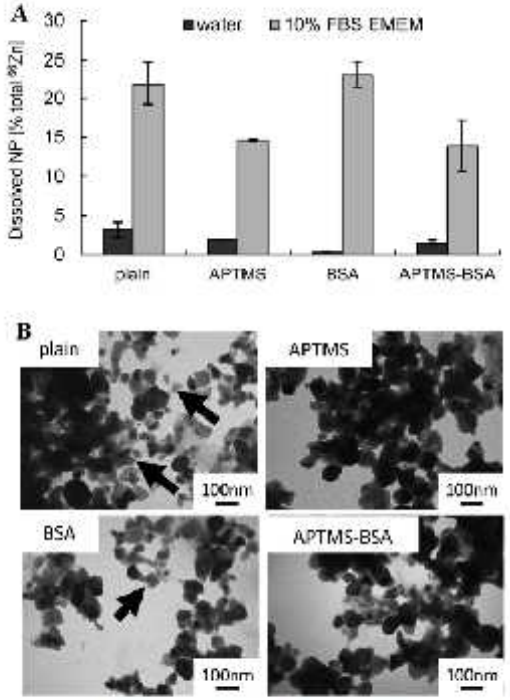


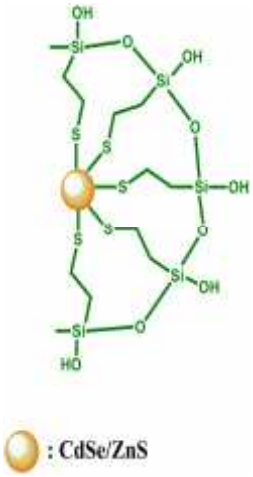
Fig. 3 Dissolution rate of ZnO NP in water and 10% FBS EMEM (A: average ± stdev, $n = 3$), determined after 24 h incubation. Representative TEM images of ZnO NP after 24 h incubation in 10% FBS EMEM (B); arrows indicate decomposed NP.

CONCLUSIONS AND RESULTS FOR ZnO NPs

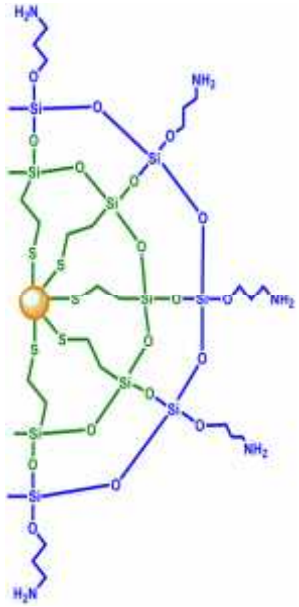
- The initial characterization of the modified ZnO NPs suggests that all surface modifications are successful.
- Both polymers and BSA modifications helped to decrease cellular toxicity according to pristine ZnO NPs.
- When the cost and availability is considered, BSA can be a good candidate as a surface modifier.
- The most important outcome is the surface modifications must be performed in extremely diluted suspensions by using excess amount of modifier to cover the whole surface area of ZnO NPs.
- However, there are still concerns about the dissolution of ZnO during the modification procedure.

Quantum Dots (QDs)

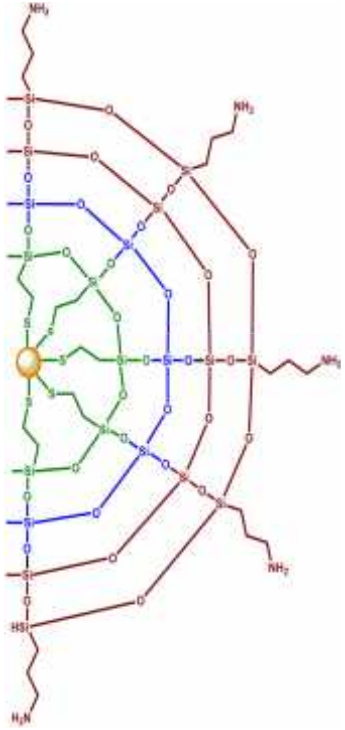
QD-Si-OH

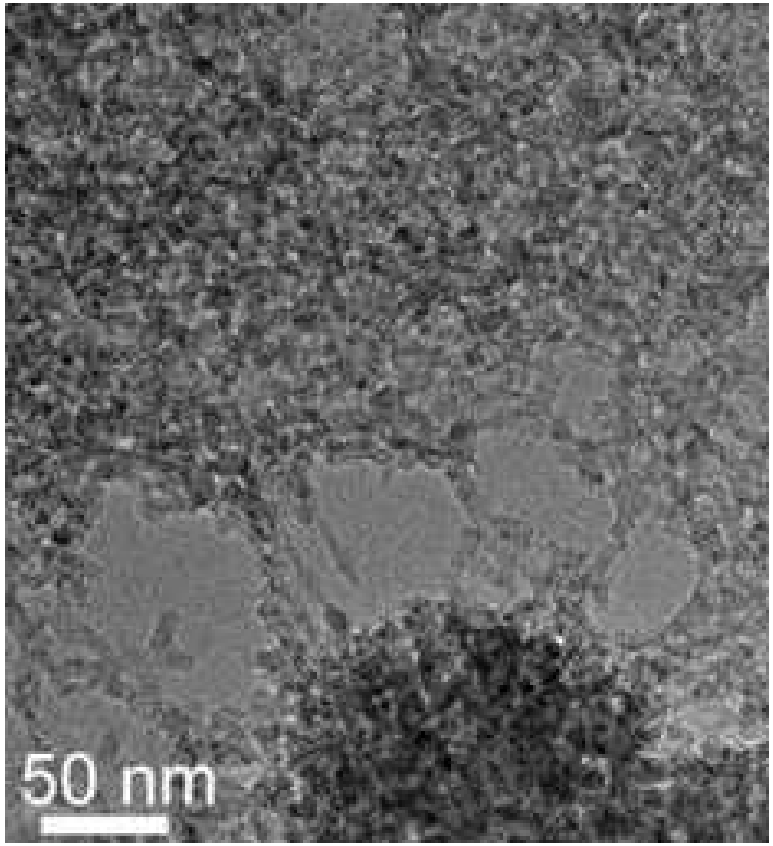


QD-Si(2)-NH_2

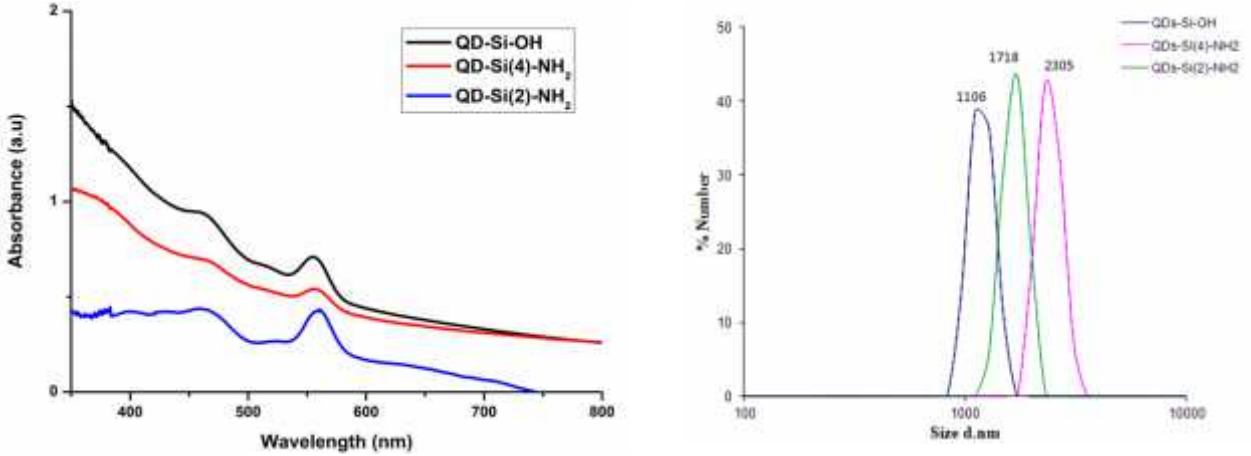


QD-Si(4)-NH_2

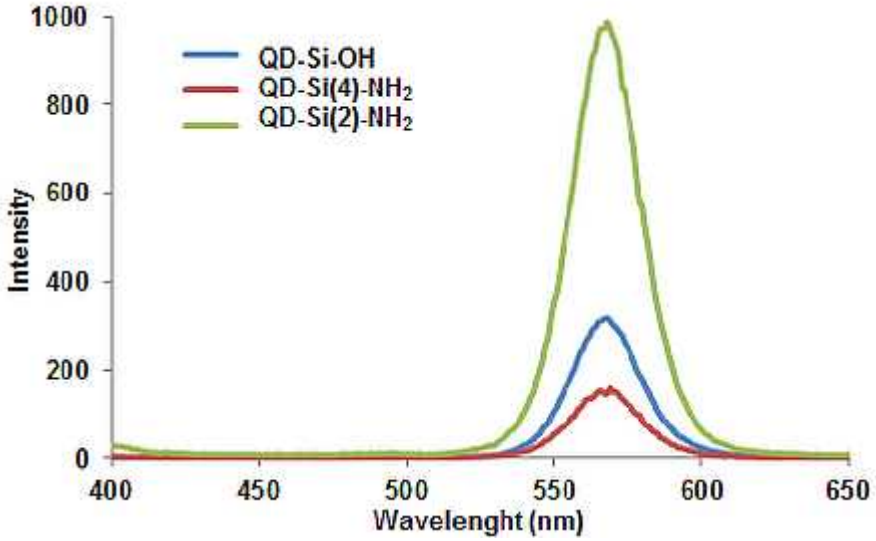




Initial Characterization of QDs

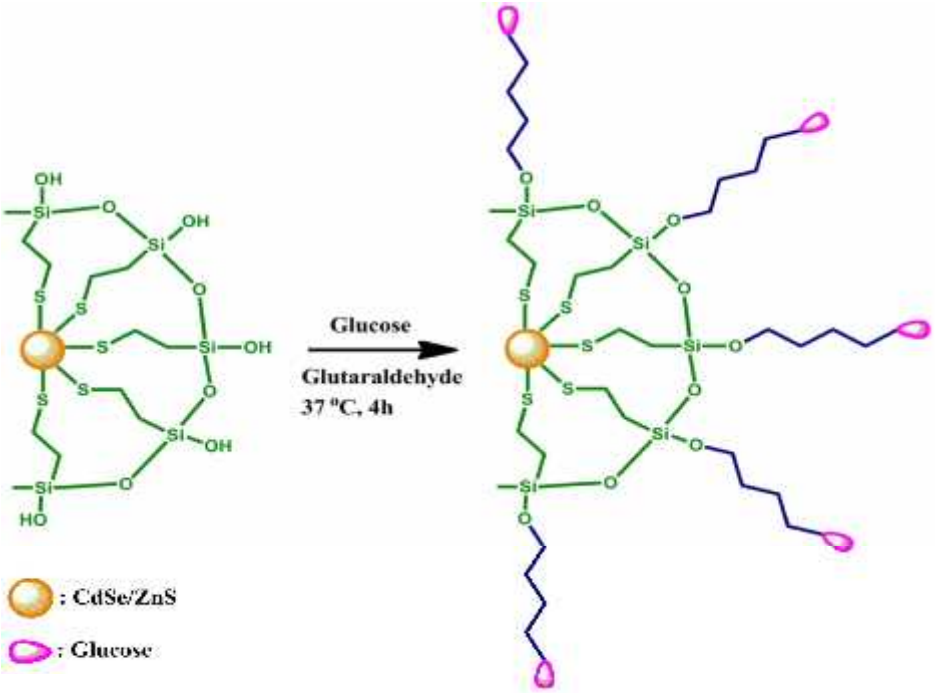


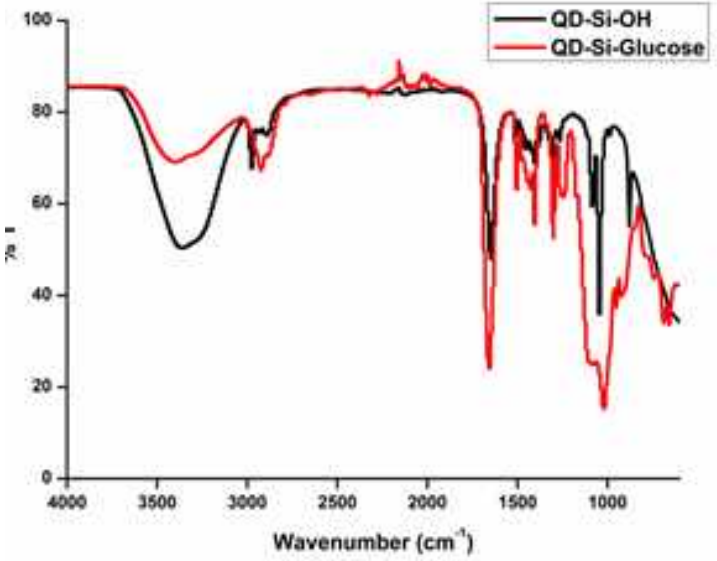
UV-Vis and DLS spectra of QD-Si-OH, QD-Si(4)-NH_2 and QD-Si(2)NH_2 (Concentrations at 0.125mg/mL)

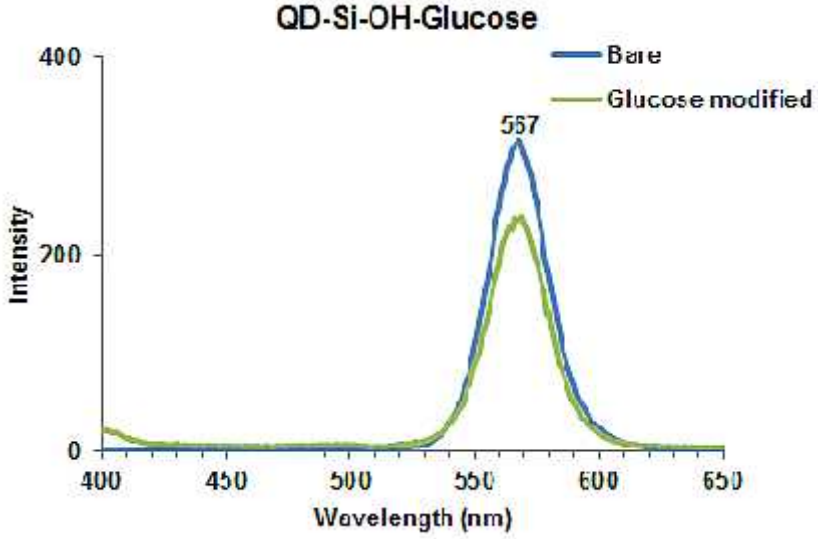


Fluorescent spectra of QD-Si-OH, QD-Si(4)-NH_2 and QD-Si(2)NH_2

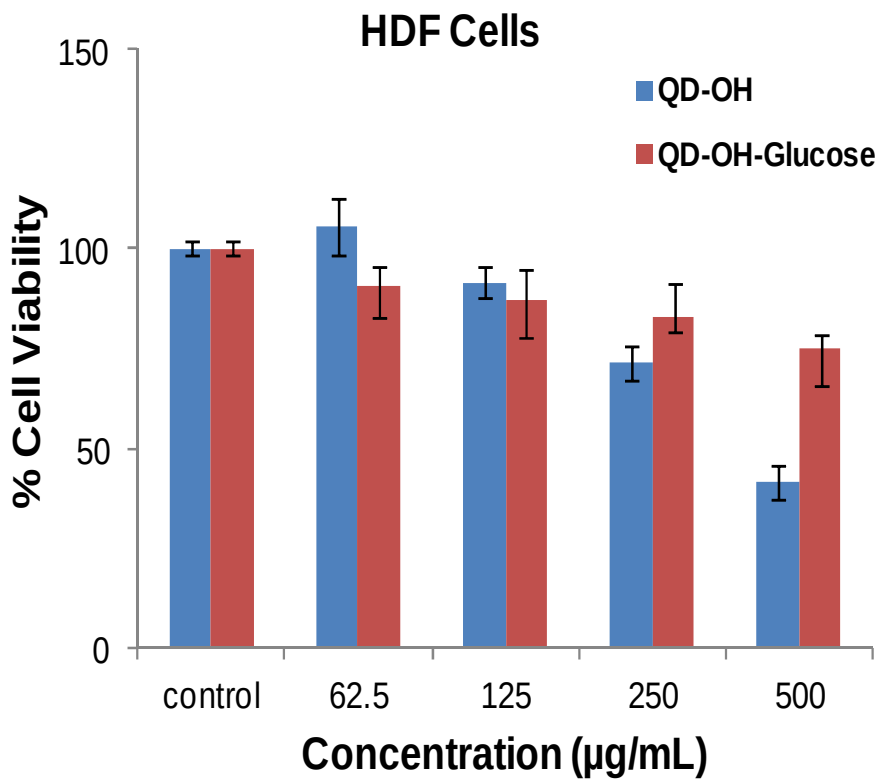
Glucose Modification of QDs-Si-OH

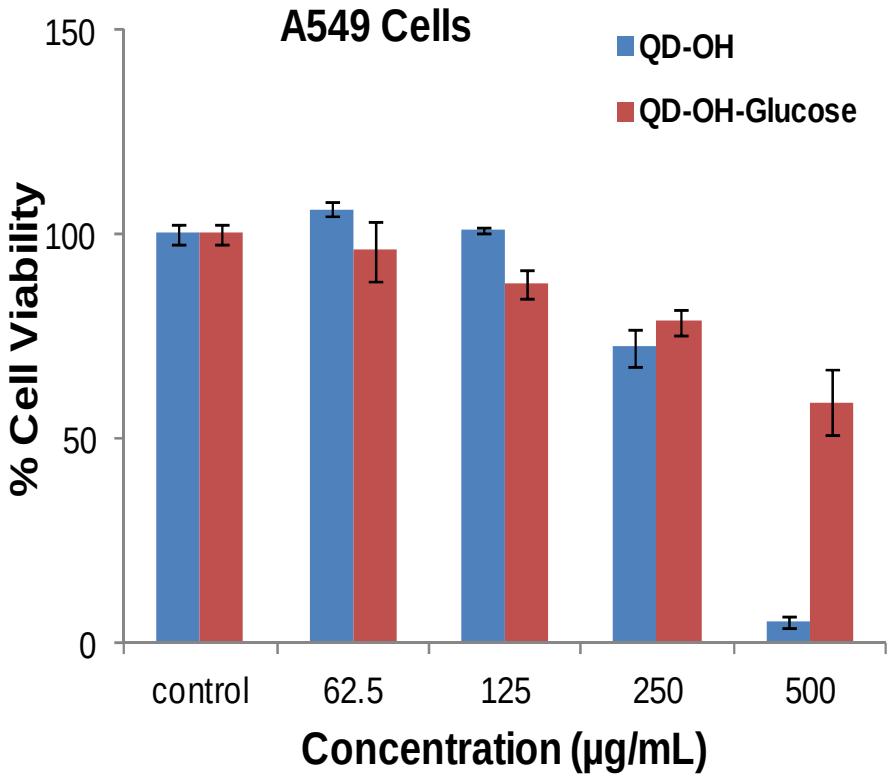






QDs-Si-OH-Glucose Cytotoxicity Assessments

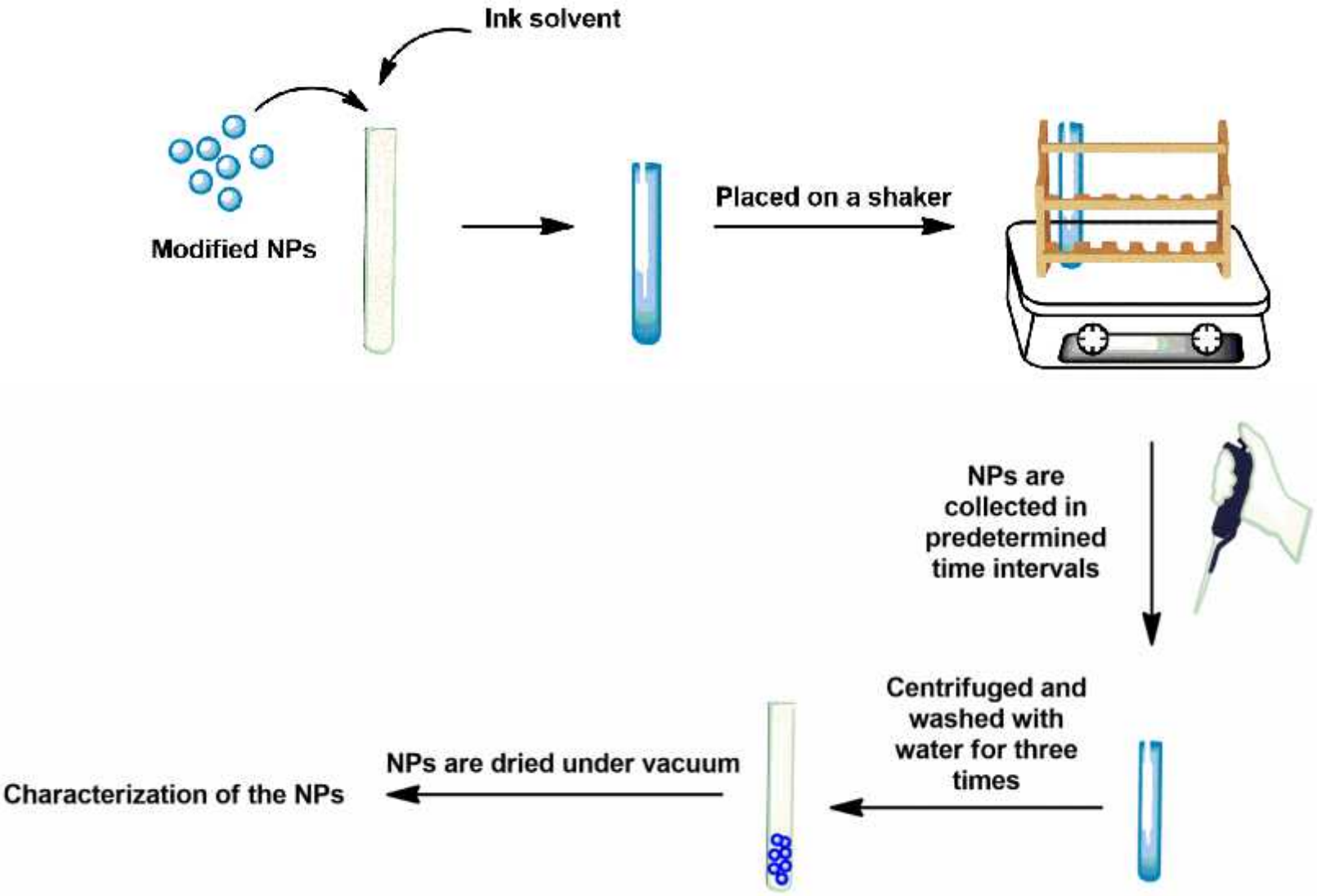




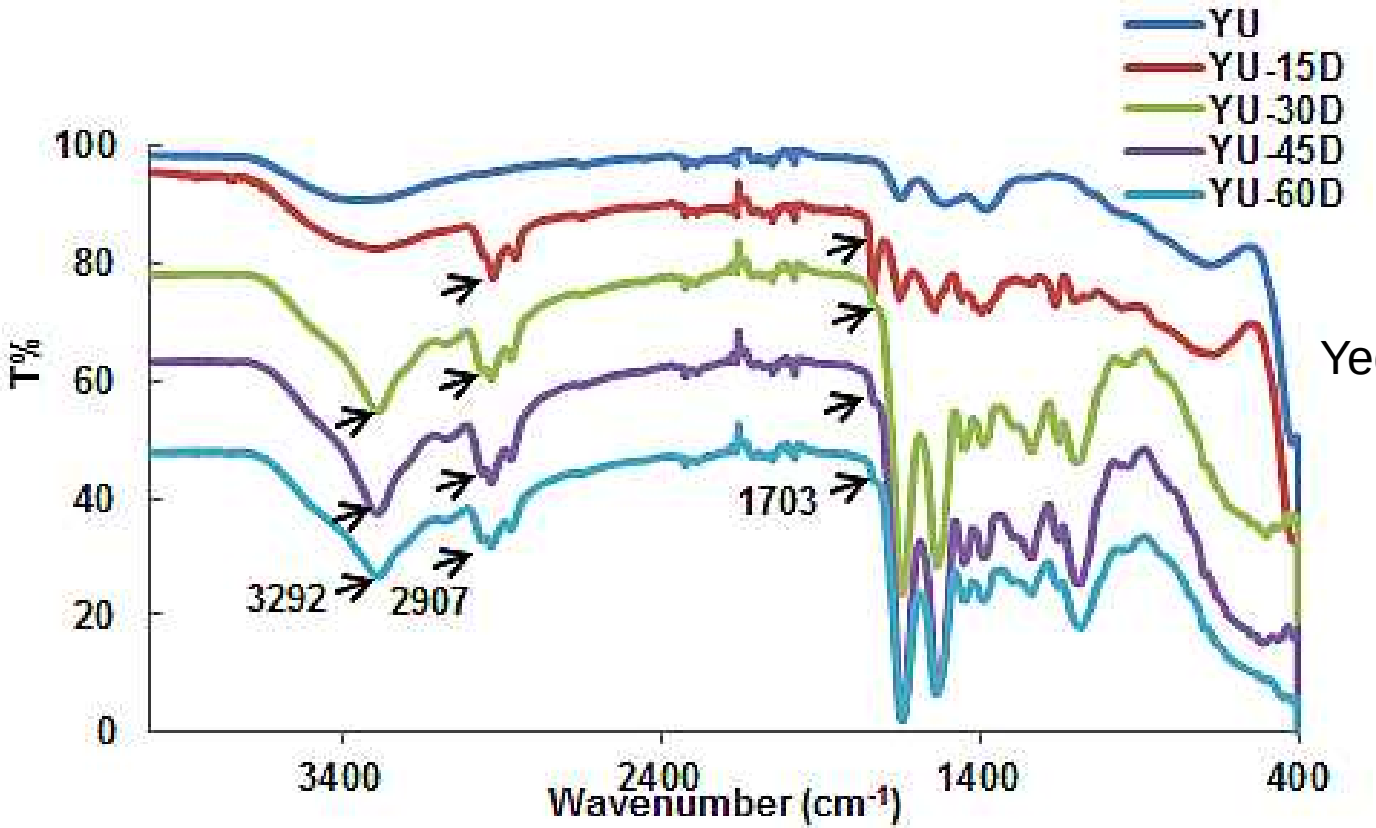
CONCLUSIONS AND RESULTS FOR QDs

- It is necessary to coat the surface of QDs with a silica layer to stop the Cd ion release into the medium.
- A strategy to modify the QDs with glucose developed.
- Glucose modification helped to reduce the cytotoxicity without altering the fluorescing properties of QDs.
- However, the need to use hydrophobic surface in the ink formulations hinders the use of QDs modified with glucose.
- A possible change in ink formulation is suggested to be able to accommodate the QDs with hydrophilic surface.

Stability Assessments of Modified NPs



Stability Assessment of ZnO NPs



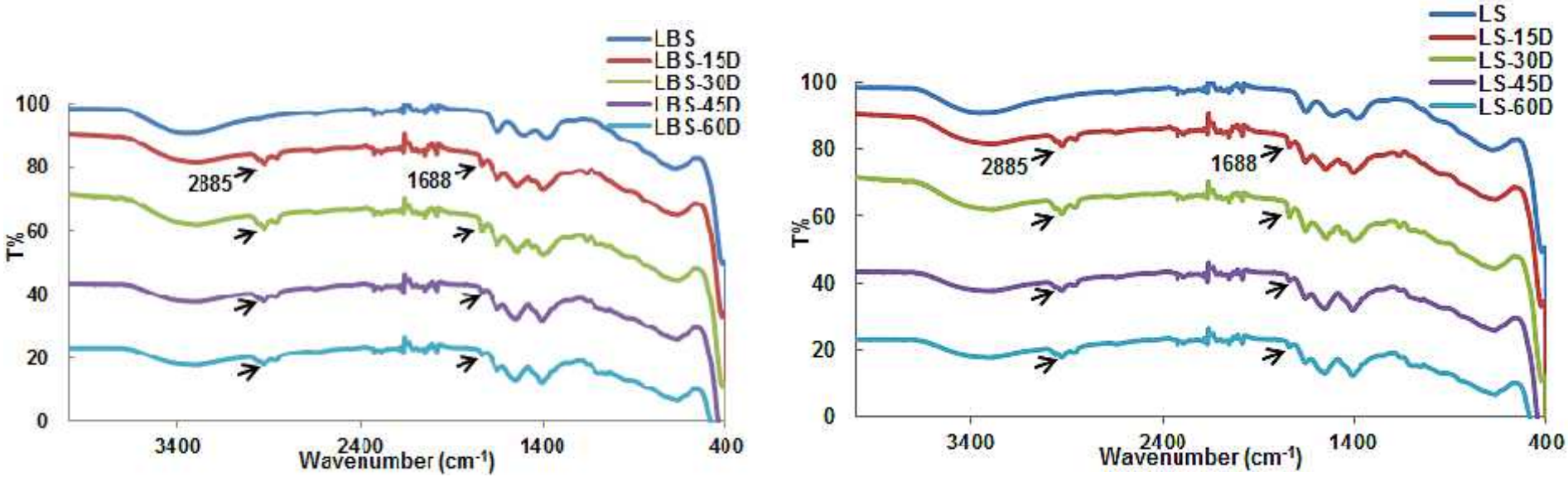
Yeditepe University: YU

- After incubation of the modified ZnO NPs in ink solvent, amide I and amide II bands of BSA near 1500 and 1600 cm^{-1} can be observed clearly, demonstrating the stability of ZnO NPs-BSA (YU).

- A carboxyl peak at 1703 cm^{-1} and a –CH peak at 2907 cm^{-1} were appeared on the spectra of BSA modified ZnO NPs. In addition, after 30 days incubation in ink solvent, the broad –OH band near 3300 cm-^{1} was turned to a sharp peak at 3292 cm^{-1} .

- The changes on the spectra pointed that the surface characteristics of BSA modified ZnO NPs were changed after incubation process due to the residuals of ink solvent.

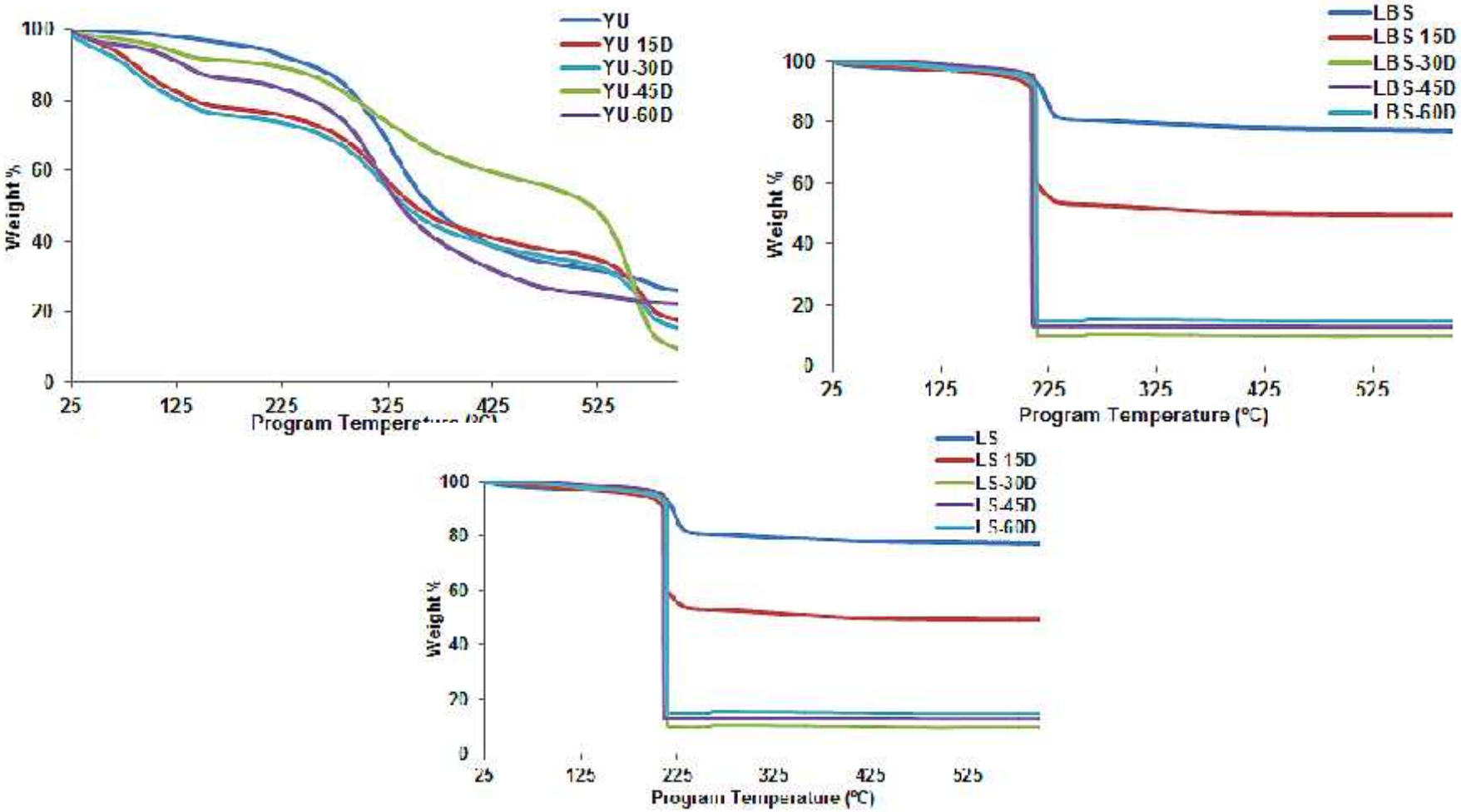
Stability Assessments of ZnO NPs (from TecStar)



LBS: Laboratory Scale, LS: Large Scale

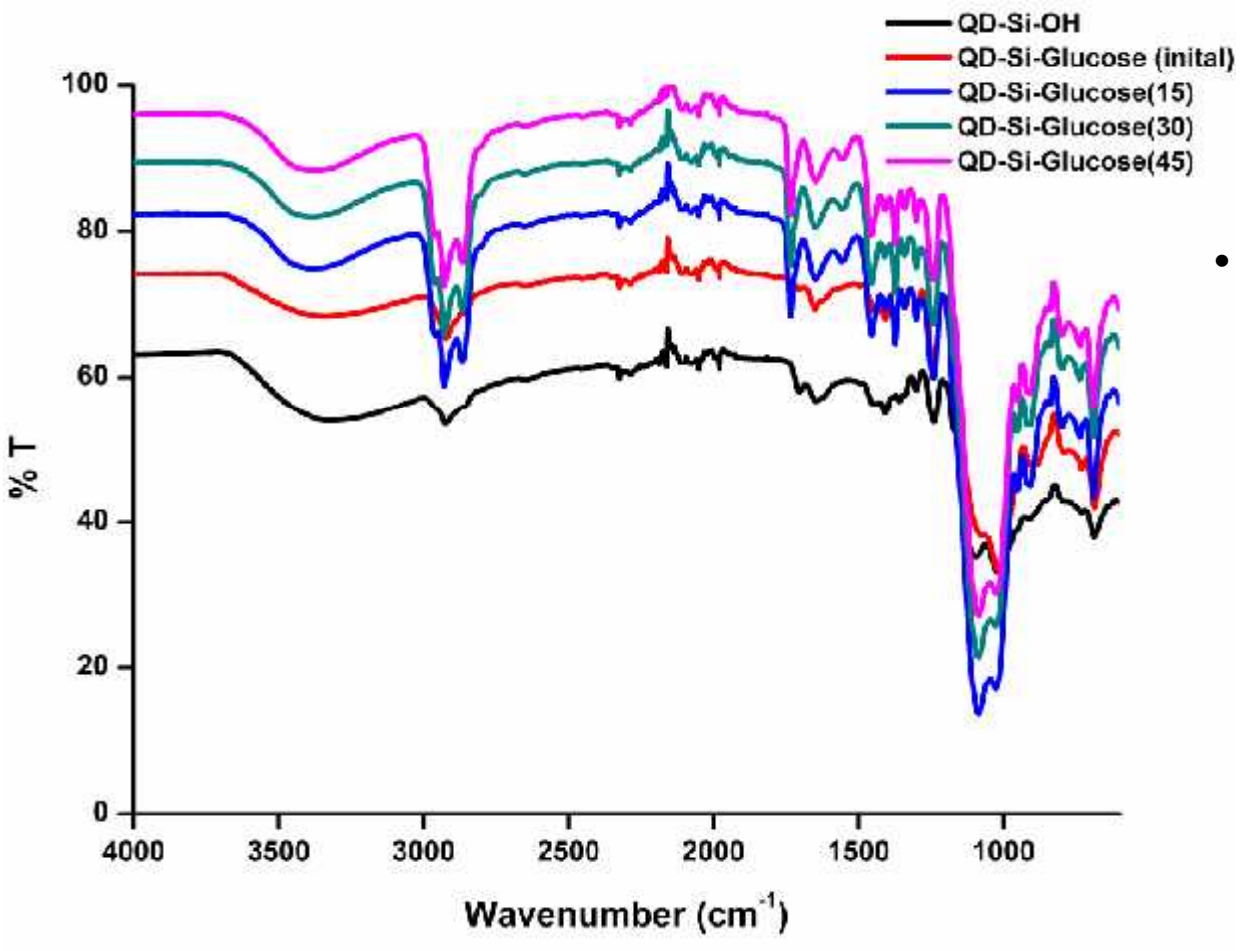
- After the incubation of the modified ZnO NPs (LBS and LS, TecStar) in ink solvent, amide I and amide II bands of BSA near 1500 and 1600 cm^{-1} can be observed clearly as evidences of the stability of the NPs.
- A carboxyl peak at 1688 cm^{-1} and –CH peaks at 2875 and 2885 cm^{-1} were appeared on the spectra of BSA modified ZnO NPs (LBS and LS).
- The appearance of new peaks may be associated with the residuals of the ink solvent.

Stability Assessments of ZnO NPs



The surface coverage of ZnO NPs-BSA (YU and LBS) did not change after the incubation of the modified NPs in ink solvent but the surface coverage of ZnO NPs (LS) increased after incubation process unexpectedly.

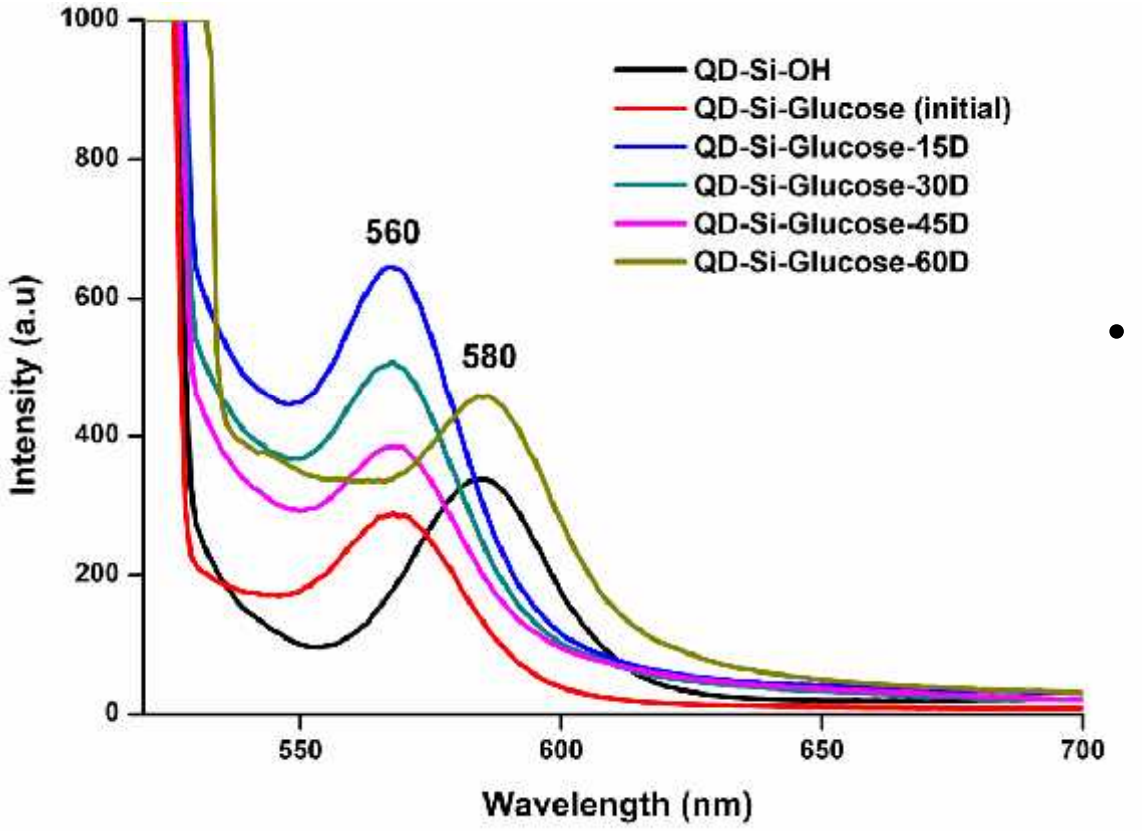
Stability of glucose modified QDs



FT-IR spectra of QDs before and during stability testing

- A decrease on the –OH band and an increase on the –CH stretching are clearly seen, indicating the decomposition of glucose by time.

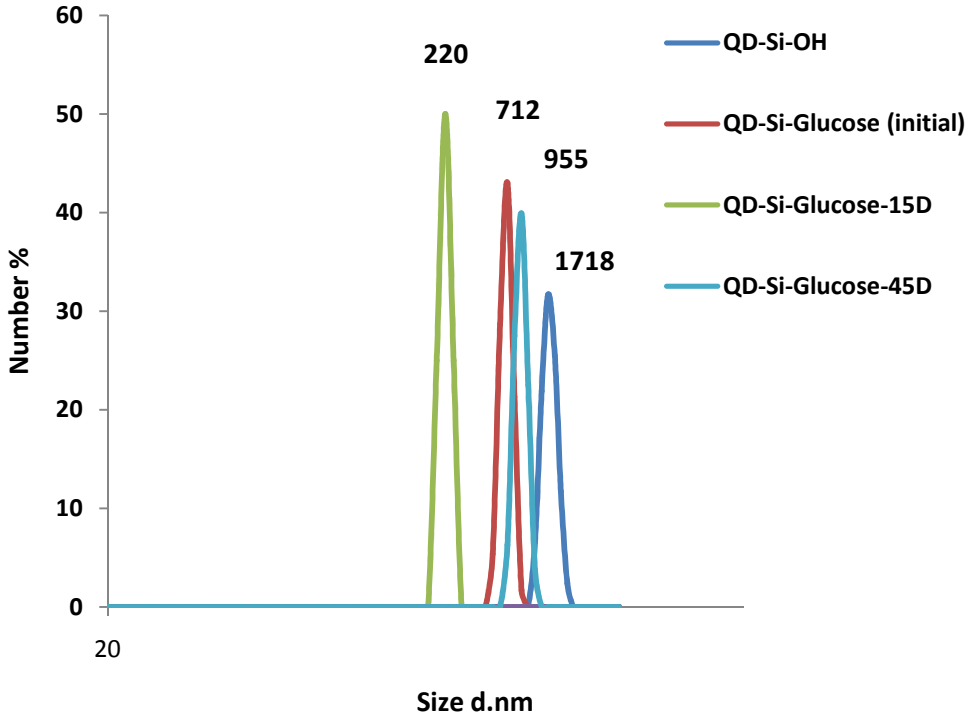
Stability of glucose modified QDs



Fluorescence spectra of QDs before and after stability testing

- A shift on the wavelength of fluorescence from 580 nm to 560 nm is seen after glucose modification.
- The similar situation is seen after the stability assessment the wavelength of fluorescence shifted to 580 nm from 560 nm at the 60th day of incubation with the decomposition of glucose on the surface of QDs.

Stability of glucose modified QDs



DLS spectra of glucose modified QDs before and after stability assessments

- As it can be seen from the DLS spectra, the size of the QDs decreased to 712 nm from 955 nm with glucose modification.
- The size of the QDs increased due to shaking during incubation. We could not get any data from the samples belong to the 30^{th} and 60^{th} days of incubation caused by the agglomeration of the QDs.

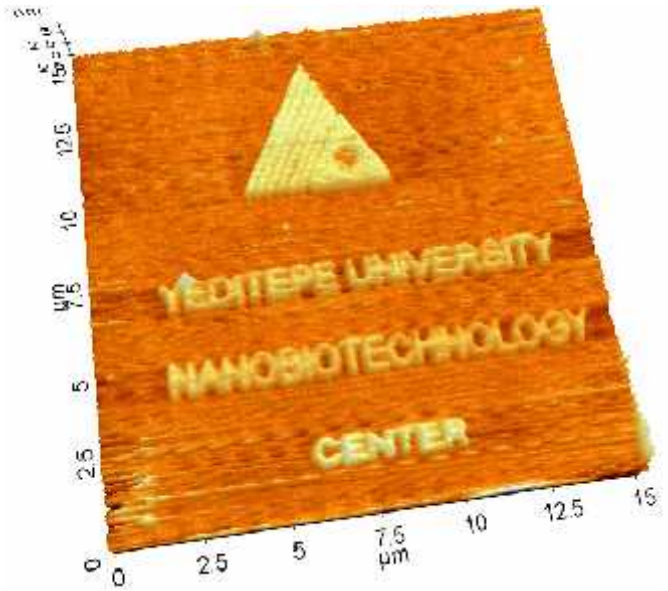
CONCLUSIONS AND RESULTS FOR STABILITY STUDY

- According to FT-IR and TGA measurements, protein modified ZnO NPs are stable for 60 days but the residuals of ink solvent are detected.

- The fluorescence spectra of QDs indicate that the NPs are stable for 45 days. After 60 days of incubation, the glucose on the surface of the NPs are decomposed based on FT-IR and fluorescence data.

Nanobiotechnology and Molecular Engineering Group

Acknowledgement



NANOMICEX FP7 Project and Yeditepe University for funding.