Electrochemistry in diagnostics

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biorecognition

• Life in molecular level is organized according to hierarchy of recognition and non-recognition. Enzyme-substrate, nucleic acids, immune reactions, etc. – called "bioaffinity".

• Currently molecules in body fluids are predominantly analyzed by exploiting their bioaffinity.
Procedure

- Label = measurable marker molecule or atom
- Number of molecules $A$ is determined with labelled antibodies binding to $A$ (Ab-L):
  - $(X$ copies of) $A$ + $(Y$ copies of) Ab-L $\rightarrow$
  - $(X)A$-Ab-L + $(Y-X)$ Ab-L ; $(Y>X)$
- Mixture is purified to contain pure $A$-Ab-L
- Number of $A$-Ab-L is measured = number of L
How L is measured?

- Fluorescent or luminescent labels are currently used and an evident choice for future.
- L is induced or "excited" to produce light emission.
- Excitation is done with light or with electrical pulses.
Basic electrochemistry

- **Electrolyte**: water solution containing salts. Salts form ions in water and allow electric current through water.
- **Electrodes** (metal or graphite) connect electricity source to electrolyte.
- Inert or reactive electrodes.
- Electric current from anode to cathode effects electrode reactions, charge or discharge of ions in solution.
- Water decomposition, example.

**Electrolysis**: Splitting water with electricity to produce hydrogen and oxygen:
Electrode reactions

- Different molecules are reduced or oxidized at different potentials on inert electrodes.
- Water decomposition starts at a certain voltage. Not possible to use this area for excitation of labels.
- Redox voltage of L must be less that that of water.
Potential window at inert anode is limited for exciting L

- Bioaffinity reaction must be done in water.
- Organic solvents can decrease decomposition of water and allow higher applied voltages to excite L.
- Still, limited number of L are measurable (Roche:rutheniumbipyridium)
- UV –excitation not possible
How to widen the potential window in water?

• Excitation of labels is carried out by very high energy, “hot electrons” at CATHODE.

• Hot electrons are achieved by forcing electrons to “jump” over an insulator barrier with pulsed voltage of 10-30 V.

• Normal electrons jump 1-2 nm BUT hot electrons jump up to 100-200nm distance from electrode. Gas evolution occurs only at high voltages → strong redox reactions possible in 100 times higher volume.
Highly reducing and oxidizing conditions are simultaneously achieved by injection of hot electrons into aqueous solution from cathodically pulse-polarized thin film-coated electrodes.
The distance from the working electrode, within which HECL occurs, has been estimated from $e_{aq}^-$ reactivity and diffusion coefficients to be on the order of 200 nm.

- Labeled-antibody: 15 nm
- CRP: 8 nm
- Catching antibody: 15 nm

Insulation layer
Cathode
IPR

• Rosche Diagnostics owns patents on excitation on inert metal anode. Applied in practise world widely.

• Labmaster Ltd. Turku, owns patents on excitation of labels on insulator-covered cathode ( >10 inventions).

• ”hot electron electrochemistry” - ”HECL”, development stage for POC-diagnostics. Very potential technology for future.
Advantages of cathodic excitation over old methods

• Water solutions are used throughout, no need to change to organic ”measuring” solutions.
• Any kind of labels can be excited to produce emission from UV to IR range.
• ”Time-resolved” technology can be used to increase sensitivity (require UV excitation and long-life luminescence label).
• Internal calibration from insulator´s fluorescence signal.
• Cathode can be cheap metal because it is covered with inert layer → single used test sticks → POC
• Multiplexing easy
• Cheap simple measuring instrument, no optics
Time-resolved measuring principle

Excitation pulse time 0.3 ms

Counting time 8 ms
Simultaneous excitation of short-lived and long-lived luminescence displaying labels using HECL

Tb(III) chelate + Ru(bpy)$_3^{2+}$

Eu(III) chelate + FITC
Cells for multiplexing and printable electrodes
Strictly Private and Confidential. Do not copy.

Analyte (sample) → Membrane → Capture Antibody → Labeled Antibody → Silicon chip

Capture Antibody

Silicon chip

PMT Measuring HECL

\( \epsilon_{hot} \) \( \epsilon_{hot} \) \( \epsilon_{hot} \)
The comparison methods: **Roche Hitachi 917 Tina-Quant® CRP** (latex) high sensitive assay and **Innotrac Aio! usCRP** immunofluorometric assay.

*Figure 1. Linearity and variation of standards usCRP assay performed by PiIA ECL analyser.*

*Figure 2. Correlation between usCRP assay performed by PiIA ECL analyser and two reference methods using plasma samples; concentration range < 10 mg/L.*

*Figure 3. Correlation between whole blood and heparin plasma samples. Whole blood samples have been corrected for hematocrit.*
What is needed for using HECL and Time-Resolved Detection in Analysis?

- A pulse generator
- Electrochemical cell (e.g. a cassette)
- Photon counter
- A laptop computer or internal microprocessor

*PiiA ECL analyser*
Thank you!
ご清聴ありがとうございました