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Sensor Technology For Rapid Environmental Ammonia Monitoring : (STREAM)

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Industrial Need for a Rapid Ammonia Test

- Ammonia is the major toxic component to be found in water
- Ammoniacal build up in the environment results from sewage effluent, land fill sites, effluent generated by paper, textile, leather, agricultural industries, including fish farming, chemical, petrochemical, pharmaceutical and metallurgic industries
- There is a real need for localised testing
- Great savings in time and effort for all water related industries will achieved if a portable field instrument measuring low levels of total ammoniacal nitrogen could be manufactured

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Project History

This project was originally formed as a collaboration between the Environment Agency, The University of West England, Gwent Electronic Materials Ltd. & Jenway Ltd. about 5 years ago.

The aim of this collaboration was to produce an electrochemical test for ammonia in water. This required the development of an accurate electrochemical biosensor which was successfully delivered by Professor John Harts Group at UWE.

The development of the base sensor material was carried out in conjunction with GEM.

The instrument development was carried out by Jenway

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Project History

3 years ago Applied Enzyme Technology became involved to solve the problem of enzyme stability

Following the proof of principle for a working stable ammonia biosensor The consortium entered a next phase of funding.

The Environment Agency, GEM and AET applied for a DTI BIOWISE Demonstrator award in order to move this project from the research phase to the mass manufacture of the biosensors.

This project started in January 2003

Further Instrument development was also initiated in January 2003 with the Environment Agency to deliver 5 handheld field instruments

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BIOWISE Demonstrator Project

Awarded in December 2002 started in January 2003
Partners AET, GEM and the Environment Agency

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Current Field Testing Technology

Ammonium-selective ion electrodes too insensitive to ammonium ions. They are subject to unacceptably high interference from sodium and potassium

Colorimetric test kits are inaccurate at low levels and impractical for field use

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Aqua System

Current State of the art, high cost multi-analyte system
Runs 24/7 all year
Needs skilled operators
High cost per test
Samples brought back to lab for analysis
Levels of ammonia change during transport

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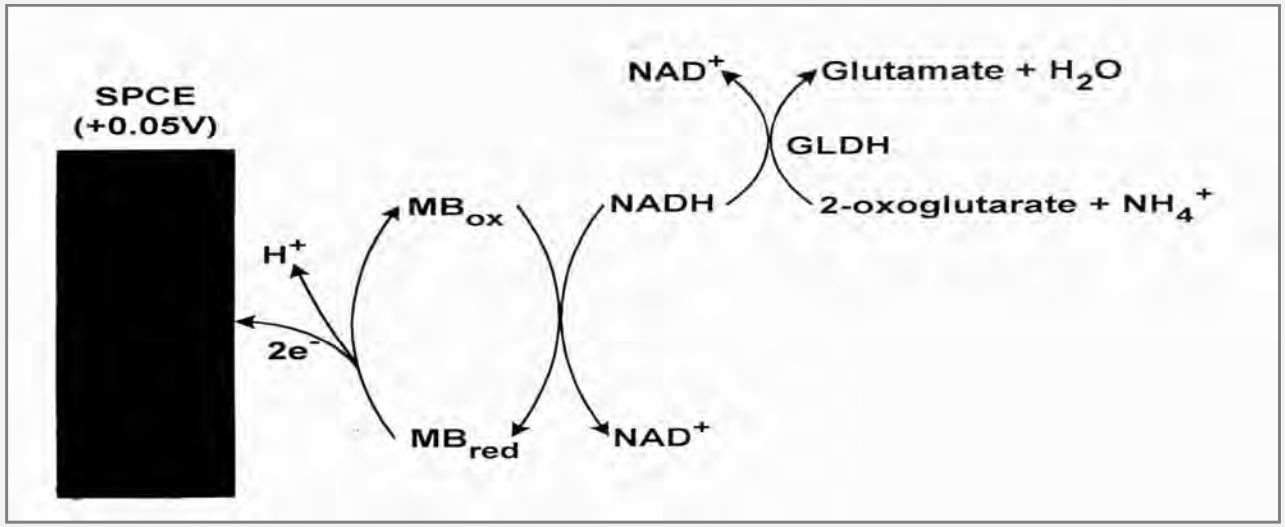
Schematic of Electrocatalytic Activity at the Ammonium Sensor Surface



- Base sensor is a screen printed carbon electrode impregnated with the electrocatalyst meldola's blue
- The MB-SPCE detects the conversion of NADH to NAD^+ when polarised at +50mV
- The electrons measured at the electrode surface result from the cycling of the MB between the oxidised and reduced states
- In the presence of ammonium ions, GLDH oxidises NADH to NAD^+ independently of the electrode potential
- This results in less NADH converted to NAD^+ by electro-oxidation route and less electron transfer at the electrode surface, resulting in a lower final current.

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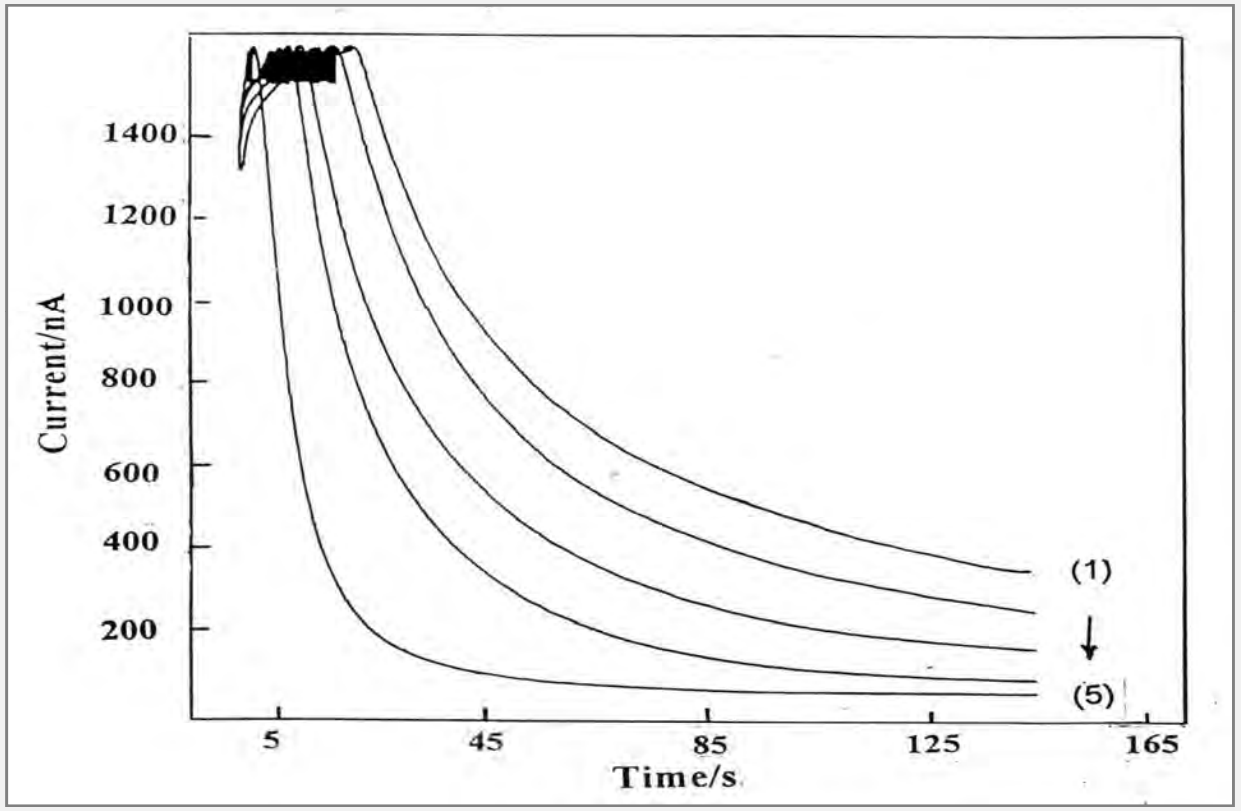
Sensitivity



- The chronoamperograms show the decrease in anodic current obtained with increasing ammonium ion concentrations [0, 0.05, 0.1, 1.0 and 2.0mM, equivalent to 0, 0.9, 1.8, 18 and 36 ppm. respectively

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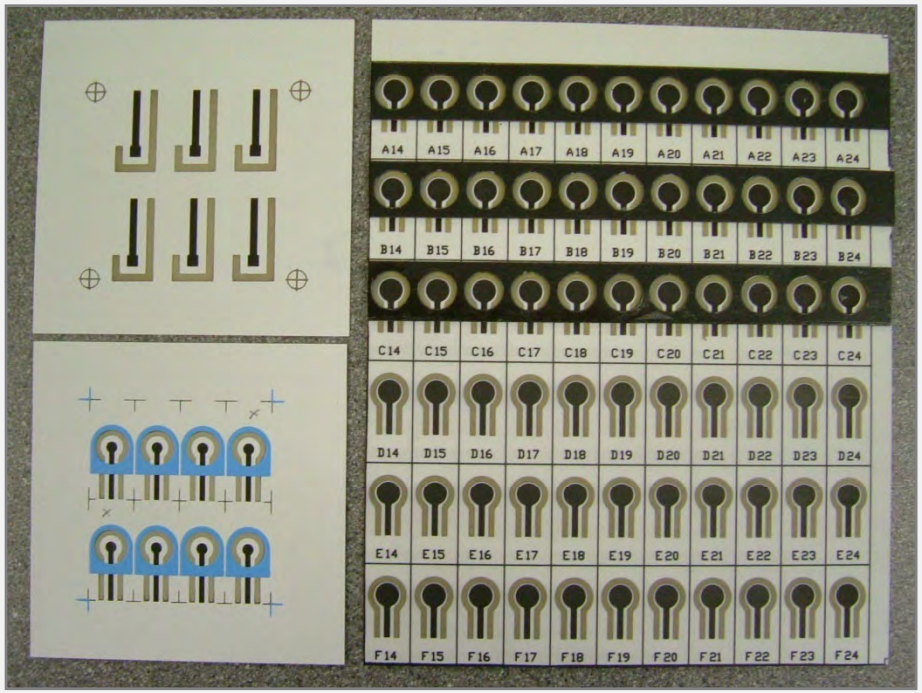
Biosensor Development



Sensor design changes over the past 2 years

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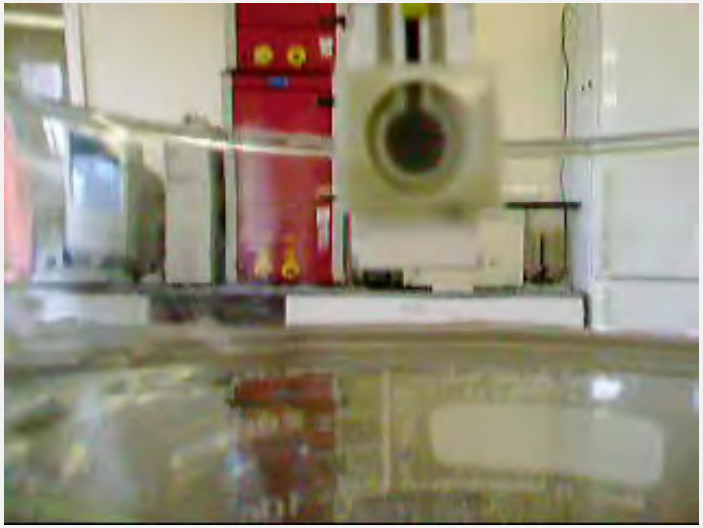
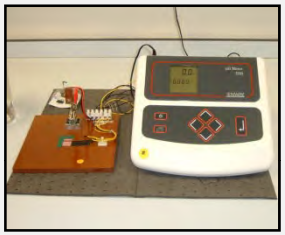
Sampling System



- Prototype biosensor dipping system enables low skill sampling
- Introduce filling system in mass manufacturing step
- Introduce mass packaging of completed biosensors

Instrumentation



Instrument array
Jan 2003

Integrated battery operated
potentiostat & Heater November 2003

Instrument array
June 2003

- Instrumentation has gone through several iterations
- OEM instrumentation in March 2003
- Delivery of battery operated heater June 2003
- Delivery of integrated potentiostat and heater November 2003

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Novel Technology

- Low skill disposable biosensor for on site testing
- High speed of test (5 minutes)
- Low cost
- Good precision (less than 10% Bias, 10% error as stipulated by EA)
- Good reproducibility
- Manufacturing quality system ISO 9001-2000
- Current dynamic range of sensor 1-10ppm
- No pre-calibration needed by operator

- Current State of the art, high cost multi-analyte system
- Runs 24/7 all year
- Needs skilled operators
- High cost per test
- Samples brought back to lab for analysis
- Levels of ammonia change during transport
- Pre-calibration required

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Instrumentation

Handi-lab October 2004

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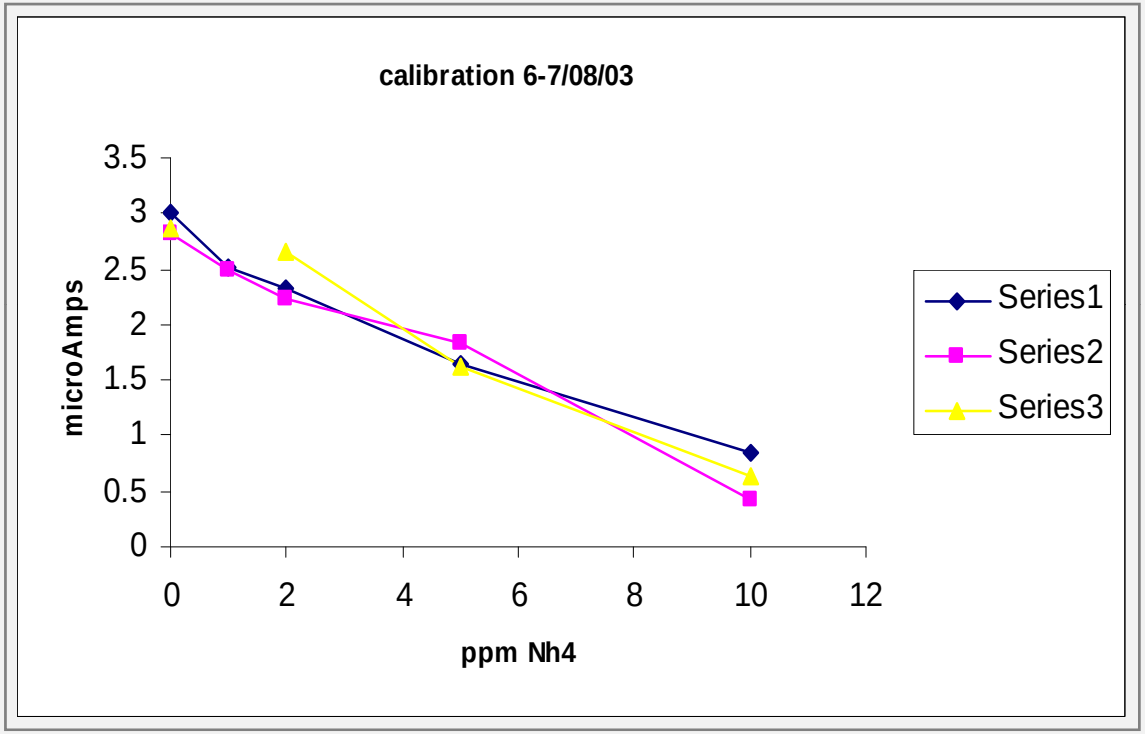
Reproducibility



- The sensor is able to give a linear calibration plot over the concentration range 1-10ppm NH4
- Calibration curves show less than 10% batch to batch variation following the manufacturing process

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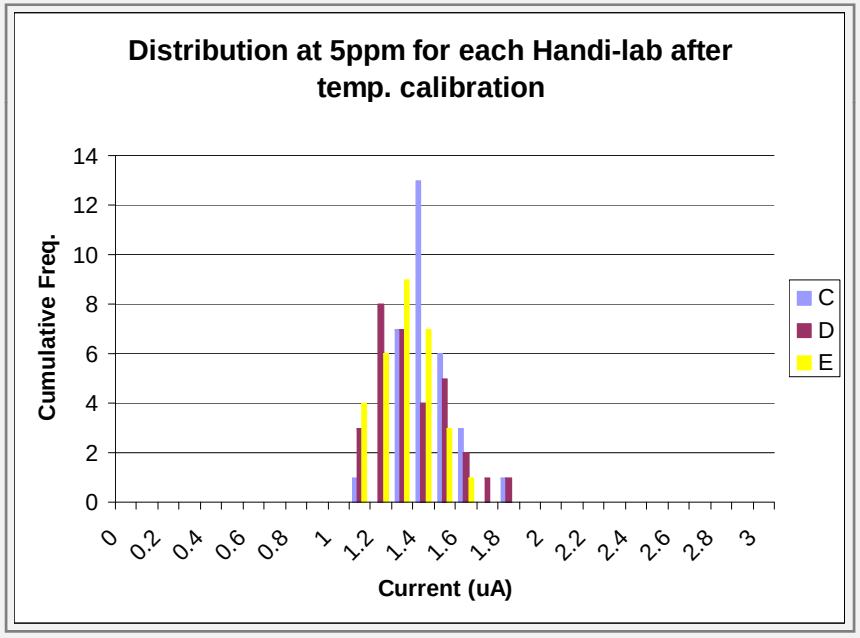
Quality Control



	C	D	E
Mean	1.366	1.300	1.256
Stdev	0.128	1.3	1.256
CV%	9.3	13.1	10.8

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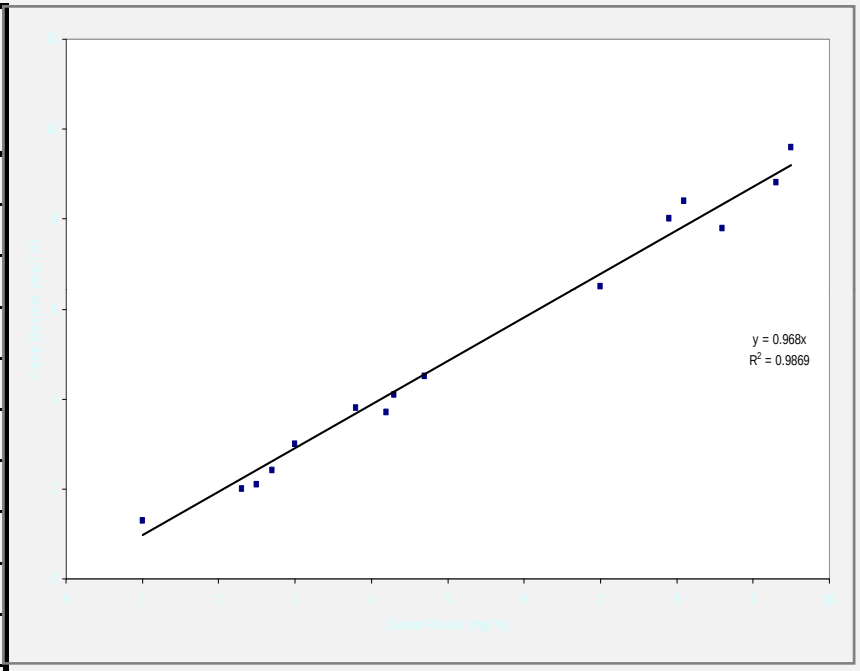
Real sample Trials at EA Starcross Laboratories

Sample	Meter Readings (uA)			Average Meter Reading (uA)	NH4 Result (mg/l N)	Lab NH4 Result (mg/l N)
	1	2	3			
419740	0.66	0.63	0.54	0.61	3.8	3.8
419745	0.31	0.35	0.28	0.31	8.6	7.8
419747	0.75	0.82	0.8	0.79	1	1.3
419751	0.69	0.77	0.66	0.71	2.5	2.1
419753	0.33	0.44	0.42	0.4	7	6.5
419754	0.27	0.34	0.16	0.26	9.5	9.6
421251	0.69	0.75	0.73	0.72	2.3	2
421256	0.38	0.31	0.33	0.34	8.1	8.4
421258	0.27	0.43	0.36	0.35	7.9	8
421183	0.58	0.56	0.48	0.54	4.7	4.5
421250	0.76	0.68	0.63	0.69	2.7	2.4
421261*	0.57	0.57	0.58	0.57	4.3	4.1
421227*	0.58	0.59	0.58	0.58	4.2	3.7
421364*	0.68	0.68	0.65	0.67	3	3
421366*	0.26	0.28	0.27	0.27	9.3	8.8

Sample Results: Batch 2030401.07
UKAS accredited analysis



Over 1000 sensors sent to Starcross Labs
Tested in parallel with Aqua lab test
Tested against unfiltered sewerage effluent
Falls within 10% bias 10% precision required by EA
r^2 values reported between 0.86 to 0.98

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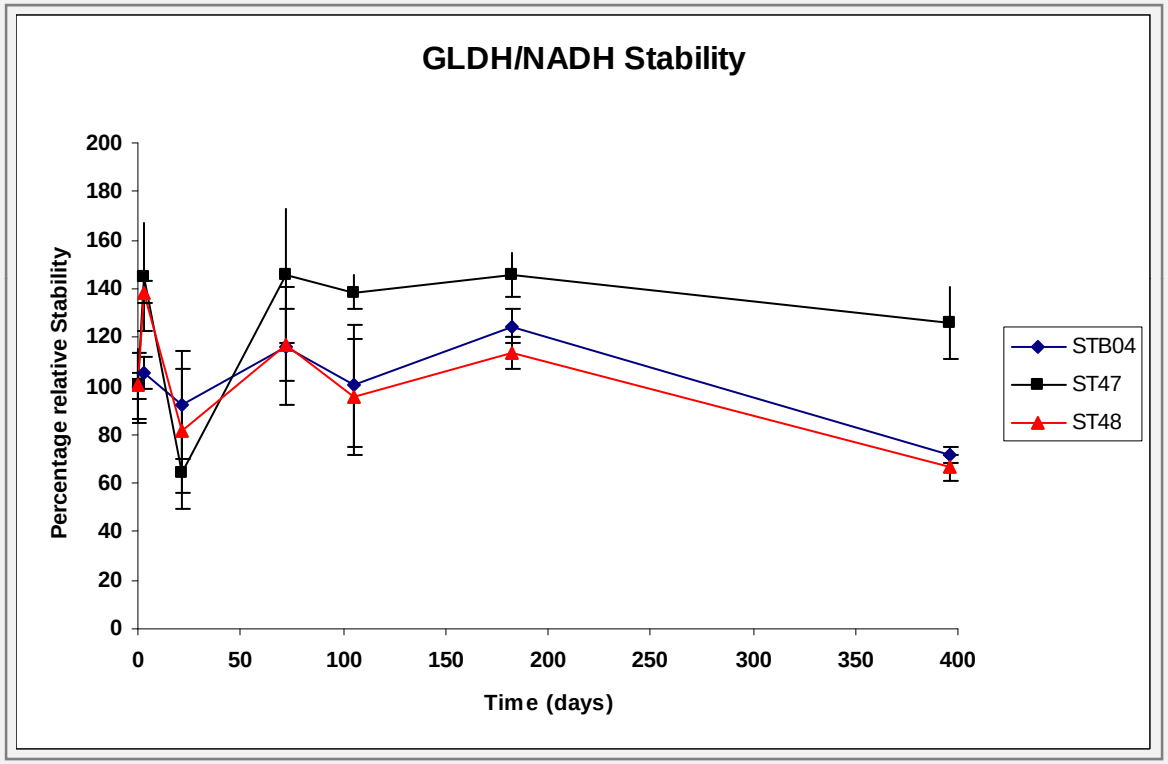
Long Term Stability of GLDH & NADH



3 stabiliser formulations were used to determine the long term stability of the biological components used on the sensor surface. One formulation shows no significant decrease in activity over 396 days at 37ºC, equivalent to 665 days at room temperature.

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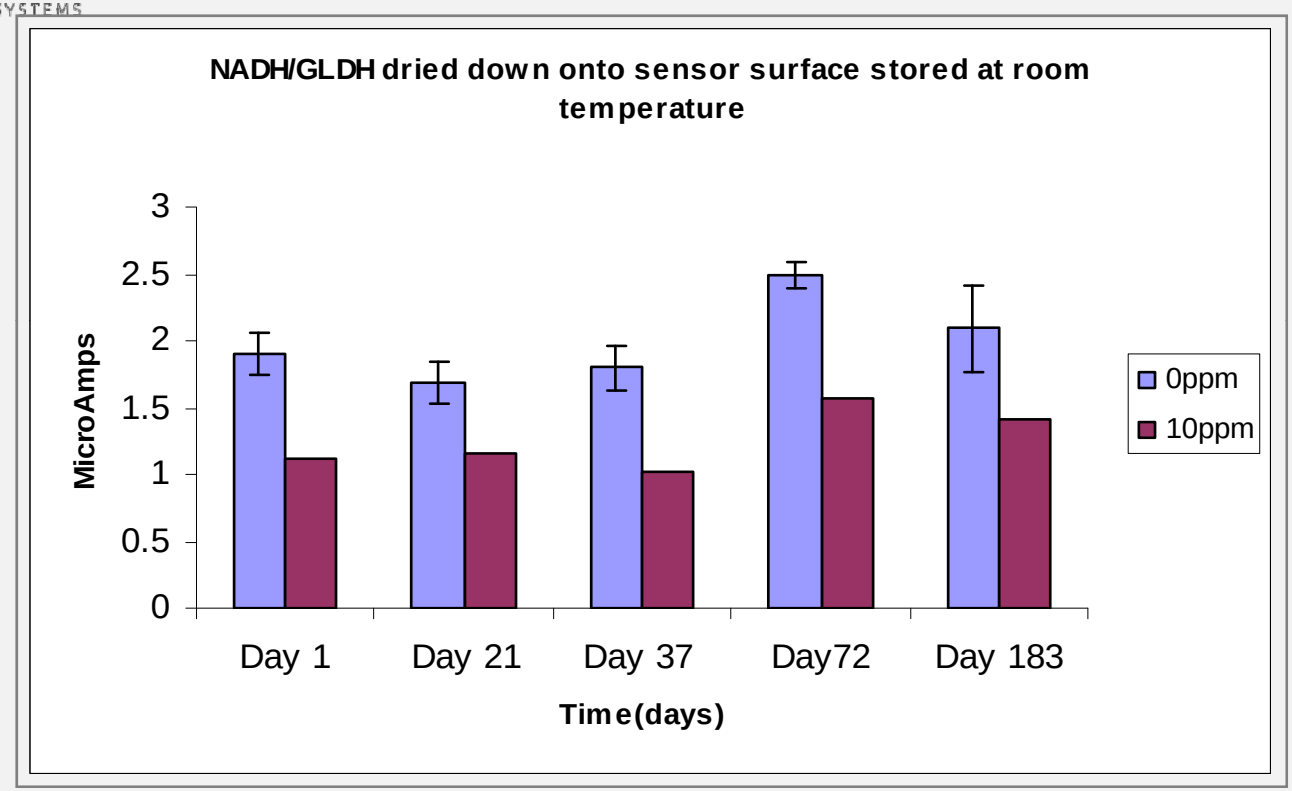
Long Term Sensor Stability



Stabilisation formulation transfer onto sensor surface shows stability of 183 days at room temperature, 6 months stability minimum requirement

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Summary

- This ammonia biosensor detects ammonia in the 1-10ppm range
- Stable for at least 6 months at room temperature, projections suggest 2 years stability
- The bias and precision of the biosensor fall within the requirement for the Environment Agency
- Good manufacturing reproducibility reported
- Sample delivery system satisfactory for field use
- Prototype handheld instruments delivery November 2003
- Successful testing of battery operated instrument and biosensors using “real-life” samples at the Starcross labs (over 5000 sensors)

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Future Work

Mass manufacture of complete biosensors, plus sampling system and packaging

Following testing phase of new instruments by the EA, extensive field testing will be initiated

Regional implementation

National implementation

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Future Technologies

Enzyme Based Sensors

Low level Ammonia detection (30 ppb)

Organophosphate (using acetylcholine esterase)

Phosphate (using pyruvate oxidase)

Nitrate (using Nitrate reductase)

Chlorine (electrochemical test)

Sulphate (microbial biosensor)

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“The Future of Environmental Monitoring is in our Hands”

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