SAFER BY MOLECULAR DESIGN APPROACH APPLIED TO CuO CASE STUDY

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OUTILINE

- SAFETY ISSUES APPLIED TO NANOMATERIALS
- SAFETY BY DESIGN (SbyD) STRATEGIES: SANOWORK PROJECT
- SbyD STRATEGY APPLIED TO CuO CASE STUDY: SUN PROJECT

SAFETY

SAFETY ISSUES APPLIED TO NANOMATERIALS……

→ **NANOSCALE REACTIVITY**

COMPOSITION ← SIZE → SURFACE

Bulk properties modified by Quantum size effects

Nanophase-heterogeneity Huge interface between nano-objects and the surrounding media.

Bulk properties modified by low range order of crystallinity: surface defects / impurities High surface energy

(Catalytic, Electrical, Magnetic, Optical, Carrier capability, Surface cross-linking)

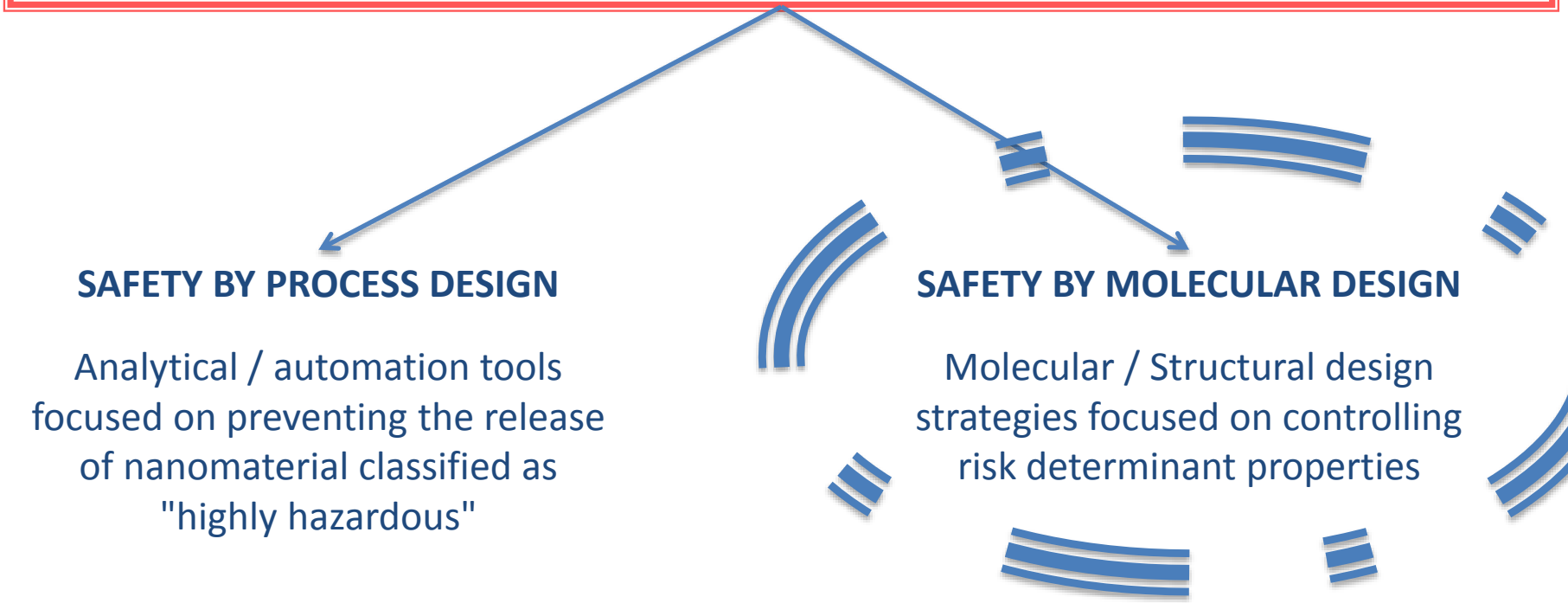
POTENTIALLY HEALTH DANGEROUS EFFECTS

NMs APPLICATION

SAFETY BY DESIGN (SbyD)

DEFINITION

ENGINEERING of **NANOMANUFACTURING PROCESS** or **PRODUCT** with specific attention to design out risks rather than address them when they occur



SAFETY BY PROCESS DESIGN

Analytical / automation tools focused on preventing the release of nanomaterial classified as "highly hazardous"

SAFETY BY MOLECULAR DESIGN

Molecular / Structural design strategies focused on controlling risk determinant properties

SOURCE TO EFFECT FRAMEWORK

SYNTHETIC IDENTITY (BORN TO BE……)

Intrinsic *physicochemical properties* ***(PCHEMS)***

DESIGN CRITERIA

(ENVIRONMENTAL TRANSPORT, BIOKINETICS, DOSIMETRY)

EXPOSURE CRITERIA

Evolution of PCHEMS in Life-cycle and Testing Media;
Exposure related PCHEMS

LIFE CYCLE ⇕ TESTING

SYSTEM IDENTITY

MODES of ACTION

ACELLULAR ⇕ CELLULAR

HAZARD CRITERIA

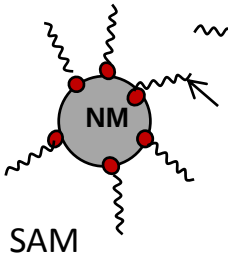
PCHEMS triggering early effects

PRO-OXIDATIVE POTENTIAL, TRANSPORT and RELEASE of transition metal ions / impurities / toxicants by the surface.
BIOPHYSICAL INTERACTION with Extra, Intra and Surface CELLULAR components

IN VITRO / IN VIVO TOXICITY

SANOWORK SAFETY BY DESIGN STRATEGIES

SURFACE COATING



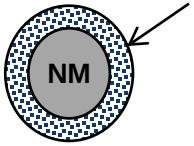
Hydrophobic tails

Polar heads

Amphiphilic coating

SAM

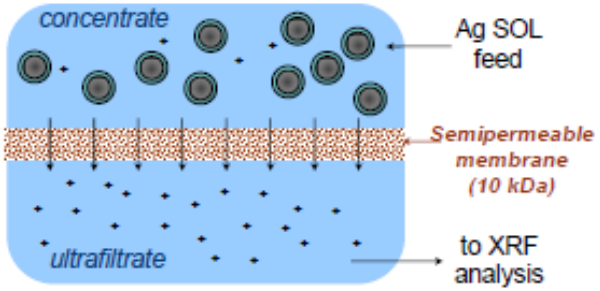
ORGANIC



Silica coating

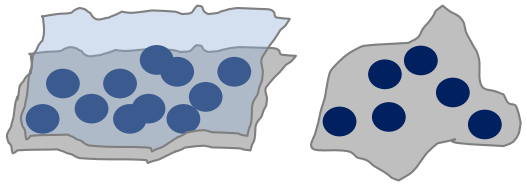
INORGANIC

PURIFICATION



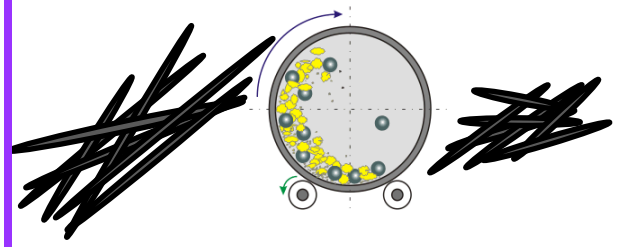
ULTRAFILTRATION TREATMENT

NANO in MACRO



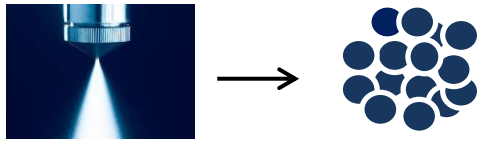
GEL COATING, IMPREGNATION ON POROUS INORGANIC STRUCTURE

MILLING



WET MILLING OF NANOFIBERS

NANO in MICRO



SPRAY-GRANULATION

TO CONTROL *PRO-OXIDATIVE POTENTIAL*
and further hazard mechanistic criteria

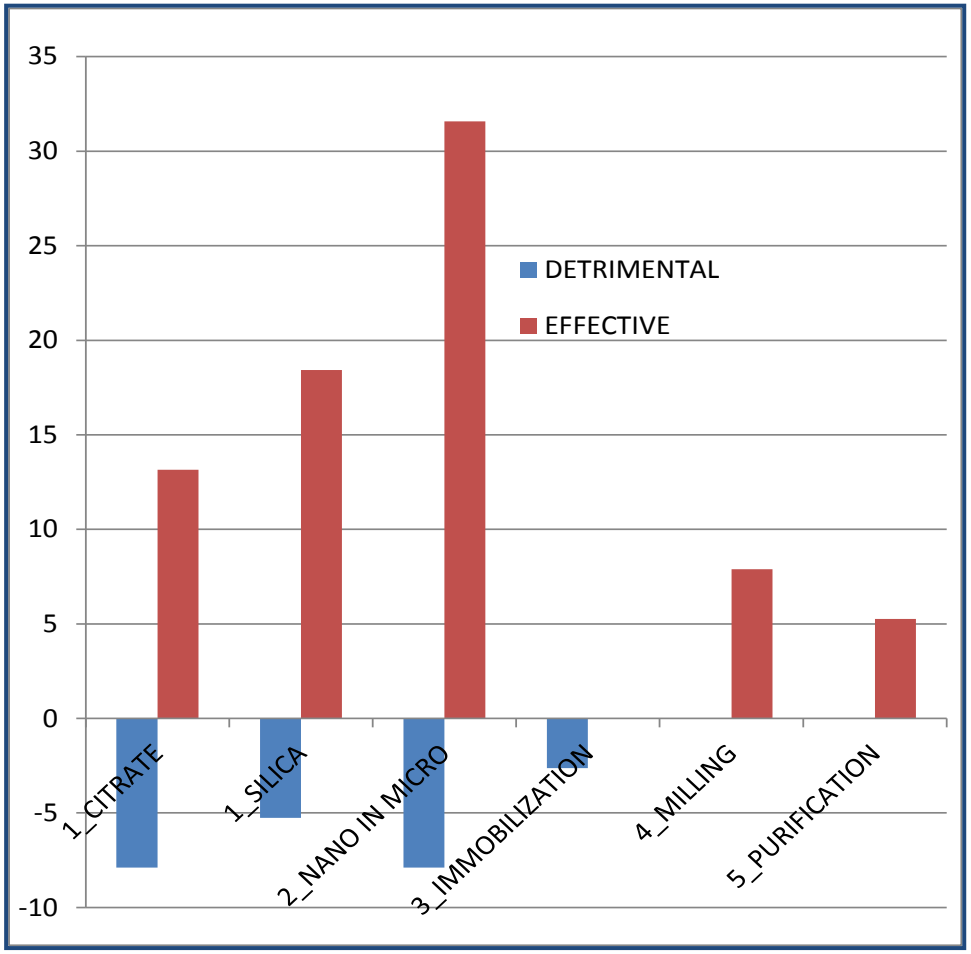
TO CONTROL *BIO-PHYSICAL INTERACTION*

TO CONTROL *EXPOSURE CRITERIA*
such as emission potential and To improve nano-product recovery and disposal

PRESERVE NANOSCALE REACTIVITY

RISK / PERFORMANCES EVALUATION

SAFETY BY DESIGN STRATEGIES EVALUATION……considering for each strategies the total amount of positive and detrimental effect due to their introduction, normalisingfor the total amount of experiments carried on



Nano to Micro / Macro *technologies (spray drying, spray-freeze granulation, forcing colloidal agglomeration, immobilization) seem to be the most promising strategies for the control of emission/exposure potential,*

as well ***Silica Coating*** *is for the control and harmonization of surface chemistry*

CuO CASE STUDY FROM SUN PROJECT

SUN

Sustainable Nanotechnologies Project

NMP4-LA-2013-604305
From Oct 2013 to March 2017

WP7: Safe production, handling and disposal

CuO NPs are synthesized by sol-gel / pyrolysis by **Plasmachem**

↓ *Formulation* (**BASF**)

↓

Antibacterial WOOD PAINTS

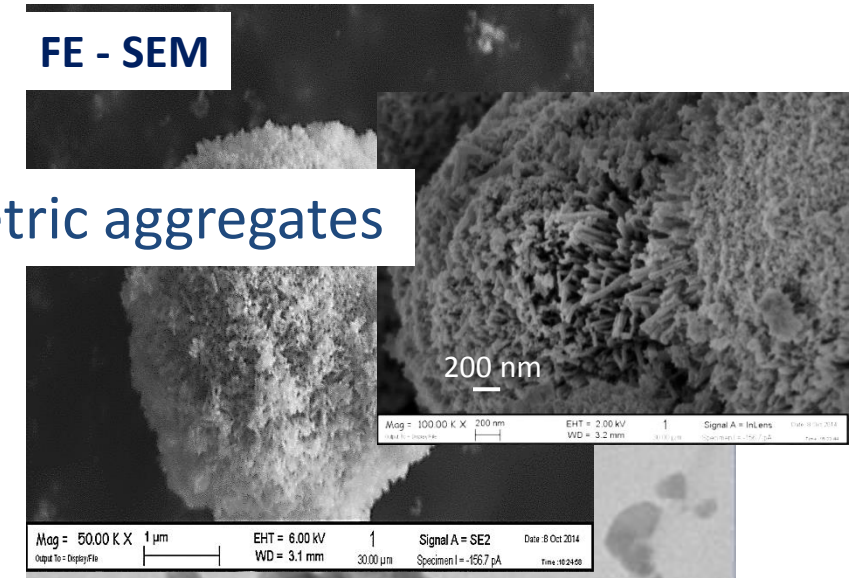
CuO INTRINSIC PROPERTIES

Properties	Results
Crystallite size, nm	ca. 15
BET, m^2/g	45 ± 5
Stabilizer	No organic stabilizer
Form	Dry powder
Colour	Black
Phase	Monoclinic

BET equivalent diameter: ≅ 20 nm
(dBET = 6/SSA • ρ)

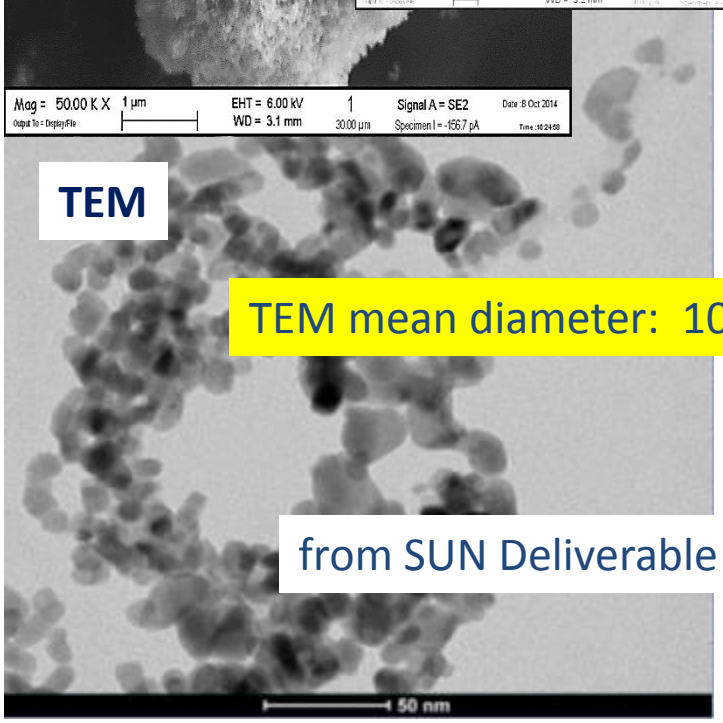
FE - SEM

Micrometric aggregates



TEM

TEM mean diameter: 10nm



from SUN Deliverable 1.4

SbyD STRATEGY APPLIED TO CuO CASE STUDY

DESIGN STRATEGY

Preparation of pristine and modified samples in **buffer phosphate (**$PO4^{3-}$ 0,05M**)**

PO_4^{3-}

Maximizing **nanofraction** and promoting adhesion of surface coating agents by **wet ball milling**

Surface modification: stock suspensions 10 g / L

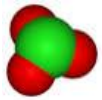
$PO4^{3-}$	$PO4^{3-}$ PVP	$PO4^{3-}$ CIT or ASC (−)	$PO4^{3-}$ PEI (+)
101	102	102, 105	104

EXPOSURE IDENTITY

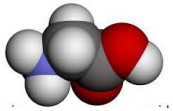
aggregation
DLS size / ELS Zeta

DILUTION (100 mg/L) in:

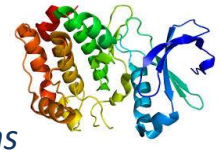
H_2O



+ **SALTS** *Ca^{2+}, Mg^{2+}, oxoanions*
Dulbecco's PBS
AFW, AMW



+ **AMINOACIDS** *Amphiphilic moieties*
and NUTRIENTS
MEM and DMEM in vitro media



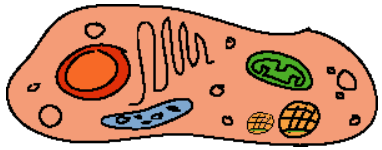
+ **SERUM** *Proteins*
COMPLETE in vitro media

HAZARD IDENTITY

(**early**, **apical** effects)

PCHEM property of early effects:
Ion/solid phase ratio
Ion speciation

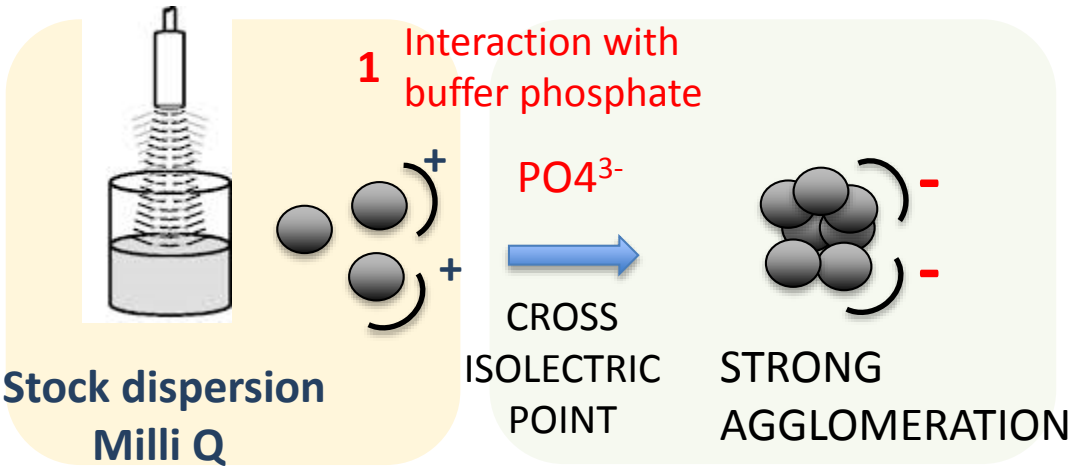
IN VITRO TESTS



IN VIVO TESTS

MAT. PERFORMANCES

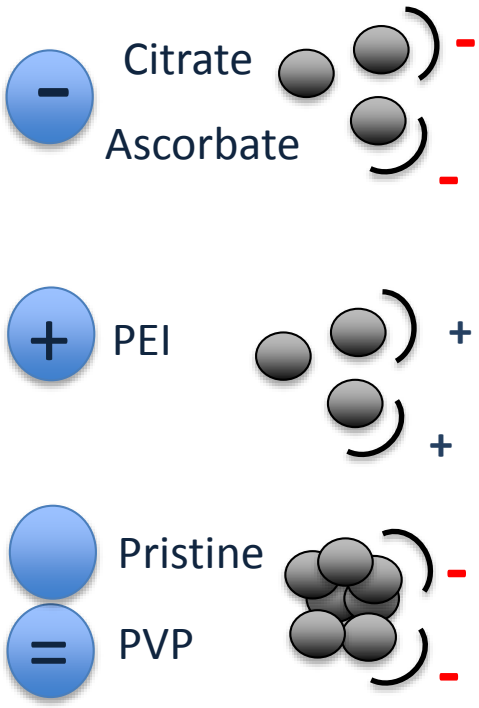
Effect of PO_4^{3-} buffer dispersing media



Z pot:	+ 34.9 mV	**Z pot:**	- 9 mV
pH :	6.8	**pH :**	6.5
Size :	330 nm	**Size :**	1093 nm

The specific interaction of PO_4^{3-} ions with CuO surface invert positive sign of CuO water dispersion, a destabilization occurred, despite to the high value of absolute Z potential

Effect of surface modifiers (H_2O medium)



Sample	pH	d_{DLS} (nm)	ζ-pot $_{ELS}$ (mV)
CuO_101	6.4	1093	-9
CuO_102_CIT	6.5	368	-18
CuO_103_PVP	6.5	797	-8
CuO_104_PEI	6.5	247	+28
CuO_105_ASC	6.5	122	-17

Samples coated by **ionic agents** (CIT, ASC, PEI) resulted better dispersed showing values coherent with the charge given by the capping agent.

Neutral PVP did not improve significantly the dispersion of CuO NPs and as expected, did not modify zeta potential of pristine sample.

Dilution in saline media

Dulbecco buffered saline (D8662) contains all the salts of the complete media excepting proteins and antibiotics

Sample	H_2O			D8662		
	pH	d_{DLS} (nm)	ζ-pot $_{ELS}$ (mV)	pH	d_{DLS} (nm)	ζ-pot $_{ELS}$ (mV)
CuO_101	6.5	1093± 50	-9.1 ± 0.4	7.5	2756± 347	-20,7± 1.4
CuO_102_CIT	6.5	368± 10	-18.0± 0.3	7.4	271± 43	-35.8± 2.9
CuO_103_PVP	6.5	797± 84	-8.1± 2.3	7.4	2765± 432	-21.1± 1.5
CuO_104_PEI	6.5	247± 14	+28.3± 0.7	7.4	209± 16	+25.4± 1.9
CuO_105_ASC	6.4	122± 1.4	-17.4± 0.3	7.4	1314± 525	-24.5± 2.8

In Buffered saline medium, the increase of ionic strength induced a **COLLOIDAL DESTABILIZATION** as confirmed by the **increased agglomeration degree** of non modified, PVP and even ASC modified sample, despite to the **increase of negative zeta potential.**

Dilution in saline media

AFW and AMW containing Mg^{2+} and Ca^{2+}, more than 10 times concentrated in AMW than AFW

Sample	H_2O			AFW, pH 8.1		AMW, pH 8.1	
	pH	d_{DLS} (nm)	ζ-pot $_{ELS}$ (mV)	d_{DLS} (nm)	ζ-pot $_{ELS}$ (mV)	d_{DLS} (nm)	ζ-pot $_{ELS}$ (mV)
CuO_101	6.5	1093	-9.1	1663± 210	-3.5± 0.4	1281± 393	+7.6± 0.4
CuO_102_CIT	6.5	368	-18.0	1050± 16	+3.6± 0.4	1062± 159	+4.5± 0.7
CuO_103_PVP	6.5	797	-8.1	1159± 256	+1.6± 0.3	1661± 580	+6.5± 1.5
CuO_104_PEI	6.5	247	+28.3	675± 199	+20.9± 0.9	1281± 168	+10.1± 1.1
CuO_105_ASC	6.4	122	-17.4	1293± 278	-8.1± 0.4	1234± 25	+2.7± 0.6

.n artificial fresh and marine water, the increase of ionic strength induced a **COLLOIDAL DESTABILIZATION** as confirmed by the **increased agglomeration degree** particularly evident for samples that **reversed Z potential crossing the i.e.p**, due to the presence of Mg^{2+} and Ca^{2+} cations specifically adsorbed on metal oxide colloidal phases.

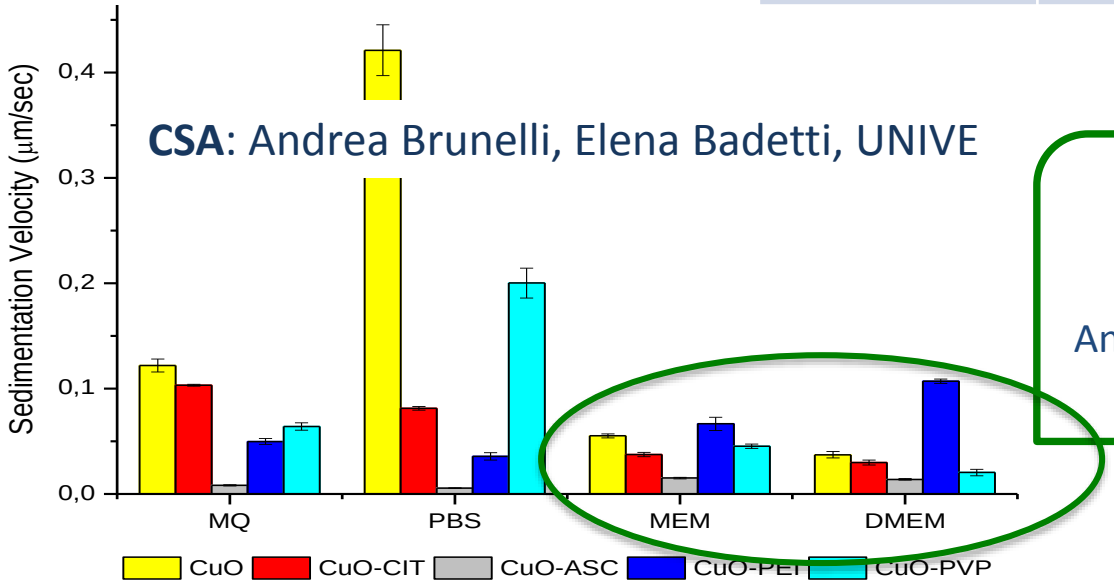
PCHEM TESTING STRATEGY FOR SbyD VALIDATION

Dilution in complete in vitro media: MEM / DMEM

Protein coating on nanoparticles

η_{DLS} and ζ_{ELS} data in MEM and DMEM e levelled off on data of media alone, but this information does not reflect potentially transformation occurred at dispersion state during evolution from synthetic to biological identity and possible consequence on the bioavailability of nano fraction.

Sample	MEM			DMEM		
	pH	d_{DLS} (nm)	ζ-pot $_{ELS}$ (mV)	pH	d_{DLS} (nm)	ζ-pot $_{ELS}$ (mV)
CuO_101	8.2	47	-10	8	55	-8
CuO_102_CIT	8.2	89	-10	7.9	37	-10
CuO_103_PVP	8.2	44	-10	7.9	53	-9
CuO_104_PEI	8.2	46	-10	7.9	45	-10
CuO_105_ASC	8.2	52	-10	7.9	73	-9
MEM	**7.6**	**21**	**-10**	**7.9**	**-**	**-11**



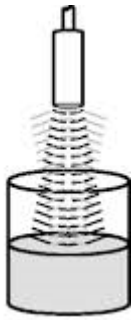
CSA: Andrea Brunelli, Elena Badetti, UNIVE

The **stabilisation** in in vitro media is confirmed by sedimentation velocity measured by Centrifugal Separation Analysi (CSA); only CuO PEI sample seems to decrease its colloidal stability

PCHEM TESTING STRATEGY FOR SbyD VALIDATION

CuO NPs

Buffer



Stock dispersion
Milli Q (10g /L)

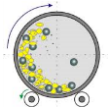
1 Interaction with buffer phosphate

$PO4^{3-}$

CROSS ISOLECTRIC POINT

STRONG AGGLOMERATION

Modifiers



BM

Citrate
Ascorbate

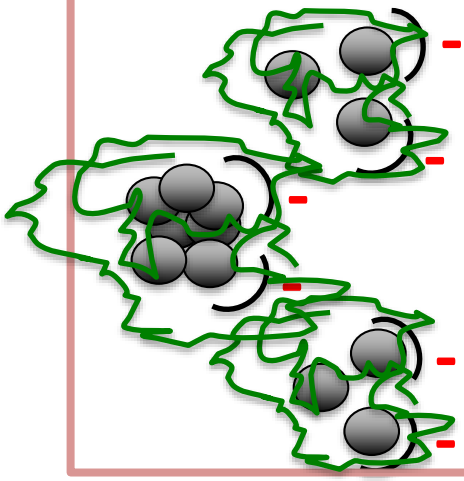
PEI

Pristine

PVP

Dilution in tox media (100mg / L)

BIO AVAILABILITY ?



= Citrate Ascorbate

CROSS ISOLECTRIC POINT PEI

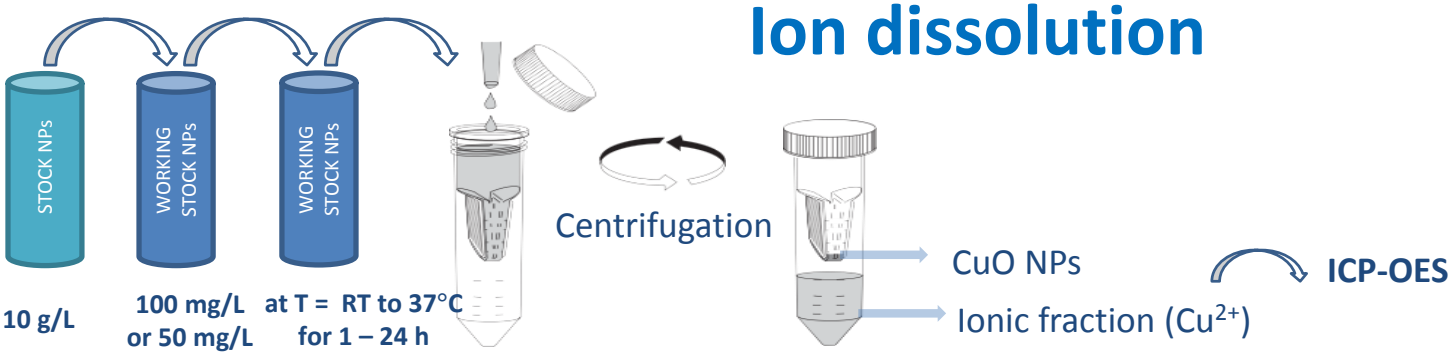
= Pristine PVP

2 Interaction with slight negative PROTEINS

PCHEM TESTING STRATEGY FOR SbyD VALIDATION

METHOD

Ion dissolution



RESULTS

Sample	Cu^{2+}/CuO (%) at 24h, 37°C			
	MilliQ	D8662	DMEM	MEM
CuO_101	0.18	<0.31	67.41	59.91
CuO_102_CIT	1.98	1.81	69.19	55.22
CuO_103_PVP	0.23	<0.33	66.93	34.00
CuO_104_PEI	2.84	2.55	66.01	43.06
CuO_105_ASC	1.99	<0.33	65.39	48.13

pH range: 6.5 - 8

In protein free media **the dissolution stays below few unit percent** with an high dissolution for ionic abilised particle.

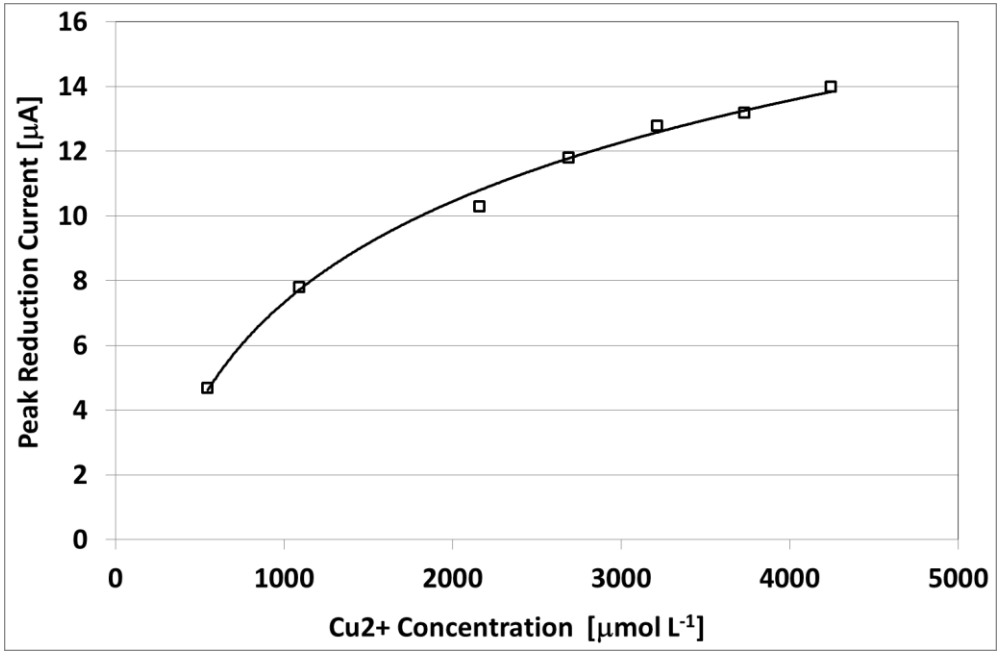
Chelating effect of ammino-acid determines an **abrupt increase of Cu^{2+} ion content**, not pH justified

Ion speciation (electroanalytical meas.)

μAutolab FRA2 Potentiostat

TECHNIQUES

- **Cyclic Voltammetry** (CV)
- **Reduction Potential Steps**
- **AGNES** (Using Hg-coated UMEs)

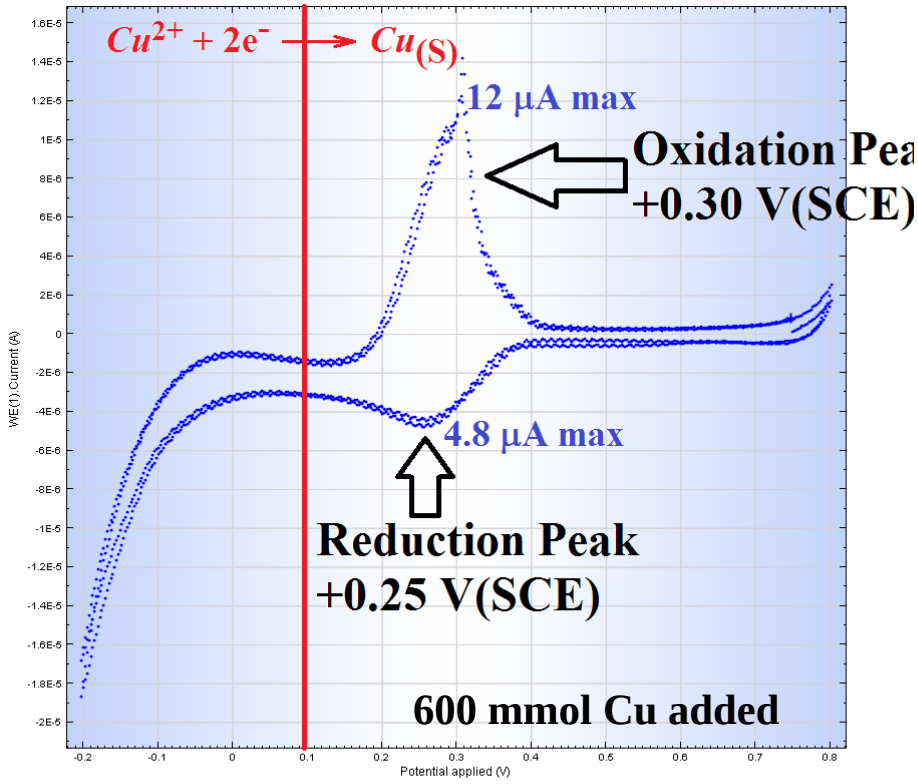


It was verified that it is possible to estimate the unknown concentration of Cu(II) ions in KCl on the 0.6 to 5 mmol L^{-1} range (10^{-2} ppm).

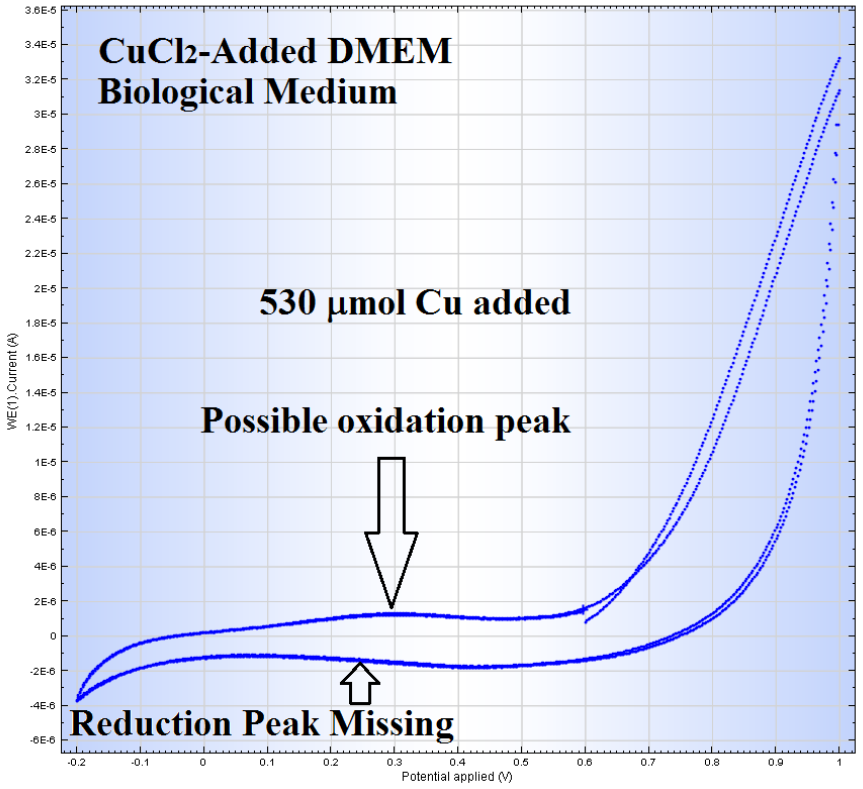
PRELIMINARY RESULTS

Ion speciation (electroanalytical meas.)

KCl at the same resistivity as DMEM + $CuCl_2$



DMEM + $CuCl_2$



Measurement made on DMEM alone did not cause any obvious peak to appear

In the presence of DMEM is necessary, up to about 5 mmol L^{-1} of Cu^{2+} ions to observe a weak oxidation peak. The very lower sensitivity in DMEM if compared with KCL can be explained by the chelating action of amminoacids vs Cu^{2+} ions

INDIRECT EVIDENCE OF IONS SPECIATION

CONCLUSION

- A source to effect framework was provided and established a platform for the definition and evaluation of SbyD strategies

- The results provided useful data to support the assessment of nano-bio interaction and make hypothesis on mechanism with the real possibility to act on molecular design and drive adverse biological effect.

- Further investigation and non testing approaches (computational modelling, read-across) needed to validate mechanicistic hypothesis and develop preventive / predictive tools

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Sanowork

SUN
Sustainable Nanotechnologies Project

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