The Stability of Enzymes in Biosensors

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Company History

- Applied Enzyme Technology Ltd was 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 Gwent Electronic Materials Ltd. and relocated June 2001
- BIOWISE Demonstrator Award 2003
- Multiple EU, DTI & DEFRA Grants
Products & Services

- Carry out contract research for the stabilisation of specific proteins
- Supply Protein Stabilisation Kits to clients to troubleshoot their own stability problems
- Supply of bulk stabiliser formulations
- Supply a range of pre-stabilised enzymes
- Design and develop biosensors for clients
- Carry out prototype and contract manufacture of low volume sensors
- Production of our own environmental & agrifood sensors
Proteins Stabilised by Applied Enzyme Technology Ltd.

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
Protein Stabilisation Kits
(product No’s STKED, STKES, STKAB, STKPH)

- 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)
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)
Acetylcholine Esterase

Dry Stability of AChE B03

280 days stability @ 37oC
Alcohol Oxidase

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

Alcohol Oxidase stability as determined by microtitre plate assay
Glucose Oxidase Stability

Solution stability trial GOX3A at 37°C

Glucose oxidase solution stored for extended time prior to biosensor construction and testing
WP3 Handi-Lab: Novel Technology for the Field Measurement of Ammonia

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.
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
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
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
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.
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
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.
Large Scale Manufacture

Large Screen

Complete Print

DEK 248 capable of screen printing 210 electrodes per sweep
Schematic of Oxidase Biosensor

\[ \text{SPCE body} \rightarrow \begin{array}{c}
\text{e}^{-} \\
\text{Co}^{2+} \\
\frac{1}{2}\text{H}_{2}\text{O}_{2} \\
\frac{1}{2}\text{O}_{2} + \text{H}^{+} \\
+ 0.5 \text{ V}
\end{array} \]

\[ \text{Immobilised GOD} \rightarrow \begin{array}{c}
\text{1/2 Glucose} \\
\text{O}_{2}, \text{GOD} \\
\text{Gluconolactone}
\end{array} \]
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.
Electrode Stability

Water based GOX electrodes printed and stored at 4oC. Electrodes tested using 2mM glucose additions.
GOX INK Aging Trials

GOX ink stable for at least 29 days at RT
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.
The Detection of the Stoichiometry of Polymer Binding to the Thermophilic Form of GLDH

GLDH → GLDH-PE Complex

Free enzyme detected (0.01% Polymer 1)
Free enzyme detected (0.01% Polymer 2)
Glucose oxidoase biodotting solution stored for up to 54 days @ 4oC prior to biosensor construction and testing
Dispensing of DNA Probes
Ink jet printing
Conclusions

• AET is able to deliver stabilisation technology both in the dry state and in solution

• AET can deliver a combination of stabilisation and immobilisation technology

• AET is currently working towards the derivatisation of surfaces for extra functionality

• All of the above technologies are applicable to a wide range of industrial applications

• The AET/GEM relationship delivers the best of both worlds:
  • Expertise in sensor materials and printing formulations, sensor design and thick film printing
  • Expertise in the stabilisation of proteins onto electrode surfaces and the immobilisation of these biomolecules onto surfaces
  • We deliver a 1 stop shop for the development and production of biosensor technology
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