Electrochemical Immunoassays

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Immunoassays

A biochemical test that measures the concentration of a substance in a biological liquid, typically serum or urine, using the reaction between antibody and its antigen.
Immunoassays
enzyme Immunoassay
radioimmunoassay
fluoroImmunoassay
metalloImmunoassays
Immunoassays

ELISA

Lateral flow strips
The point of care device has to be:

- Easy to use
- Small and inexpensive
- Portable
- Specific
- Robust
- Fast
- Working with low volume and turbid samples

The new electrochemical immunoassay has the potential to fulfil all the above requirements !!!
Electrochemical Immunoassays with gold nanoparticle

HBr/Br₂ → Au³⁺
Electrochemical Immunoassay Format
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Electrochemical Immunoassay Format
Electrochemical Measurement Process:

\[ \text{Ag}^+ \times 10^6 ! \]

ASV
Magnetic Particle – Antibody Conjugate

Amide bond
Silver Sol – Antibody Conjugate

**Mechanism:**

Proteins absorb to silver surfaces through:

1. **Hydrophobic interaction** (Tyr, Trp, His and Phe)

2. **Electrostatic interaction** (carboxylates of Asp, Glu and C-termini)

3. **Dative bonds** through sulphur atoms (Cys and Met) or nitrogen atoms (Trp and His)
Silver Sol – Antibody Conjugate

poor-quality conjugates:

Wrong orientation

Aggregation

Denatured/Inactive antibody
Silver Sol – Antibody Conjugate optimisation:

1. Silver sol : antibody ratio
2. Buffer type (PBS, TBS, Borate)
3. Buffer molarity
4. Buffer pH
5. Blocking agent
Performance of 6 different silver conjugates

- 0.2M H3BO3 pH 6.5
- 0.4M H3BO3 pH 6.5
- 0.1M H3BO3 pH 7.5
- 0.01M H3BO3 pH 7.5
- 0.1M H3BO3 pH 8.5
- 0.01M H3BO3 pH 8.5

Log (Myoglobin [ng/mL]) vs. Microcoulombs [µC]
Electrochemical Measurement Process:

\[ \text{Ag}^+ \times 10^6 \]

ASV
Silver Sol Dissolution

\[ Ag^0 + Fe^{3+} \rightarrow Ag^+ + Fe^{2+} \]

\[ Ag^+ + m(NH_4^+) + n(SCN^-) \rightarrow [Ag(NH_4^+)_m(SCN^-)_n]^{m-n+1} \]
ASV scan of Silver Colloid with and without Fe (III)
Silver Sol can be measured with no oxidant!
Silver Sol in the presence of thiocyanate forms aggregates with the SCN- layer surrounding it.

\[
\text{NH}_4\text{SCN} \quad \rightarrow \quad \text{Ø} = 40 \text{ nm} \quad \text{Ø} = 400 \text{ nm}
\]

\[
\text{Zeta Potential} = -50 \text{ mV} \quad \text{Zeta Potential} = -10 \text{ mV}
\]
UV-Vis spectrum of Silver Colloid
Electrochemical Measurement Process:

\[ \text{Ag}^+ \times 10^6 \]

ASV
Carbon Screen Printed Electrodes

- Working Electrode
- Reference Electrode
- Counter Electrode
Carbon Screen Printed Electrodes
The voltage value at WE during different steps of ASV

Nucleation Step

Accumulation Step (Reduction)

Stripping step (Oxidation)

pre-treatment

0.4 V

-1.6 V

-1.2 V

time [s]
The influence of pre-treatment potential (t=10s) on current signal. Signal from Ag+ is potential-independent; signal from silver sol is potential-dependent.
The influence of pre-treatment time (at $U = +0.4V$) on current signal. Signal from Ag+ is time-independent; signal from silver sol is time-dependent.
Pre-treatment: THIOCYANATE MOLARITY

Ag+ vs Silver Sol in 1M NH₄SCN

The influence of NH₄SCN molarity on current signal. Signal from Ag+ is basically constant; signal from silver sol is thiocyanate-dependent. (Pre-treatment time t=10s at 0.4V).
Electrochemical reactions at the WE:

1. Silver sol dissolution and formation of electroactive chelate: ammonium thiocyanatosilver(I) at +0.4V

\[
Ag^0 \leftrightarrow Ag^+ + e^-
\]

\[
Ag^+ + m(NH_4^+) + n(SCN^-) \rightarrow [Ag(NH_4^+)_m(SCN^-)_n]^{m-n+1}
\]

2. Accumulation step of ASV (reduction) at –1.2V

\[
[Ag(NH_4^+)_m(SCN^-)_n]^{m-n+1} + e^- \rightarrow [Ag(NH_4^+)_m(SCN^-)_n]^{m-n}
\]

3. Stripping step of ASV (oxidation) -1.2V → +0.1V

\[
[Ag(NH_4^+)_m(SCN^-)_n]^{m-n} \rightarrow [Ag(NH_4^+)_m(SCN^-)_n]^{m-n+1} + e^-
\]

NPL

National Physical Laboratory
Anodic Stripping Voltammetry

0.4 V/15s
Anodic Stripping Voltammetry

\[ + + + + + + + + + 0.4 \text{ V} \]
Anodic Stripping Voltammetry

- - - - - - - - - - - - - - -1.2 V
Anodic Stripping Voltammetry

-1.2 V, t=55s
Anodic Stripping Voltammetry

Analytical Signal

\[ \text{Analytical Signal} \]

\[ \begin{align*}
\text{Ag}^+ & \rightarrow \text{Ag}^+ \\
\text{Ag}^+ & \rightarrow \text{Ag}^+ \\
\text{Ag}^+ & \rightarrow \text{Ag}^+ \\
\text{Ag}^+ & \rightarrow \text{Ag}^+ \\
\text{Ag}^+ & \rightarrow \text{Ag}^+ \\
\end{align*} \]
Electrochemical signal is related to analyte concentration

\[ Q = I \times t \]

[Ag⁺]

[Analyte]
Assay for Myoglobin

![Graph showing the relationship between Log Myo (pM) and Microcoulombs (µC)].

- **Absorbance**
  - **Argento**: Pink squares with error bars.
  - **ELISA**: Blue diamonds.

**Legend**:
- Argento
- ELISA

**Axes**:
- **Log Myo [pM]**
- **Microcoulombs [µC]**
- **Absorbance**
Multiplexing

Au
Ag
Cu
Multiplexing

ASV scan of 40ppm Ag⁺ and 40ppm Au³⁺ in 0.1M HCl
Summary

1. A new sensitive platform technology. Myoglobin down to 0.2 ng/mL.

2. The problematic oxidative dissolution step eliminated.

3. The assay can be used in Point of Care devices.
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