Investigations into the use of a Thionine/PEDOT Electroactive Layer as an NADH Electrocatalyst, with Applications in Glutamate and Lactate Sensing

Susan Warren

Presentation Outline

- Aims and Introduction
- Electrochemical biosensing of neurochemicals in vivo
  - Formation of redox dye (thionine) in conducting polymer (PEDOT) layer on electrode surface

1. Film Studies
2. Electrocatalysis of NADH
3. Glutamate and Lactate Biosensors

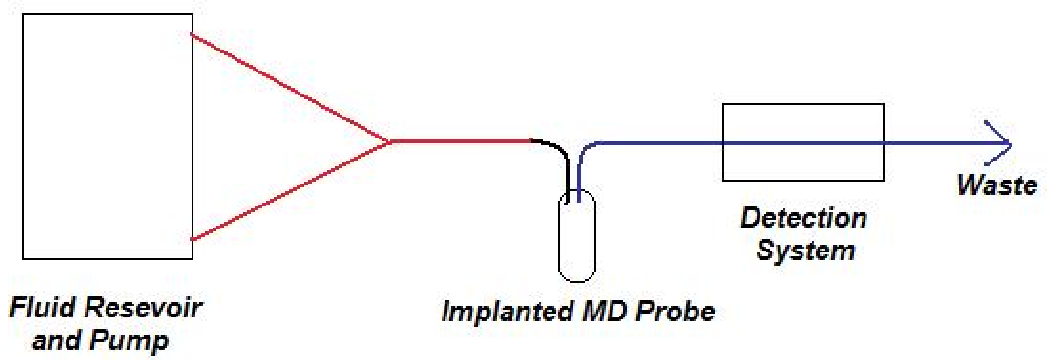
Aims of this Project

1. Develop a Sensors capable of analysing *neurochemicals* of interest:

Glutamate — not intrinsically electroactive

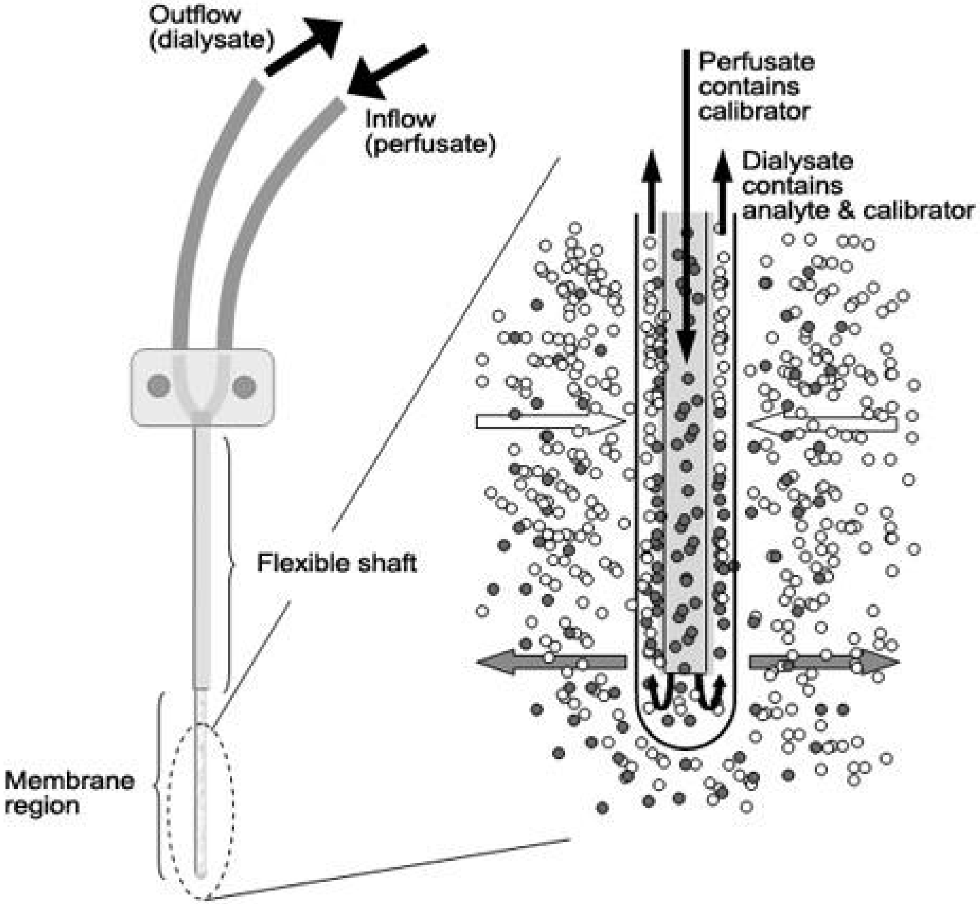
Lactate — will require specific enzyme.

2. Transfer technology to a microelectrode and couple with a ***microdialysis*** setup.



Microdialysis Sampling Tool

- *In vivo* sampling with online detection
- A probe is inserted into the brain region of interest
- The tubular, semi-permeable membrane in conjunction with a perfusion system samples the extracellular fluid.
- The membrane omits any large biological molecules while allowing smaller molecules, e.g. neurotransmitters, through to the detection system.



http://www.aapsj.org/articles/aapsj0901/aapsj0901006/aapsj0901006_figure1.jpg

Analytes of Interest

- Neurochemicals – detection in the brain
  - Challenging environment;
  - Low concentration of analytes, fluctuating levels;
- Presence of interferences.

- **Glutamate** – excitatory neurotransmitter, involved in cognitive functions;
  - Excess glutamate in the ECF is related to strokes, retardation and Alzheimer's disease etc.
  - ECF level : **~10μM**
- **Lactate** – concerned with energetics and metabolism of brain systems.
  - ECF level: ~ **1mM**

Dehydrogenase Enzyme Systems

- Biological catalysts (proteins) that are specific to one substrate only.
- The enzyme possesses a redox moiety i.e. can be oxidised and reduced
    - ➢ Monitor reaction of Enzyme/Substrate electrochemically

- Redox moiety – cofactor (Nicotinamide Adenine Dinucleotide)
    - **NAD^+/NADH**

$$\text{L Lactate} + NAD^+ \xrightarrow{\text{LDH}} \text{Pyruvate} + NADH$$

creat Centre for Research in Electroanalytical Chemistry

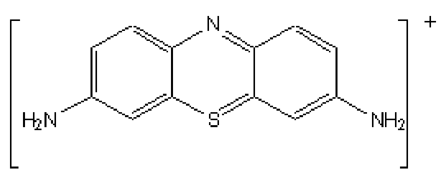
ITT DUBLIN

Formation of redox dye (thionine) in conducting polymer (PEDOT) layer on electrode surface

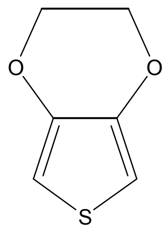
1. Film Studies
2. Electrocatalysis of NADH
3. Glutamate and Lactate Biosensors

Immobilisation of EDOT in the presence of Thionine Acetate

- Immobilized an organic dye, Thionine, with 3,4,-ethylenedioxythiophene (EDOT) via electropolymerisation on both GCE and Pt macro electrodes.



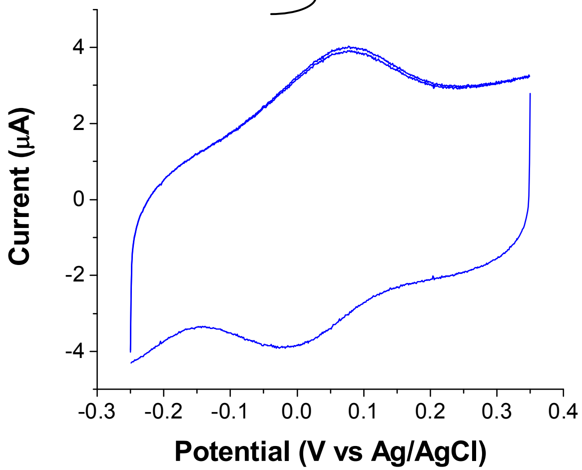
Thionine (Th)



EDOT

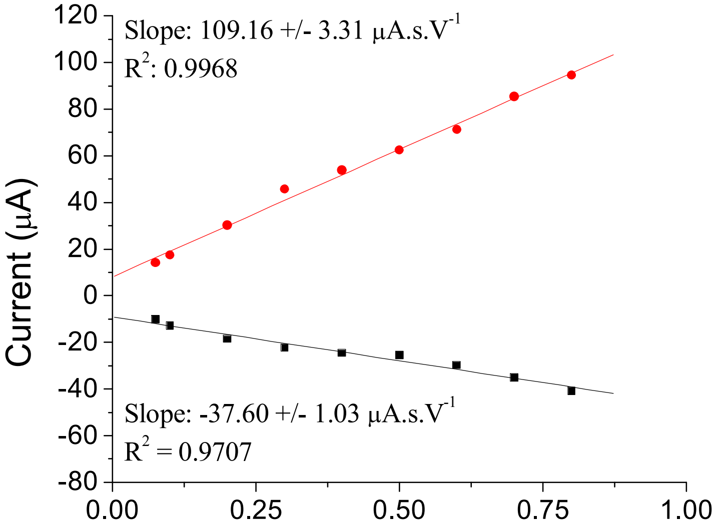
- Optimised method:
  - 10mM Thionine
  - 15mM EDOT
  - 10mM $LiClO_4$

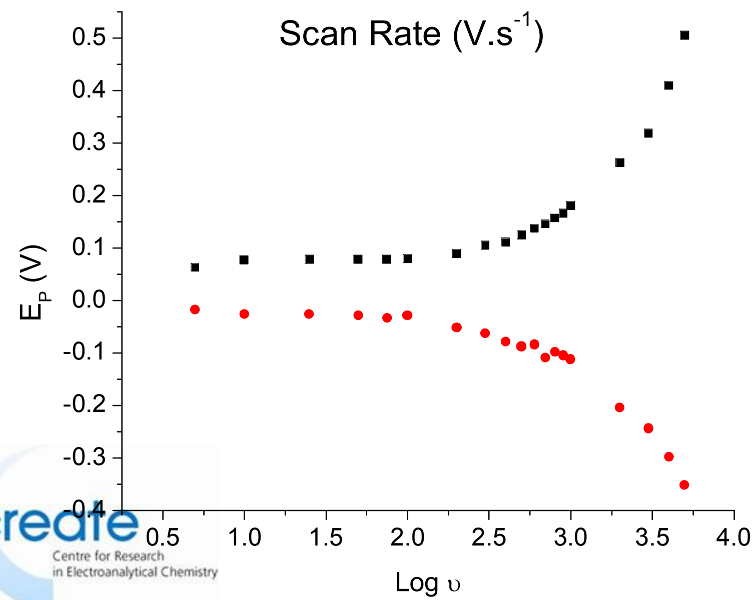
5mC film 0 – 1.2 V vs Ag/AgCl



- Thionine/PEDOT film
- $E_{1/2}$ +0.08 V. vs Ag/AgCl

Film Kinetics





- Scan Rate study at pH 6 from 5 – 1000 mV.s^{-1}
- Τ – surface coverage 1.82 x 10^{-8} mol.cm^{-2}

$$Slope = \frac{n^2 F^2 A \tau}{4RT}$$

- Laviron method [1]: log v vs E_P

$$\log k_s = \alpha . \log(1-\alpha) + (1-\alpha)\log\alpha - \log\frac{RT}{nF\upsilon} - \frac{\alpha n_\alpha F \Delta E_P (1-\alpha)}{2.3RT}$$

- **α** - transfer coefficient – 0.26
- **k_s** – CT rate constant – 280 s^{-1}

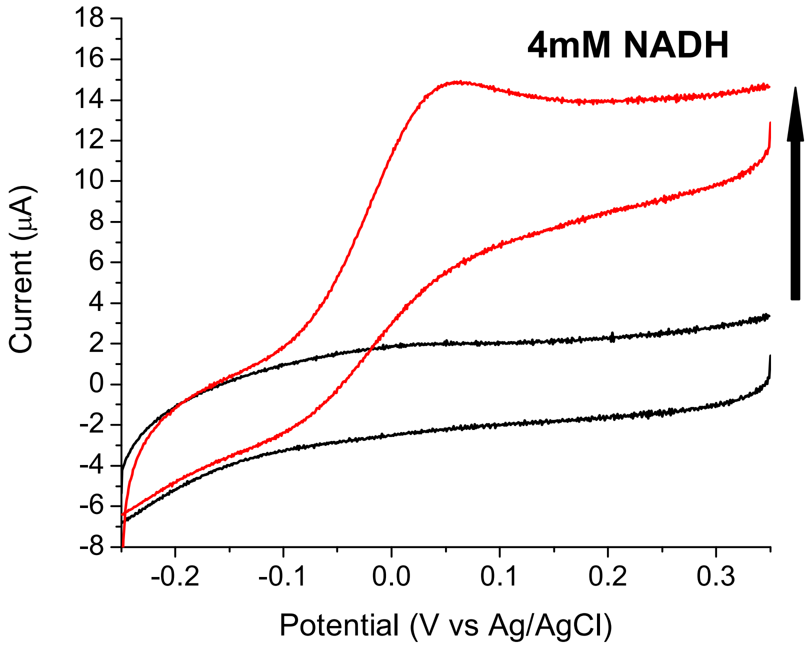
$$Th_{RED} \xrightarrow{k_s} Th_{OX}$$

[1]E. Laviron, J. Electroanal. Chem. 101 (1979) 19-28.

NADH Electrocatalysis using Cyclic Voltammetry (CV)

• CV of the modified electrode in the absence and presence 4mM NADH @ 0.75mV.s^{-1}

• Increase in Oxidation and decrease in Reduction currents – indicative of an electrocatalytic response.



•$Th_{RED} \xrightarrow{k_s} Th_{OX}$ **E**

•$Th_{OX} + NADH \xrightarrow{k_{CAT}} Th_{RED} + NAD^+$ **C**

• $Th_{RED} \rightarrow Th_{OX} + 2e^- + H^+$

Rotating Disc Electrode (RDE): Catalytic Studies

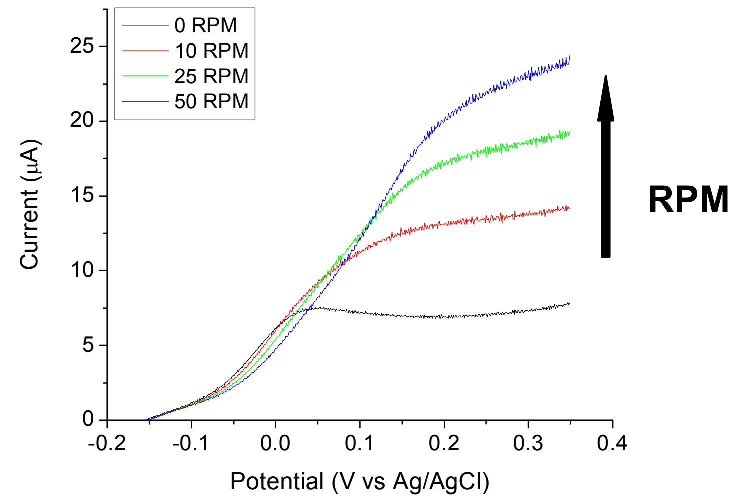
- The catalytic rate constant (k_{CAT}) associated with the electrocatalytic oxidation of NADH was carried out using a RDE Pt electrode.

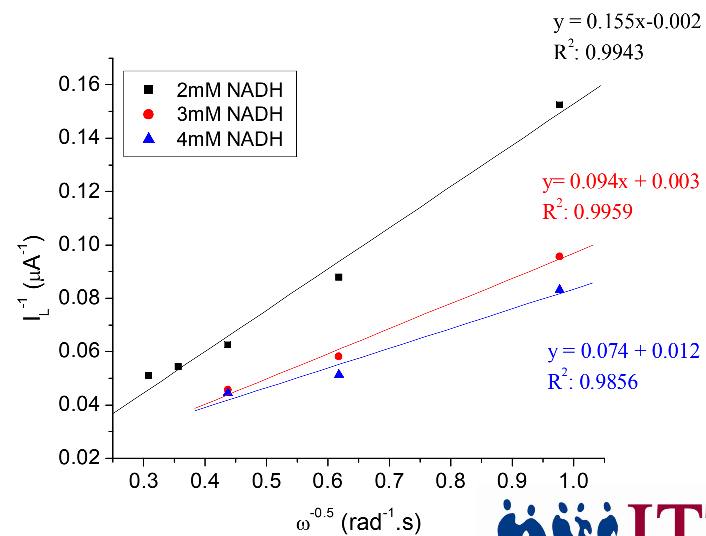
- The modified electrode was rotated at different speeds in the presence of varying NADH concentrations, 2 – 4mM, @ 0.75 mV.s^{-1}.

- Koutcky-Levich plot :

$$[I_L]^{-1} = [nFACk_{CAT}\Gamma]^{-1} + [0.620nFAD^{2/3}v^{-1/6}C]^{-1}.\omega^{-1/2}$$

- Intercept: k_{CAT} 1.86×10^{3} mol^{-1}.L.s^{-1},
- Slope: $D_{APP(NADH)}$ 5.61×10^{-7}cm^{2}.s^{-1} in good agreement with literature [2].

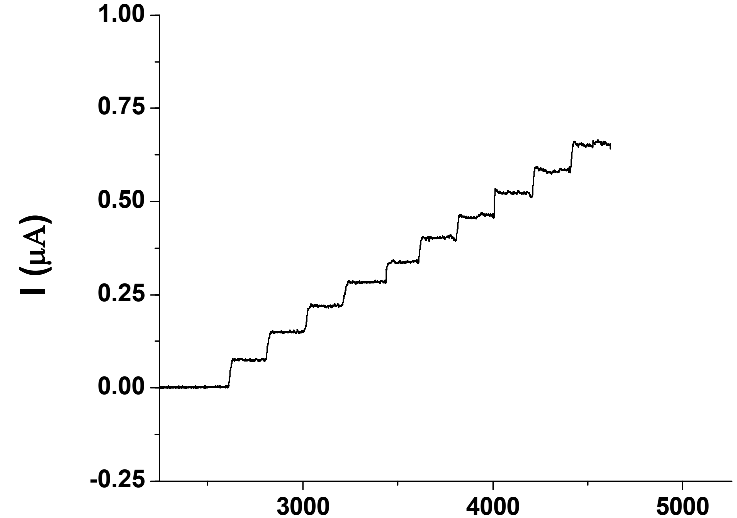




[2] P.R. Lima *et al* Biosensors and Bioelectronics 24 (2008) 448-454.

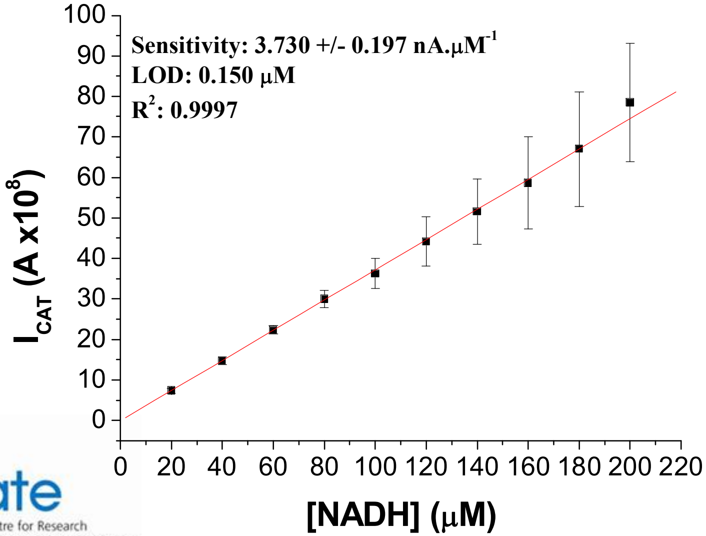
NADH Electrocatalysis using Amperometry

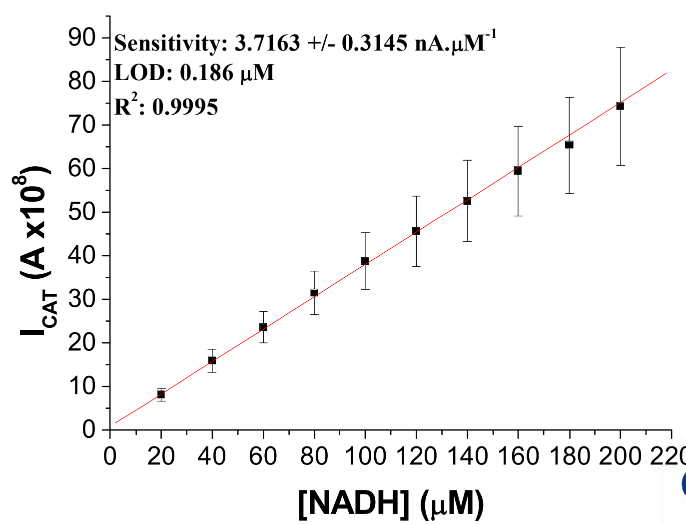
- Amperometry: E_{APP} +0.12 V vs Ag/AgCl
- 20 µM NADH additions
- (a) Intra and (b) inter precision (n=5).
- Good stability and reproducibility



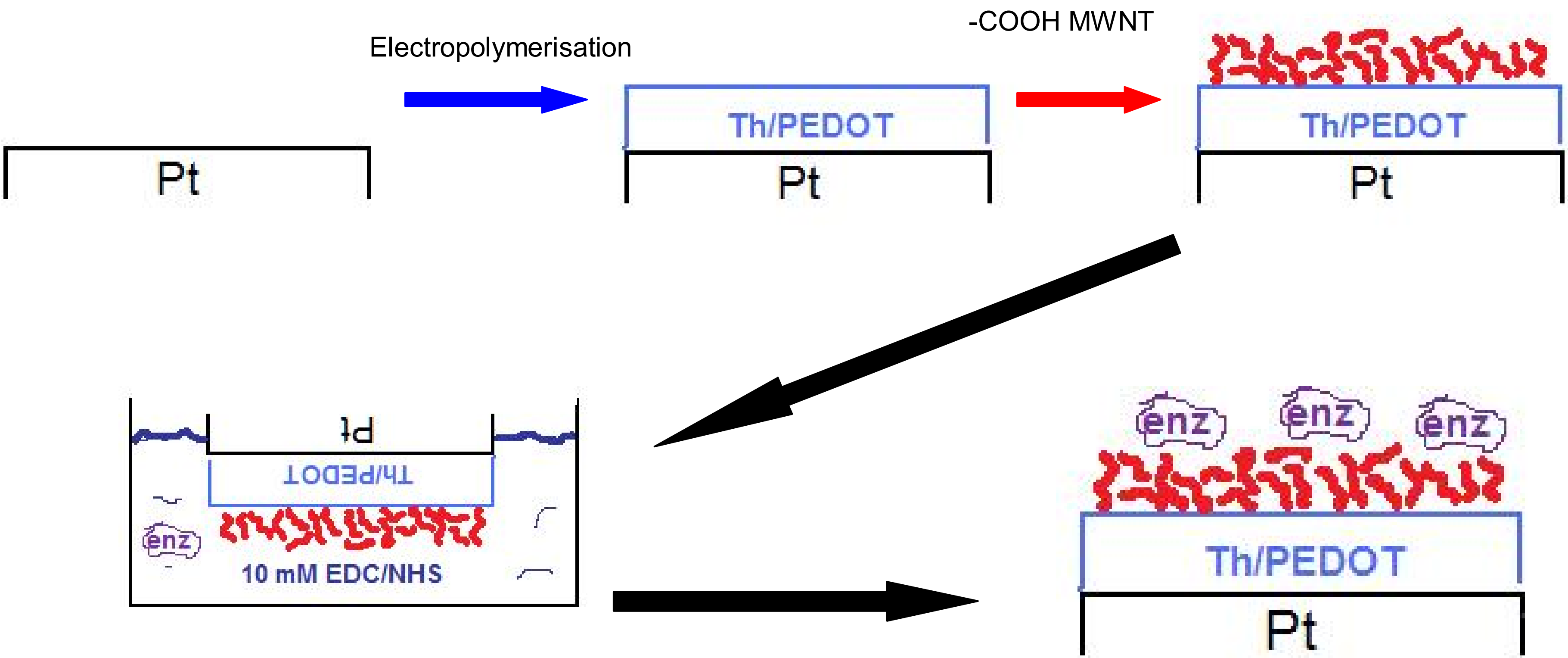
(a)

(b)





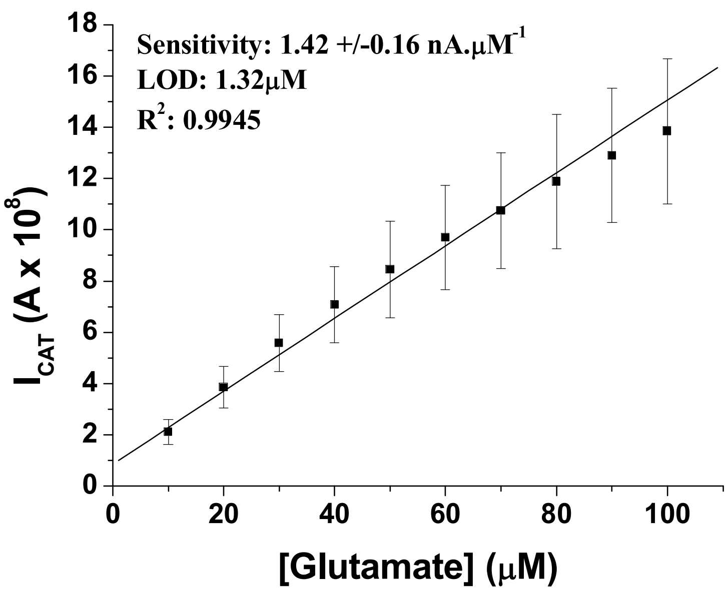
Enzyme Immobilisation – Glutamate and Lactate Dehydrogenase

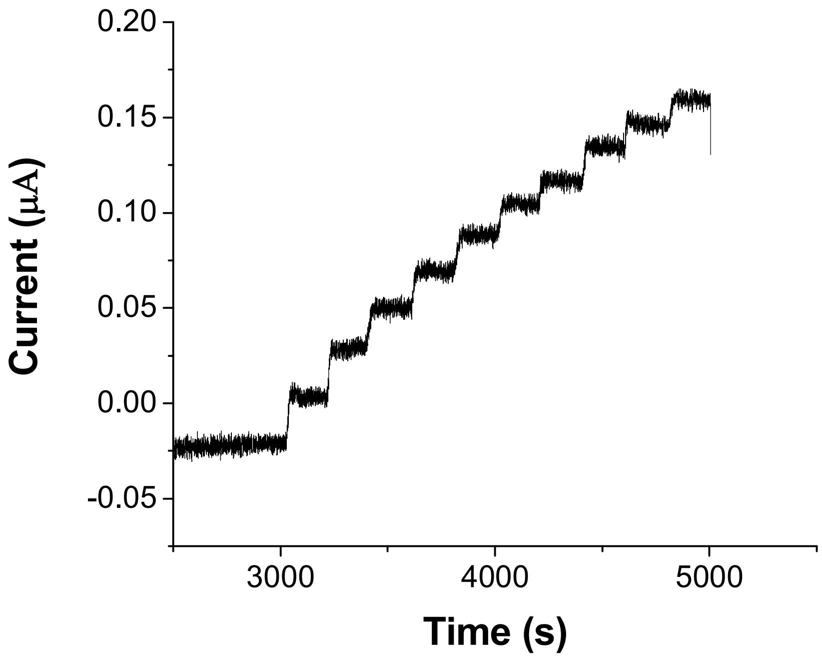


- **All enzyme studies done @37^{0}C in 1mM NAD$^+$/PBS**

Glutamate Sensor

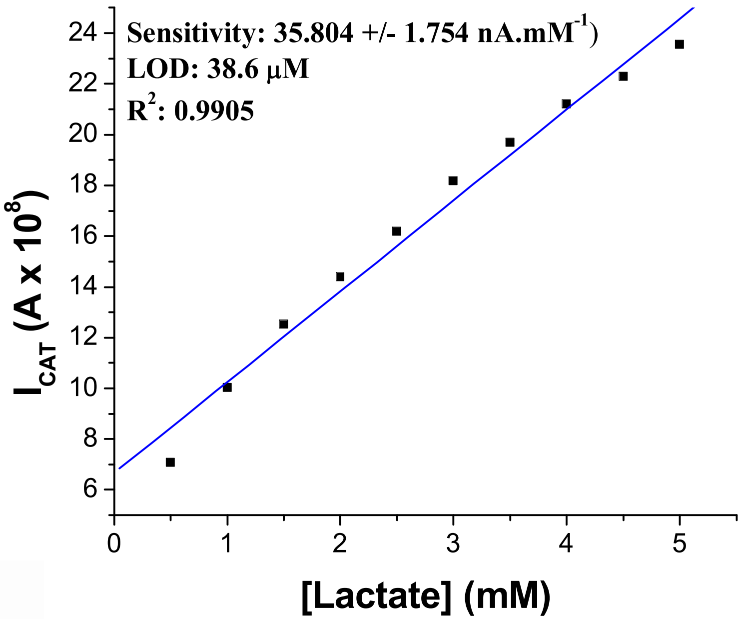
- E_{APP} + 0.12 V vs Ag/AgCl;
- **10 µM** Glutamate additions (n=5)
- Good stability

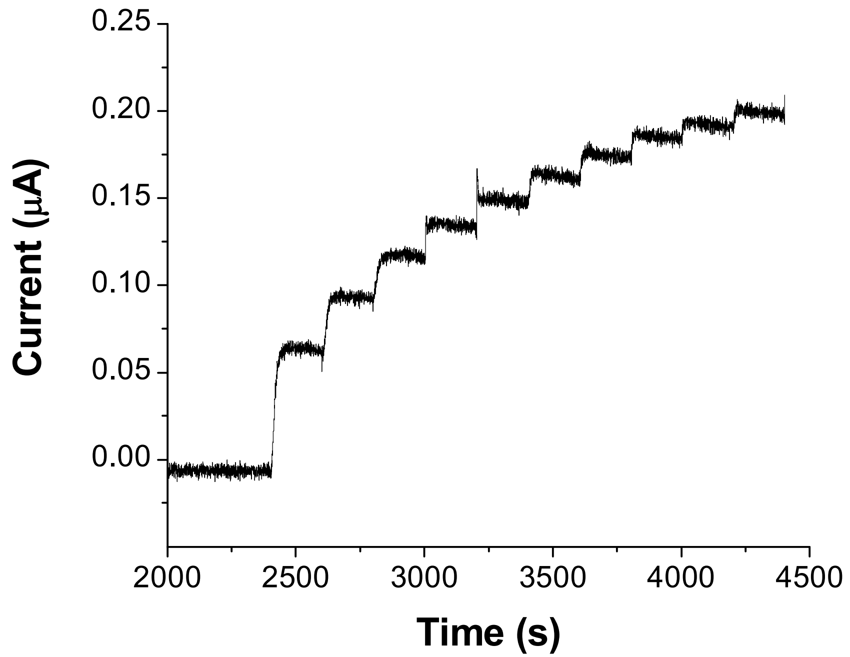




Lactate Sensor

- E_{APP} +0.12 V vs Ag/AgCl;
- **0.5 mM** Lactate additions
- Attempts to improve linearity





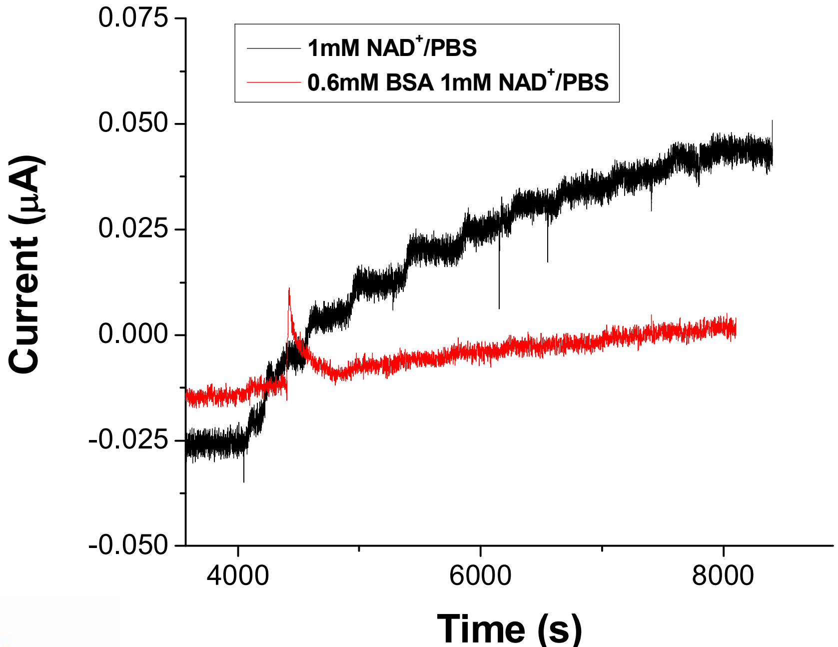
Lactate Biosensor in BSA

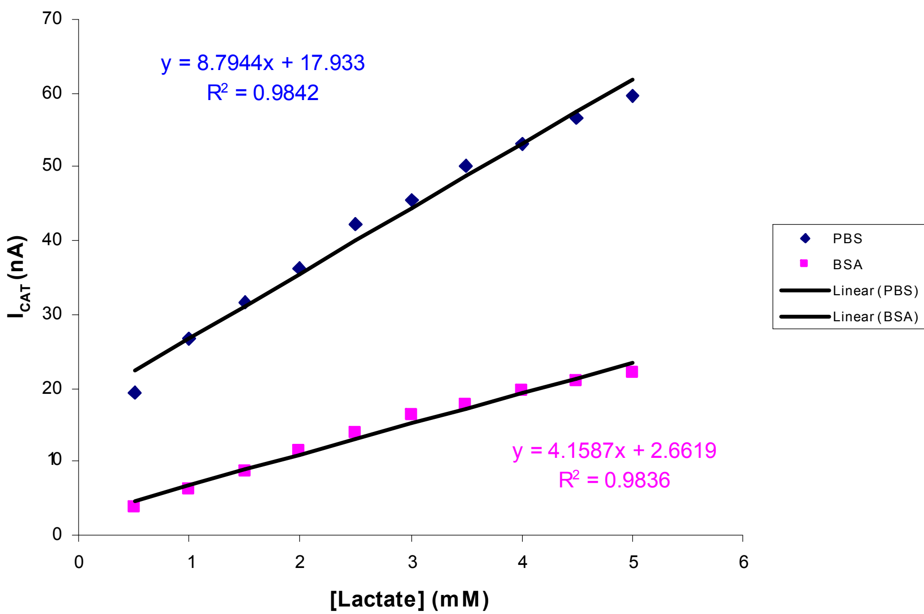
- Amperometry - 10 additions of 0.5 mM Lactate in PBS followed by analysis in background solution of physiological level BSA (0.6mM)

- The protein effectively blocks the catalytic response.

CMA 7 MD Probe
Cutoff: 6kDa

BSA ~ 66 kDa



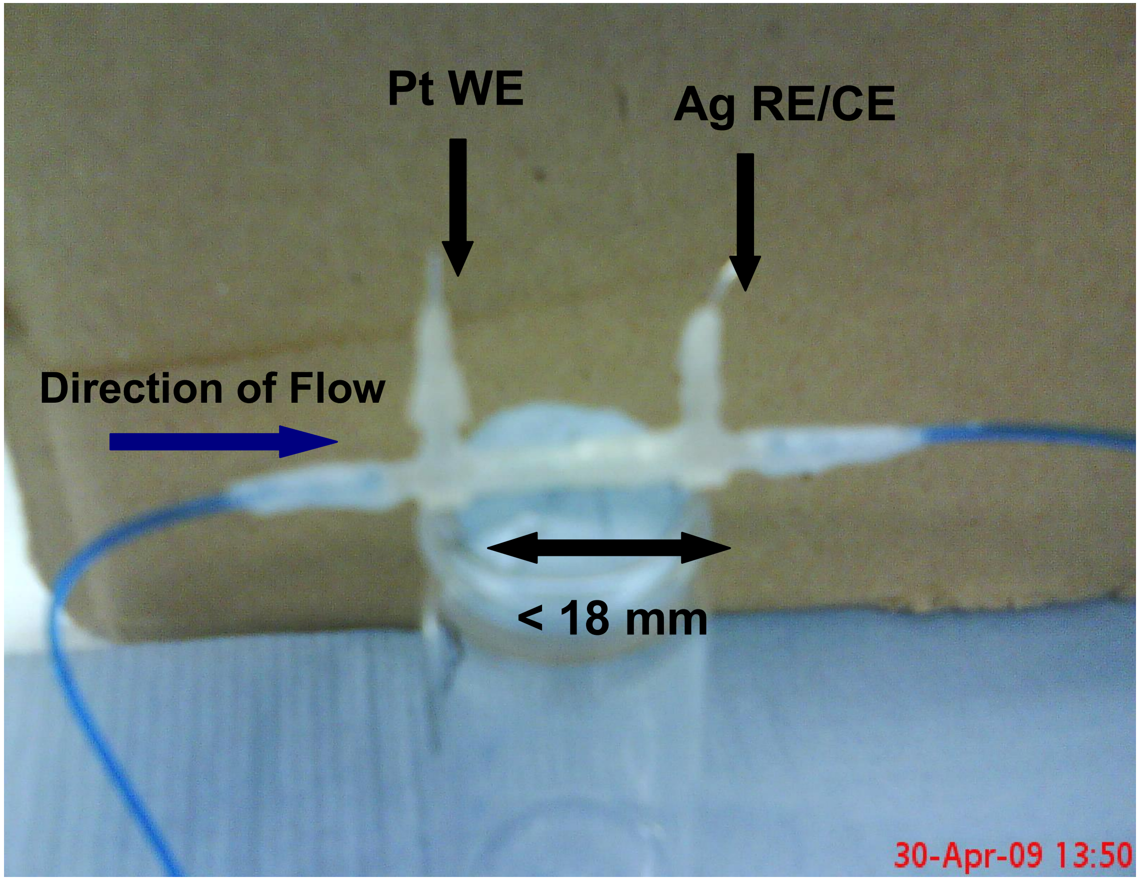


Microdialysis Setup

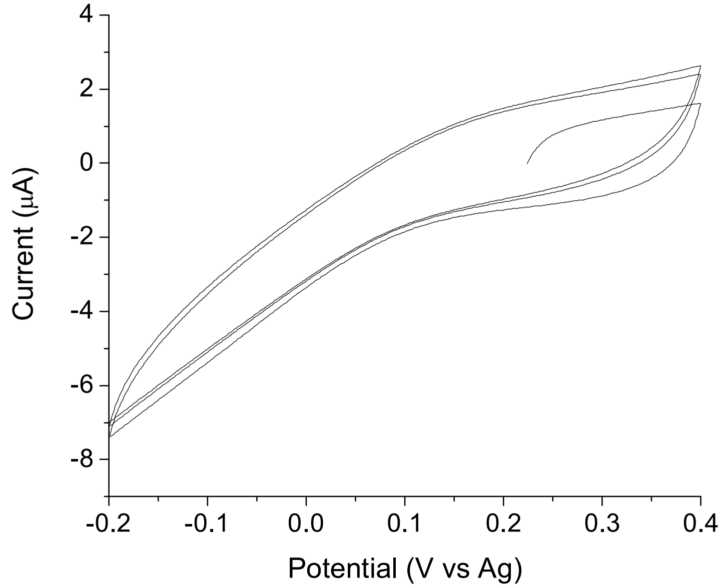
Centre for Research in Electroanalytical Chemistry

ITT DUBLIN

Prototype 2 Electrode Flow Cell



Preliminary Testing - K_3FeCN_6



CV of 2mM FeCN 20µl.min^{-1} @ 500µm Pt wire (WE) and 500µm Ag wire (RE/CE)

Conclusions and Future Work

- Developed a novel Thionine/PEDOT Film with good
  - pH stability;
  - reproducibility;
  - reactivity towards NADH oxidation;
- Kinetic evaluation carried out using CV and RDE
- Successful attachment of GlDH and LDH with NAD^+ in solution;
  - Interference and stability studies to be complete
  - Apply biosensor system to Pt wire and couple with microdialysis.
  - Develop a sensor for Dopamine and H_2O_2

create
Centre for Research in Electroanalytical Chemistry

ITT DUBLIN

Centre of Applied Science for Health

- Supervisors: Dr Eithne Dempsey and Dr Tim McCormac (DkIT)
- Prof. John Lowry (Maynooth)
- Dr Gerry Ryder
- Lab colleagues in the Synergy Centre and Lab 125.

The Centre of Applied Science for Health

is Funded Under the

Programme for Research in Third Level Institutions Cycle 4

Ireland's EU Structural Funds Programmes 2007 - 2013

Co-funded by the Irish Government and the European Union

EUROPEAN UNION STRUCTURAL FUNDS

HEA

Higher Education Authority

An tÚdarás um Ard-Oideachas

Thank you for listening!

create

Centre for Research in Electroanalytical Chemistry

ITT DUBLIN