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ADVANCED MATERIAL SYSTEMS

The Stability of Enzymes in Biosensors

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Company History

- **Applied Enzyme Technology Ltd formed in 1994**
- **Smart Awards 1994 & 1995 to develop protein stabilisation technology**
- **Patents for dry stabilisation fully granted in 1995 and 1996**
- **Patent for solution stabilisation fully granted in 2000**
- **AET acquired by LRH Ltd. and relocated June 2001**
- **BIOWISE Demonstrator Award 2003**
- **Multiple EU, TSB, Home Office & DEFRA Grants**



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Products & Services

- **Contract research for the stabilisation of specific proteins**
- **Supply Protein Stabilisation Kits for stability troubleshooting**
- **Supply of bulk stabiliser formulations**
- **Supply pre-stabilised enzymes**
- **Design and develop biosensors**
- **Carry out prototype & contract manufacture of low volume sensors**
- **Production of our own environmental & agrifood sensors**



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Proteins Stabilised AET

Stabilised over 50 proteins

Horseradish Peroxidase-Conjugated antibodies

Alkaline Phosphatase conjugated antibodies

Monoclonal antibodies

Esterases

Hydrolases

Kinases

Lipases

Luciferase

Oxidases

Oxidoreductases

Peroxidases

Phosphatases

Proteases

Most contract research has led to the generation of stable enzyme formulations from between 50 days to over 18 months at temperatures of up to 50°C



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Protein Stabilisation Kits



- The result of years R&D into the stabilisation of proteins
- Incorporates AET'S "Next Generation" of stabiliser molecules
- AET manufactures 4 kits designed to stabilise enzymes in the dry state (STKED), enzymes in solution (STKES), antibodies (STKAB) and pharma approved formulations (STKPH)



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Markets

- **Agricultural Industries (Food Additives)**
- **Biocatalysis Industry**
- **Biosensor Industry (human healthcare, environmental monitoring)**
- **Cosmetics Industry**
- **Diagnostics (Diagnostic kit development)**
- **Household Products (Biosterilisation, Laundry)**
- **Hygiene Industry (hygiene test kits)**
- **Pharmaceutical Industry (Drug development inhaled & topical application)**



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Why Do we need Stability?

Shelf Life

- **All products require both shelf life and operational stability**
- **Most biosensors require at least 6 months shelf life, in fact most specifications require between 1-2 years storage stability**
- **If a sensor cannot be stored without refrigeration for extended periods that sensor will never become a viable product**



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Why Do we need Stability?

Operational Stability

- **Operational stability is dependant on the type of biosensor.**
- **Disposable sensors can be active from seconds to several minutes. Reusable sensors may require several days to several months stability (reusable glucose sensor)**
- **The stability of a sensor may be the difference between a research prototype and a commercial sensor**



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Why Do we need Stability?

Solution Stability

- **Solution stability is required during the manufacturing process of the biosensor**
- **Whether the sensor is laid down by screen printing, biodotting or ink jetting proteins can be extremely labile in solution for extended periods**
- **Surviving the laying down process**
- **The drying process, i.e. extraction of the moisture from the enzyme solution on the sensor surface, is probably the major process step which will lead to the inactivation of the majority of proteins.**



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Mechanism of Stabilisation

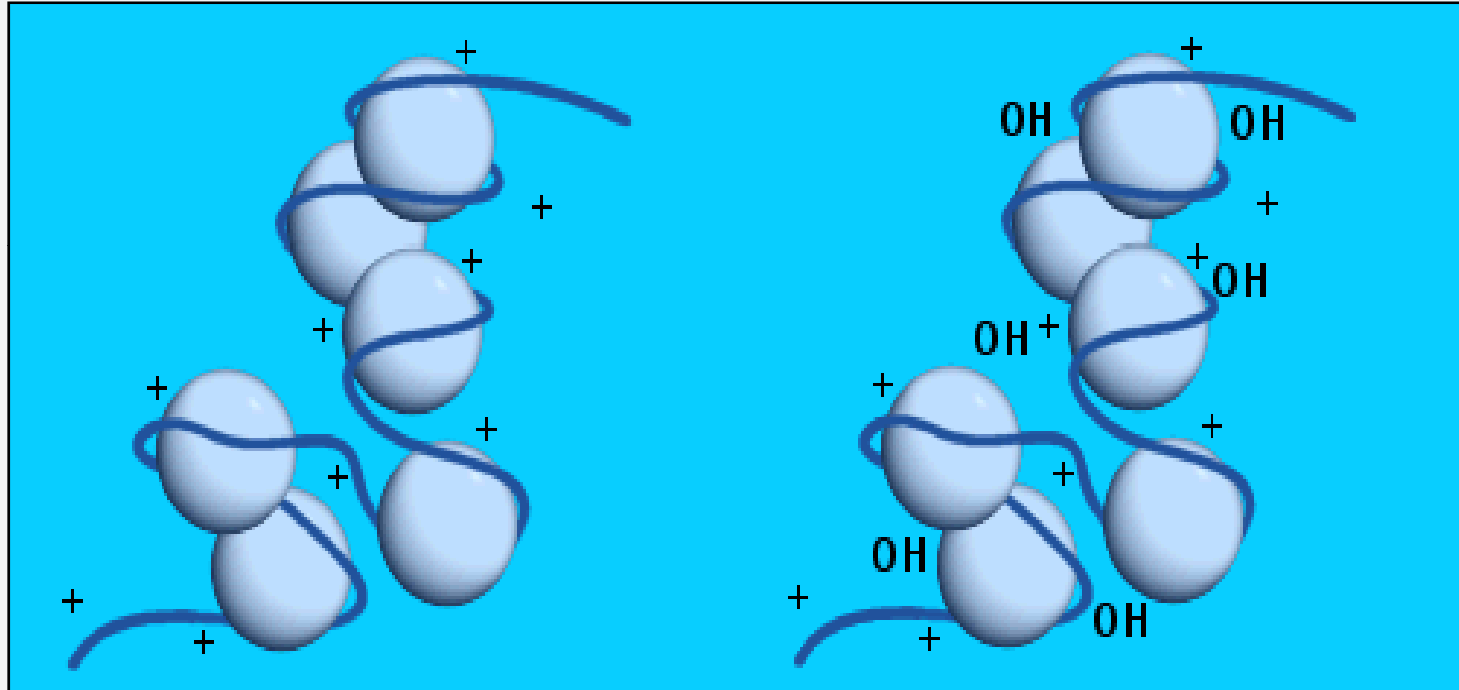
Stabiliser Combinations

- Where polyelectrolytes and polyalcohols are combined a synergistic effect is usually observed
- Ratios of polyelectrolyte to polyalcohol are extremely important in the overall stabilisation of proteins
- The buffer type, pH, ionic strength, concentration and ratio of stabilisers to protein/enzyme all play crucial roles in protein stabilisation both in the dry state and in solution



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The Mechanism of Stabilisation

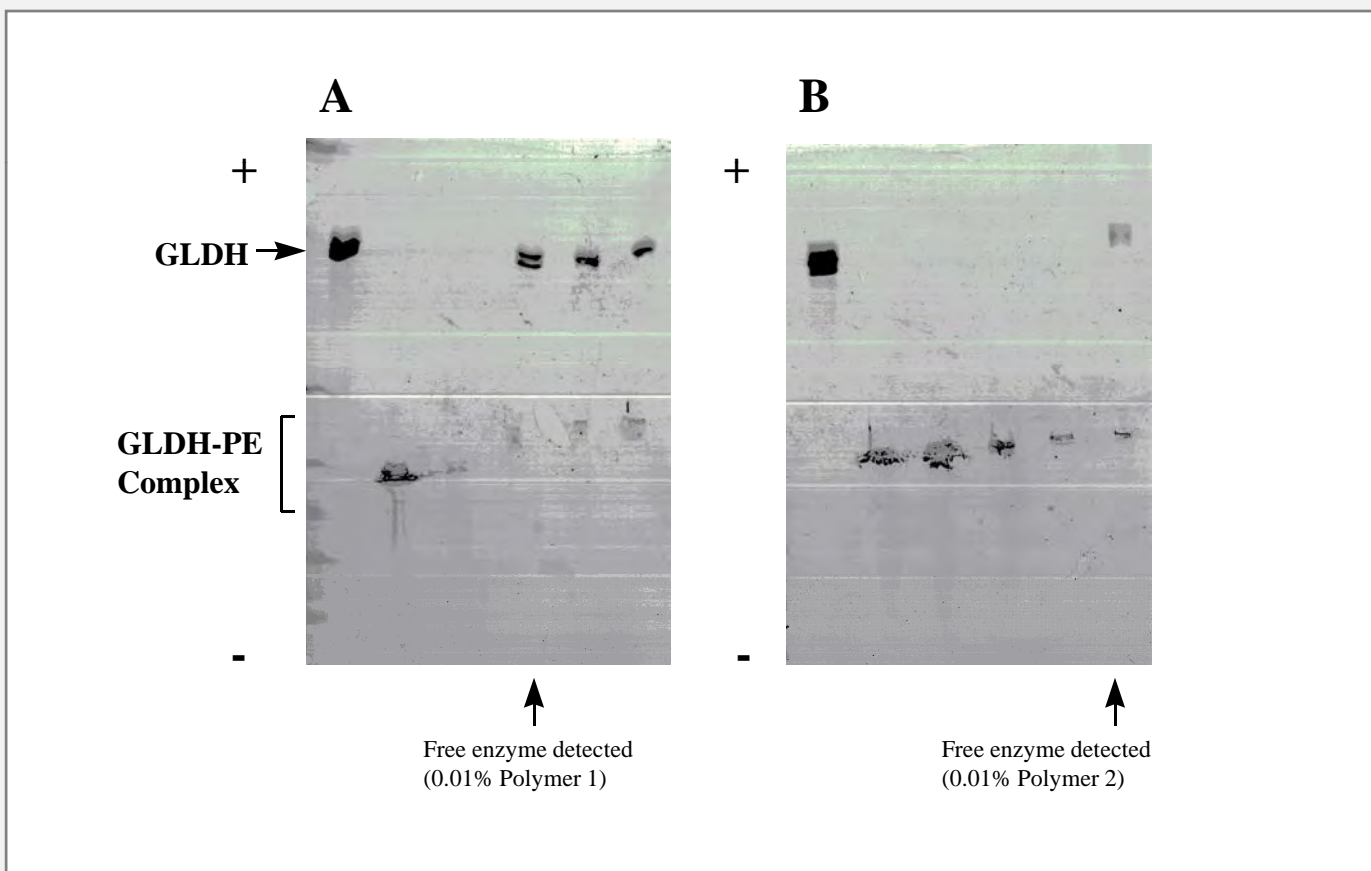


The addition of polyelectrolytes to solutions of proteins promotes the formation of soluble protein/polyelectrolyte complexes by electrostatic interaction. Polyhydroxyl compounds are then able to penetrate the structure more effectively leading to stabilisation



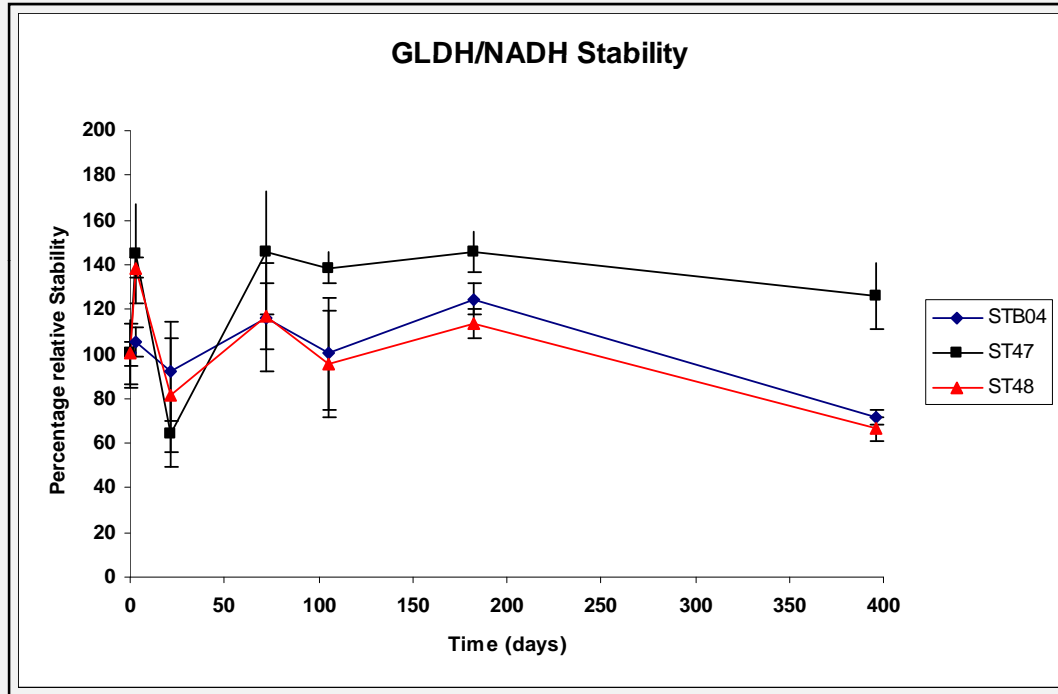
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The Detection of the Stoichiometry of Polymer Binding to the Thermophilic Form of GLDH





Long Term Stability of GLDH & NADH

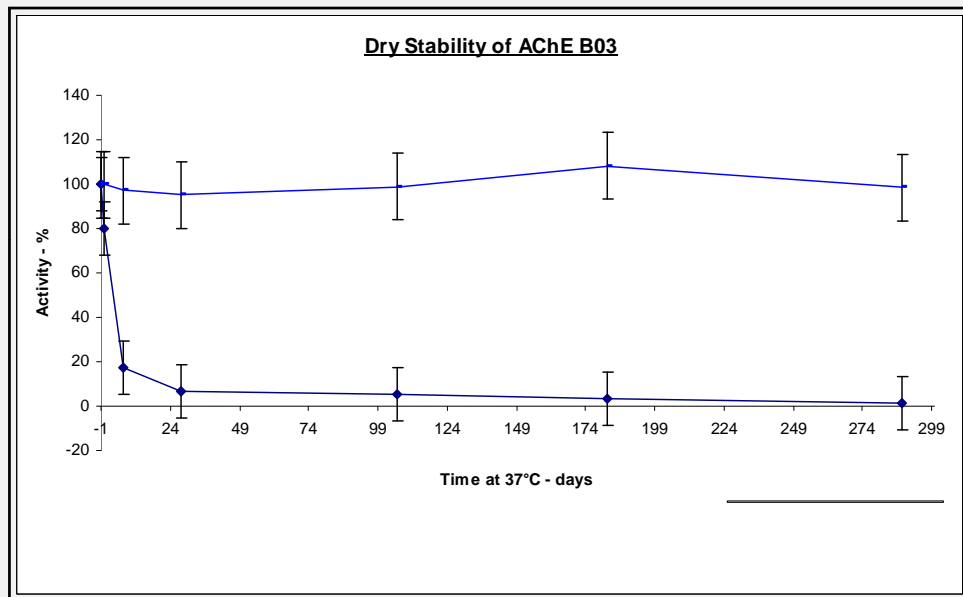


3 stabiliser formulations were used to determine the long term stability of the biological components used on the sensor surface. One formulation shows no significant decrease in activity over 396 days at 37°C, equivalent to 665 days at room temperature.

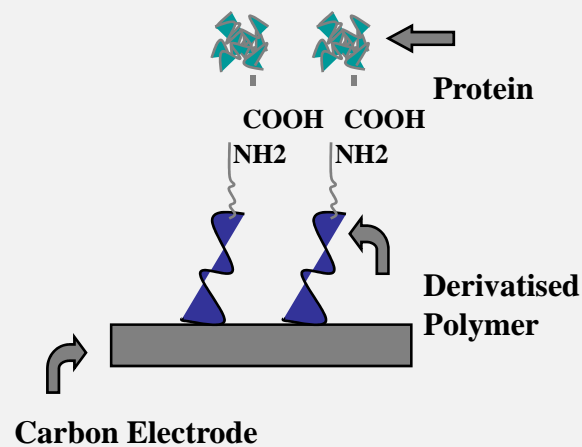
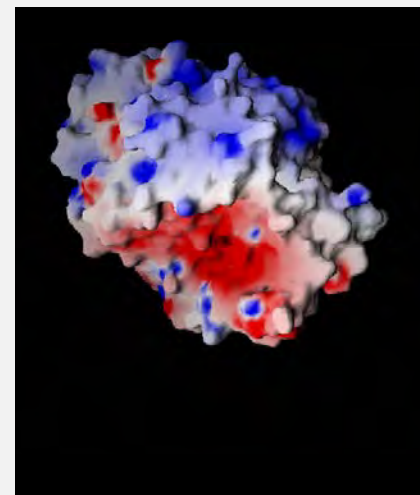


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Acetylcholine Esterase



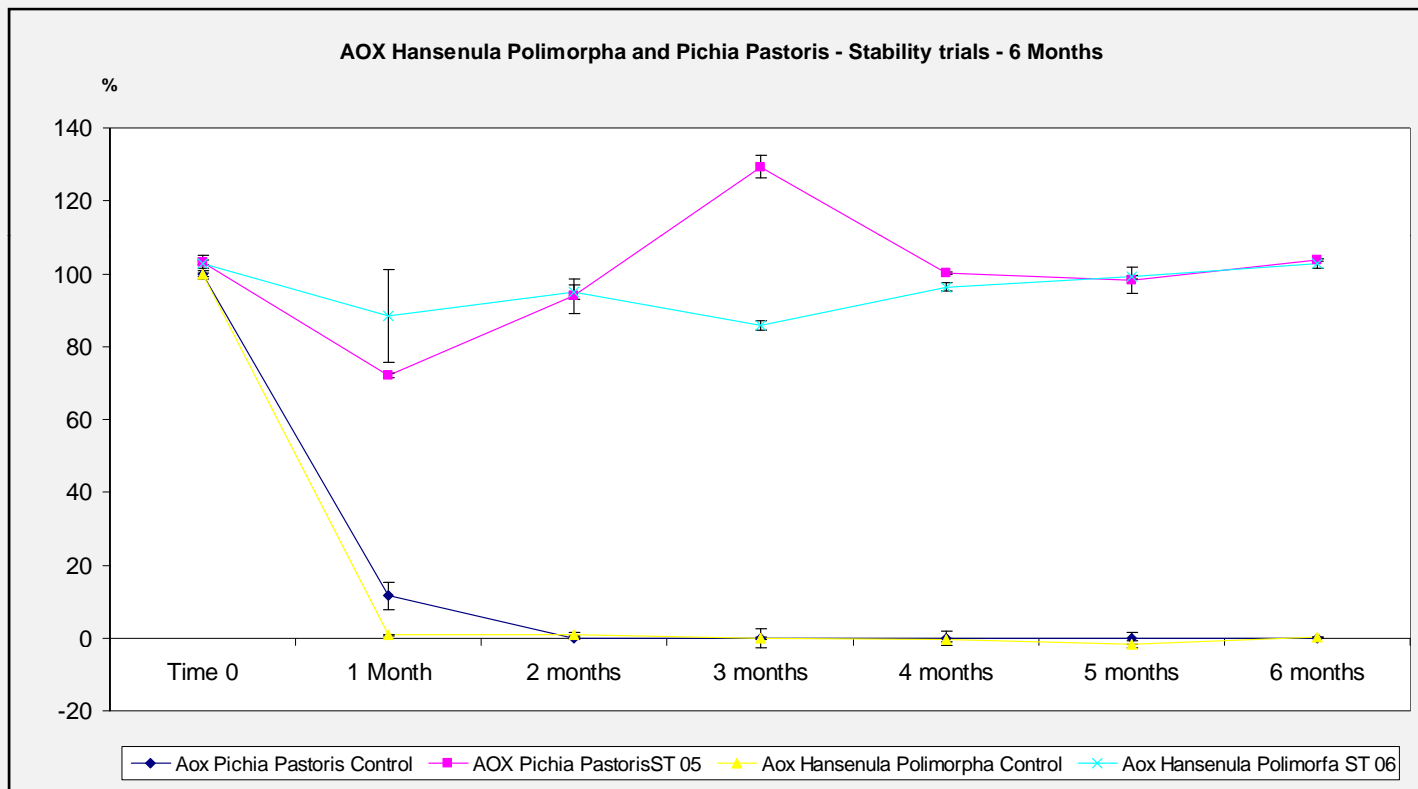
280 days stability @ 37oC





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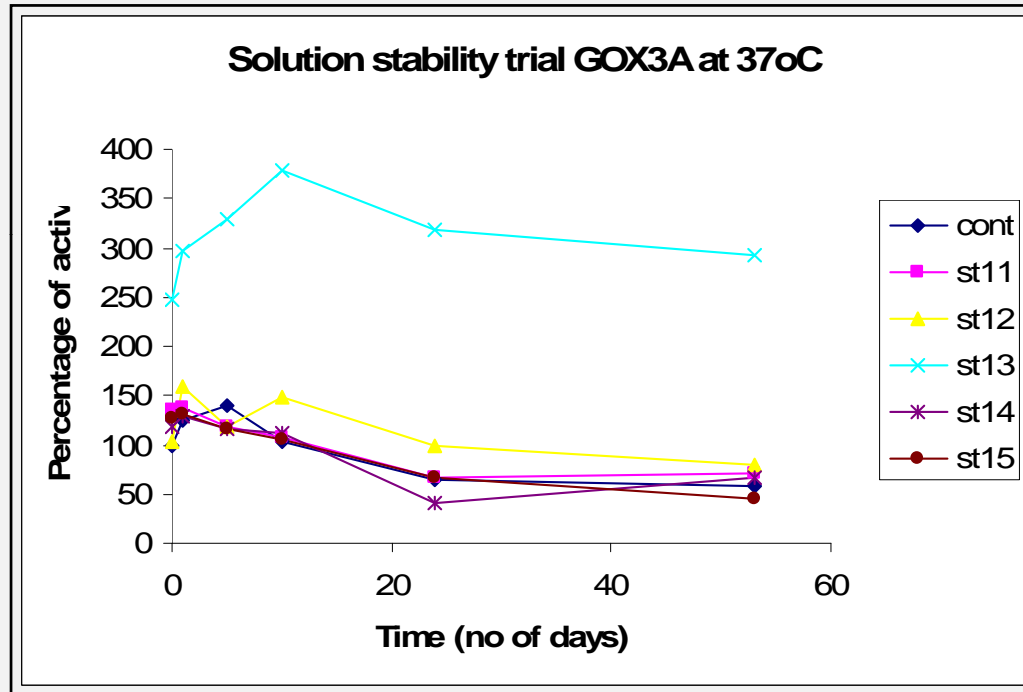
Alcohol Oxidase



Alcohol Oxidase stability as determined by microtitre plate assay



Glucose Oxidase Stability

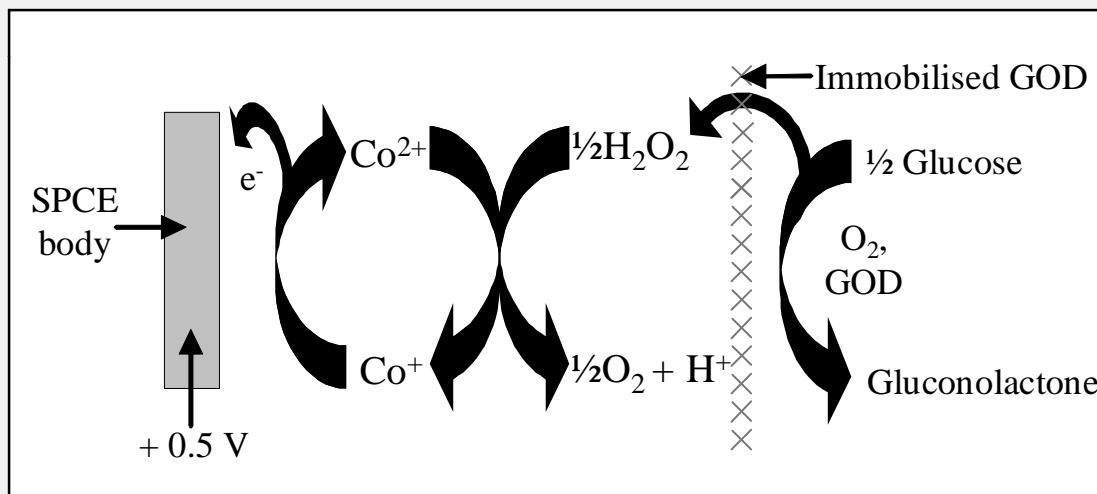


Glucose oxidase solution stored for extended time prior to biosensor construction and testing



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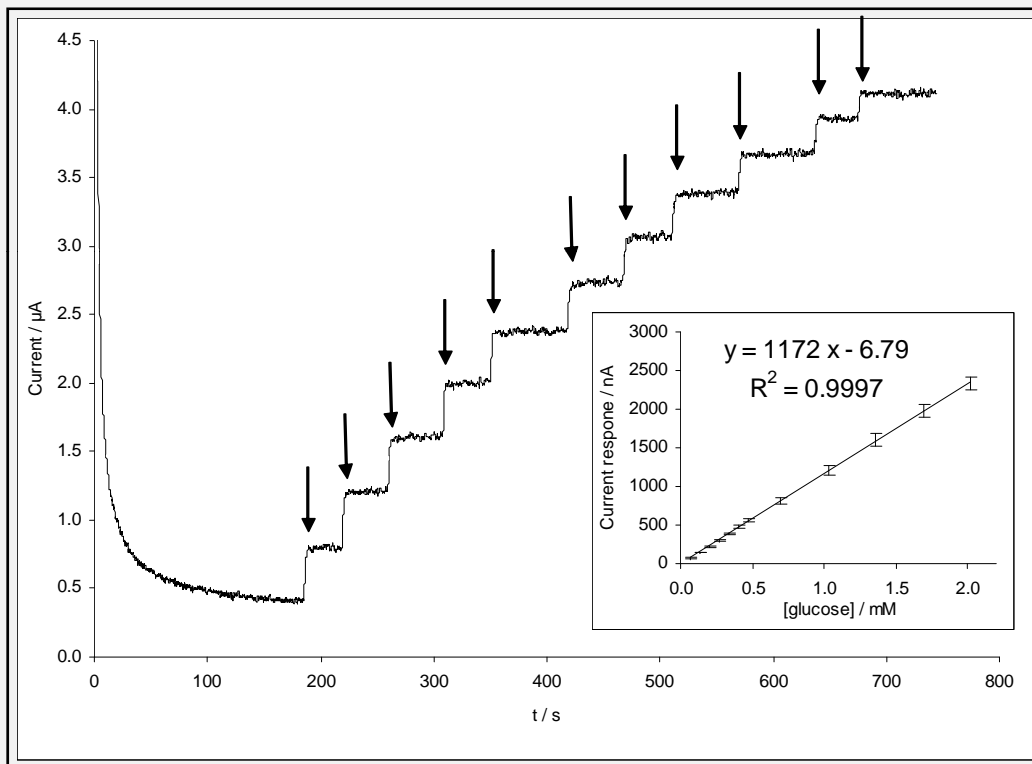
Schematic of Oxidase Biosensor





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Water Based GOX Ink

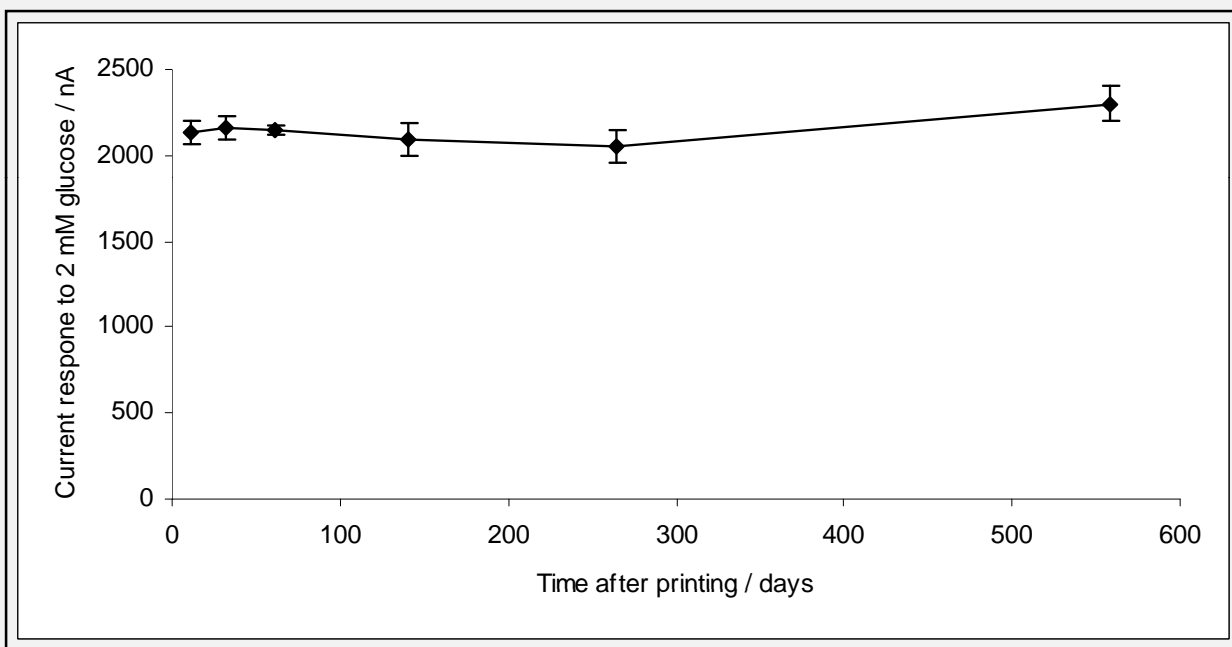


Amperogram obtained using glucose biosensors in stirred solution. Arrows correspond to 70 µl additions of 50 mM glucose to 10 ml supporting electrolyte.



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Electrode Stability

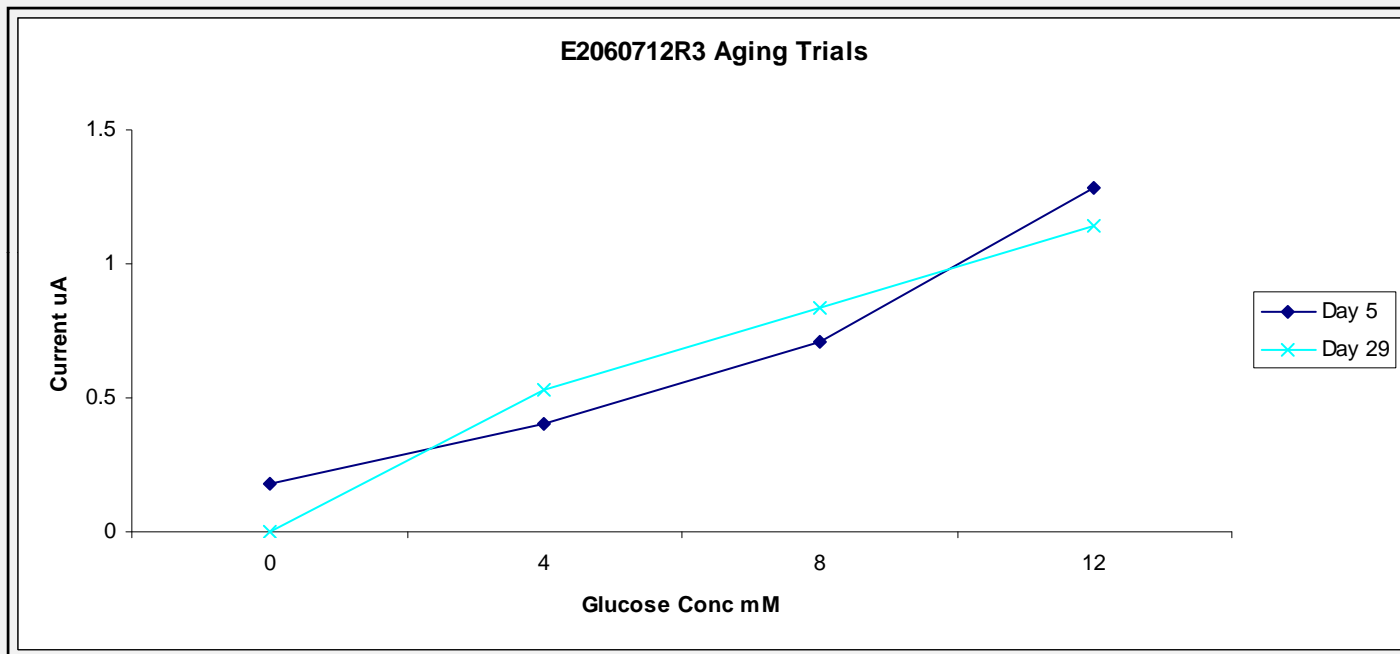


**Water based GOX electrodes printed and stored at 40°C.
Electrodes tested using 2mM glucose additions**



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GOX INK Aging Trials



GOX ink stable for at least 29 days at RT



Lactate Biosensor

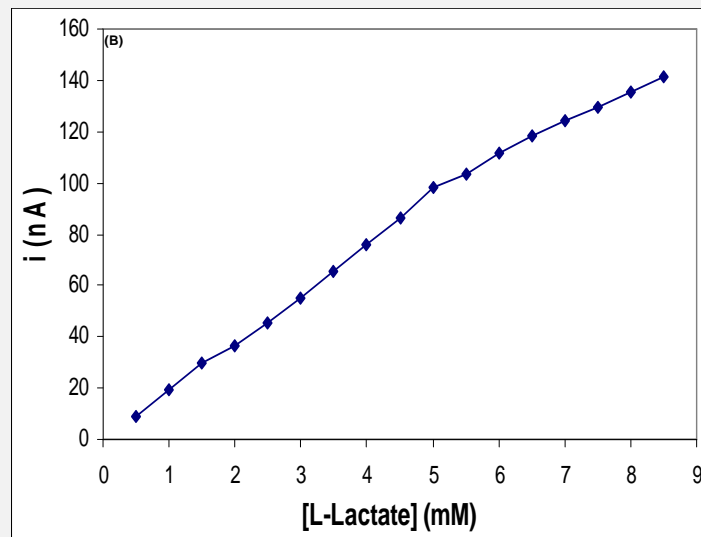
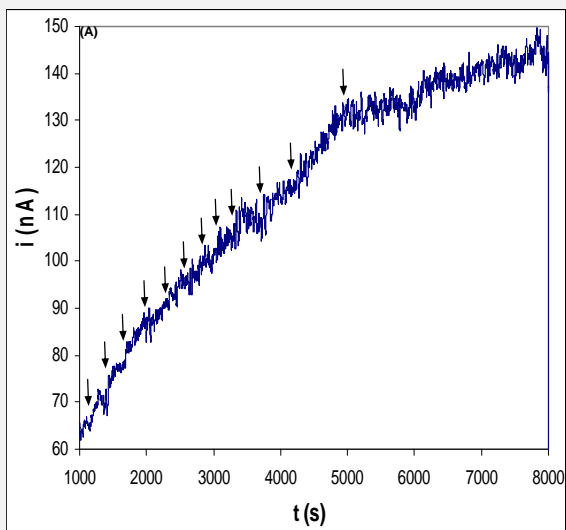
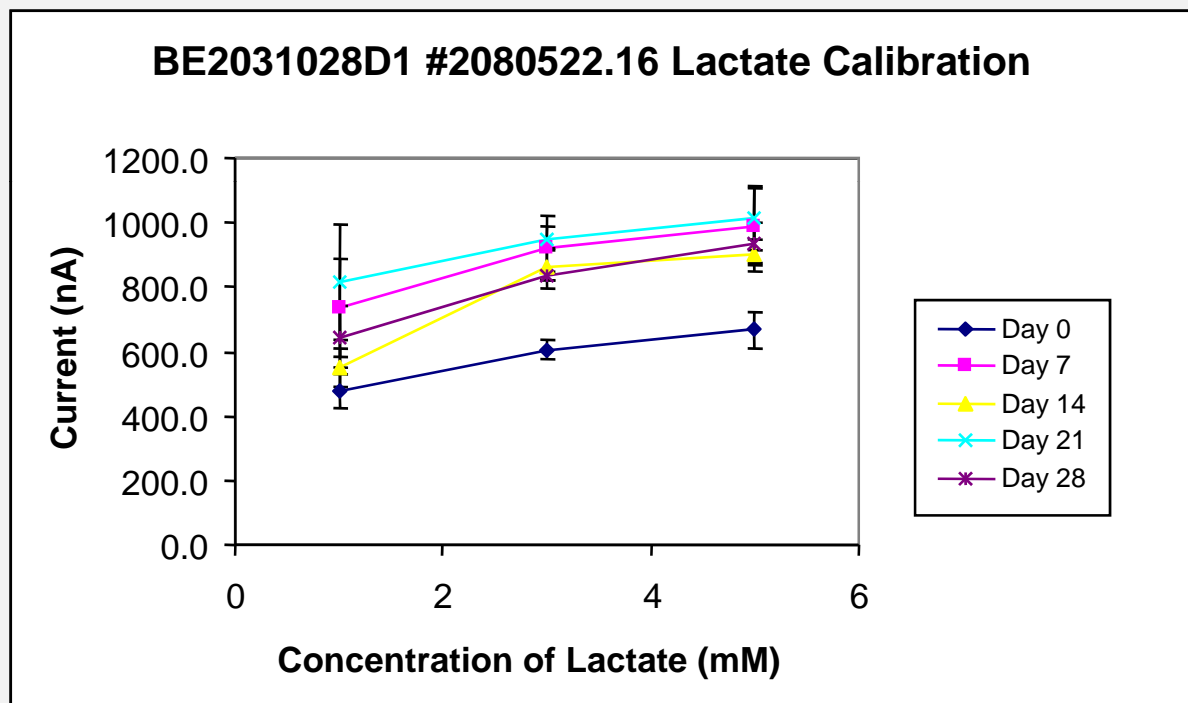


Fig.5. (A) amperogram and (B) calibration plot for L-lactate in culture medium using a microband biosensor at 37°C.



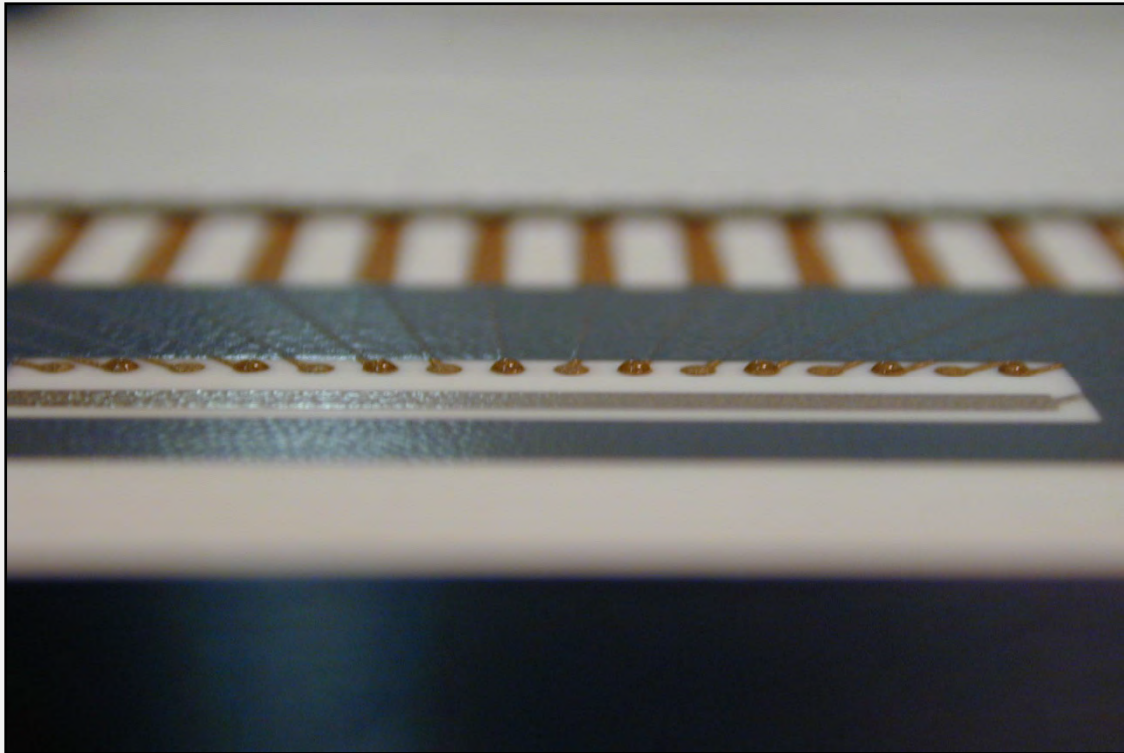
Lactate sensor stability





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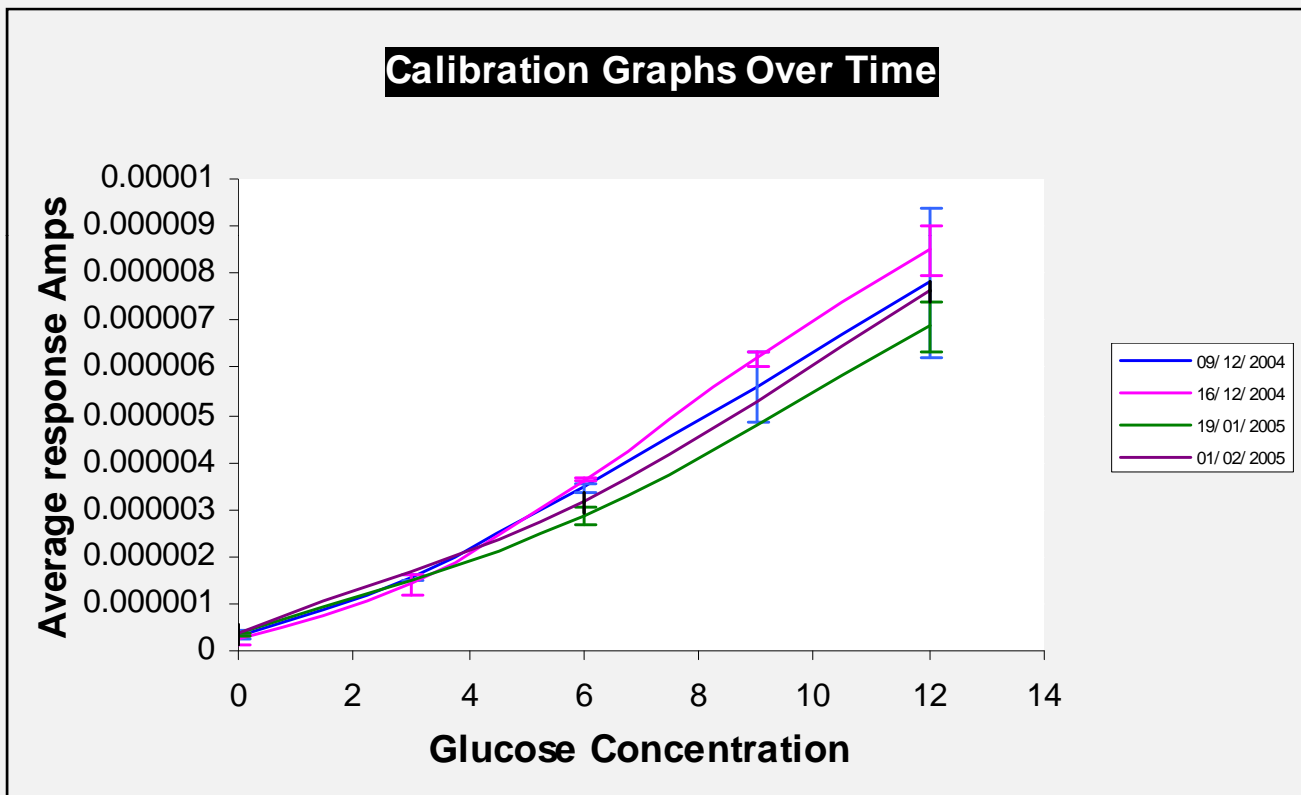
Dispensing of DNA probes





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Glucose Biosensor

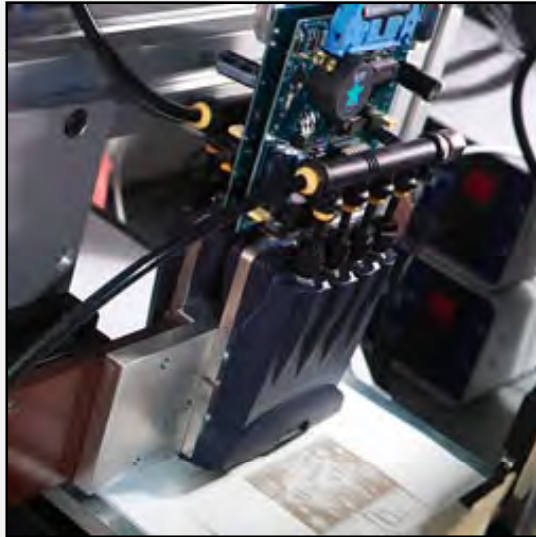


Glucose oxidase biodotting solution stored for up to 54 days @ 4oC prior to biosensor construction and testing



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Ink jet printing





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Conclusions

- **AET is able to deliver stabilisation technology both in the liquid & Dry states.**
- **The Gwent Group has a number of printable enzyme formulations**
- **The Gwent Group has a platform technology for screen printing water based carbon/enzyme inks**
- **The Gwent Group can deliver expertise in:**
 - **sensor materials and printing formulations, sensor design and thick film printing**
 - **The stabilisation and immobilisation of proteins onto electrode surfaces**
 - **We deliver a 1 stop shop for the development and production of biosensor technology**
- **The Gwent Group are further developing:**
 - **Water based carbon/enzyme inks**
 - **Biodot compatible enzyme mediator formulations**
 - **Ink-jetable enzyme formulations**



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