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# **Enzyme Cocktails & Bio-Stabilisation on Biosensors**

**Applied Enzyme Technology Ltd.**

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# Outline of Presentation

- **Why do we need stabilisation of proteins?**
- **Choice of stabilisers**
- **Influencing factors**
- **Examples of stabilised proteins**
- **Protein immobilisation and stabilisation**
- **AET Products**



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## Proteins Stabilised by AET Ltd.

**21 proteins stabilised in the dry state**

**28 proteins stabilised in solution**

**Horseradish Peroxidase-Conjugated Antibodies**

**Alkaline Phosphatase conjugated antibodies**

**Esterases**

**Kinases**

**Luciferase**

**Oxidoreductases**

**Phosphatases**

**Hydrolases**

**Lipases**

**Oxidases**

**Peroxidases**

**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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# 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**
- **The stability specification must be determined early on in development**



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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 or via dotting proteins can be extremely labile in solution for extended periods.**
- **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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**Shelf life**  
**Operational stability**  
**Solution stability**

**Via the introduction of the right  
stabiliser formulation and protocol all of  
these stability issues can be solved!**

**We can help!!**



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# Stability Troubleshooting

- **Stabiliser pre-screening is carried out using optical methodology and or electrophoretic techniques, these can interrogate large numbers of stabiliser combinations.**
- **Choice of stabilisers, buffer, pH, ionic strength, protein concentration are critical.**
- **Dry Formulation (air dry, vaccum dry, freeze dry). Dependant on sample volumes and equipment available.**
- **Store at elevated temperature in order to increase speed of degradation (preferred temperature 37°C).**
- **Ensure storage in low humidity and low oxygen environment.**
- **Measure enzyme activity regularly.**
- **Transfer enzyme/ stabiliser formulation to biosensor for further investigation.**





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# Choice of Stabiliser

- **Determine the isoelectric point of your protein**
- **This will enable you to identify the type of polyelectrolytes that will bind your protein of interest**
- **If you are unable to do this you can either use gel electrophoresis to determine the binding avidity of the protein/polyelectrolyte complexes**
- **Or use a small panel of positively, negatively or neutrally charged polyelectrolytes**
- **The recommended polyols are less easy to determine. However there are a small number of standard molecules which are quickly screened**



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## Enzyme Stabilisation: Influencing factors

***The single most important parameter in promotion of structural stability of proteins is retaining the surface water activity of the protein. By adding polyhydric alcohols the surface water activity is modified relative to the absolute concentration of the additive.***



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## **Enzyme Stabilisation: Influencing factors**

**When used in combination with additives promoting electrostatic interactions (polyelectrolytes) or surface chemical interactions leading to immobilisation or crosslinking, the efficacy is usually enhanced significantly, indicating synergy of action at the surface of the protein structure thereby stabilising the protein overall.**



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## Enzyme Stabilisation: Influencing factors

**Some additives such as metal ions are directly related to enzyme structure and as such are not strictly surface interactions. Addition of dilute solutions of metal salts eg. magnesium, calcium often stabilise proteins to a high degree and act synergistically with polyelectrolyte combinations.**



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# The Mechanism of Stabilisation

## 1. Polyalcohols

- The polyalcohols include sugars and sugar alcohols
- Polyalcohols modify the water environment surrounding a protein thus replacing and competing for free water within the system
- This modified hydration shell confers protection to the protein maintaining 3D structure and biological activity
- This enables storage of biological materials both in solution and in the dehydrated state



# The Mechanism of Stabilisation

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## 2. Polyelectrolytes

- **Polyelectrolytes include numerous polymers of varying charge and structure**
- **The interactions between proteins and polymers are electrostatic**
- **These form large protein-polyelectrolyte complexes which retain full biological activity**
- **At AET we have the ability to calculate the stoichiometry of protein/polymer binding which we have determined as critical for maintaining enzyme activity in stabiliser formulations**



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# The Mechanism of Stabilisation

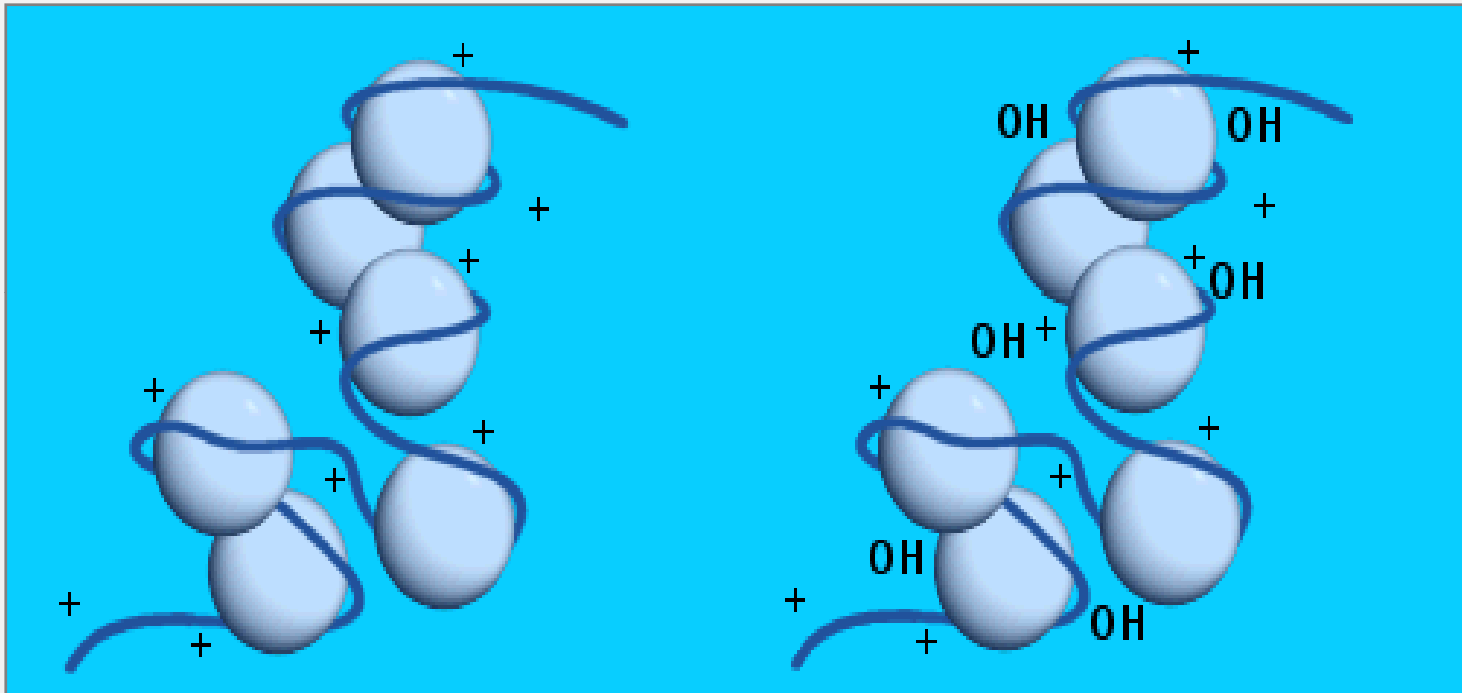
## 3. Stabiliser Combinations

- 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



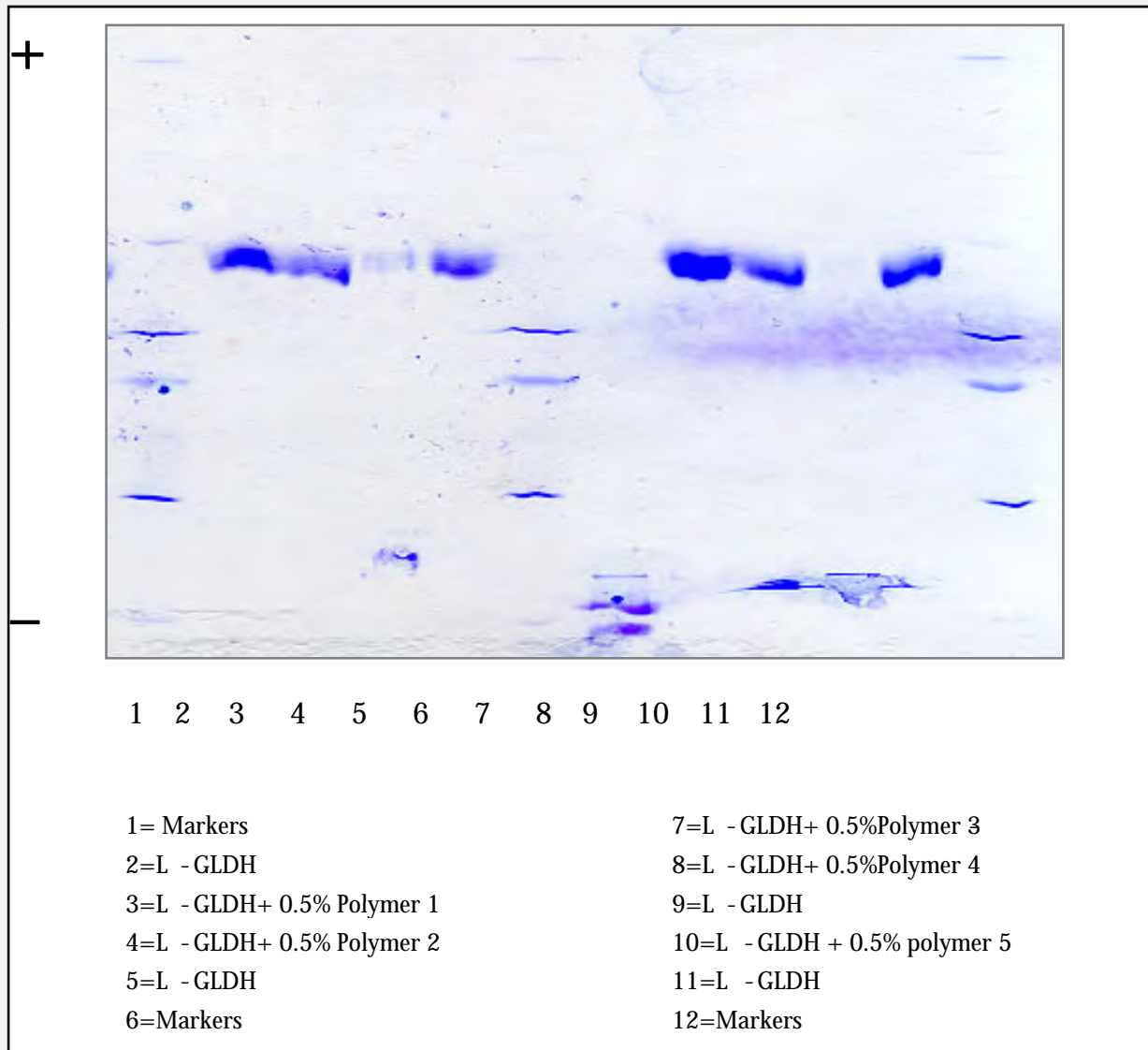
The addition of polyelectrolytes to solutions of proteins promotes the formation of soluble protein/polyelectrolyte complexes by electrostatic interaction. Polyhydroxyl compounds are then able to penetrate the structure more effectively leading to stabilisation





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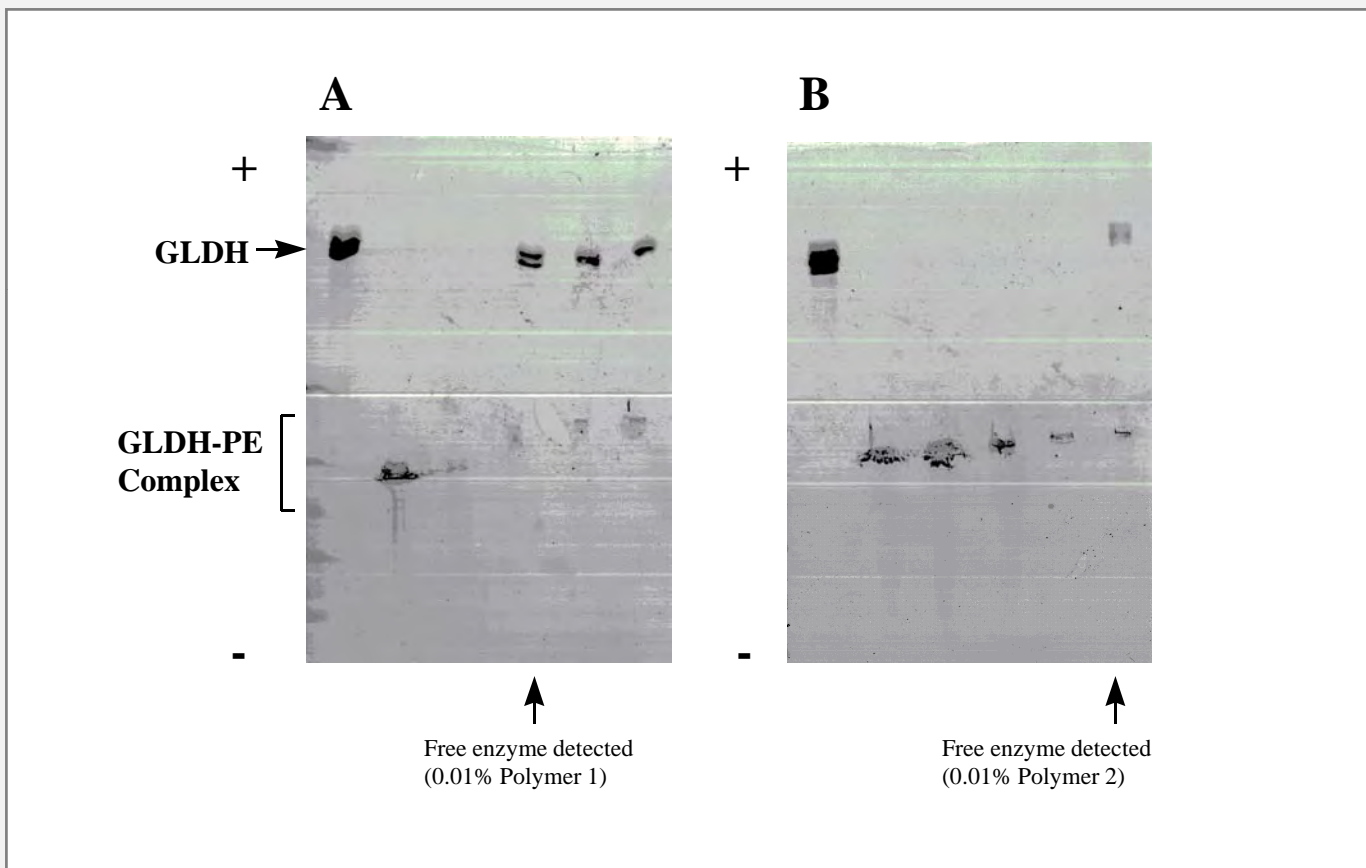
## The Detection of Protein/polymer Complexes by Isoelectric Focusing





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

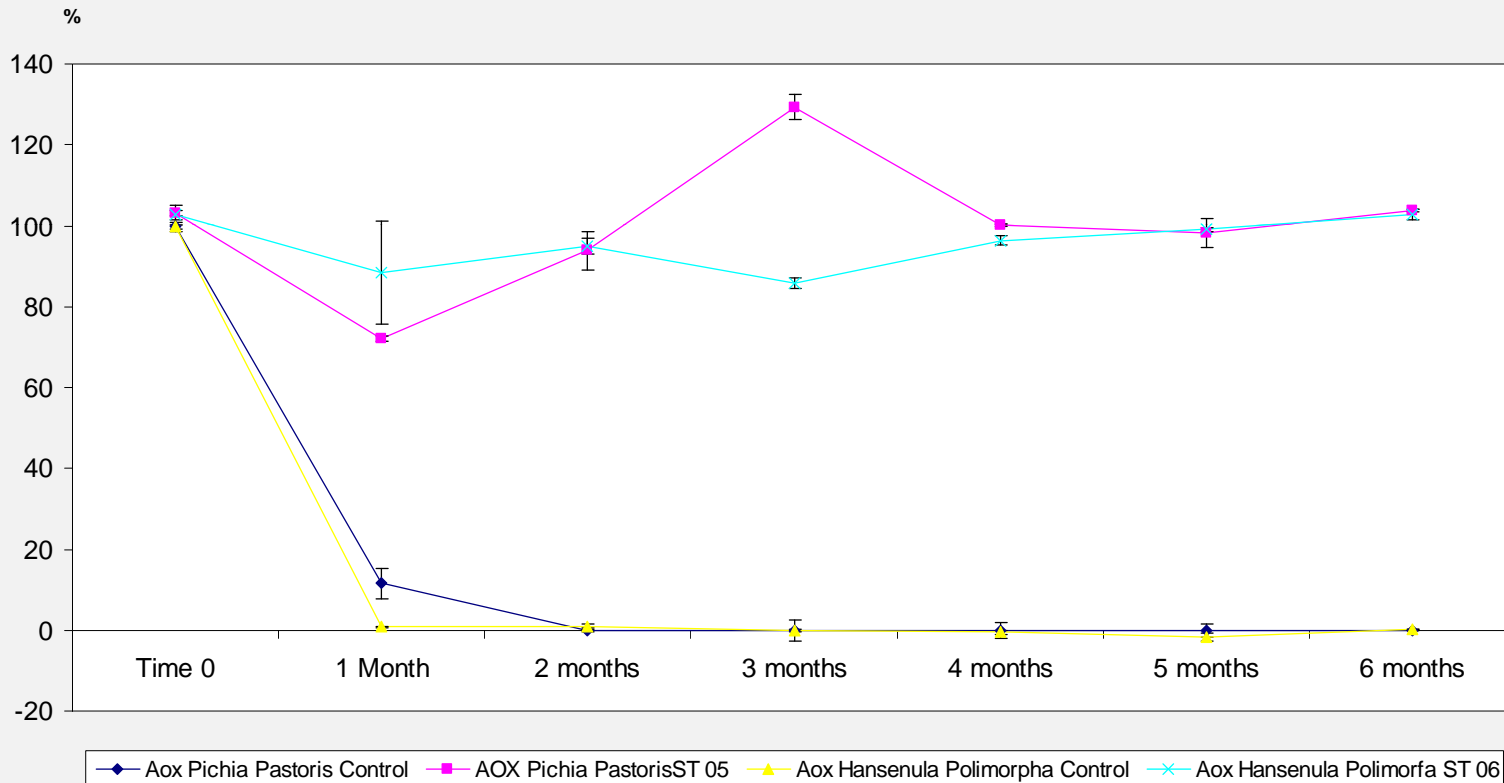




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# Alcohol Oxidase

AOX Hansenula Polimorpha and Pichia Pastoris - Stability trials - 6 Months



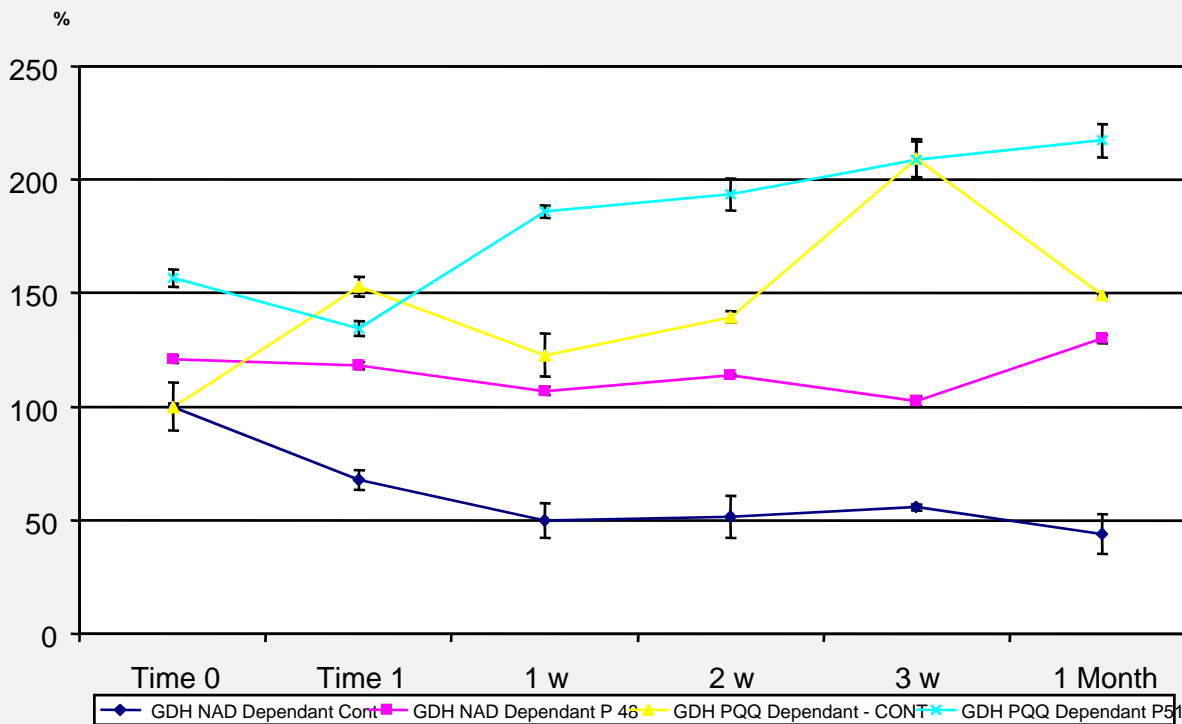
**Alcohol Oxidase stability as determined by microtitre plate assay**



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# Glucose Dehydrogenase

GDH NAD Dependant and PQQ Dependant - Stability trials

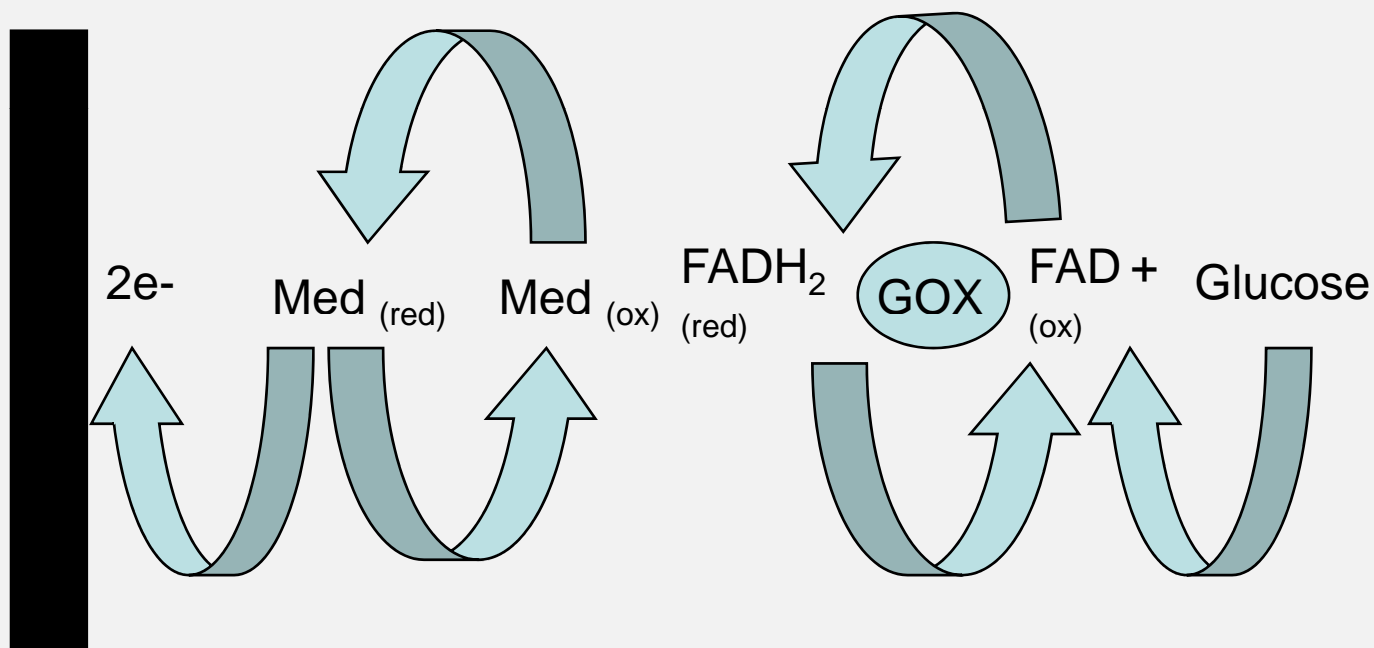


**Glucose dehydrogenase stability as determined by microtitre plate assay**



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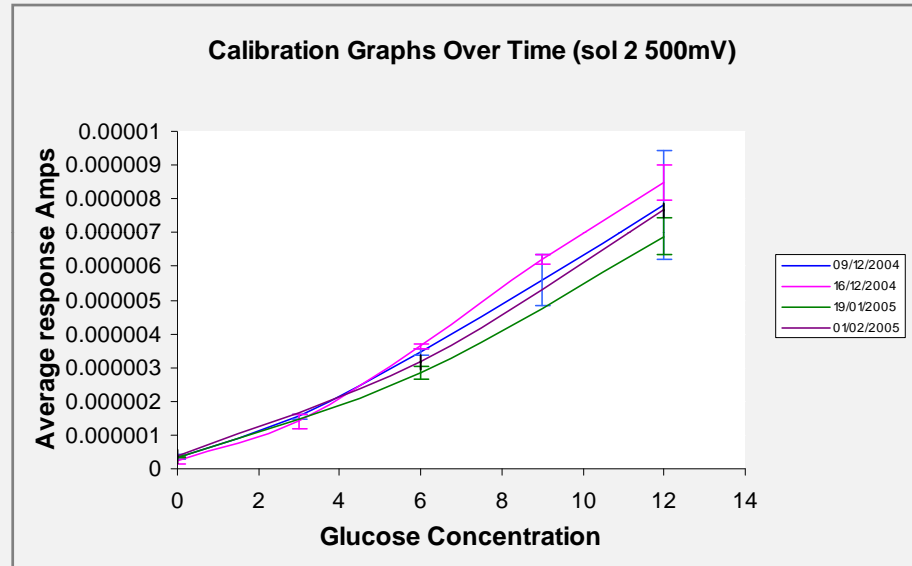
# Printed Electrode with Mediator: Processes at Sensor Surface





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# Glucose Biosensor



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



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# The PolyEnz Process

**This process produces pre-stabilised enzymes which can then be immobilised onto or into a multitude of matrices.**

**This methodology can be applied to:**

- **The adhesion and stabilisation of biomolecules to biosensor surfaces**
- **The production of stabilised biocatalysts involved in the biotransformation of targets for the Pharmaceutical & Chemical Industries**
- **The stabilisation of enzymes & microbial targets used in bioremediation**

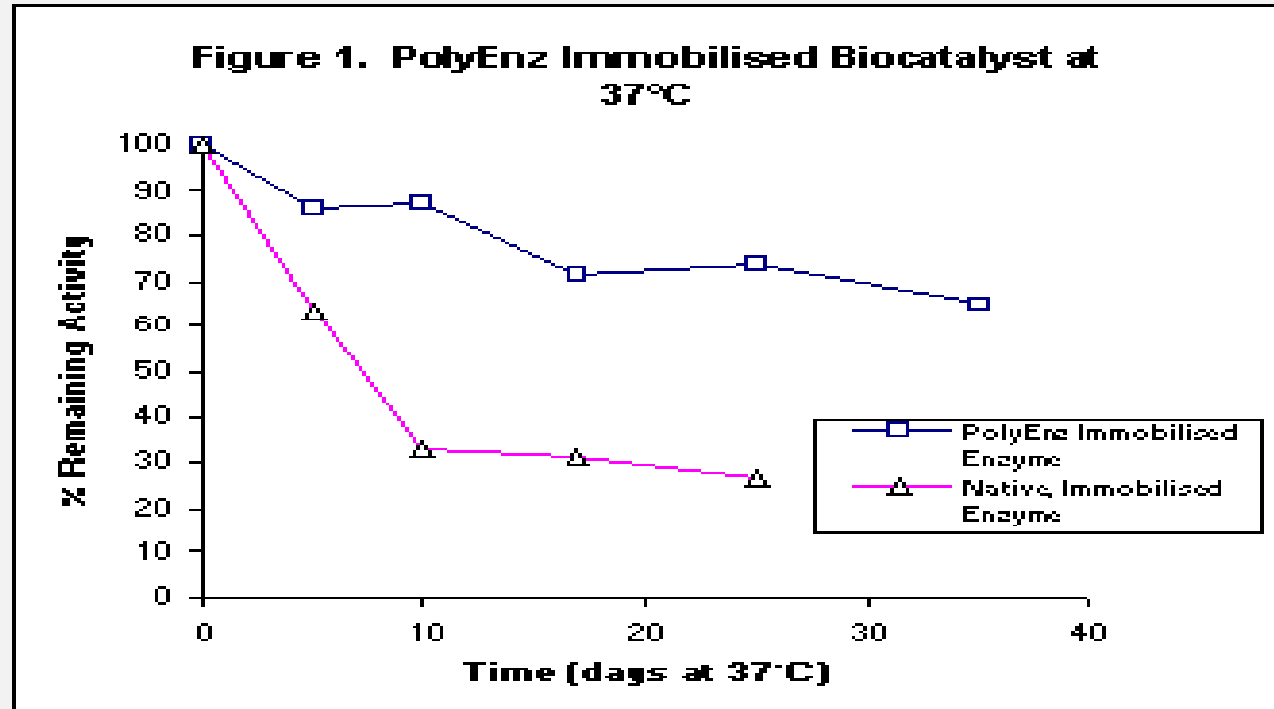
**This process has been demonstrated on solid activated particulate supports**

**Significant improvements in the shelf-life of the immobilised biocatalyst have been recorded**



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# The PolyEnz Process



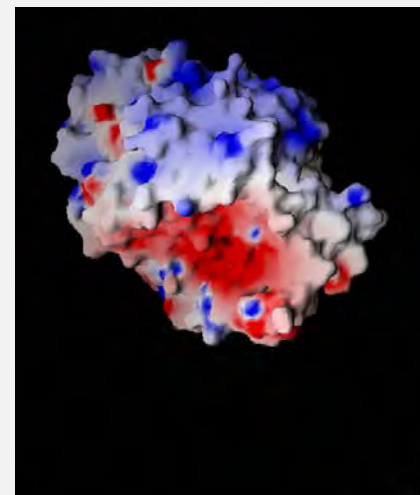
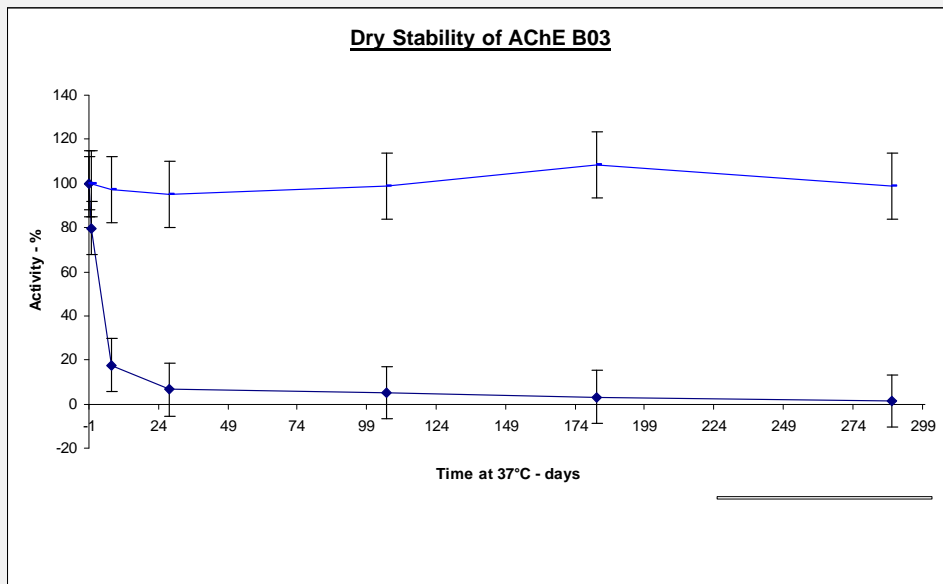
- The PolyEnz Process changes the microenvironment of the immobilised biocatalyst, which can lead to higher enzyme activity levels
- Improved production process, longer shelf life and operational stability
- Improved process efficiency and reduced production costs



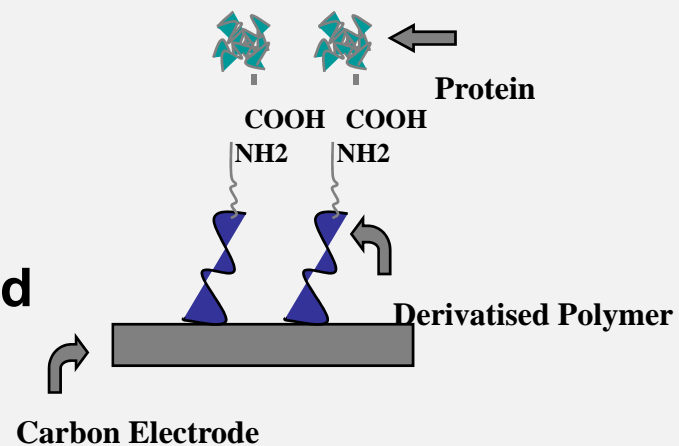


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# Acetyl Choline Esterase



**Acetyl choline esterase stability determined by microtitre plate assay**





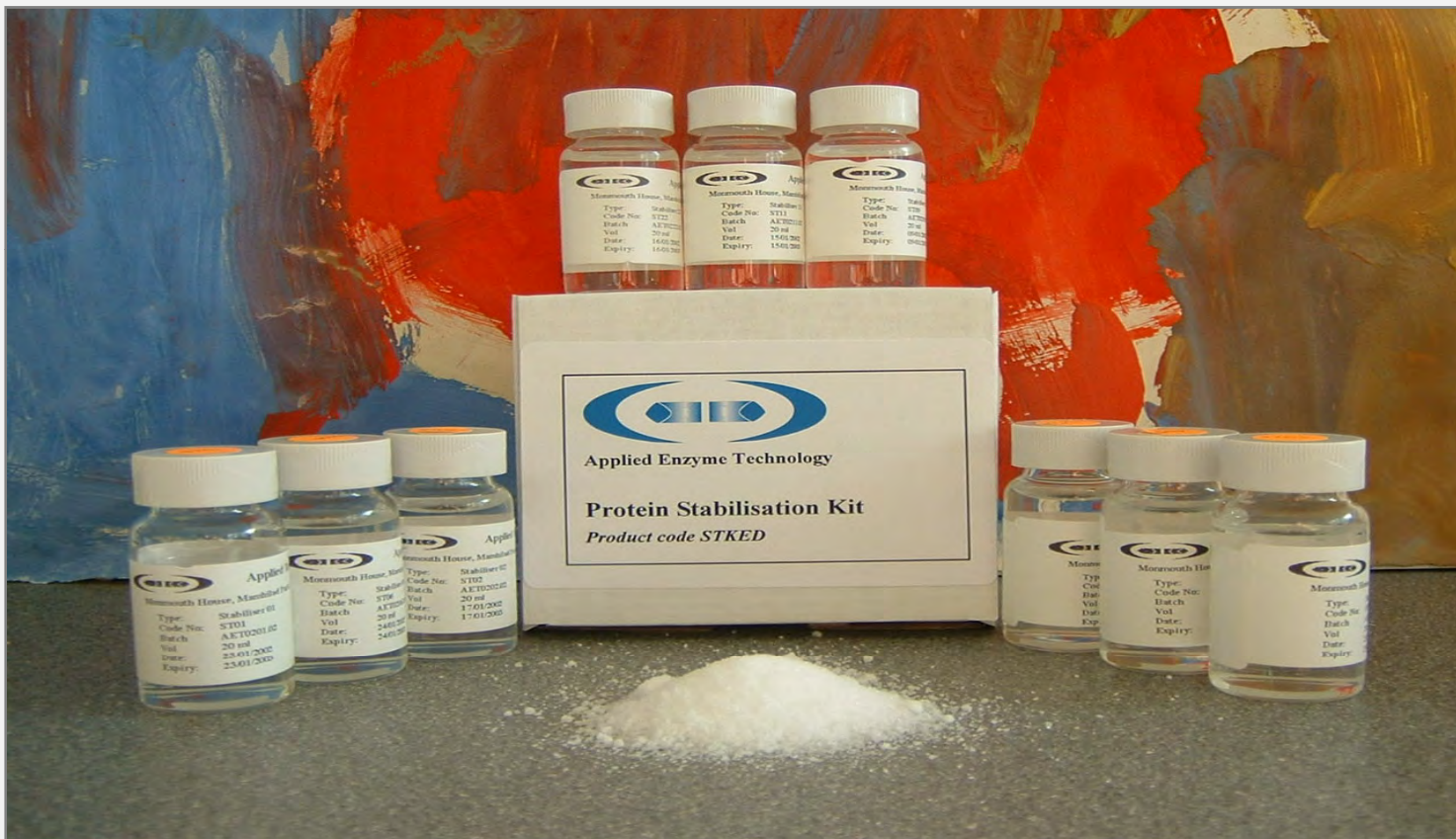
# Conclusions

- **Stabilisation of enzymes is crucial for the production of a commercially viable biosensor.**
- **The choice of stabilisers can be quickly determined by optical assays.**
- **Forming cross-linkable complexes in the presence of stabilisers can be used to manufacture stable disposable and reusable sensors.**
- **Determining the pI of your protein, optimal pH, buffer type and ionic strength of the environment required all go a long way to stabilising your protein of interest.**
- **The introduction of cofactors and metal ions into your stabilisation formulation will also enhance specific enzymes significantly.**
- **Maintaining a low water and oxygen environment during the storage of the complete biosensor is essential for long term stability**



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# Protein Stabilisation Kits



**AET manufactures 5 kits**



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# Biosensor Trouble shooting Kit



**Contents:**

**Stabiliser Formulations**

**Pre-stabilised Enzymes**

**Surfactant**

**Complete biodotting formulation**

**Array of screen printed sensor designs  
Using different electrochemical  
materials**

**Electrochemical protocols**



# Handi-Lab: Novel Technology for the Field Measurement of Ammonia

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The Handi-Lab delivers a low skill, low cost, fast quantitative measurement of ammonia in water samples.

One shot disposable sensors.  
Integrated sampling device.  
Recyclable materials.



**Digital output within 5 minutes.**

**No pre-calibration required.**

**Measurement range 1-10 parts per million of ammonia.**

**Automatic adjustment for sample and ambient temperature.**

**Fully portable and battery operated.**

**On board data storage and down loading facility.**

**Measures within 10% bias 10% error.**

**Allows on site testing and 'live' mapping of pollution events.**

**Biosensors stable for at least 6 months at room temperature .**





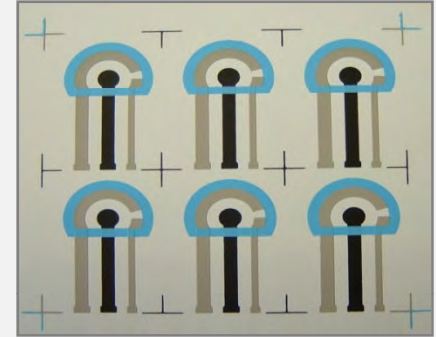


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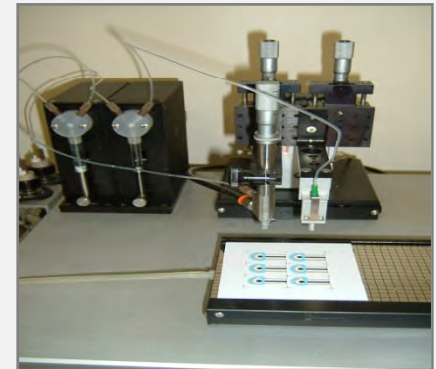
# Electrode Manufacture & Enzyme Deposition



**GEM Clean Room Printing Facility**



**Screen Printed Electrodes**



**AET Biodot Facility**



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# The Gwent Group

- **Can provide a unique range of materials and services for all biosensor systems**
- **GEM produce materials and base electrochemical transducers**
- **AET provide biostabilisation services and biosensor fabrication**



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