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Lactate Thick-Film Screen Printed Biosensor

SAFEGARD: Sensor Arrays for Environmental, Generic and Routine Detection

Lactate Thick-Film Screen Printed Biosensor

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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

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- 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

Background to Lactate Sensor Development

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- 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

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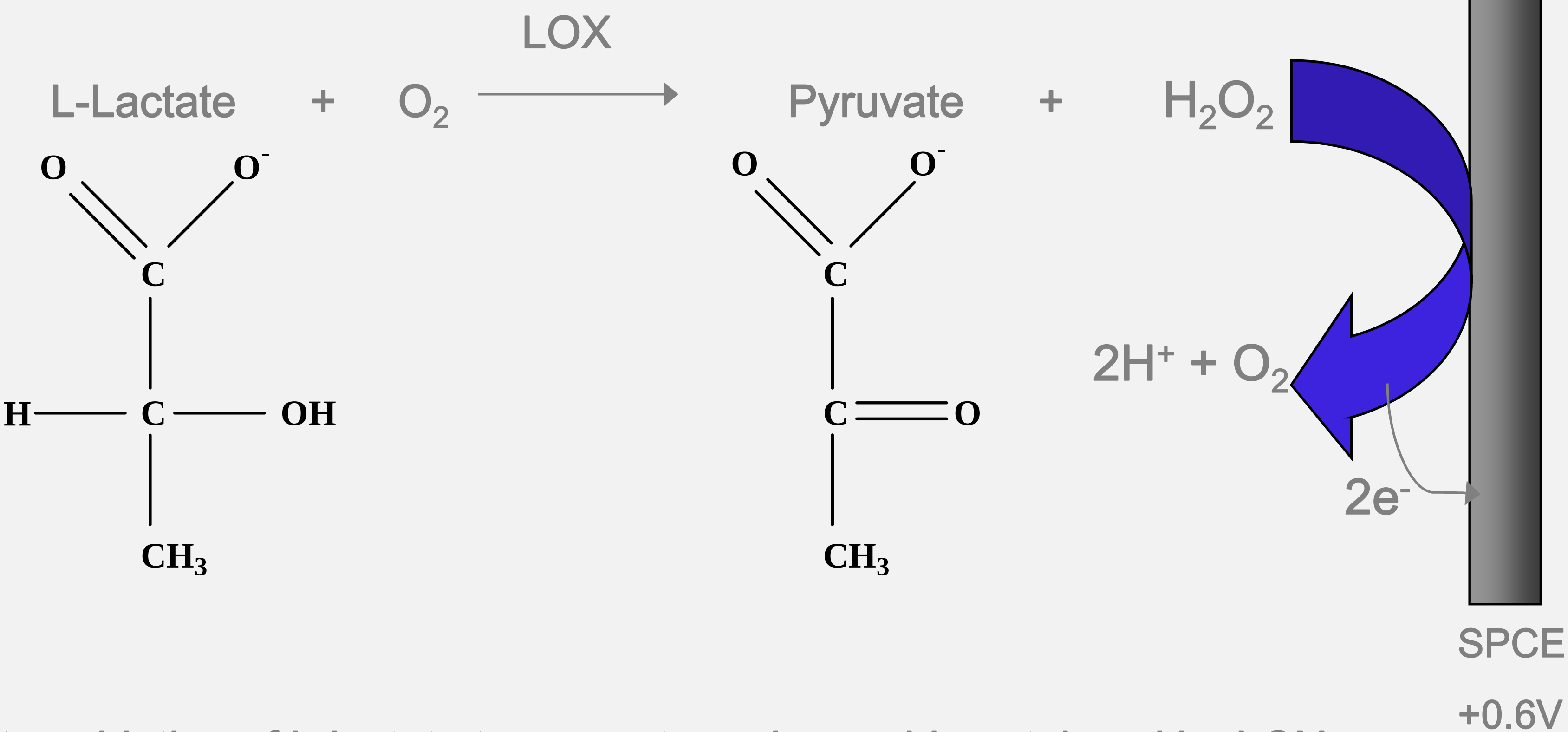
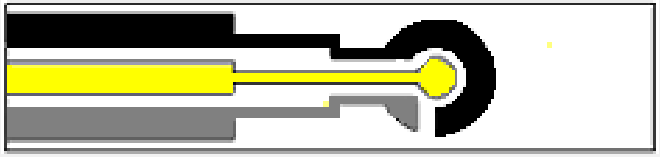


- oxidation of L-lactate to pyruvate and peroxide catalysed by LOX
- peroxide oxidation at SPCE polarised at +0.6V *vs.* Ag/AgCl
- electron flow at electrode surface proportional to L-lactate in sample

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

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

- manufacture of screen-printed base electrodes

Role of AET in Sensor Development

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- enzyme formulations:
  - stability
  - immobilisation on electrode surface
  - optimisation of enzyme loading

Role of AET in Sensor Development

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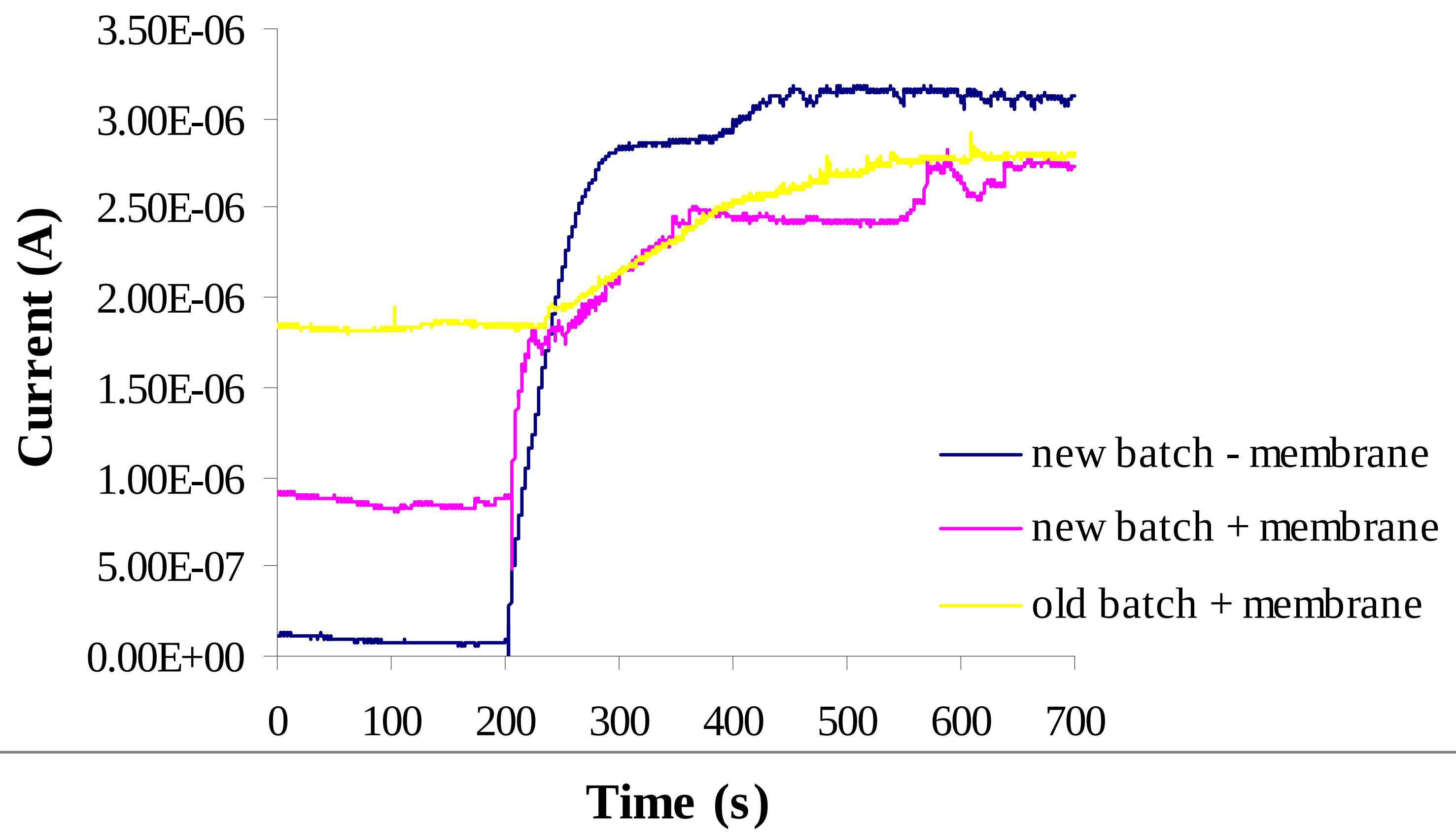
- outer membrane – enzyme protection / diffusion limitations
- electrochemical measurement
  - working potential
  - signal-to-noise ratio

Sensor Response with 1mM Lactate



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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

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- 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

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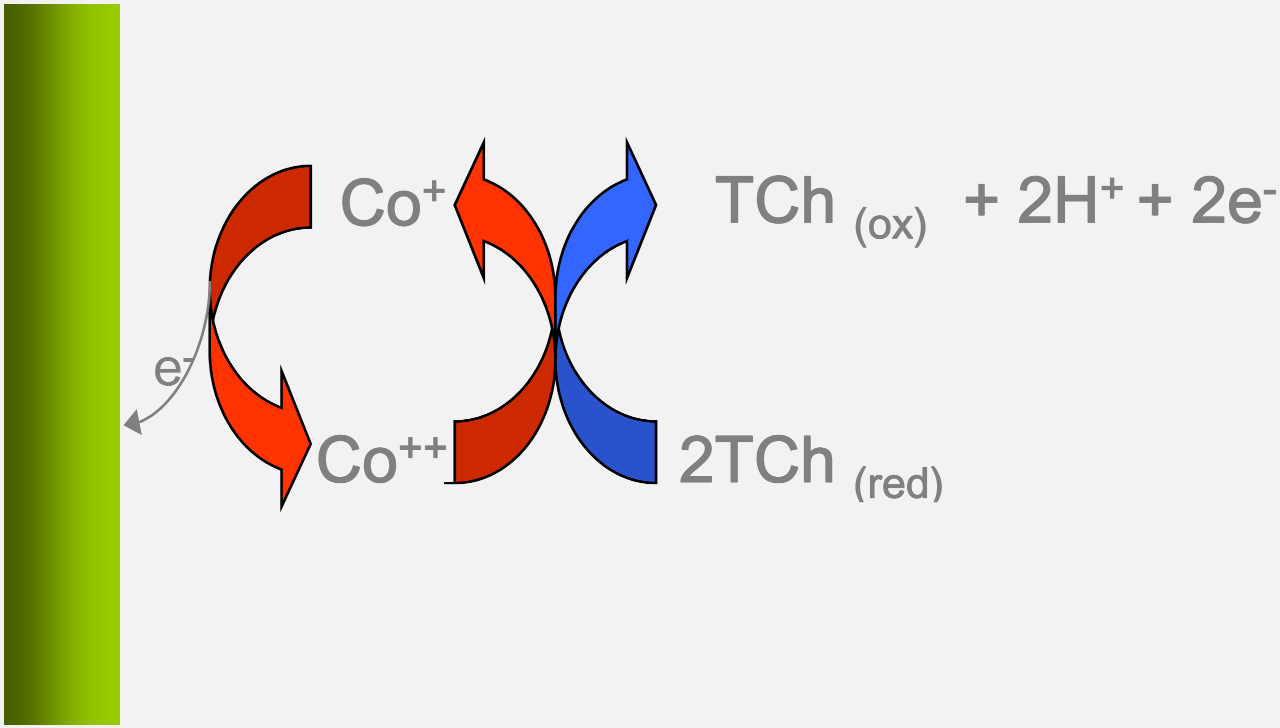
- extensively used in modern agricultural techniques
- control of insect infestation and damage
- excess of active ingredients used as large amounts are washed off/absorbed onto surfaces contacted
- contamination and severe toxicological effects, particularly to CNS, arise as a result
- EU directives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

Current Research Trends

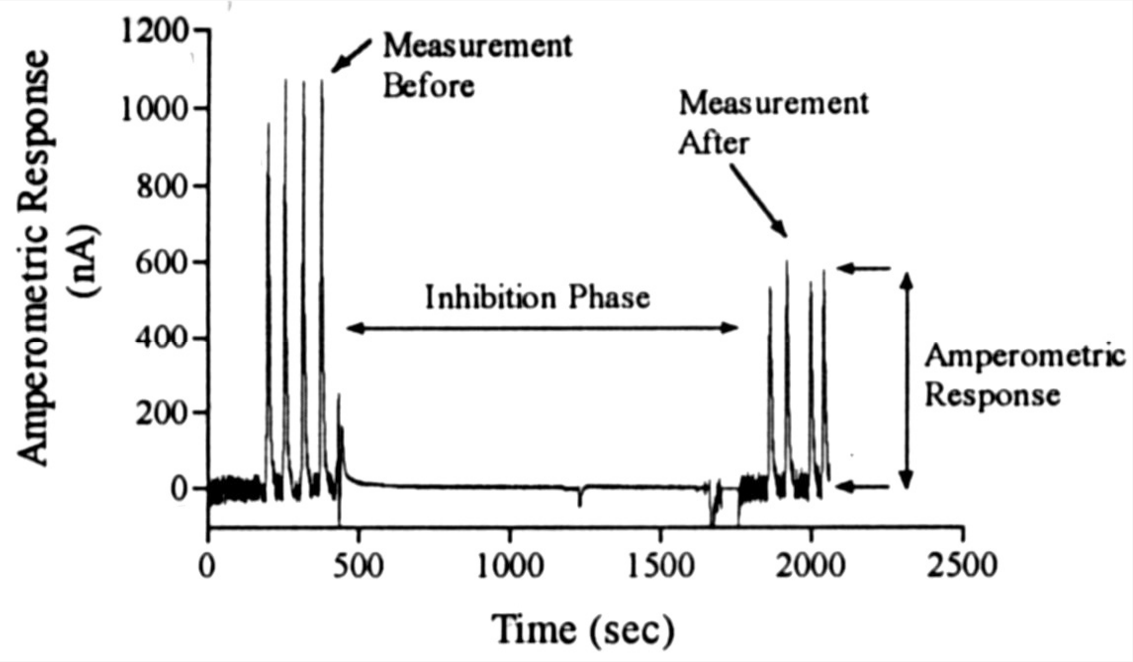
- potentiometric/amperometric techniques largely employed
- based on utilisation of inhibition enzymes, usually cholinesterases, particularly acetylcholinesterase
- enzyme activity measurements before and after exposure to pesticides $\Rightarrow$ difference in values directly related to pesticide concentration 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



(J.J.Rippeth, Univ. Leeds, 1999)

- measurements performed within flow-injection system
- decrease in amperometric response upon injection of pesticide sample

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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

The Gwent Group,
Monmouth House,
Mamhilad Park,
Pontypool NP4 0HZ.
United Kingdom.
sales@gwent.org
http://www.gwent.org
Tel: +44 (0)1495 750505
Fax: +44 (0) 1495 752121