Lactate Thick-Film Screen Printed Biosensor

SAFEGARD: Sensor Arrays for Environmental, Generic and Routine Detection
Lactate Thick-Film Screen Printed Biosensor
Why Lactate?

- early diagnosis of mastitis in dairy cows
- current enzymatic detection methods mainly use lactate oxidase or lactate dehydrogenase
- require lengthy and mechanically complex preparatory stages
- enzyme based sensors could provide rapid, simple alternative
Requirements for Lactate Sensor

- simple, economical manufacturing process *e.g.* screen printing technology

- rapid, accurate measurements in highly humid environment with large temperature variations

- no sample pre-treatment or requirement for trained operators

- adaption to on-line analysis system
Hart et al (1996) - screen printed enzyme electrodes for lactate detection

- lactate oxidase / graphite mixture printed onto electroplated graphite pads
- based on electrochemical detection of hydrogen peroxide

- commercialisation supplier of SPCEs GEM / AET
Electrochemical Detection of Hydrogen Peroxide

- L-Lactate + O₂ → Pyruvate + H₂O₂
- Oxidation of L-lactate to pyruvate and peroxide catalysed by LOX
- Peroxide oxidation at SPCE polarised at +0.6V vs. Ag/AgCl
- Electron flow at electrode surface proportional to L-lactate in sample
Role of GEM in Sensor Development

- electrode design
  - three electrode setup
  - working electrode potential more stable

- batch manufacture simplified
  - cost reduction
  - high reproducibility
- smaller, well-defined geometric area
Role of GEM in Sensor Development

- ink formulations
  - mediated carbon
  - Ag/AgCl
  - dielectric

- manufacture of screen-printed base electrodes
Role of AET in Sensor Development

- enzyme formulations:
  - stability
  - immobilisation on electrode surface
  - optimisation of enzyme loading
Role of AET in Sensor Development

- outer membrane – enzyme protection / diffusion limitations
- electrochemical measurement
  - working potential
  - signal-to-noise ratio
Sensor Response with 1mM Lactate

![Graph showing sensor response with 1mM Lactate over time. The x-axis represents time in seconds ranging from 0 to 700, and the y-axis represents current (A) ranging from 0.00E+00 to 3.50E-06. Three curves are shown: blue for new batch - membrane, pink for new batch + membrane, and yellow for old batch + membrane.](image-url)
Development

- optimisation of enzyme loading on sensor surface
- effect of temperature on enzyme performance
- evaluate different membrane formulations / methods of application
- short-term stability trials
- initiation of long-term stability trials
- evaluate requirement of inner-membrane
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Project Overview

- EU-funded as part of Fifth Framework “Quality of Life and Management of Living Resources” Program
- AET / GEM sub-contractors
- GEM supplier of screen-printed transducers
Role of AET in SAFEGARD

- enzyme stabilisation
  - mutant cholinesterases for higher sensitivity and specificity

- development of derivatised surfaces
  - towards specific immobilisation of enzymes on different electrode surfaces
Importance of OP Detection

- extensively used in modern agricultural techniques
- control of insect infestation and damage
- excess of active ingredients used as large amounts are washed off/absorbed onto surfaces contacted
- contamination and severe toxicological effects, particularly to CNS, arise as a result
- EU directives in force for monitoring / measurement
Current Research Trends

- Potentiometric/amperometric techniques largely employed
- Based on utilisation of inhibition enzymes, usually cholinesterases, particularly acetylcholinesterase
- Enzyme activity measurements before and after exposure to pesticides ⇒ difference in values directly related to pesticide concentration in sample
- Allows for quantitative, sensitive detection systems
Thiocholine Oxidation at CoPC-Modified SPCE

Co electrocatalyses oxidation of TCh at low working potential (+100mV)

reduction in enzyme activity after exposure to OP decreases production of TCh(ox) - hence lower signal
Typical Amperometric Response

- Measurements performed within flow-injection system
- Decrease in amperometric response upon injection of pesticide sample
Conclusions

- GEM and AET involved in collaborative EU and commercial projects
- GEM – manufacturer and supplier of screen-printing materials and electrodes
- AET – supplier of stable enzyme / protein systems
- Synergy between GEM and AET – focus towards development of commercially viable biosensor systems