PATROLS
Advanced Tools for NanoSafety Testing

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PATROLS Standard Operating Procedures (SOP)

Electrochemical measurement of the redox potential of nanoparticles in biological media

This is a SOP used by members of PATROLS only

Adapted from the NanoImpactNet SOP, Clift *et al* (Deliverable 5.4 under the European Commission's 7th Framework Programme, Grant Agreement 218539).

Authored by:

Anna Luisa Costa[1], Felice Simeone[1], Lara Faccani[1], Ilaria Zanoni[1] and Marina Serantoni[1]

[1]CNR-Institute of science and technology for ceramics (CNR-ISTEC), 48018 Faenza, Italy

Reviewed by:

Anna Luisa Costa[1], Ilaria Zanoni[1]

[1]CNR-Institute of science and technology for ceramics (CNR-ISTEC), 48018 Faenza, Italy

Document History:

Version	Approval Date	Description of the change	Author(s) of change
1.0	15/04/2021	Initial Document	Ilaria Zanoni
1.1	16/04/2021	Revised Document	Marina Serantoni
1.2	21/04/2021	Revised Docment	Felice Simeone
1.3	26/04/2021	Final Document	Anna Costa

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1 Introduction:

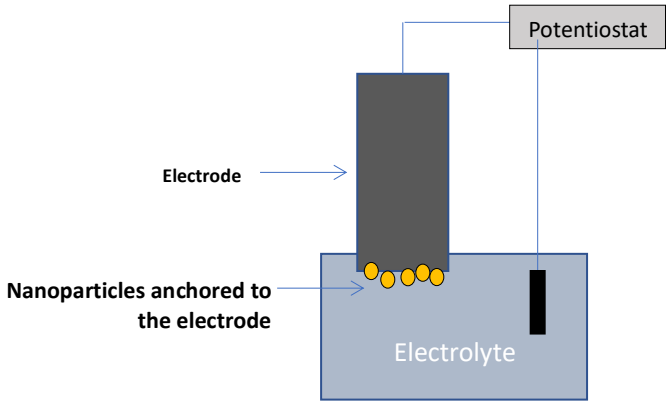
DOMAIN: Material characterization.

This document includes a description of the setup and standard operation procedure for conducting direct measurements of ENM redox potential, calculated from the reduction and oxidation peak that appear in a cyclic voltammogram.

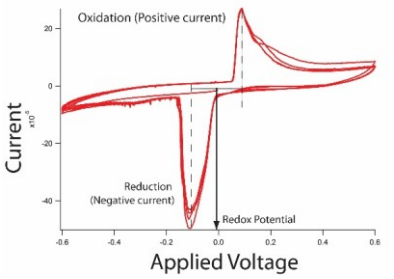
The redox potential of an ionic couple (i.e., oxidized + reduced form) can be calculated from the reduction and oxidation peak that appear in a cyclic voltammogram.[1] *A cyclic voltammetry is just a current-voltage plot for a circuit that include a solution of ions that can exchange electrons with the electrodes.*

A reduction is defined as the transfer of electrons from the electrode to a dissolved species; analogously, an oxidation occurs when electrons flow from dissolved species toward the electrode. Reduction and oxidation take place at applied potentials (voltages) characteristic of the species and generate peak of current in a current-potential plot of a cyclic voltammetry(negative for reduction; positive for oxidation) in a cyclic voltammogram

Cyclic voltammetry cannot be applied directly nanoparticles mainly because dispersion of nanoparticles are unstable, or become unstable when a support electrolyte (necessary to run electrochemical experiments) is added. By anchoring the nanoparticles on an inert electrode, cyclic voltammetry of nanoparticles can be executed independently of the composition of the support electrolyte.



In this configuration, the redox potential of the nanoparticles can be calculated from the positive and negative peaks of current.



We used this method to determine the redox potential of ENMs in different biological relevant media: DQ water + KCl, DMEM, Gamble's and PSF. The observed ENMs redox potential was then compared with redox potential range of cells, determined by the redox potential of NADPH and that of dissolved of O_2. We considered, as range limits, the nicotin amide adenine dinucleotide phosphate (NADPH/ NADP1) couple, which has a value of approximately -0,400 mV, and the couple 2SGH/GSSG has a redox potential of -0,265 V [2,3]; the redox potential of for the dissolved O_2/H_2O couple is approximately +0,800 V.

1.1 Scope and limits of the protocol

Kommenterede [IZ1]: Check

The scope of the research protocol is to provide a thermodynamical measure of the reactivity of ENM in an biological environment; in this context, we define "reactivity" as the ability of transformation of chemical species by exchanging electrons. By definition, the redox potential is a measure of the energy required to add electrons to a species; this energy is measured against the redox potential of hydrogen to which is assigned, conventionally, the redox potential 0 V; the higher the value of the redox potential (i.e., the more positive with respect to the redox potential of hyfrogen), the easier is to add electrons to the species (in other words, the more positive the redox potential, the higher is the affinity or the reactive species for electrons.) By comparing the redox potential of an ENM with the redox potential of the reactive species of the cells, it is possible to establish if the ENM will react with the cell by exchanging electrons.

The values of the redox potential of an electro-active species can be dermined from cyclic-voltammetry, which is a standard electrochemical method. Cyclic-voltammetry

records the current flowing across an electrode in contact with an interfase containing electroactive species. Anodic current peaks (conventionally positive) appear in correspondence of applied voltages that oxidize the species (strip electrons); analously, negative current peaks appear at voltages (i.e., potentials) that lead to the reduction (i.e., addition of electrons) to the species. Theory of cyclic voltammetry predicts that the value of the redox potential of an electroactive species is the half-way point between the potential (voltage) of the anodic peak, and the potential of the cathodic (negative) current peak.

The direct determination of the redox potential of ENM by cyclic voltammetry described in this document, however, does not provide any kinetic information about the redox reactions.

1.2 Validation state of protocol

Level of advancement towards standardization	**Level reached** (please mark only one with "X")
Stage 1: Internal laboratory method under development	
Stage 2: Validated internal laboratory method	X
Stage 3: Interlaboratory tested method	
Stage 4: Method validated by Round Robin testing	
Standardisation plans	
Is the method considered for standardisation (OECD SPSF or similar)?	N
Has the method been submitted for standardisation (to OECD, CEN, ISO,…) in its own right or as part of another standardisation project?	N
Is the method included in an existing standard (or ongoing standardisation work)	N

2 Terms and Definitions:

Nanomaterial
Material with any external dimension in the *nanoscale* or having internal structure or surface structure in the nanoscale.

Note 1 to entry: This generic term is inclusive of *nano-object* and *nanostructured material*.

[SOURCE: ISO/TS 80004-1: 2016, definition 2.4]

Engineered nanomaterial
Nanomaterial designed for specific purpose or function

[SOURCE: ISO/TS 80004-1: 2016, definition 2.8]

Particle
Minute piece of matter with defined physical boundaries.

Note 1 to entry: A physical boundary can also be described as an interface.

Note 2 to entry: A particle can move as a unit.

Note 3 to entry: This general particle definition applies to *nano-objects*.

[SOURCE: ISO 26824:2013, 1.1]

Substance
Single chemical element or compound, or a complex structure of compounds.

[SOURCE: ISO 10993-9:2009, definition 3.6]

Redox potential (Eh)

Redox potential is a measure of the tendency of a chemical species to acquire electrons and thereby be reduced. Redox potential is measured in volts (V), or millivolts (mV). For the generical species A, the redox potential is determined for the half-reaction

$A + ne^- = A^{n-}$

Where n is number of electrons exchanged, and, conventionally, the half reaction is completed by the oxidation of hydrogen to which is assigned the redox reference potential 0 Volt (thi half-reaction of hydrogen is never shown but implicitly assumed.) For a generic redox reaction, the redox potential of A is given by the Nernst equation:

$E_h = E_h° - 0.0592/nF \cdot \log([A^{n-}]/[A])$

Where F is the Faraday, that is, the charge of a mole of electrons, and Eh° is the standard redox potential obtained for $[A^{n-}]$ and [A] = 1M. It can be demonstrated that Eh° is the half-way potential between the potential of the peak of oxidation and the potential of peak reduction in a cyclic voltammetry.

[ISO ?]

Kommenterede [IZ2]: @Felice: Aggiungere ISO o riferimento normative per la definizione di REDOX

Potentiostat / Gavanostat instrumentation

Description

[ISO ?]

Kommenterede [IZ3]: @Felice: Aggiungere ISO o riferimento normative per la definizione di REDOX

Cyclic voltammetry

Cyclic Voltammetry records the current flowing through a solution contining electroactive species, that is, species that can exchange electrons with two metallic electrodes in contact with the solution. The voltage applied to the electrodes is varied cyclically and linearly between two values that should include the voltage at which the dissolved species tranfer or accept electrons to or from the electrode. In the present context, nanoparticles are the species that tranfer electrons; because they are anchored to the surface electrode (working electrode), diffusion rates within the solution do not contribute to variations of the current.

To fine control the voltage applied to the electrode loaded with the nanoparticles, a three-electrode configuration is adopted; this configuration makes it possible to change the voltage applied to the working electrode by connecting it to a reference electrode, which is fabricated in a way that its voltage (i.e., potential) cannot be changed. Finally, the circuit is close by a third electrode, the counter electrode whose beahvior is not relevant for the study of the redox potential of the ENP.

[ISO ?]

Kommenterede [IZ4]: @Felice: Aggiungere ISO o riferimento normative per la definizione di REDOX

Working Electrode

3 Abbreviations:

ENM	Engineered nanomaterial
CV	Cyclic voltammetry
Eh	Redox potential

SCE	Saturated calomel electrode
NMs	Nanomaterials
NADPH	Nicotinamide adenine dinucleotide phosphate
2SGH/GSSG	?

Kommenterede [IZ5]: completare

4 Principle of the Method:

The method is based on the direct measurement of NMs redox potentials. The value of Eh, also termed the electromotive force, is a quantitative measure of the tendency of a redoxactive molecule to donate or accept electrons, expressed relative to a standard electrode.

5 Description of the Method:

5.1 Chemicals and reagents used:

Chemicals and reagents used in the protocol are reported in Table 1.

Table 1 List of chemical and reagnets used in the protocol.

Substances	CAS#	Supplier
KCl	text or no.	text or no.
DMEM	text or no.	text or no.
	Special line to be highlighted or subtitles	text or no.
	text or no.	text or no.
	text or no.	text or no.
	text or no.	text or no.

Kommenterede [IZ6]: @Felice & Lara & Marina aggiungete qui tutte in fo richieste

5.2 Apparatus and equipment used:

The SOP is based on the use of a Potentiostat / Gavanostat instrumentation with 3-electrodes cell. The equipment requires a current values of maximum 2 A, an applied potential range from -10 V to +10 V, and a dedicated acquisition software.

Concerning instrument calibration, Potentiostat / Gavanostat functionality is periodically verified by dommy cell. The calibration procedure depends on the instrument model and is reported in the instrument manual.

Kommenterede [IZ7]: @Felice / Marina giusto?

5.3 Health and safety precautions:

Training of personnel should be completed before any person is working with this SOP and Potentiostat instrumentation, considering all potential risks associated with chemicals / substances use and electric voltage applied during electric measurements, in compliance with national regulation.

5.4 Applicability:

This SOP will be use for the determination of redox potentioal of oxide NPs / NMs in different media. In principle, there are no specific resctictyions in media used or particles kind.

5.5 Reagent preparation:

WORKING ELECTRODE PREPARATION PROCEDURE

A piece of graphite rod (diameter 3 mm, 2 cm length) is used as working electrode:

1) Cut one end of the bar with a cutter to have an uncontaminated surface.

2) Sonicate for 10 minutes in ultrapure water (18,2 MOhm cm) to remove the debris.

3) Boil for 1 hour in ultrapure water and rinse with fresh ultrapure water, let dry.

Kommenterede [IZ8]: @Felice lo lasciamo? Possiamo anche ometterlo

4) Stock and electrode preparation: starting from NMs at power-state, 0,5 microliter of NMs ethanol solution (2560 ppm), containing the nanomaterial to be examined, is

dropped on the base of the electrode; (the bar will have a lateral notch near the end not added with NPs).

5) Allow the deposited solution to dry so that the NPs adhere to the surface.

6) Store the modified electrode in a sealed container.

5.6 Procedure:

EXECUTION OF THE REDOX POTENTIAL MEASUREMENT

A. Remove the naturally dissolved oxygen from the measuring solution (medium) by bubbling nitrogen for 10 minutes;

B. Insert the reference electrode (calomel, SCE) and the counter electrode (platinum spiral) into the electrochemical cell;

C. The modified graphite electrode is the working electrode. It can be kept in place inside the measurement cell by using a crocodile clip. In order to maintain the working electrode area as constant as possible, attention in paid in inserting the electrode in the solution only for the length needed to form a meniscus;

D. Perform from 2 to 6 cycles of voltammetry between -1.5 V and + 1.5 V vs SCE to identify redox peaks;

E. Compare the voltammetry obtained with the modified electrodes to that obtained with the electrode without NPs (baseline);

F. Once any peaks have been identified, optimize the voltammetry potential range to highlight the identified peaks.

G. After the measurement, remove the graphite electrode from the cell, wash it with deionized water and store it for measurement in another medium.

5.6.1 Testing for nanomaterial interference:

As described in the working electrode preparation, particular care was taken in testing only one NP / NM in a new graphite road electrode, avoinding any possible contaminations from previous analysis.

5.7 Quality control & acceptance criteria:

The base line was produced for each media using a new graphite electrode without nanoparticles.

6 Data Analysis and Reporting of Data:

Each CV produced was analysed to read the position of the redox reaction and NPs were considered active only if their redox potential was included in to the potential window of cellular activity.

7 Publications:

8 References

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