Fabrication, Characterisation and Applications of a Glucose Biosensor Based on a Screen-Printed Carbon Electrode Incorporating Insoluble Meldola`s Blue and Glucose Dehydrogenase.

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Outline of the talk

The Aim *of the study was to produce an amperometric Glucose biosensor based on NADH detection to be used for Flow Injection Analysis (FIA)*

Biosensor with Glucose dehydrogenase
Oxygen independent

Use of SPCEs in FIA system
Cheap device, easy to modify and adapt to detectors

Meldola`s Blue Reinecke Salt (MBRS) as mediator for NADH
MBRS mediator less soluble, electrodes can be used in FIA

Characterisation of a glucose biosensor based on MBRS mediator

Biosensor introduction

Glucose biosensor: *Why glucose??*
one of the most important measurements required in clinical analysis, and food industries.

Self monitoring glucose device and laboratory glucose assays use:

1. **Glucose oxidase,**
2. **Glucose dehydrogenase PQQ dependent,**
3. **Glucose dehydrogenase NAD^+ dependent.**

We choose to use *GDH NAD^+ dependent* enzyme:

✓**Advantages:** **Oxygen independent**
Low cross reactivity with maltose

✓**Disadvantage:** **Less stable than GOX**

Glucose dehydrogenase Enzymes

- **Systems with GDH PQQ dependents can not distinguish maltose from glucose**
- **GDH PQQ methods provide higher current in the presence of maltose**
- **Using GDH PQQ based monitoring system patients might receive high doses of insulin.**

High insulin doses can cause brain damage, or death of patients

GDH NAD^+ dependent is not affected by icodextrin or maltose

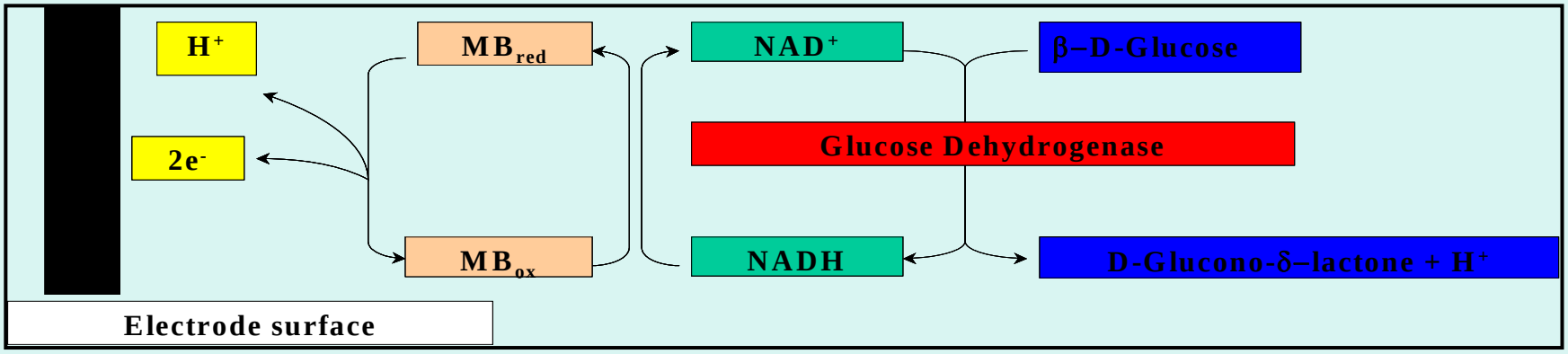
Substrate	Relactive Activity (%)	
	NAD^+	PQQ
D-Glucose	100	100
D-Maltose	6	61

Data from Genzyme Diagnostic

Ref.:

1. **T. Ghys et al, Clinica Chimica Acta 386 (2007) 63-68;**
2. **http:www.fda.gov/cdrh/oivd/news/glucosefalse.html**

Glucose Biosensor



Reaction occurring during the operation of the biosensor

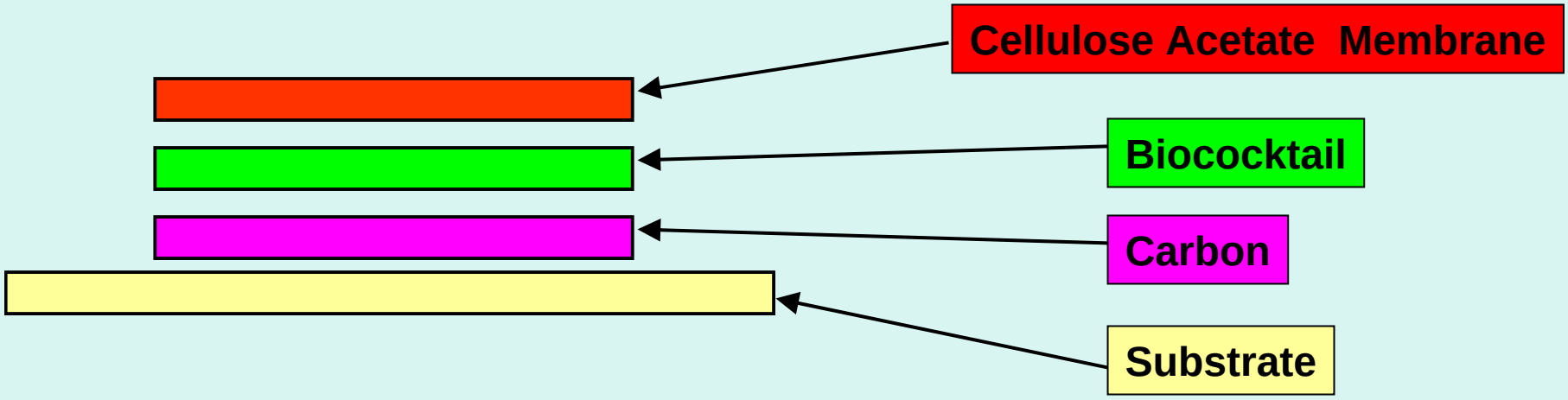
Glucose dehydrogenase enzyme

Glucose Dehydrogenase from ***Bacillus sp.***

Molecular Weight: 105kDa Isoelectric point: 4.5*

***Data from Genzyme website.**

Introduction to materials under investigation



Type of carbon used in the SPCE

Stabilisation of Glucose Dehydrogenase enzyme (GDH)

Loading of enzyme for the Biosensor manufacturing

Cellulose Acetate Membrane optimisation for Flow Injection Analysis

Flow injection analysis (FIA)

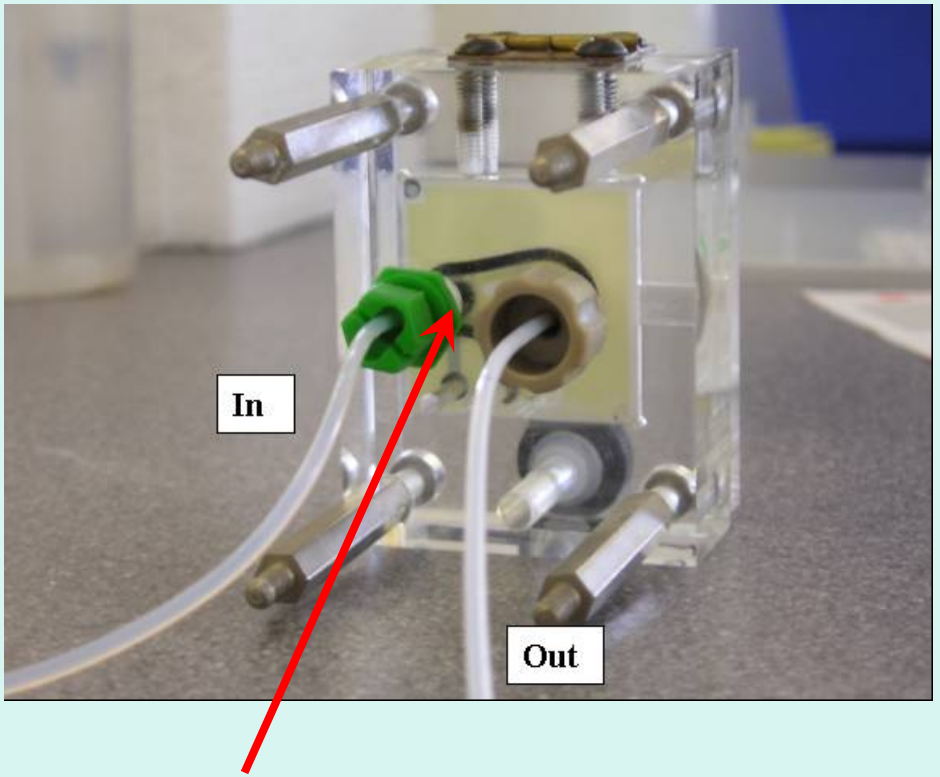
Flow injection analysis (FIA) is a continuous flow technique which is ideally suited to rapid automated analysis of liquid samples

Flow injection analysis:

- **a large number of determinations performed in a short time**
- **lower cost per test when compared to single shot measurements.**

Easy to automate, extensively used in brewing industries

Flow Cell:



Electrode in position in the flow cell

uniscan instruments

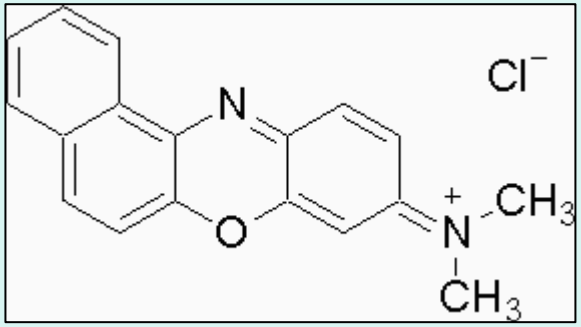
Flow cell manufactured by Uniscan Instruments Ltd

MBRS Carbon

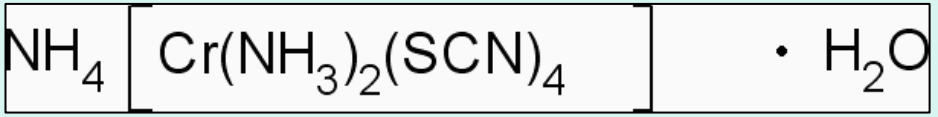
The complex of Meldola`s Blue and Reinecke Salt (MBRS) is less soluble than Meldola`s Blue

The procedure by Schumann et al was used as a starting point for the manufacturing process of the MBRS dye complex

MBRS and MB dyes were incorporated into the carbon paste.



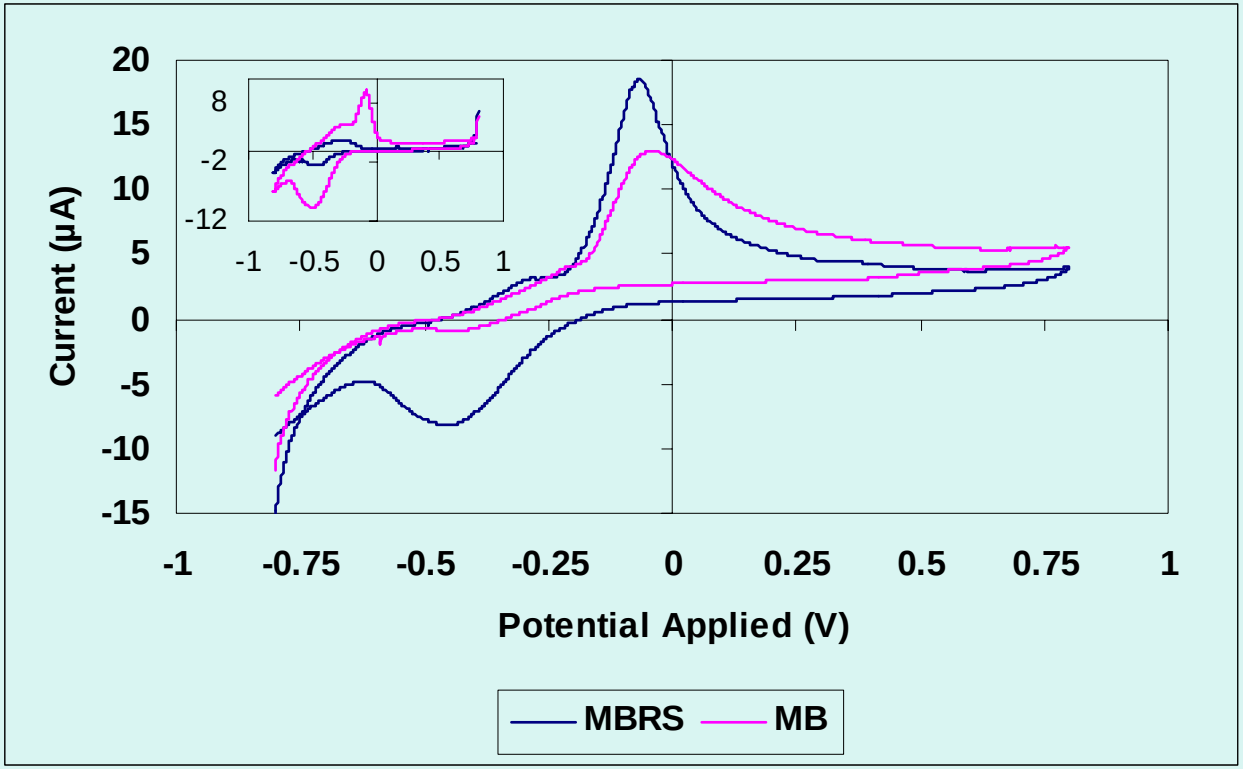
Meldola`s Blue



Reinecke salt

Ref: W. Schumann, J. Huber, H. Wohlschläger, B. Strehkitz, B. Gründig – Journal of Biotechnology 27 (1993) 129-142

Electrochemical characteristics of MB and MBRS Electrodes.



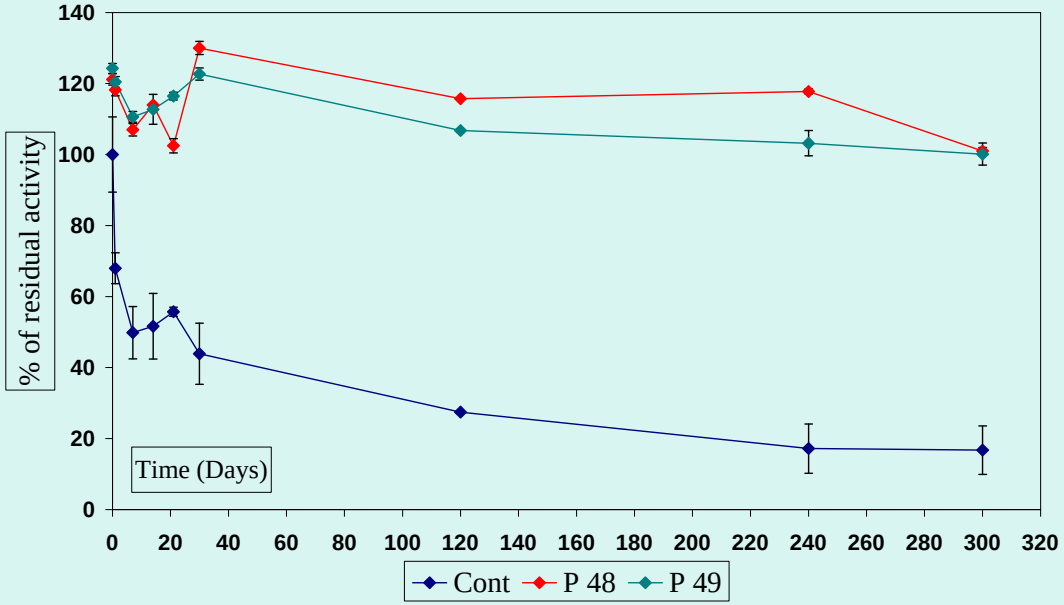
MB 1.82 fold increase in NADH detection

MBRS 7.28 fold increase in NADH detection

Electrodes tested with 1mM NADH in 0.05M PB pH 7.6

Cyclic Voltammetry, scan rate 50mV/s

Enzyme stability: Stabilisation of GDH enzyme in dry state



Microtitre plate analysis at 37ºC, 15% humidity
Plates vacuum dried

9 stabilisers tested, different combinations of sugars and polymers
AET propriety formulations

After 300 days storage at 37ºC:

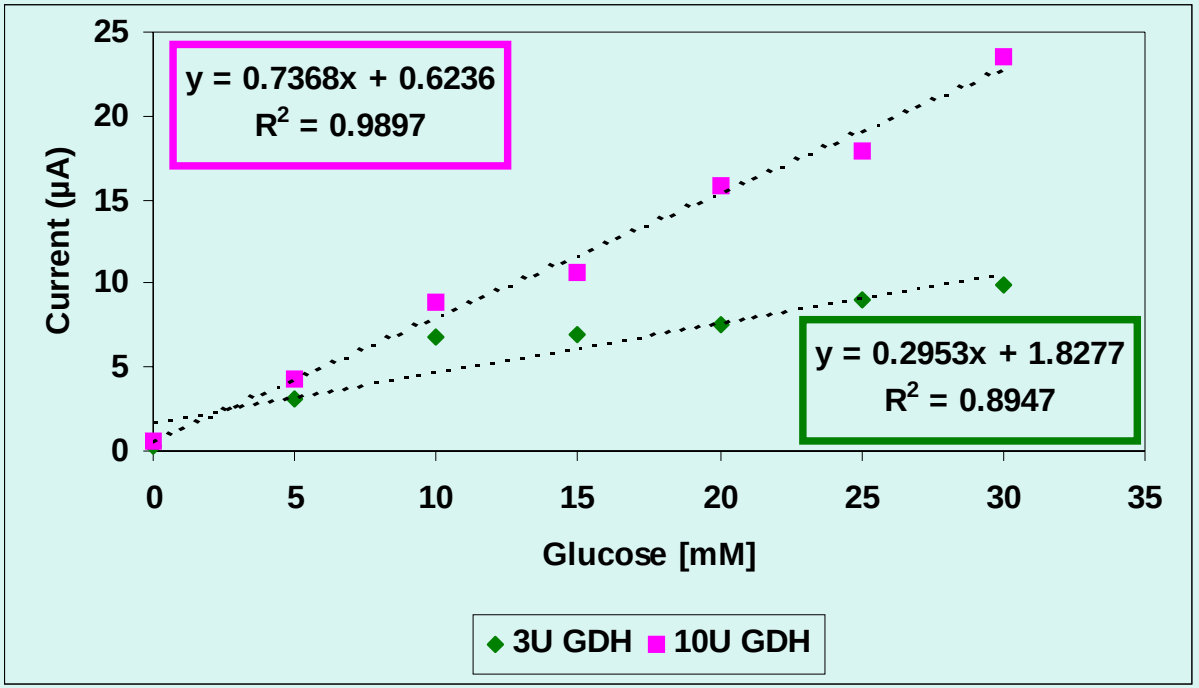
Enzyme test with no stabiliser: retained 16.76% of activity

Q2030317P48, P49 retained 100% activity,

Q2030317P47, P50 and P52 retained > 80% activity

The Q2030317P48 will be included in the biococktail formula.

Biosensor: Optimisation of GDH loading



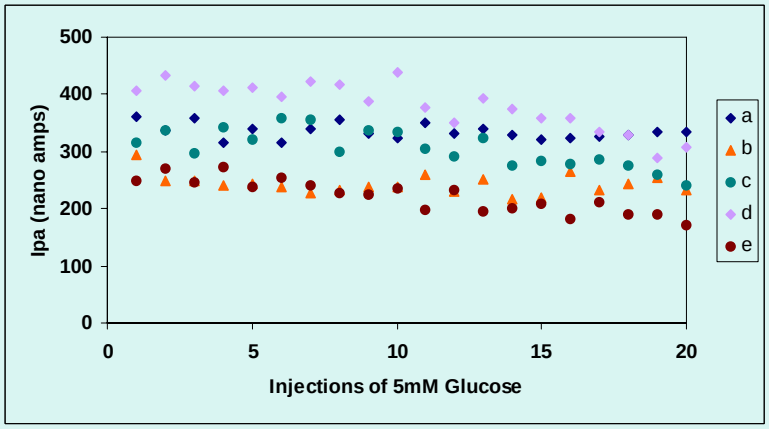
Complete biosensor tested by chronoamperometry in static mode

10 U GDH:
High sensitivity: 0.7368 µA/mM
Best correlation: R^2 =0.9897

The optimal loading for the biosensor was determined to be 10 units of GDH

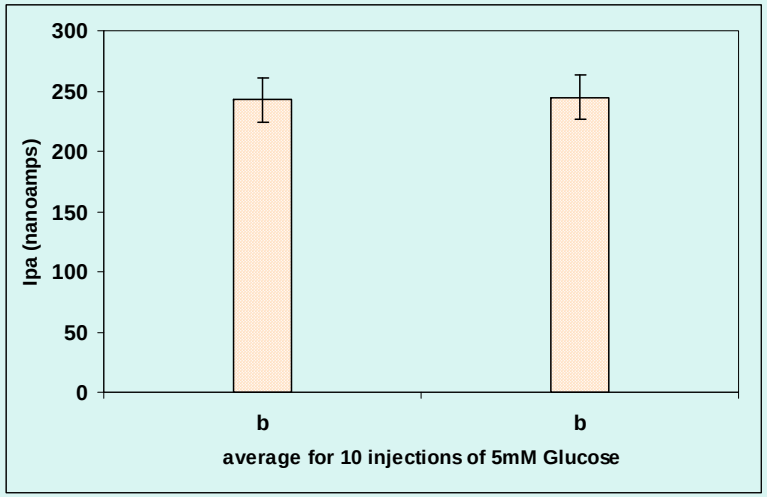
Reproducibility of cellulose acetate membrane:

Inter batches reproducibility



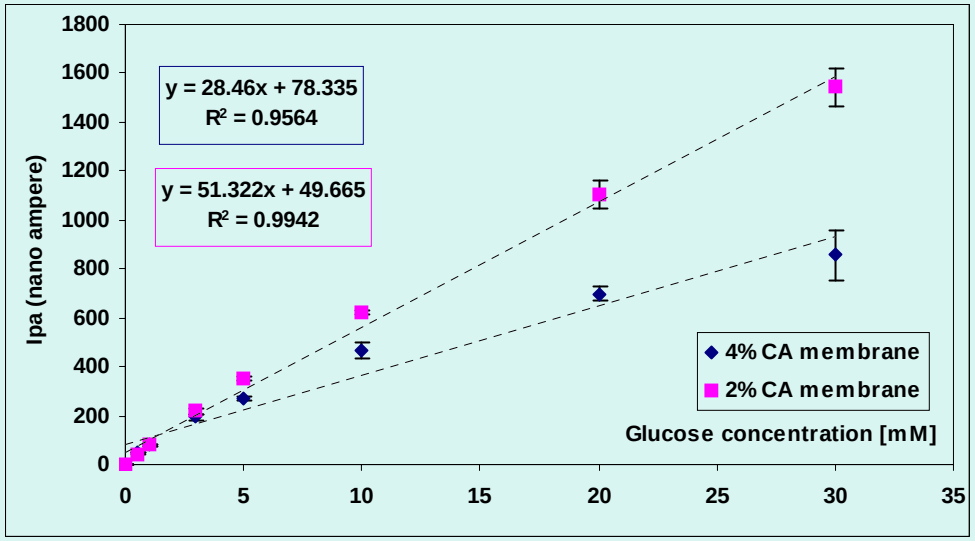
2% CA membrane	a	b	c	d	e
Ipa in nanoamps	334.60	242.65	305.70	380.25	221.65
St Deviation	12.96	17.26	32.28	42.14	29.67
CV%	3.87	7.11	10.56	11.08	13.39
n = 20 injections of 5 mM Glucose					

Intra batches reproducibility



Batch B	Sensor 1	Sensor 2
Ipa (nanoAmps)	244.90	242.63
St Deviation	18.55	16.53
CV%	7.57	6.81
For n = 10 injections of 5mM Glucose		

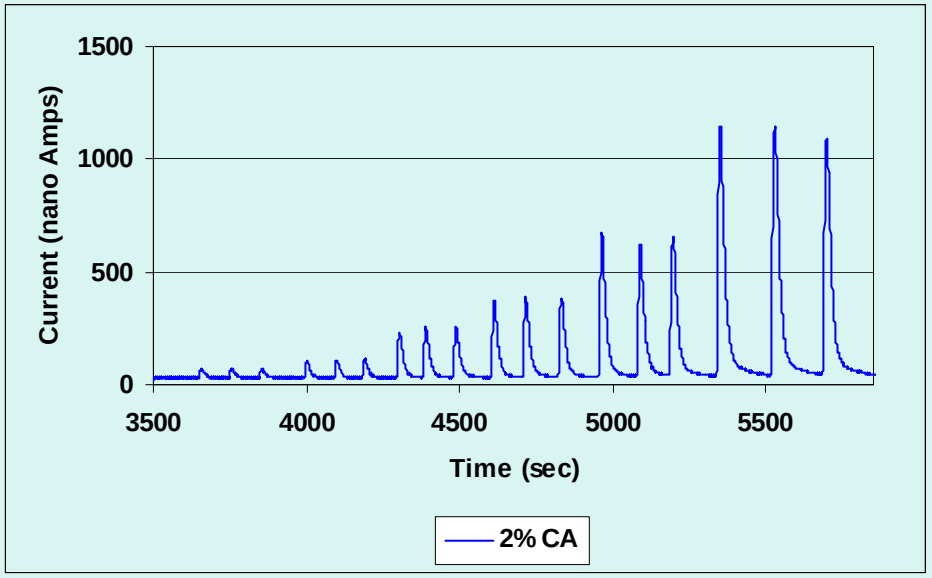
Glucose calibration limit of detection

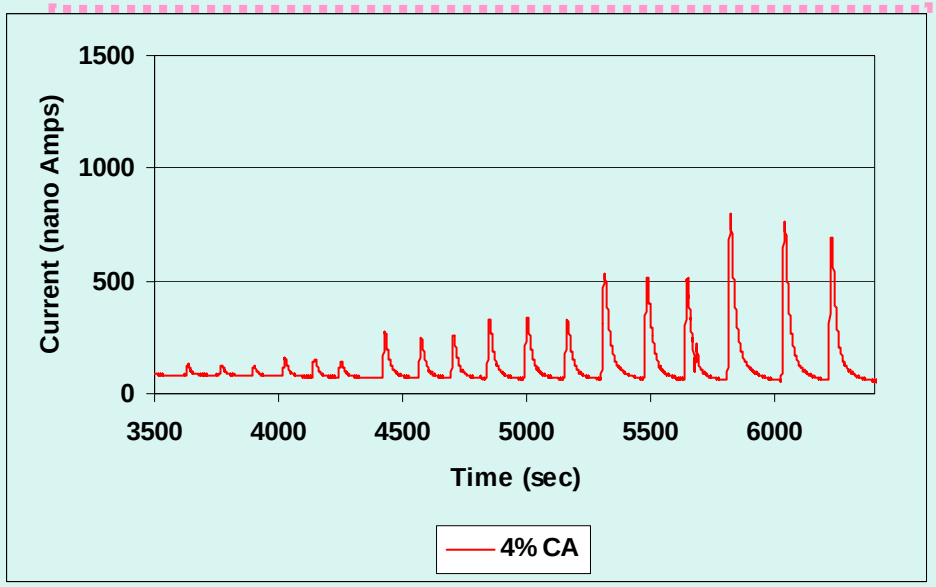


Test conditions:

Flow rate: 800 µl/min
Injection loop: 65µL
Applied potential: 50mV
10 Units GDH; NAD^+: 75mg/ml
25% Stabiliser P48; 0.5% Glutaraldehyde
2% Cellulose Acetate Membrane

Limit of detection with 2.0% Cellulose Acetate Membrane:
0.075 mM Glucose





Analytical applications

Injection	Native [mM]	Added mM	Found [mM]	% Recovery
1	10.87	5	15.02	82.90
2	10.87	5	15.02	82.90
3	10.87	5	14.71	76.89
			Average	80.90
			St Dev	3.47
			CV%	4.29

Multiple injections of Calf Serum, no pre-treatment to the sample

Response in mM Glucose, on single biosensor in FIA

Conclusions:

- **Cyclic voltammetry confirmed that MBRS under our test conditions showed a 4 times higher turnover for NADH detection compared to MB .**

- **Glucose dehydrogenase enzyme has been stabilised for 10 months in dry state at 37ºC = 444 days at room temperature.**

- **2 stabiliser formulations have been found to retain 100% activity,(Q2030317P48 and Q2030317P49).**

- **Other 3 further formulations retained more than 80% of enzyme activity (Q2030317P47, Q2030317P50, Q2030317P52).**

Conclusions:

- Good sensitivity and good linear range (0 - 30mM Glucose) was exhibited with the Flow Injection System (0.513μAmM^{-1} – R^2 = 0.9942)
- The optimised biosensor showed a limit of detection in FIA of 0.075mM of glucose
- The biosensor work at a low applied potential (50mV), therefore oxidation of interferences may be avoided
- Analytical application of the Biosensor in FIA using Calf Serum show that the proposed method has promise for the determination of glucose in complex samples.
- Advantage of fast response, in less that 10 second from injection.
- No alteration to the sample before analysis.

Future work

Operational stability of the biosensor in FIA

Analytical Application: Food analysis (Fruit juices)

Application of the same flow injections system to detect lactate and alcohol using dehydrogenase enzymes

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