



Electrochemical Characterization of Suspensions of Oxidic Nanoparticles in Biological Media

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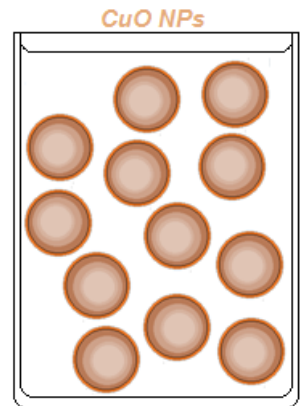
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- Bioceramics
- **Nanotechnologies**

Biological Medium



NPs suspension

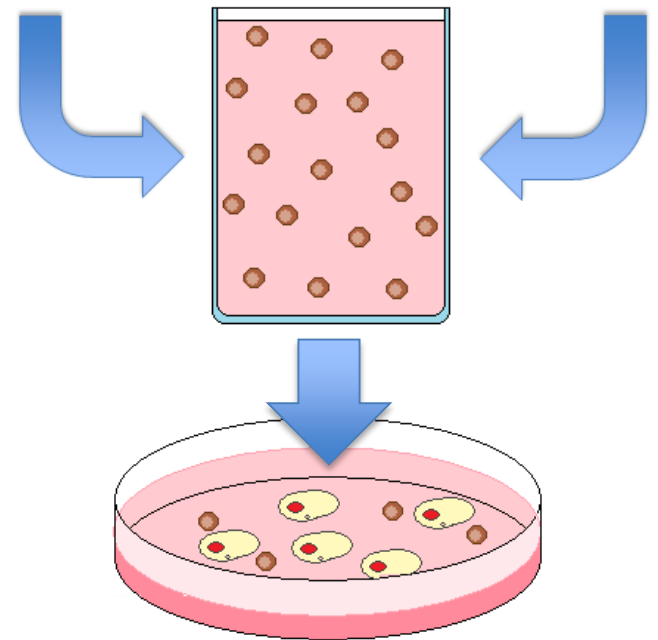


NP-added biological media such as DMEM are being used as cell culture substrates in order to assess the biological effects of NPs.

Adding copper (II) oxide (**CuO**) NPs to cell cultures has been demonstrated to induce cellular damage and eventually cells death.

Common experimental protocols include mixing aqueous suspensions of NPs with culture media.

The chemical stability of NPs in such media may be assumed based on the slightly basic pH (7.4).

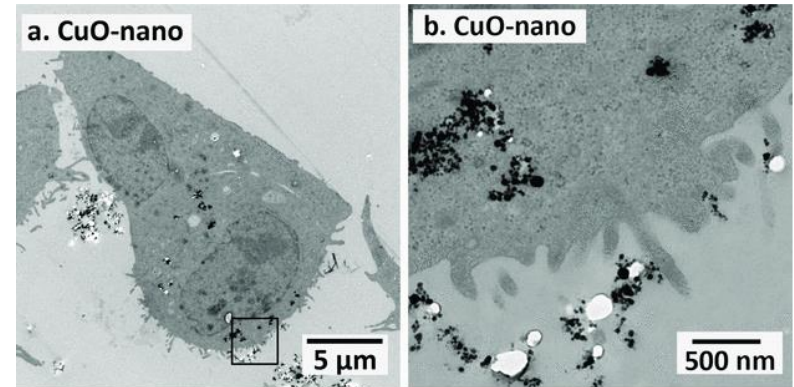


NPs-enriched cell culture medium

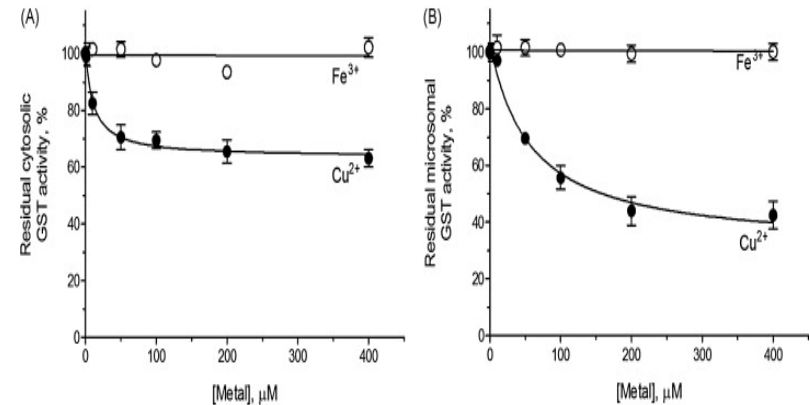
Intracellular uptake of **CuO** nanoparticles induces cell death.
Intracellular dissolution of CuO NPs was observed.

Troian horse mechanism

Cu²⁺ binds to protein thiol groups and displays pro-oxidant activity.

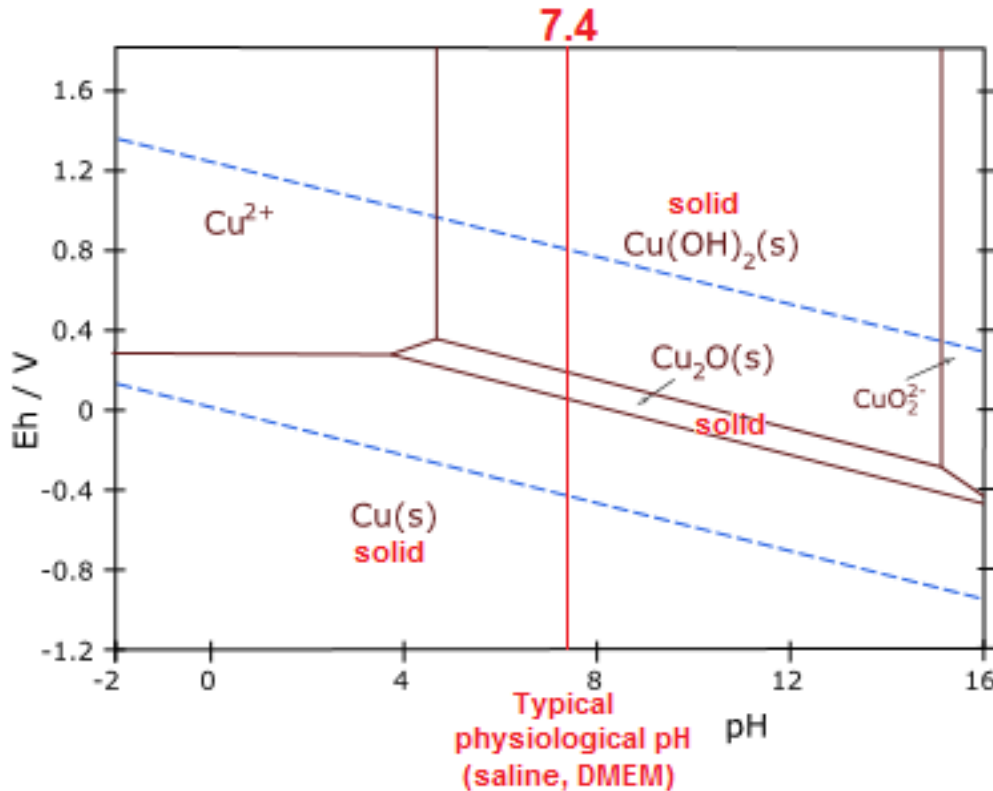


P Cronholm *et al.*, *Small* 2013 , 9, No. 7 , 970-982



M. E. Letelier *et al.*, *Chemico-Biological Interactions* 188 (1) 2010

At no potential is the cupric ion expected to be stable in aqueous environment at pH about 7.4.



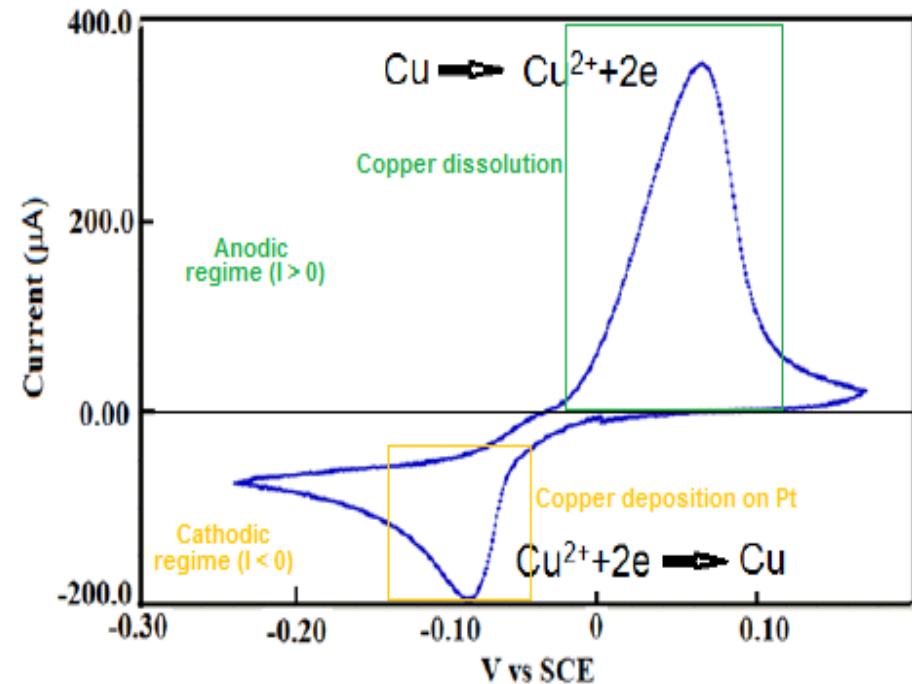
M. Pourbaix, *Atlas of Electrochemical Equilibria in Aqueous Solutions*, NACE, Houston 1974

Based on standard thermodynamics, all copper compounds are expected to be present as solid phases at physiological pH, either as oxide (Cu_2O , CuO) or hydroxides ($\text{Cu}(\text{OH})_2$), or elemental Cu

Electrochemical detection of Cu^{2+} performed at inert (mostly noble metal) electrodes



should not be possible due to Cu^{2+} ions not being available at physiological pH



Cu^{2+} ions in aqueous solution can be detected by cycling the potential of a noble metal electrode. This causes a series of deposition/stripping events to take place.

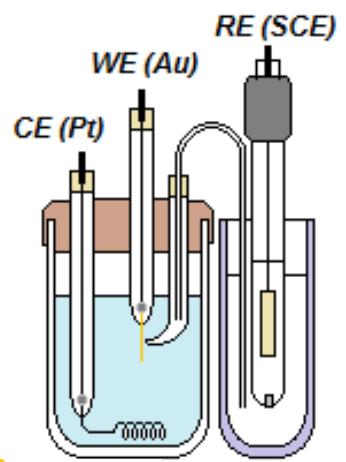
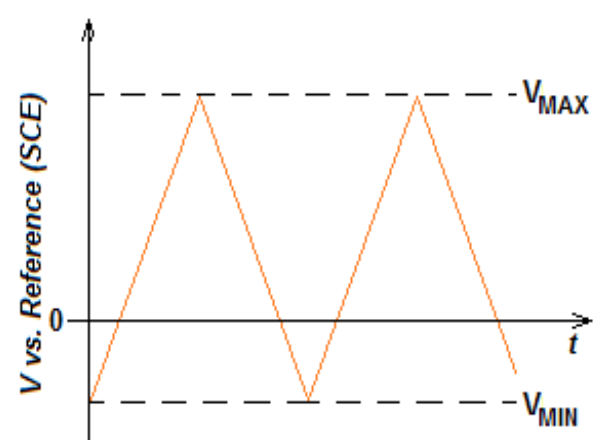
The height of the peaks correlates with the amount of copper being deposited/stripped

The voltammetric response due to copper is easy to single out in simple solutions (typically copper sulphate in a diluted solution of sulphuric acid)

In chemically complex systems the response of copper may be dwarfed by that from other electrochemical processes

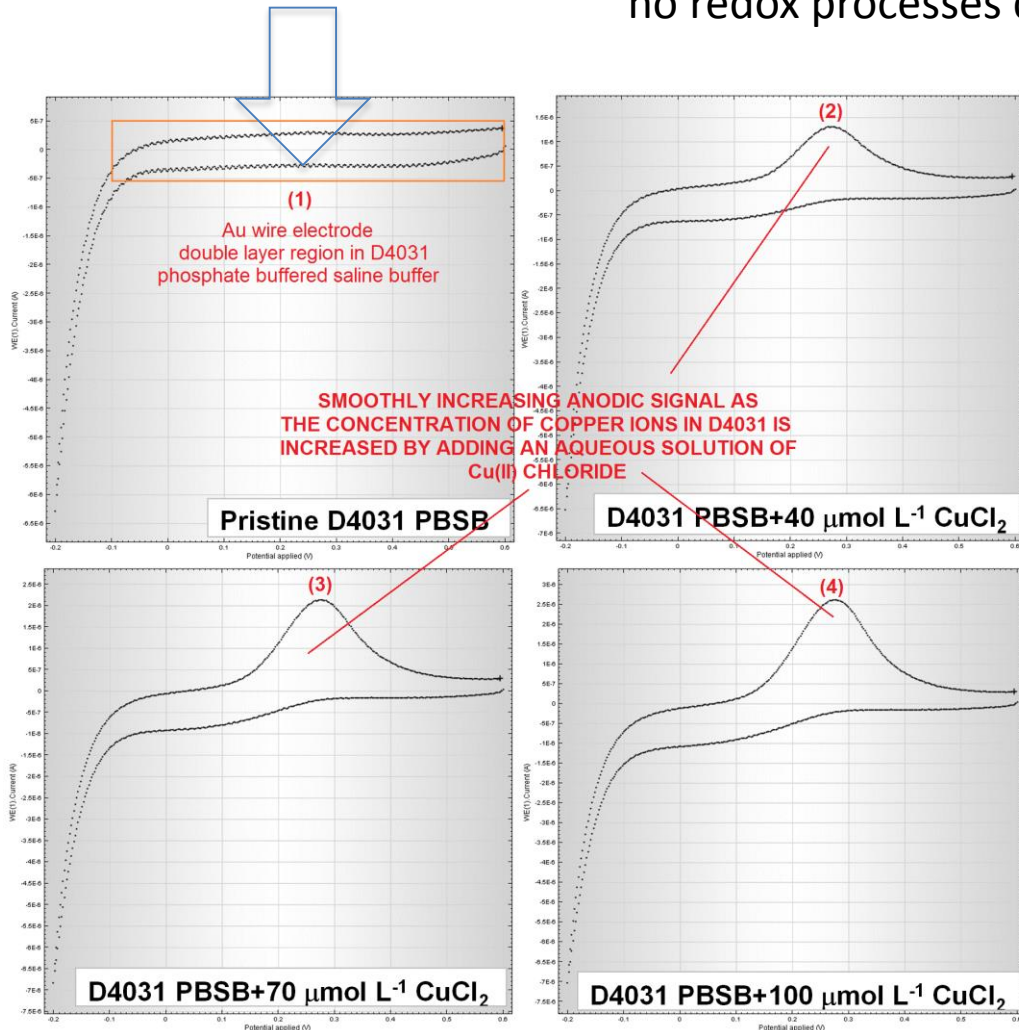
Bio-media are VERY complex systems

A. Jaikumar *et al.*, *ECS Transactions*, **66** (30) 55-64 (2015)



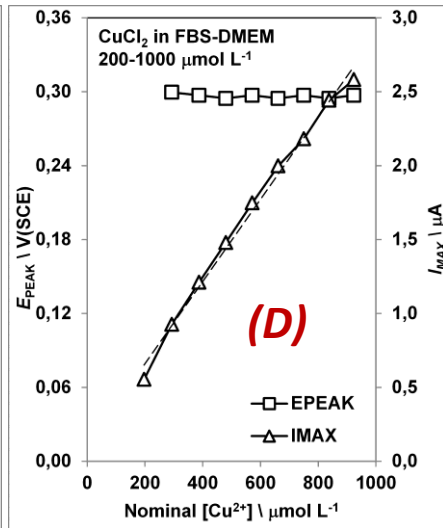
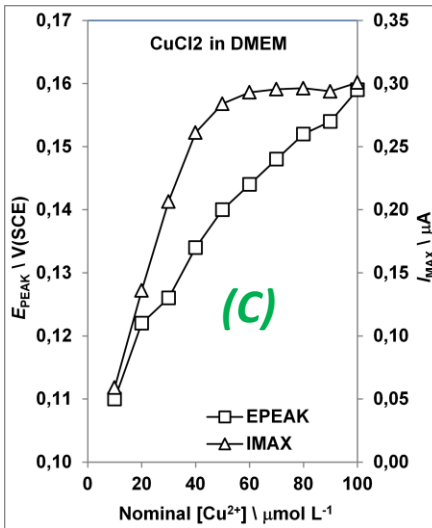
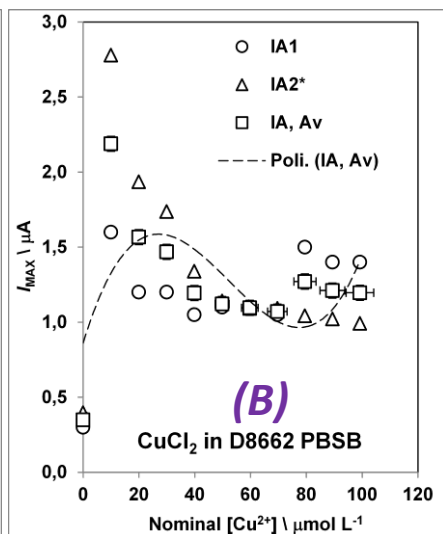
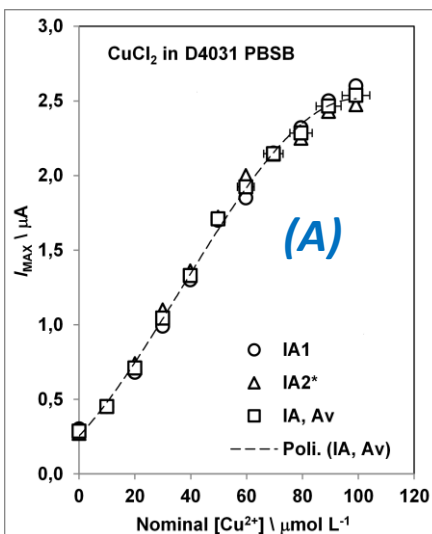
DOUBLE LAYER REGION

a potential interval over which - in pristine copper-free media - no redox processes do occur



Au wire features a **double-layer region** in saline buffers and DMEM at potentials at which the signal from Cu is expected to appear based on thermodynamic grounds

Working over the double layer region of an electrode prevents major alterations of the electrode's surface
RELIABLE QUANTITATIVE ANALYSIS



Known amounts of Cu²⁺ ions were gradually introduced in four different media as **aqueous CuCl₂ solution**:

- **ALDRICH D4031** saline buffer **(A)**
- **ALDRICH D8662** saline buffer **(B)**
- **THERMO FISCHER DMEM** high glucose, pyruvate **(C)**
- **Fetal bovine serum-added DMEM** (FBS-DMEM) **(D)**

Increasing the amount of introduced Cu²⁺ ions causes the peak anodic current I_{MAX} to increase in all media but D8662 saline buffer (see **(B)**)

Type of correlation varies, generally non-linear

Reproducibility is fair to good (within 10%) for all media but D8662 (see **(B)**). Excellent for D4031, see **(A)**

In FBS-DMEM a tenfold increase of concentration is required for a measurable signal to be seen (see **(D)**)

Baldisserri C., Costa A.L., *J Nanopart Res* (2016) 18:96



Aminoacids and proteins have a strong effect on the electrochemistry in the presence of copper

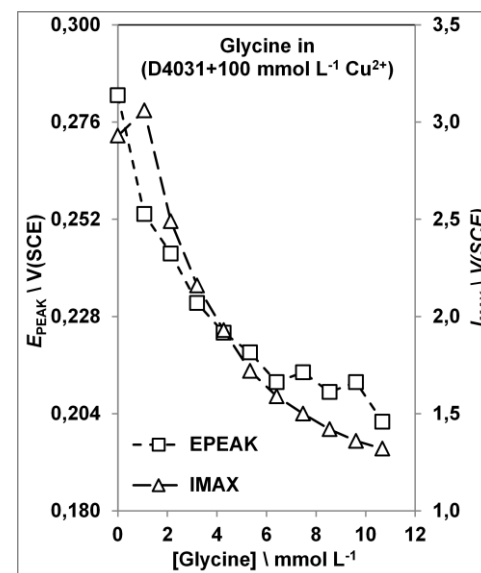
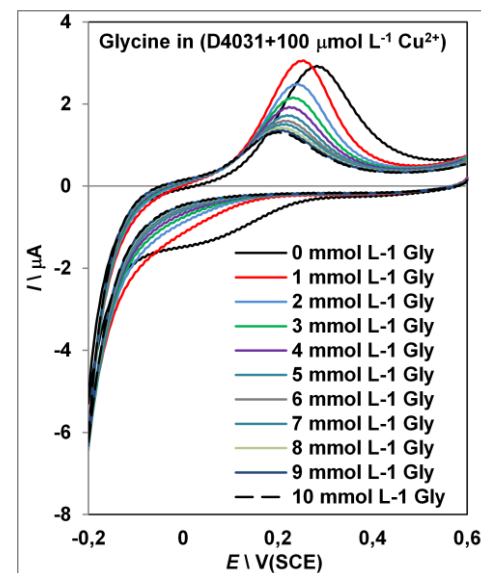
In **D4031 saline buffer** to which **100 $\mu\text{mol L}^{-1}$ of CuCl_2** had been added, stepwise addition of **glycine** up to **$10^{-2} \text{ mol L}^{-1}$** causes the strong anodic copper stripping peak to decrease, as well as the position of the peak to shift in the cathodic direction

The following factors must be considered

Aminoacids and other complex molecules **chelate Cu^{2+}**

Amino acids are known to **self-assemble** at the **electrode**, thus modifying its properties

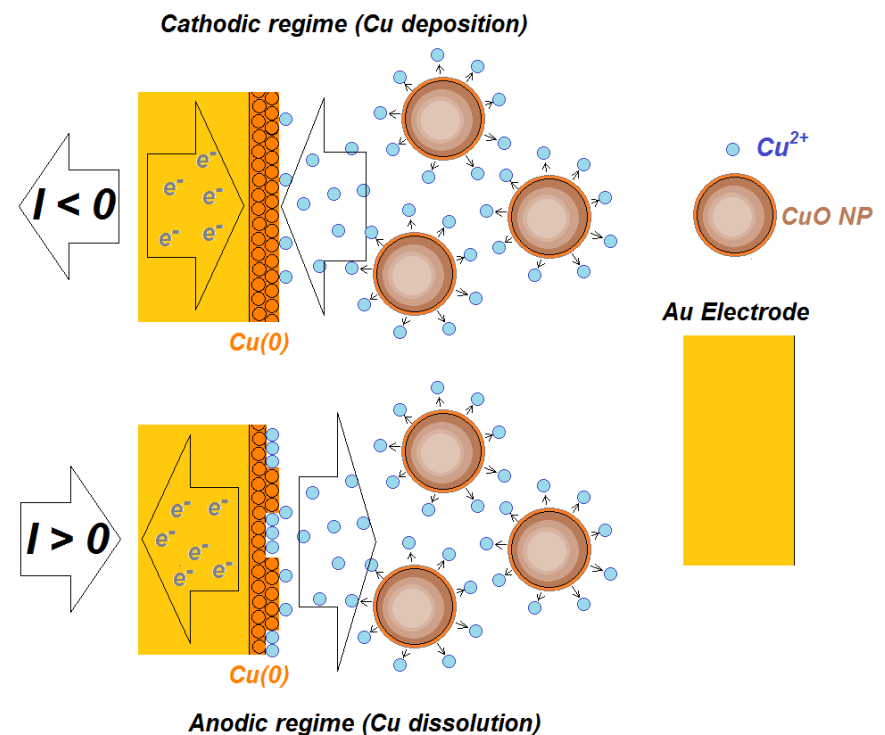
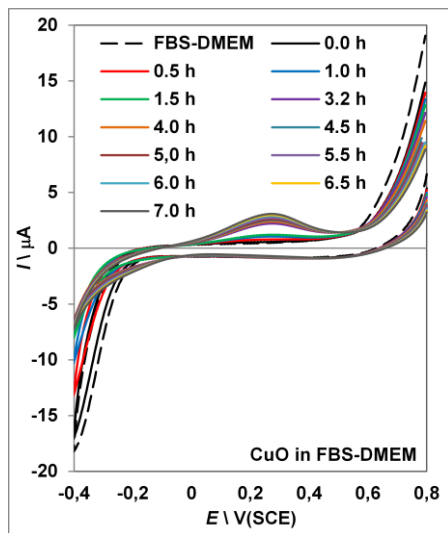
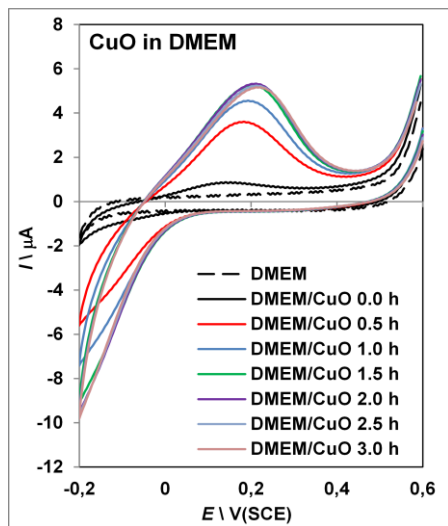
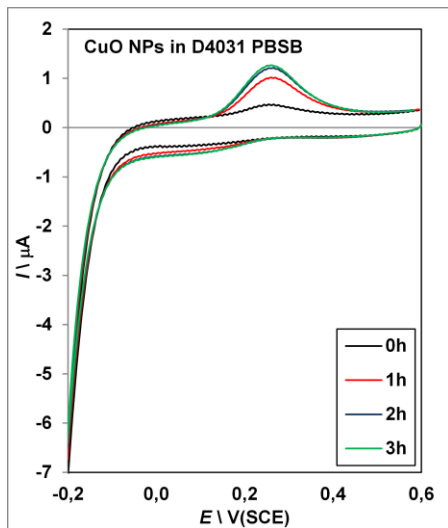
Both DMEM and FBS-DMEM contain a large amount of amino acids and preservatives, which makes it difficult to identify the precise electrochemical mechanisms that contribute to the overall current response in CV experiments



CuO nanoparticles were added to D4031 saline and DMEM and FBS-DMEM biological media. A single addition of CuO nanoparticles was made by injecting small volumes of aqueous CuO suspension

By running **cyclic voltammetry** scans at regular time intervals after injection, it was observed that similar anodic features to those observed after CuCl_2 addition appeared, a strong indication that electroreactive copper was being released into media

Both cathodic (Cu deposition) and anodic (Cu stripping) features were observed to grow stronger as the time elapsed from CuO injection increased



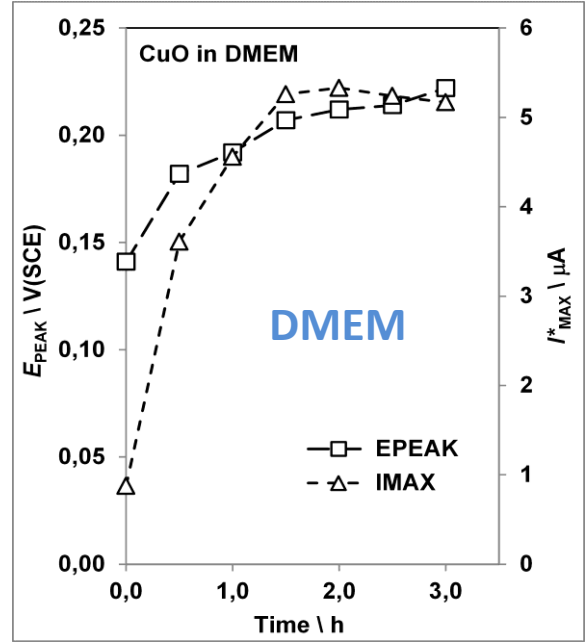
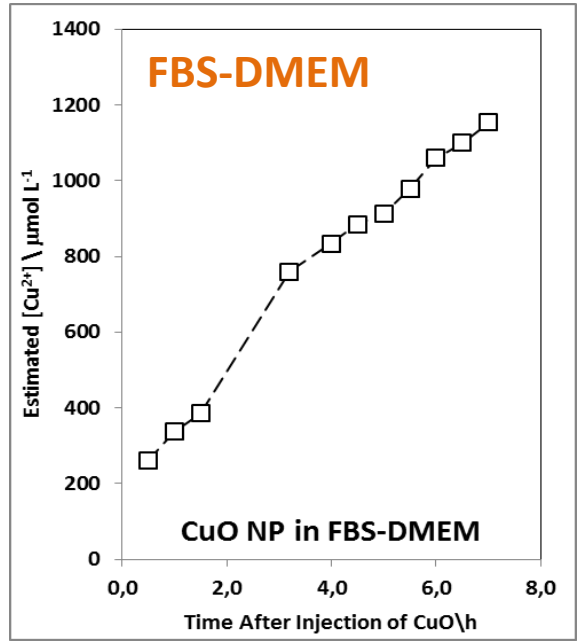
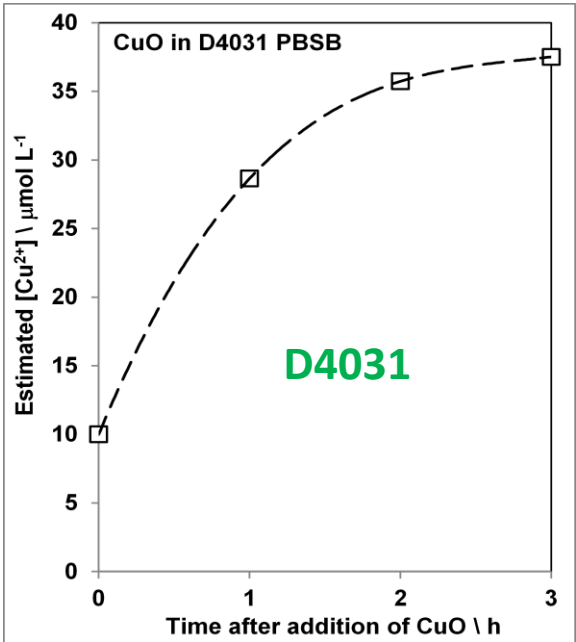
Baldisserri C., Costa A.L., *J Nanopart Res* (2016) 18:96



The amount of Cu released from CuO particles vs. time may be estimated by using the above calibration data to rescale maximum anodic current values to concentrations

This is easier in **D4031** and **FBS-DMEM**, since calibration curves do not saturate at high concentration. Amount of dissolved Cu never exceeds that from total dissolution

In **DMEM**, strong saturation of the calibration curve at high concentration hinders a reliable determination of the amount of released Cu in that medium. Current data indicate that the amount of Cu released in DMEM was much higher the calibration range.



Dissolution of Cu is strongly enhanced in DMEM and FBS-DMEM as compared to saline buffer

- Detection of Cu in can be accomplished by standard cyclic voltammetry at the Au electrode in CuCl_2 -added saline buffer and biological media
- Detection is possible down to nominal Cu^{2+} concentrations of a few $\mu\text{mol L}^{-1}$ using standard electrochemical gear and a gold wire electrode
- Adding CuO nanoparticles to the same media brings about the same effects as adding aqueous CuCl_2 , providing evidence of continued ion leaching from CuO NPs even at $\text{pH} = 7.4$
- Using calibration curves obtained in CuCl_2 -added D401, DMEM and FBS-DMEM media allows obtaining estimates of the amount of copper dissolved from CuO NPs dispersed in the same media. All estimates are below the concentration that would result from complete dissolution of CuO. CuO dissolution was estimated as 60% in FBS-DMEM 8 h after injection
- In all investigated media, a substantial fraction of the copper introduced as CuO NPs appears to undergo dissolution at physiological pH. Toxicological effects of CuO NPs may well be due to Cu^{2+} ions rather than CuO NPs.

The sensitive response afforded by electrochemical measurements is promising for the *in situ* detection, speciation, and biodistribution of ions.



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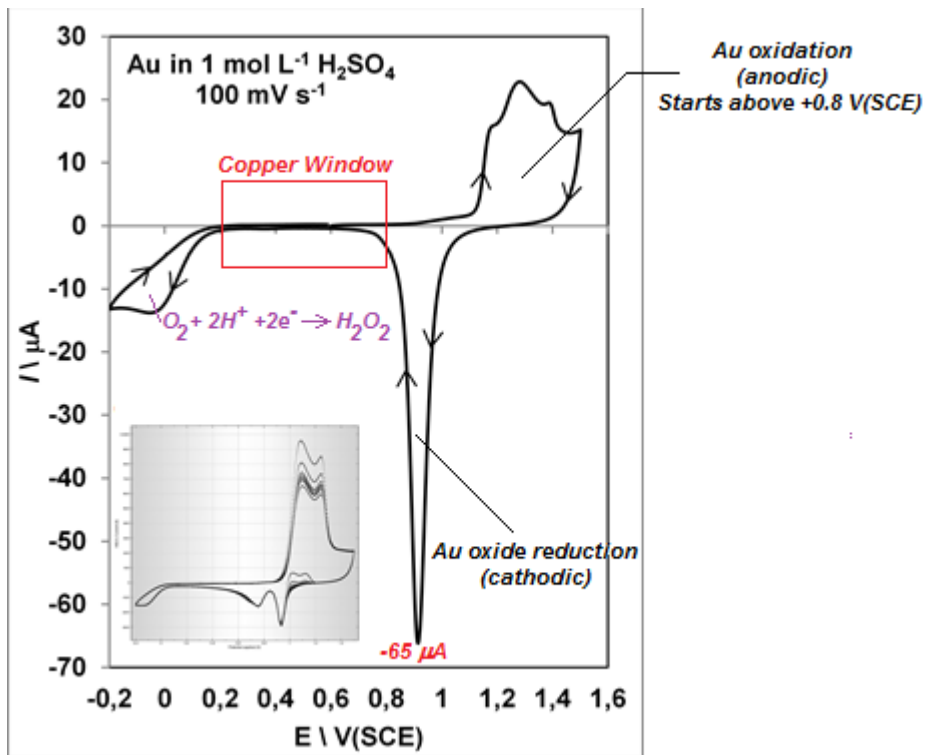
ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA



THANK YOU

Potentiostat:	Metrohm μAutolab FRA2 – Type III EcoChemie, The Netherlands
Reference electrode:	Saturated Calomel Electrode (SCE), +0.241 V(SHE) AMEL srl, Milan (Italy), type 303/SCG/12, kept in a separated vessel filled with 3M KCl; connected to the cell via an Agar/3M KCl polyethylene salt bridge
Working electrode:	Au wire, 200 μm diam., 14 mm length Goodfellow, 99.99% purity
Counterelectrode:	Coiled Pt wire, 500 μm diam., 100 mm length Goodfellow, 99.99% purity
Electrochemical Cell:	three-neck round bottom borosilicate glass flask

Phosphate buffered saline buffers:	ALDRICH D4031 used without modifications ALDRICH D8662 used without modifications
Biological media:	ThermoFisher Scientific 11995-065 Dulbecco's Modified Eagle's Medium (DMEM) ThermoFisher Scientific 11995-065+10% FBS DMEM: as above Fetal Bovine Serum (FBS): GIBCO Cat. # 10270-106
CuO suspension:	PlasmaChem CuO NPs, ca. 15 nm Lot# YF131107, 1 g L ⁻¹ in water
CuCl₂:	Obtained as a 1.67 mol L⁻¹ aqueous solution by reacting CuO (as above) with HCl (SIGMA ALDRICH 2258148, Lot# SZBD1160, 37% assay) , diluted as required
Water:	Milli-Q, 18.2 Ω cm



Cycling (10-20 times) the Au electrode in 1M H₂SO₄ causes the voltammetric curve to approach the shape shown in the figure, which is the standard in non-purged H₂SO₄ solutions

Only Au oxidation and reduction features should appear at potentials anodic of +0.8 V(SCE) in non-contaminated solutions.

The copper window is largely confined to the double-layer region of the electrode

The height of the cathodic peak is a good indicator of the electrochemical area of the electrode

Contamination of the solution brings about non-standard features (see inset). The chloride ion (Cl⁻) is a very effective contaminant, and may leak into the solution from KCl-filled salt bridges.

Table 1 Summary of experimental and derived parameters for Cu²⁺-D4031 PBSB, Cu²⁺-DMEM, Cu²⁺-FBS-DMEM, CuO-D4031 PBSB, CuO-DMEM, CuO-FBS-DMEM, and glycine in D4031 PBSB + 100 μmol L⁻¹ CuCl₂

System	Initial [Cu ²⁺] (μmol L ⁻¹)	Final [Cu ²⁺] (μmol L ⁻¹)	ΔE _{PEAK} (mV)	ΔI _{MAX} (μA)	ΔI _{MAX} /Δ[Cu ²⁺] (μA/μmol L ⁻¹)	ΔE _{PEAK} /Δ[Cu ²⁺] (mV/μmol L ⁻¹)	Linear range (μmol L ⁻¹)	Fig
CuCl ₂ in D4031 PBSB	0	100	0	2.25	2.3 × 10 ⁻²	0	0–70	4
CuCl ₂ in DMEM	10	100	+50	0.25	2.8 × 10 ⁻³	+0.6	10–50	9
CuCl ₂ in FBS-DMEM	200	900	0	2.0	2.8 × 10 ⁻³	0	200–1000	16
System	Initial [Cu ²⁺] (μmol L ⁻¹)	Final [Cu ²⁺] (μmol L ⁻¹)	ΔE _{PEAK} (mV)	ΔI _{MAX} (μA)	Δ[Cu ²⁺]/Δt (μmol L ⁻¹ /h)	ΔE _{PEAK} /Δt (mV/h)	Leached cu (%)	Fig
CuO in D4031 PBSB	10	37	0	+0.9	+12.3	0	2.9 over 3 h	7
CuO in FBS-DMEM	230	1114	0	2.45	+136	0	60 over 3 h	18
System	Initial [Cu ²⁺] (μmol L ⁻¹)	Final [Gly] (mmol L ⁻¹)	ΔE _{PEAK} (mV)	ΔI _{MAX} (μA)	ΔI _{MAX} /Δ[Gly] (μA/mmol L ⁻¹)	ΔE _{PEAK} /Δ[Gly] (mV/mmol L ⁻¹)	Fig	
Gly/ D4031+100 μmol L ⁻¹ Cu ²⁺	0	10.68	-84	-1.82	-0.17	-7.9	11	