Non-invasive measurements in sport: Biosensors and the detection of physiological biomarkers for health and fitness

Vesa Virtanen, Research director, PhD, Docent
Laboratory of biotechnology
Kajaani University Consortium
Sotkamo

UNIVERSITY of OULU
OULUN YLIOPISTO

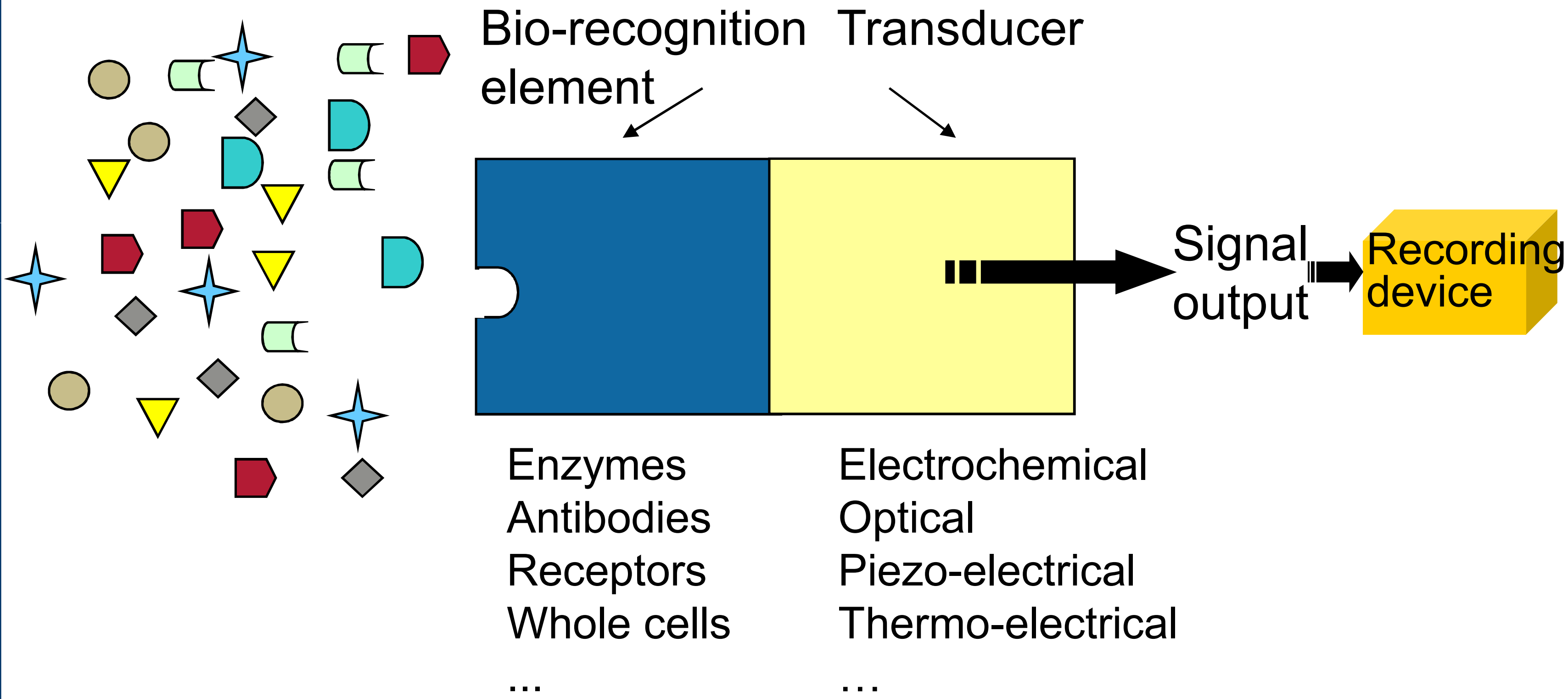
What is a Biosensor: Definition

”**A** biosensor is a compact analytical device incorporating a biological or biologically-derived /mimicking sensing element intimately integrated with a physicochemical transducer. The general aim of a biosensor is to produce either discrete or continuous digital electronic signals which are proportional to a single analyte or a related group of analytes”

What is a Biosensor?

A biosensor is a analytical device that converts a biological response into an electrical signal.

The “Classical” Definition

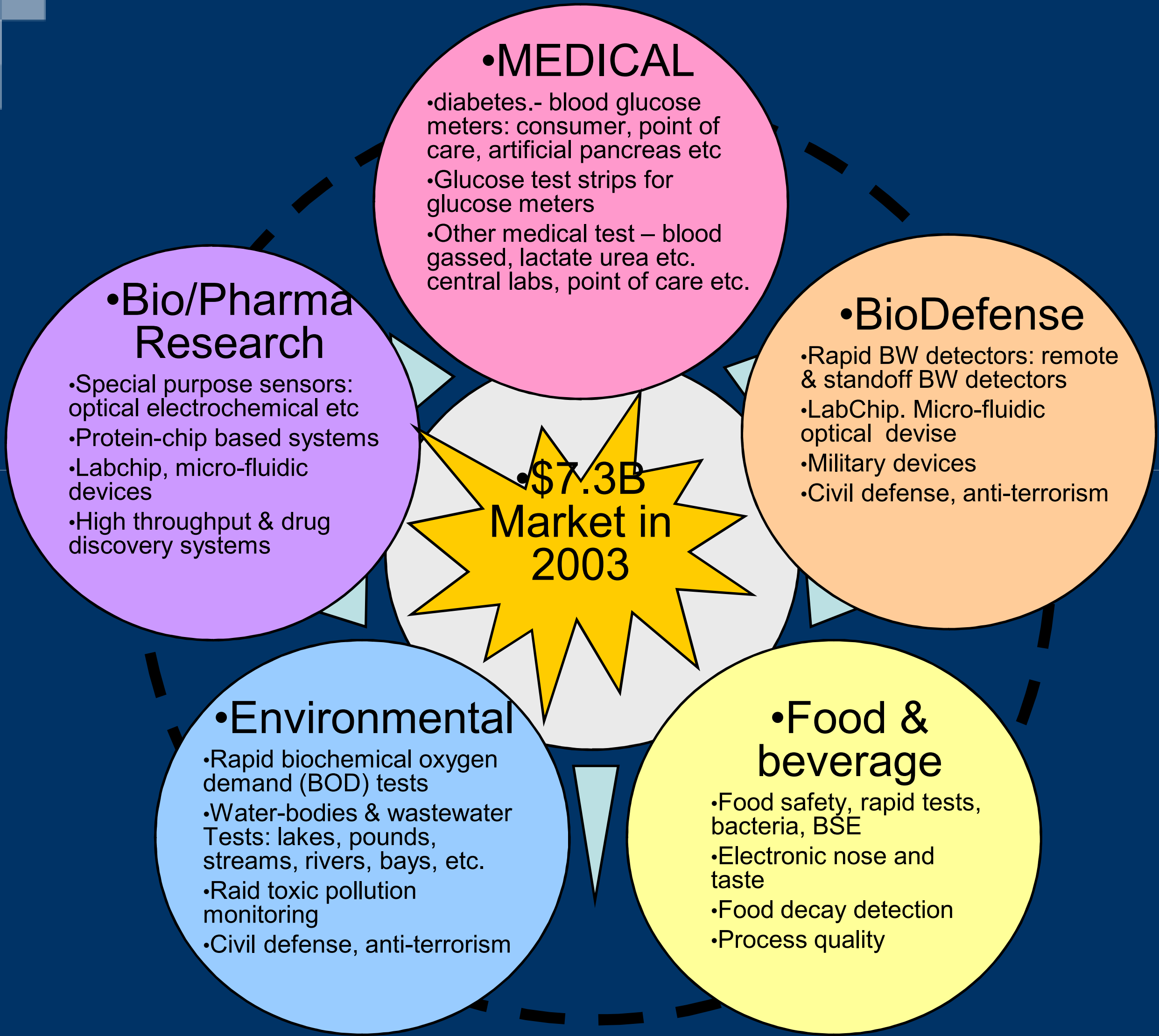


Why is there an interest in biosensor?

Biosensors are:

- Important tools in clinical and environmental diagnostics, medical monitors, food safety and warning systems for warfare agents (security)
- Important devices offering simplicity both in and outside the analytical laboratory
- Selective, rapid and sensitive instruments for detection of chemical and biochemical targets

Biosensors Market share



$7.3 billion in 2003

10.2 billion in 2007

A growth rate of approx. 10.4%

BIOSENSORS: History

- 1956: Oxygen electrode (Clark)
- 1962: Enzyme electrode
- 1972: Commercial glucose sensor (YSI)
- 1975: Microbe-based biosensor
- 1983: Surface plasmon resonanse (Biacore)
- 1987: Screen printed enzyme electrode (MediSens)

Present applications of biosensors

- Medical care and diagnostics (both clinical and within the laboratory)
- Determination of food quality
- The detection of environmental pollutants
- Industrial process control

Tomorrows application of biosensors

- Personalised point of care medical devices
- Dynamic wearable and implanted sensors
- Personalised health and fitness monitors
- Interactive environmental sensors and active protection devices

Endless possibilities

Modern biosensor research is highly multi-disciplinary. However, evolved from two disciplines

– Biochemistry
– Information technology

New research openings are being created with the increase acceptance of the analytical techniques and cross over of different fields of thought

Why are we interested in Biosensors?

- To develop biosurfaces to be used in different types of biosensors and biosensor devices that can measure important physiological markers related to health, fitness and performance.
- Also detection of pharmaceuticals and markers for environmental surveillance.

Physiological changes and biomarkers

- During pre-longed physical activity physiological changes in the bodies' biological chemistry occur
  - (e.g. Cortisol, Lactate, α-Amylase, Anti-bodies, Proteins, Electrolytes)
- The concentration of these analytes can give a good indication of the health, fitness and performance of an athlete.
- Theses analytes can be measured in blood, saliva and urine.

Physiological measurements on human; Why Saliva ?

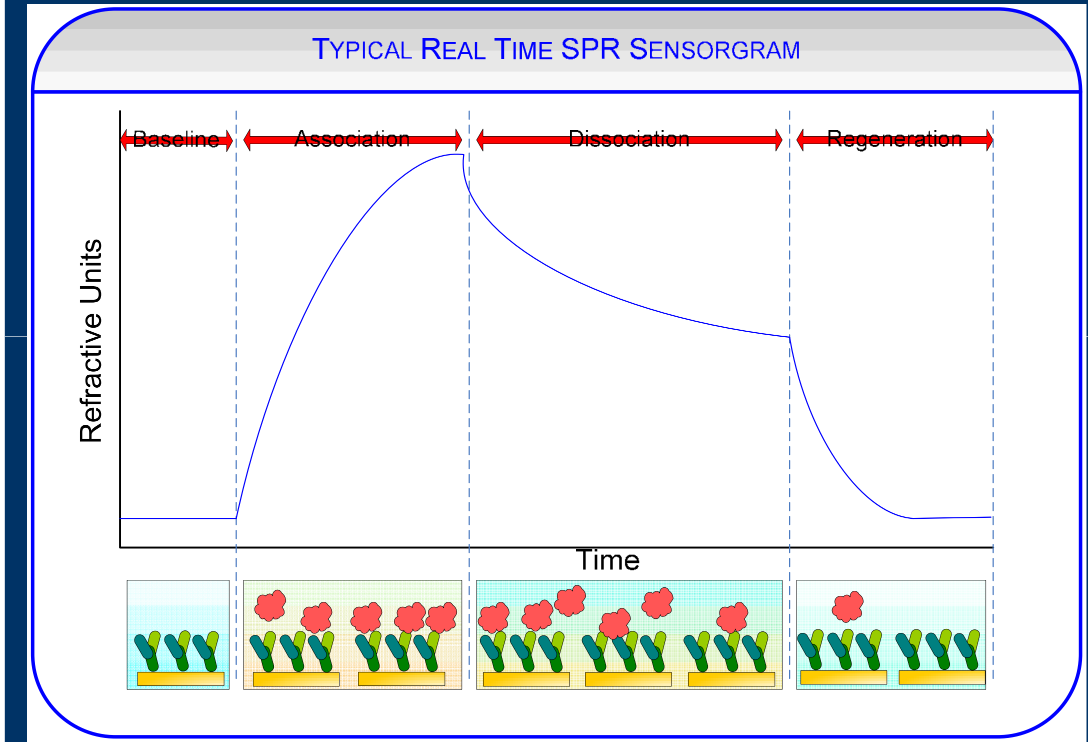
- Easily sampled non invasively without much discomfort or stress to the athlete/individual.
- Shows good correlation to whole blood and serum.

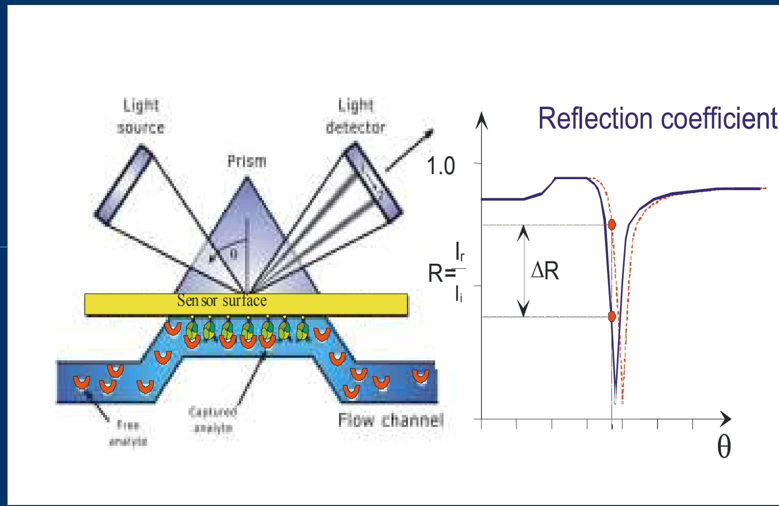
Compounds of interest	
Antibody IgA	Indicates infections and compromised immunity
a-Amylase	Relates to anaerobic threshold and stress recovery
C-Reactive protein (CRP)	Inflammation bio-marker
Cortisol	Stress recovery and over-reaching
Lactate	Anaerobic and aerobic threshold

Surface Plasmon Resonance Biosensing (SPR)

- Highly sensitive method
- Can be small and portable/hand-held
- Real time analysis, no need of label required

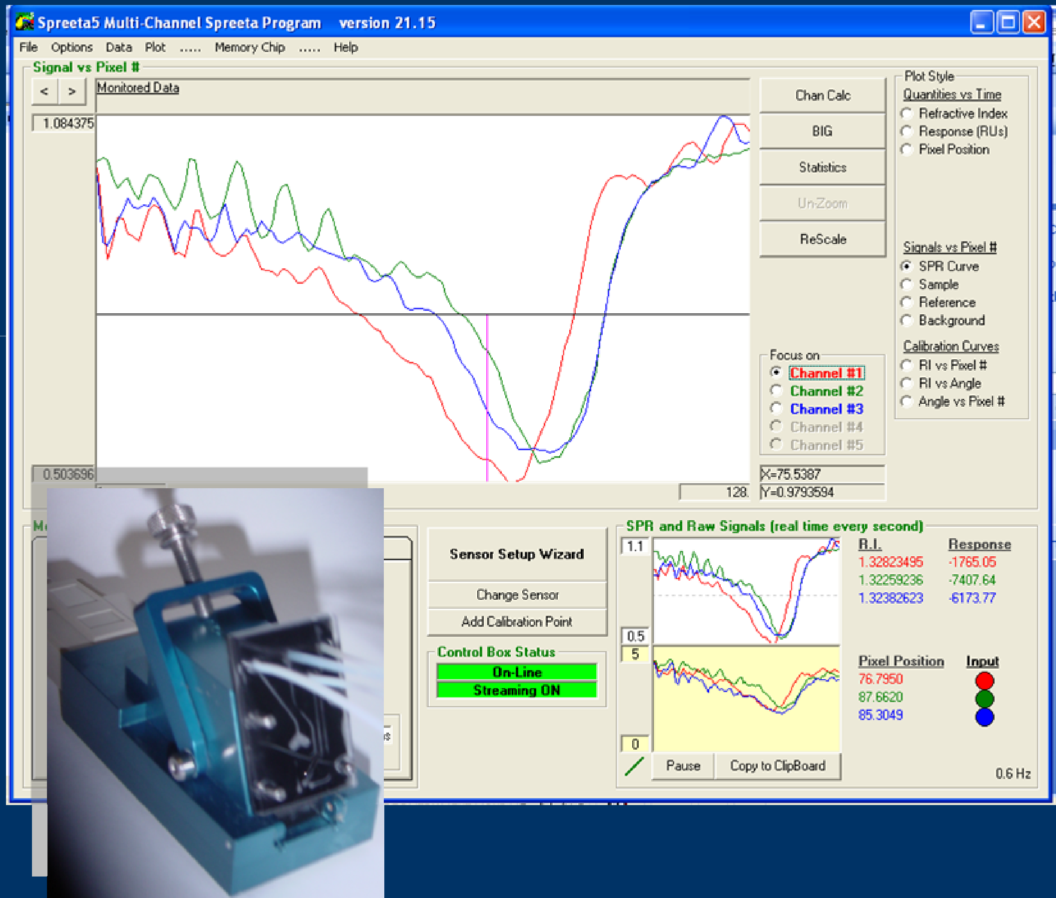
Surface Plasmon Resonance Biosensing (SPR)



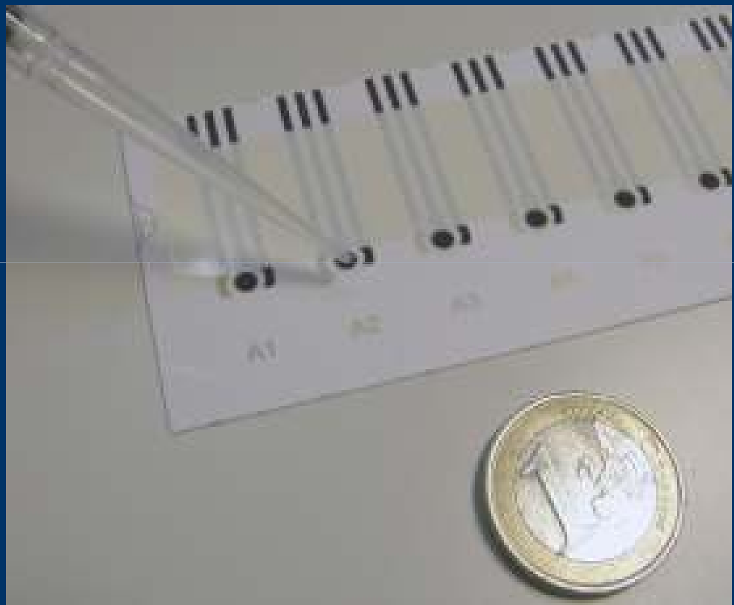


Label free, real time analysis

Surface Plasmon Resonance Biosensing (SPR)



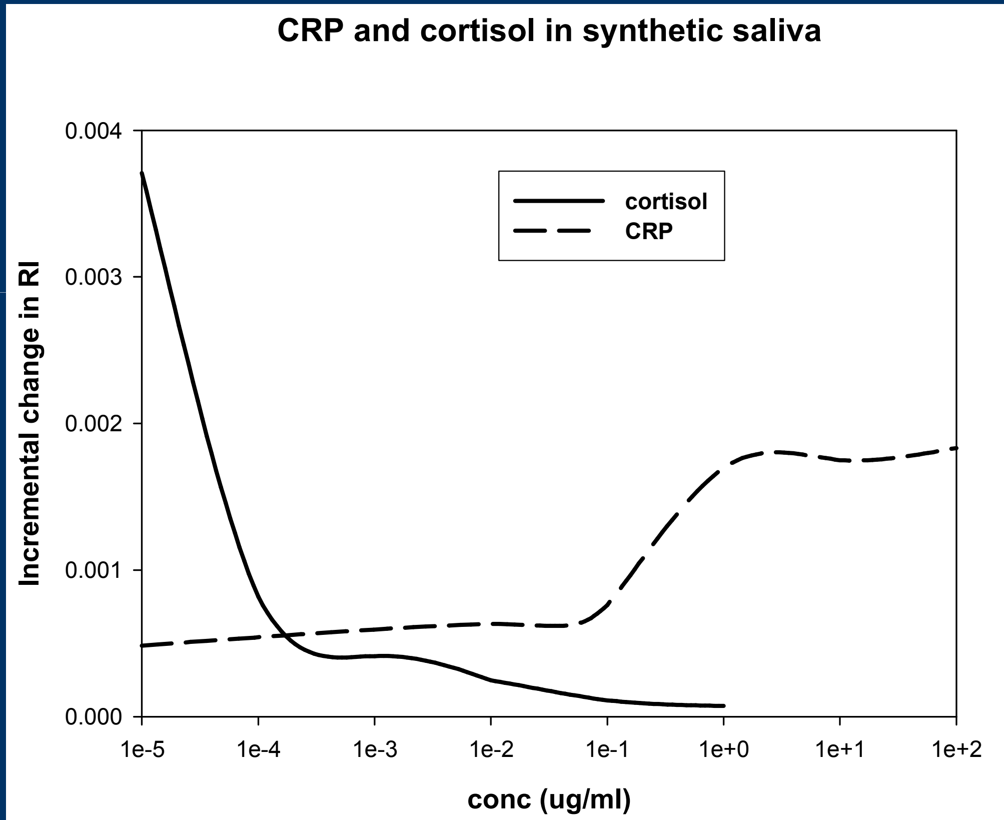
Electrochemical Bio-sensing



Screen printed electrode

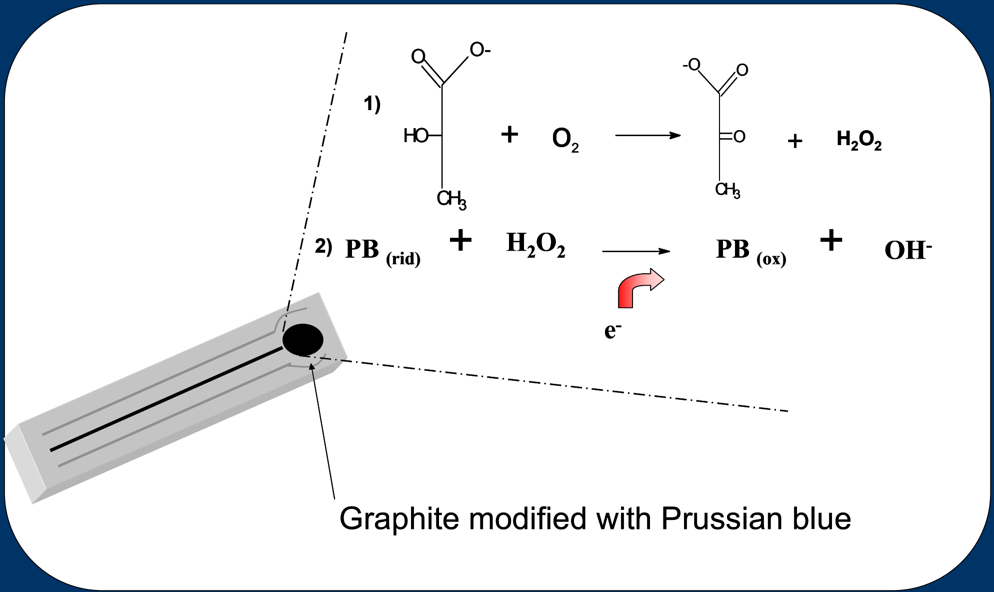
Portabel Potentiometer

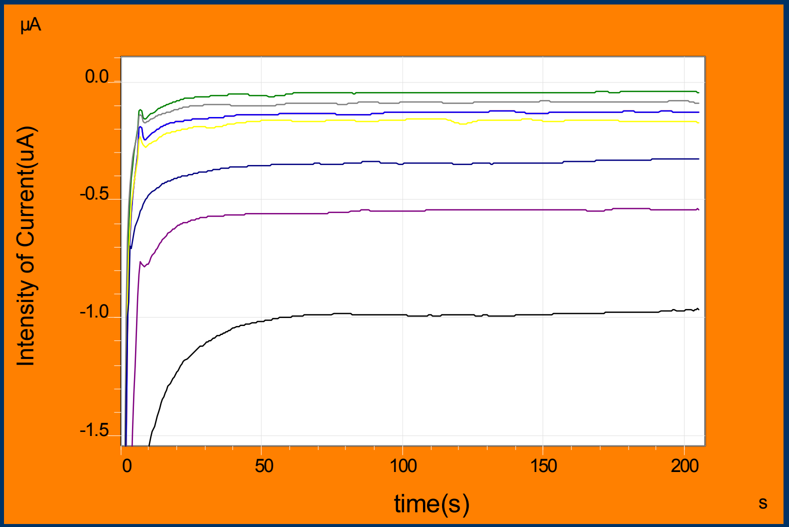
SPR Simultaneous detection of C-reactive protein (CRP) and cortisol



Determination of L-Lactate using Lactate Oxidase

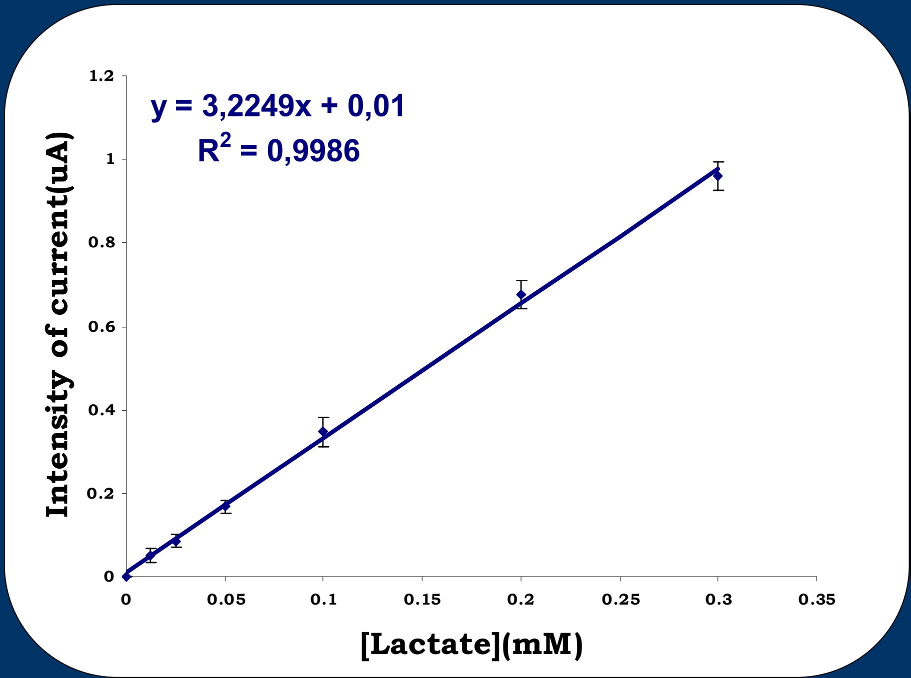
$$\text{LACTATE} + O_2 + H_2O \xrightarrow{\text{LOX}} \text{ACID PYRUVIC} + H_2O_2$$



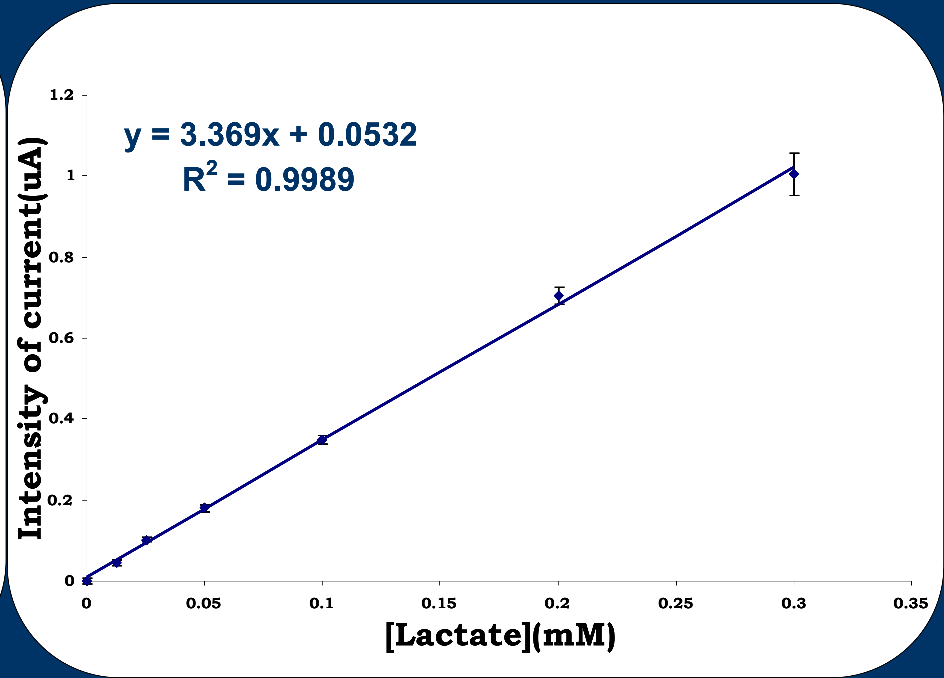


Calibration sensorgram curve as shown from the PalmSens instrument

L- Lactate : linearity



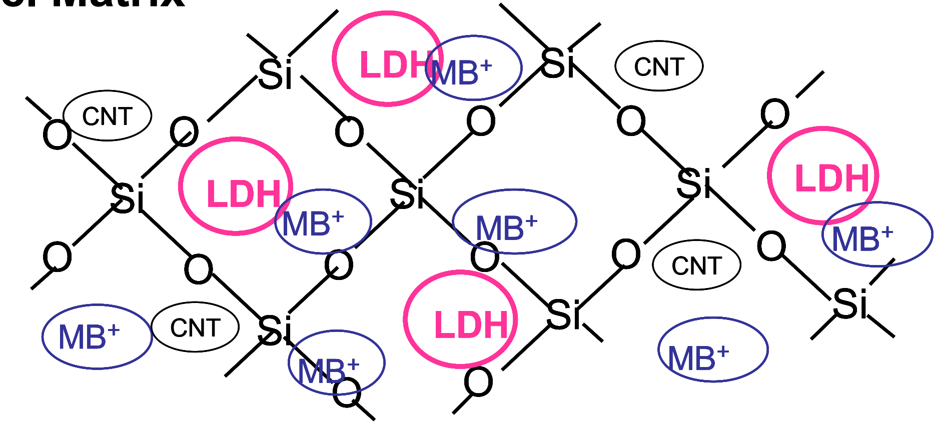
Inter electrode repeatability n=5



Intra electrode repeatability, n=3

Screen Printed Electrode Modified with a Nanocomposite Material

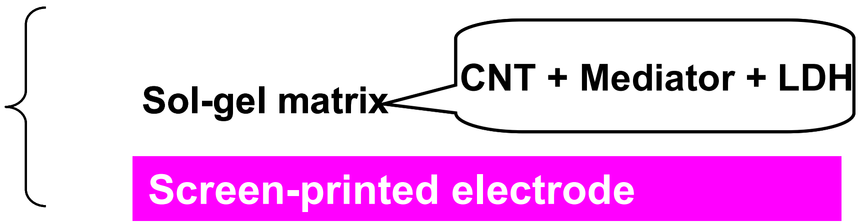
Sol-Gel Matrix



LDH = Lactate dehydrogenase

MB = Mendola's Blue

Electrode Configuration



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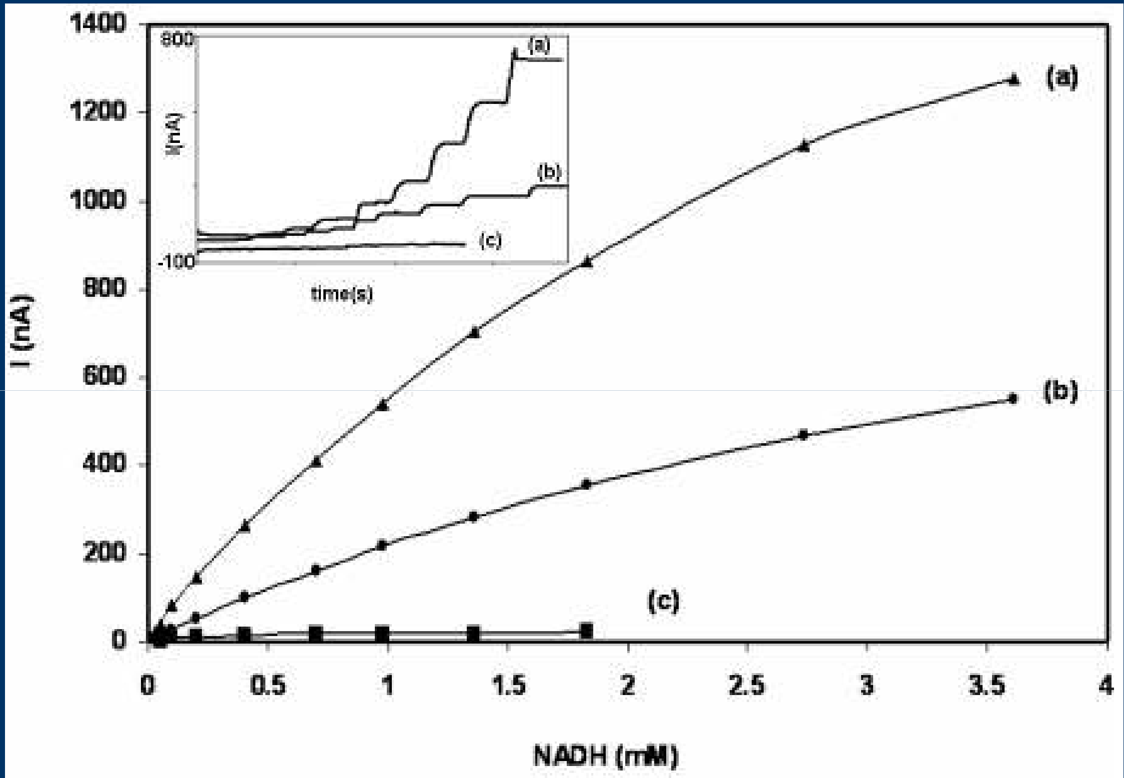
Calibration plots for NADH obtained with sol-gel based composite material



•:(a) MB-SWNT-SG/SPE, (b) MB-SG/SPE and (c) SWNT-SG/SPE; E=-50 mV;.

•Inset: Amperometric response of (a) MB-SWNT-SG/SPE, (b) MB-SG/SPE and (c) SWNT-SG/SPE for successive additions of NADH 50 mM in PBS solution, pH 7.5.

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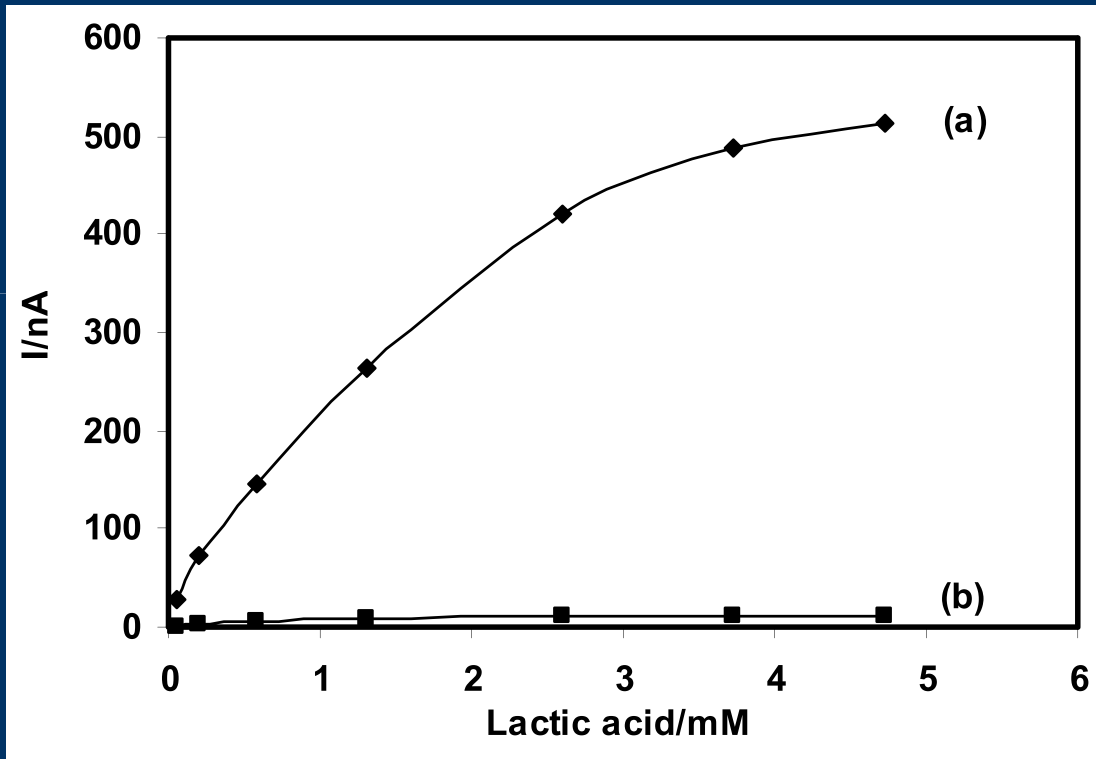
D-lactate measurements



•Calibration curves for D-lactate obtained for SPE modified with 0.78 UI LDH immobilized in composite material: (a) with SWNT and (b) without SWNT;

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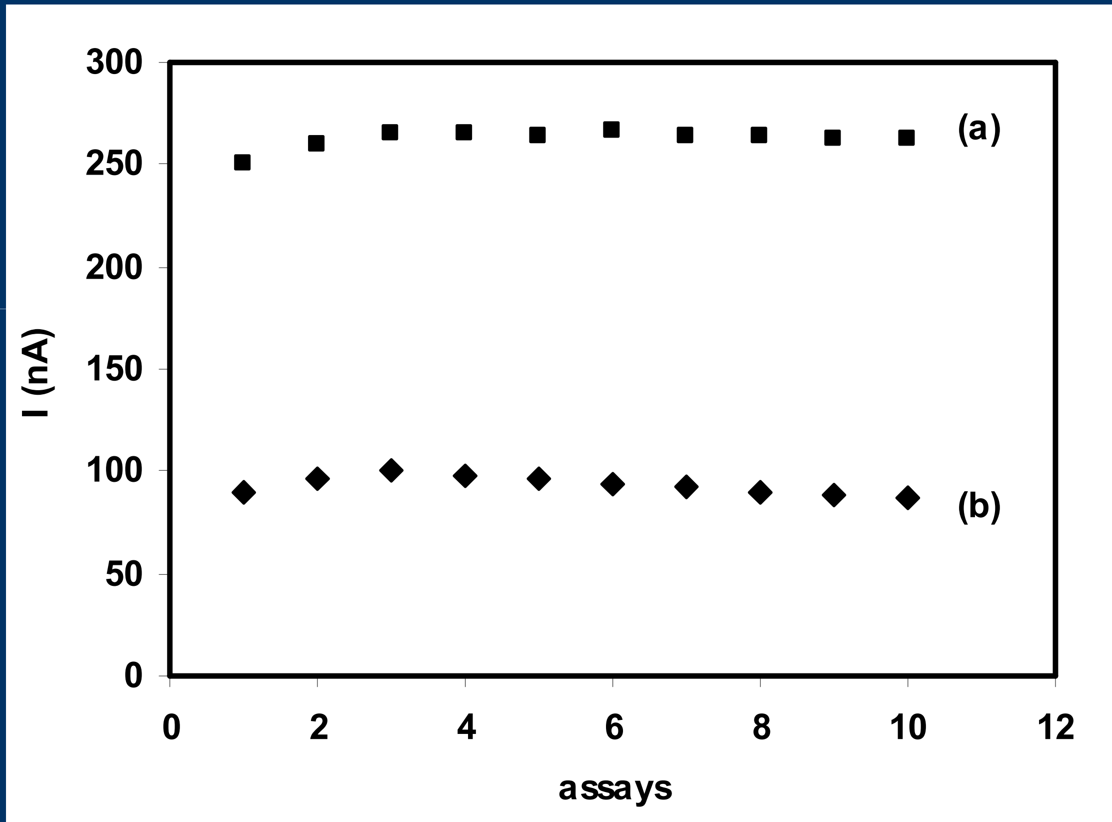
Stability of the composite material toward oxidation



•(a) MB-SWNT-SG/SPE and (b) MB-SG/SPE.

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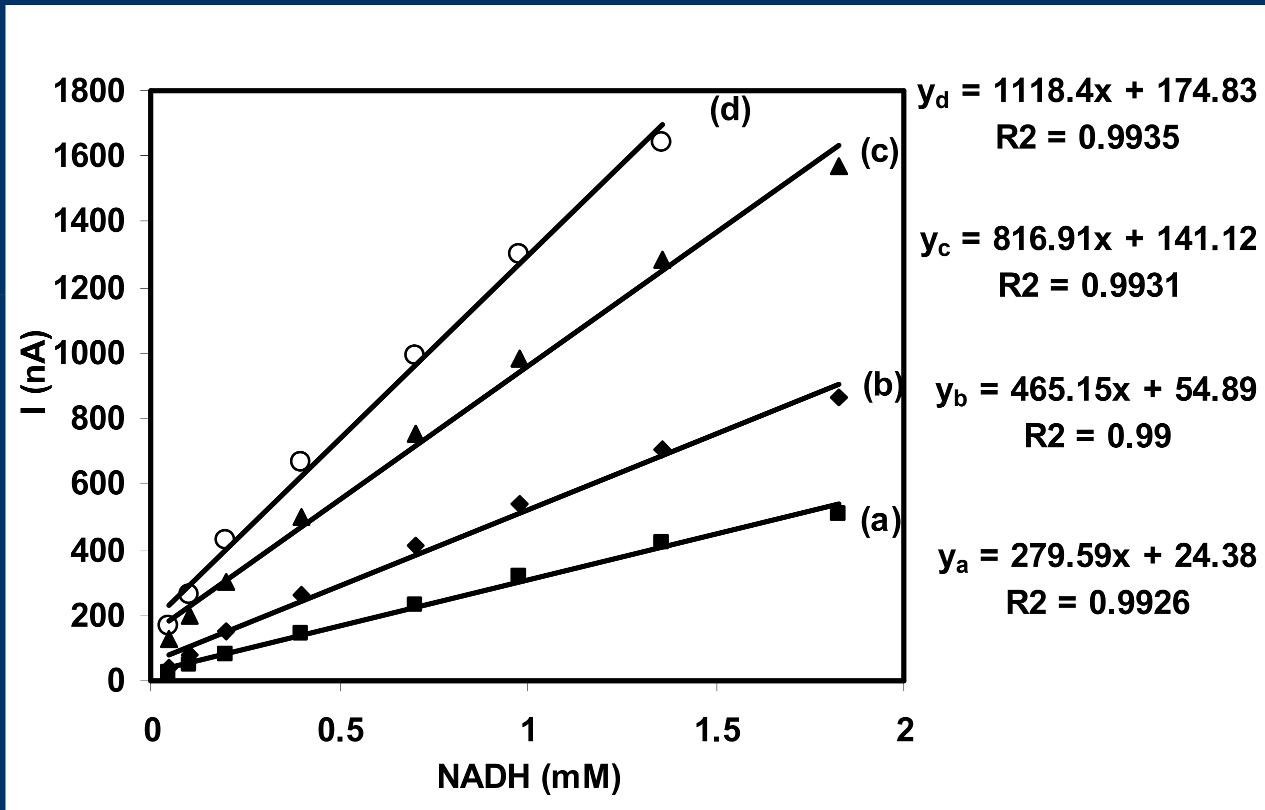
Effect of SWNT amounts included in the composite material



•(a) 1.5, (b) 2, (c) 3 and (d) 5 mg/mL.

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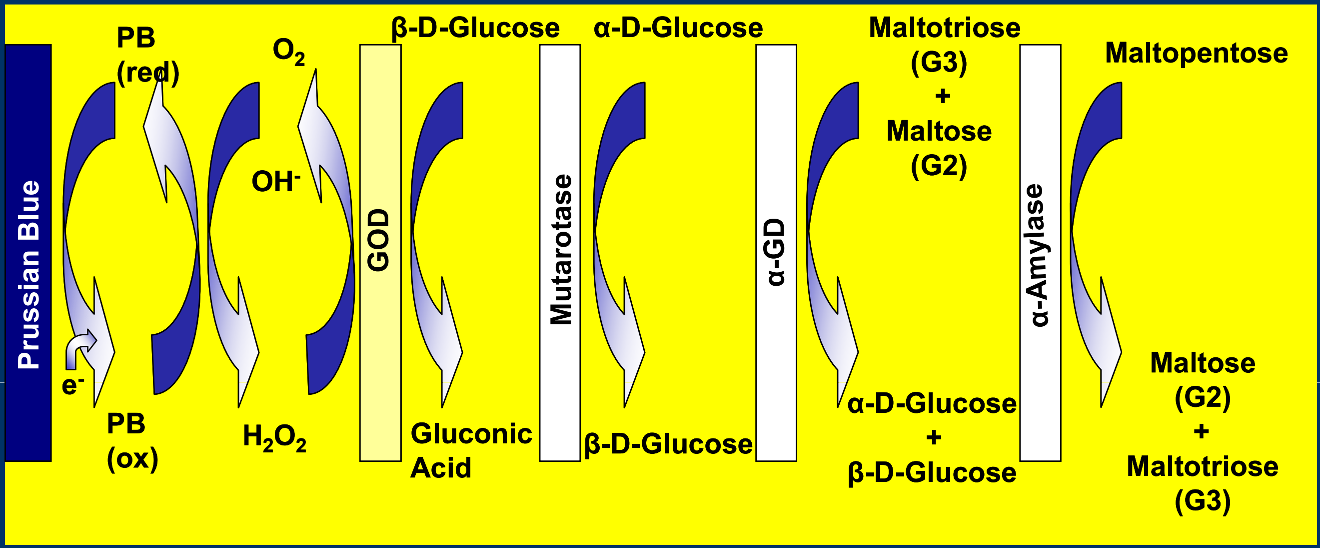
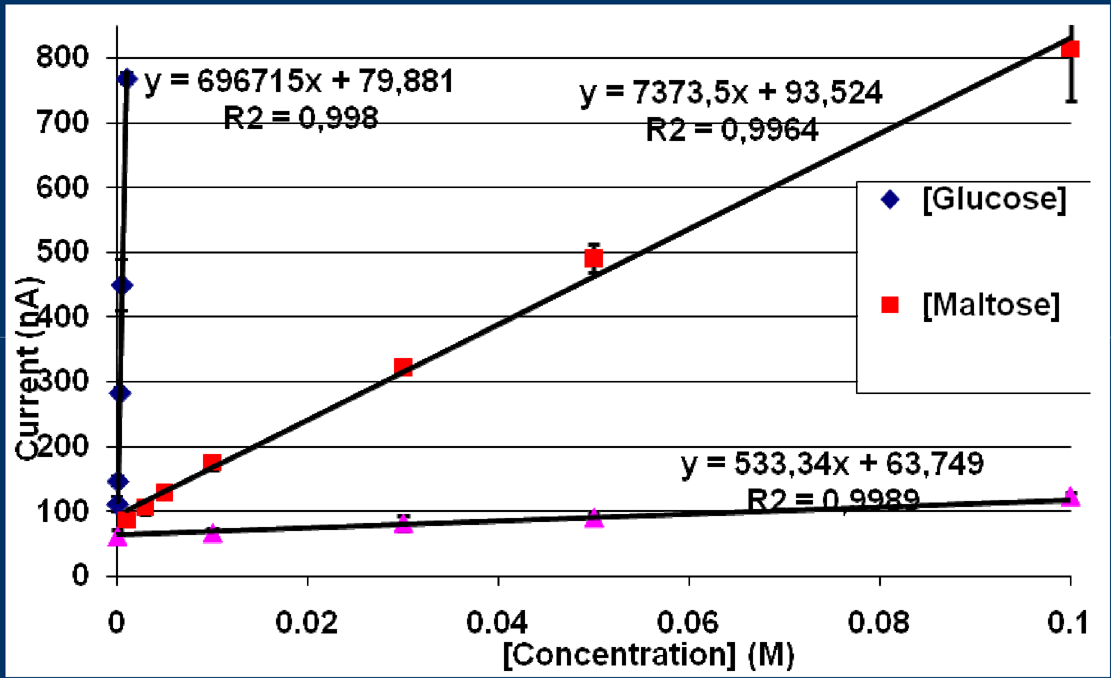
Determination of α-Amylase



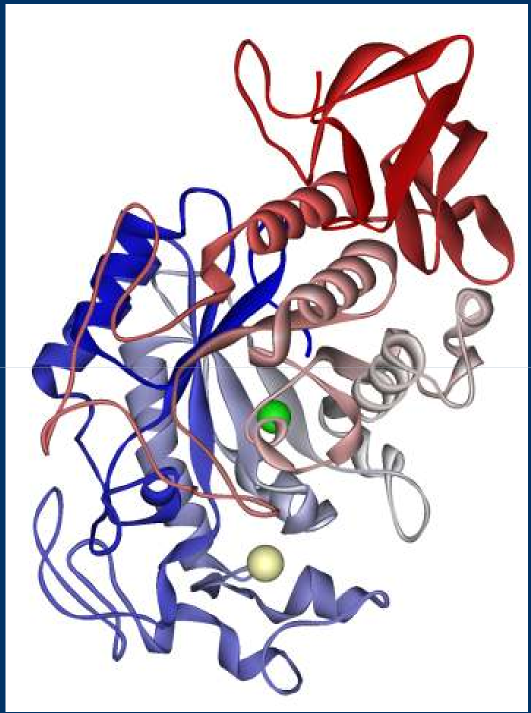
Scheme of entire reaction chain exploited in amylase measurement.

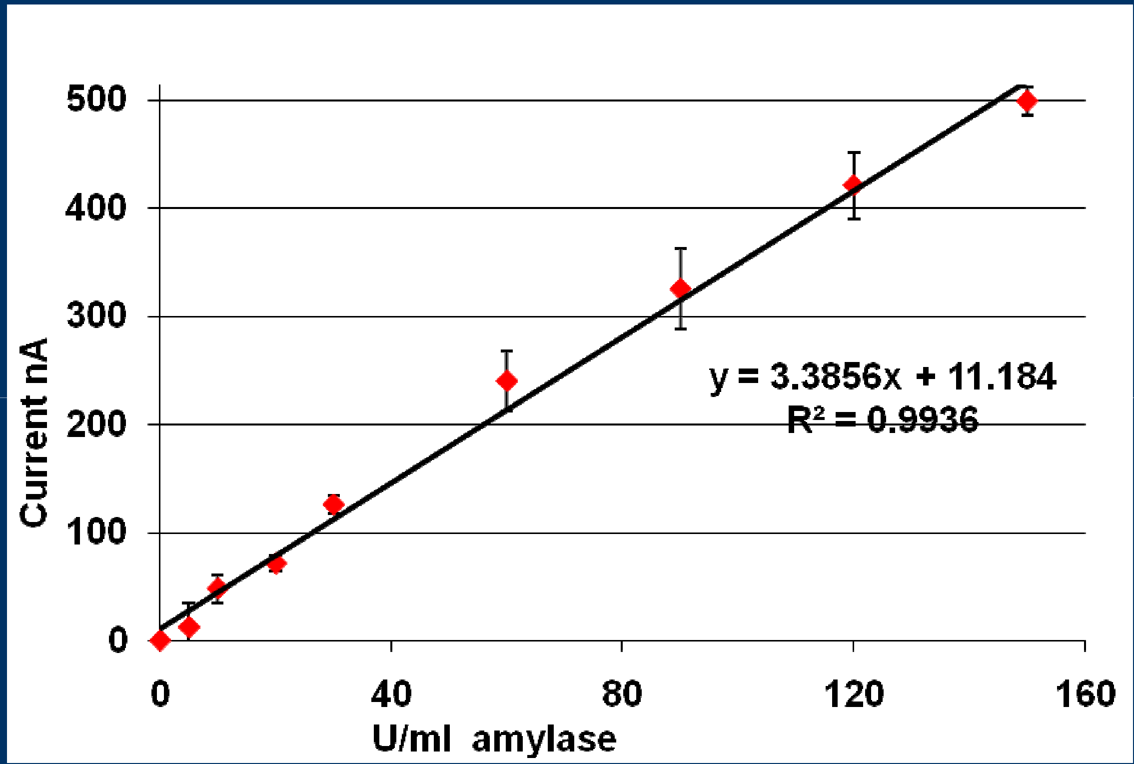
The amount of final product, hydrogen peroxide is proportional to the current detected on the electrode. The signal was recorded as a change of current at an applied potential of -25mV by chrono amperometry method. The presence of the Mutarotase is important as it catalyzes the mutarotation of α-Glucose into β-Glucose, the substrate of GOD. This reaction allows us to determine all the glucose obtained from the division of the Maltopentose and to accelerate the final reaction to obtain better linearity at a wider linear range. This will allow us to measure a-Amylase in human saliva without having to dilute the sample.

Calibration curves for maltotriose, maltose and glucose



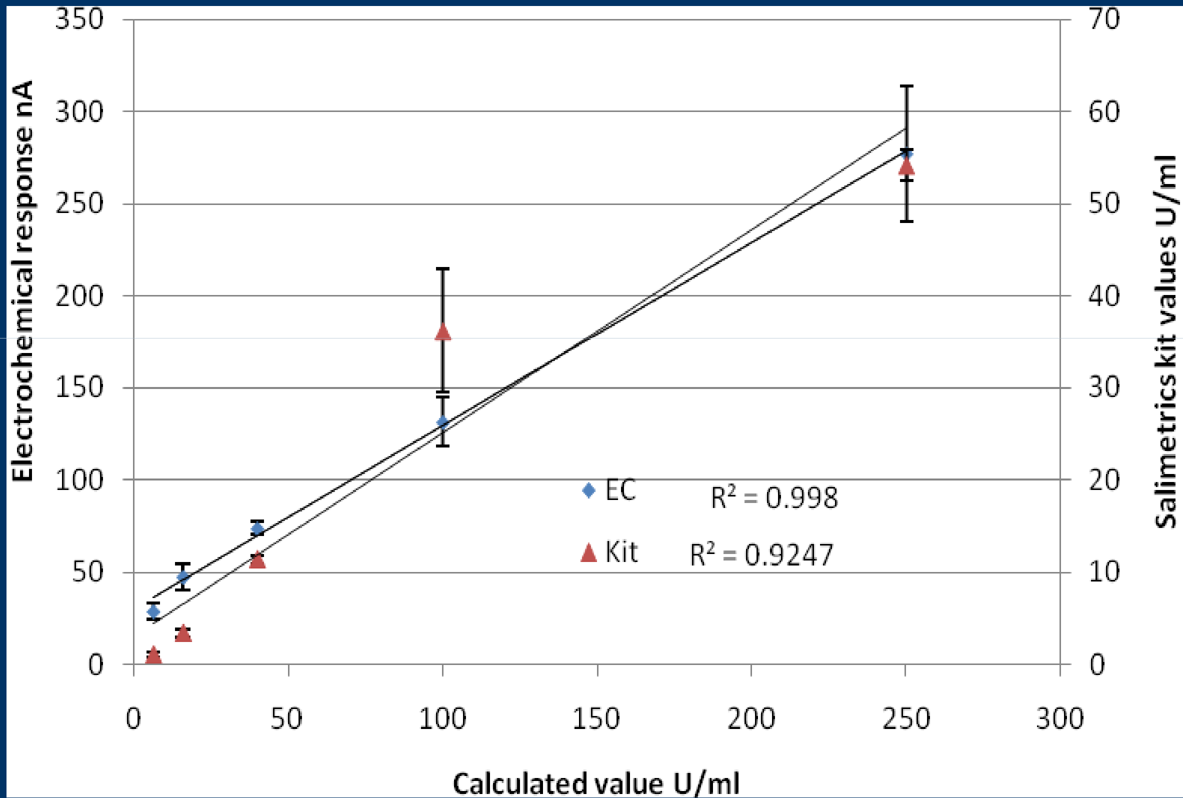
Results for α-Amylase





Intra-electrode calibration curve n=4

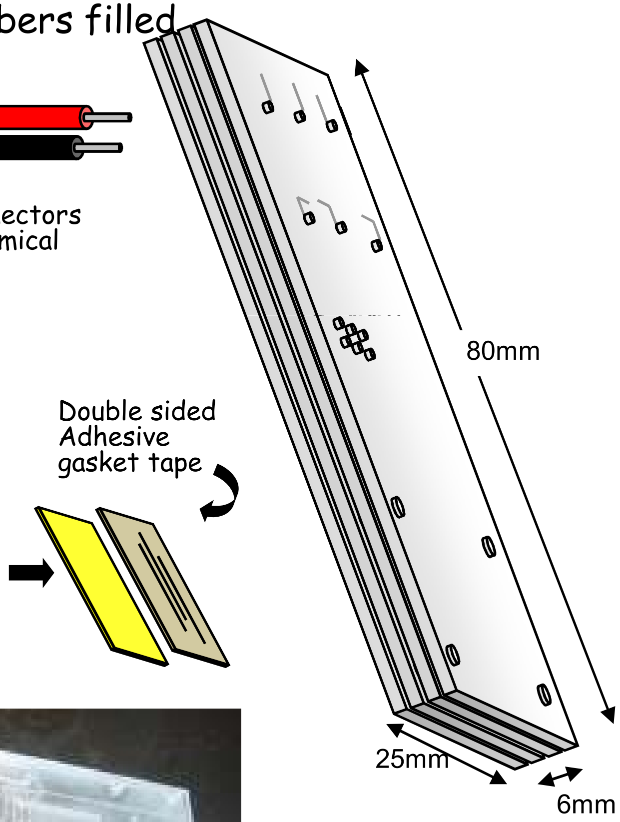
EC method vs commercial kit

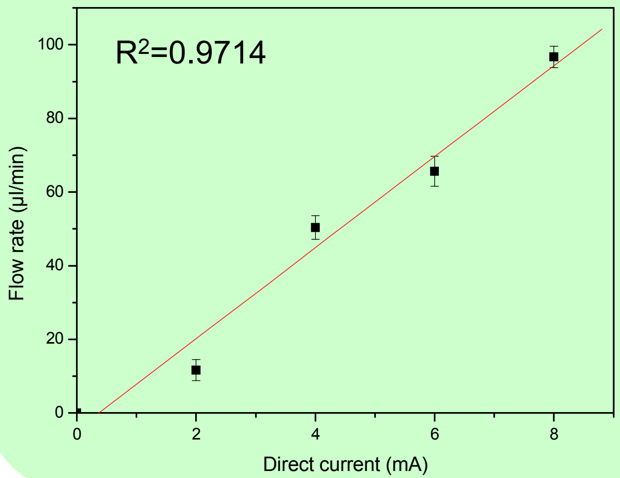


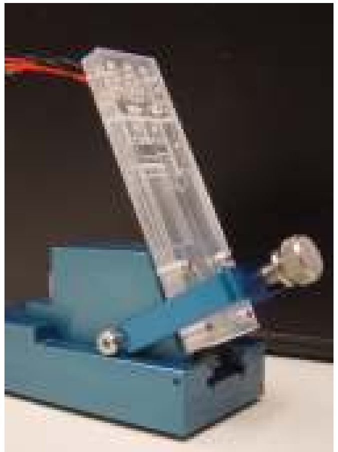
•A comparison graph of the results obtained with EC method and kit in correlation to calculated values

Assembly of the Integrated Cartridge

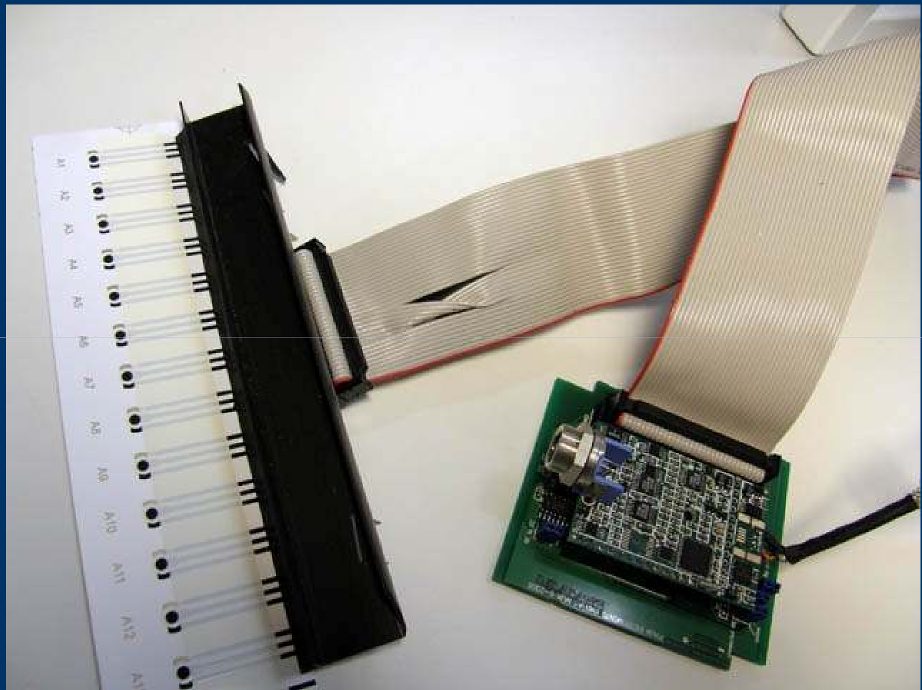
- Reservoir filled with $NaSO_4$
- Sample chambers filled







Multi-element integrated sensor



Environmental samples with EC Determination

- Determination of Heavy metals (Pb, Cd, Zn, Cu, Ni, Co, Mn etc) with electrochemical method:
- fast
- sensitivity
- Selectivity
- Simple
- cheap
- Online-measurement
- Applicable for field use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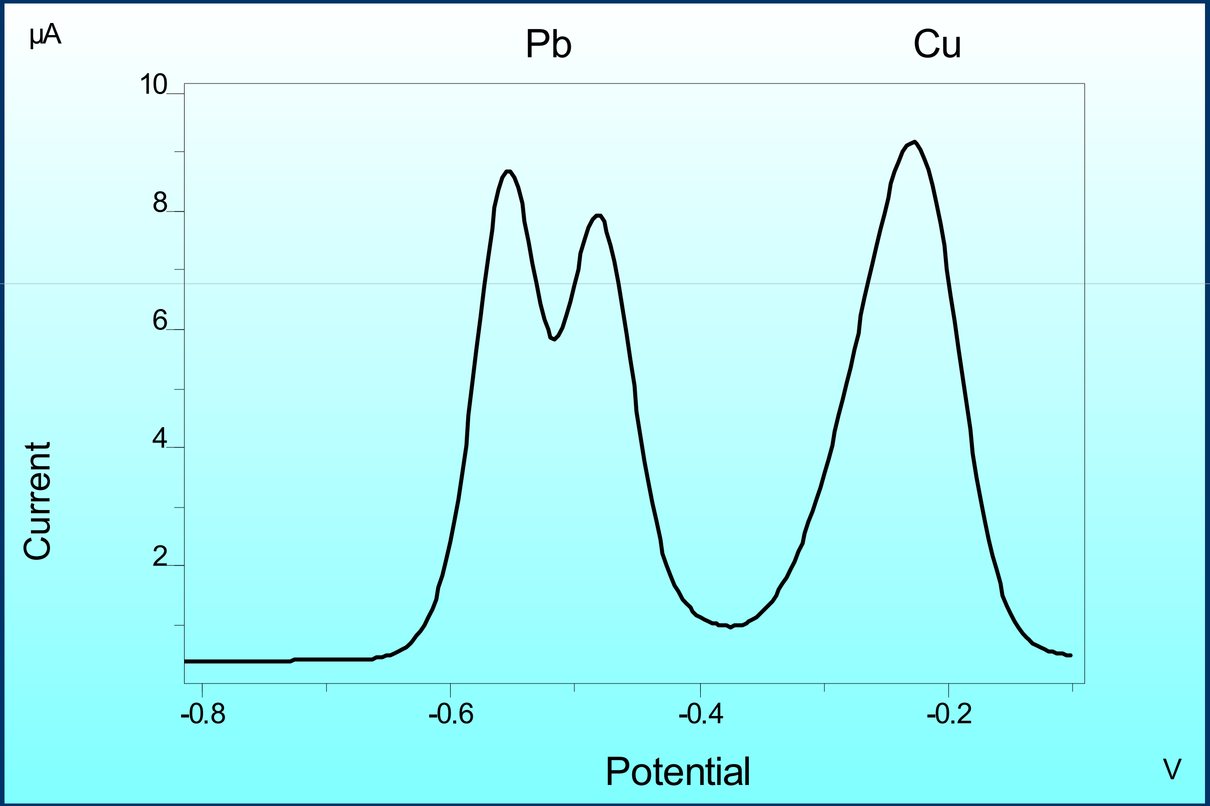
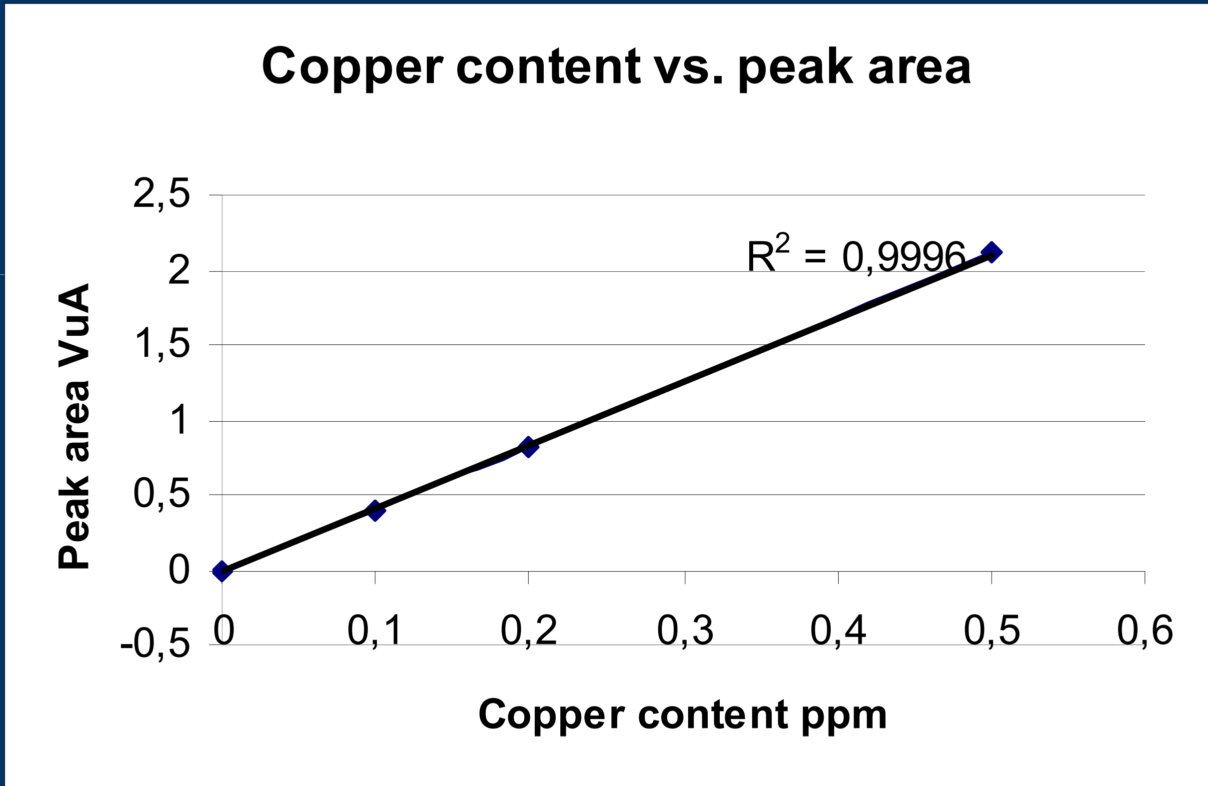
Cu, Pb: Square wave anodic stripping voltammetry (SWASV)



Linearity for copper



Ni, Co: Differential Pulse Adsorptive stripping voltammetry (DPAdSV)

- Method used for specific metals (Ni and Co), which do not form amalgame with quicksilver
  - → need for additional complexation agent (dimethylglyoksime, DMG):
  - **Ni2+ + 2 DMGH2 + 2 e- → Ni(DMG)2**
- the formed complex adsorbs to the amalgame

Future

- Biosensing is becoming more and more used
- Application area wide
- On-line systems for physiological and environmetal surveillance

Acknowledgements

- Group:
  - Ph.D. Adama Sesay, project manager
  - Ph.D. Pekka Kilpeläinen
  - M.Sc. Mika Mahosenaho
  - M.Sc. Satu Pääkkönen
  - M.Sc. Susanna Sonny
  - M.Sc. Pirkko Tervo
- Visiting scientists:
  - Ph.D Adina Arvinte
  - Ph.D. Laura Micheli
  - M.Sc. Flavio Pino
  - M.Sc. Felice Caprio