The Abdus Salam
International Centre for Theoretical Physics

United Nations
Educational, Scientific
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International Atomic
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SMR.1670 - 15

INTRODUCTION TO MICROFLUIDICS

8 - 26 August 2005

Electrical and Electrochemical Detection

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4. Electrical and Electrochemical Detection

Topics in this lecture

Sensing principles
A great many number of electrical sensors exists, here we discuss on principles that were used in integrated microfluidic devices.

Miniaturization
The introduction of miniaturized sensors in microfluidics is driven by materials and techniques based on expertise in microelectronics. These developments are collectively addressed by the expansion of µTAS research.

Applications
Examples related to specific measurement problems will be given.

- Introduction
- Strategic developments of electrical and electrochemical detection methods
- Tackling integration
- Examples of sensing devices
- Outlook: Future developments
- Summary

Introduction

Topics in this section

Downstream processing analysis
Analysts at their work place along side a process-line.

Downstream Processing
Column Chromatography
HPLC, Dialysis, etc.

Integrated electrical sensor systems
Working principles of electrical measurement systems suitable for stand alone operation

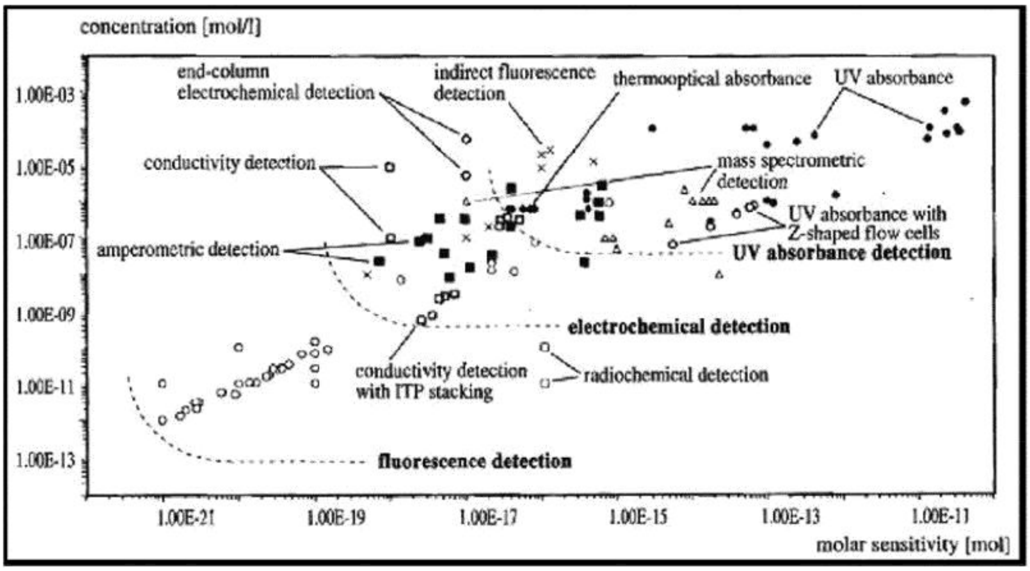
- Integrated analysis
  - Overview detection techniques
  - Principles of electrical and electrochemical detection
  - Systems

Objectives for process line monitoring

- Detection and quantification of components using electrical means is often carried out to reduce operator influence as known from optical microscopy, e.g. in the hospital environment.
- Integration of the information obtained with other process parameters available in real-time and thus utilization for process control loops.

4.1. Introduction

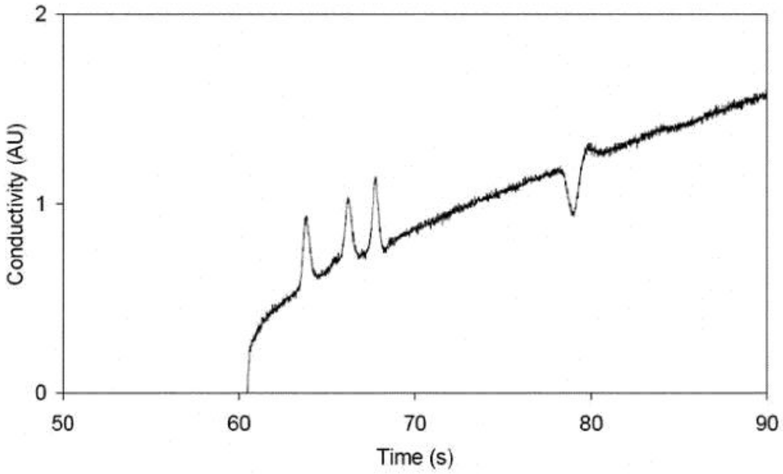
Detection principles



*From: J.P. Landers, ed., **Handbook of capillary electrophoresis**, 2nd ed., 1997, CRC Press, Inc., ISBN 0-8493-2498-X*

How does conductivity detection work?

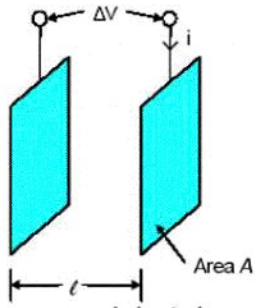
- Sensor registers a change in electrical resistance compared to a background signal.



Theory of conductivity detection

- When the electric field E=dV/dx is linear between two electrode plates with electrode area A and distance ℓ, the electronic current can be obtained:

$$i = JA = FA\frac{\Delta V}{\ell}\sum_{i=1}^{n}|z_i|\mu_i C_i \qquad A$$



- Now, the conductance L can be expressed as

$$L = \frac{i}{\Delta V} = \frac{FA}{\ell}\sum_{i=1}^{n}|z_i|\mu_i C_i \qquad \Omega^{-1}$$

- The conductance L is the variable that can be measured, but depends on the dimensions of the electrodes that determine the cell-constant, k. Therefore, the conductivity is defined:

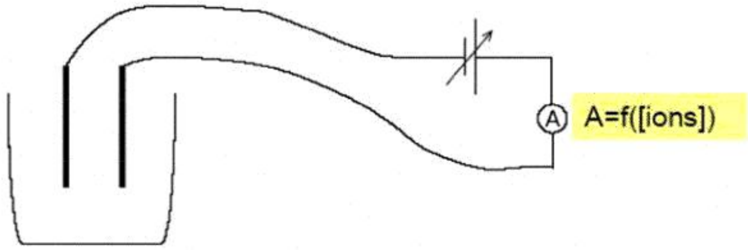
$$\kappa = L \cdot k = F\sum_{i=1}^{n}|z_i|\mu_i C_i \qquad \Omega^{-1}m^{-1}$$

Here: $k = \ell/A$ [m^{-1}]

Slide prepared by W. Olthuis, University of Twente

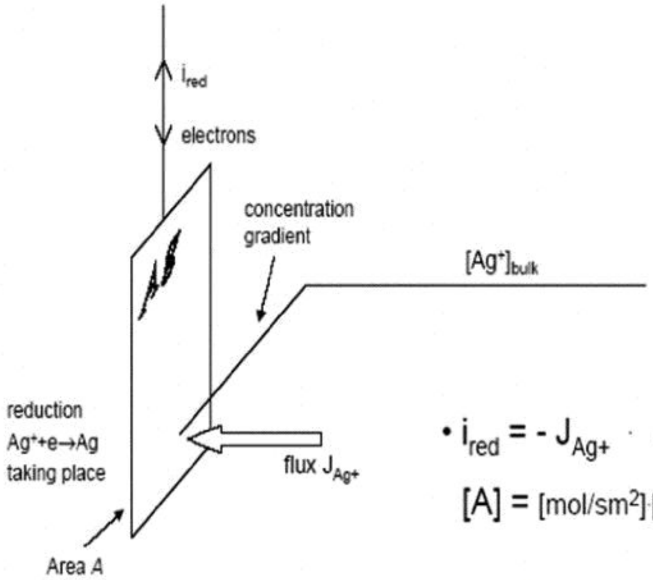
How does amperometry work?

Amperometry is a technique of measuring the concentration of an electro-active species via the electrical current that occurs due to the electrochemical reaction of that species at an electrode



Slide prepared by W. Olthuis, University of Twente

Theory of amperometric detection



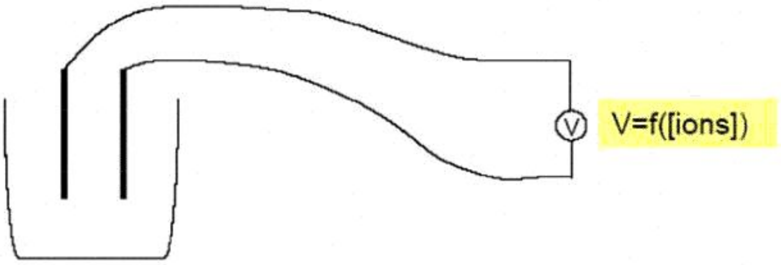
- $i_{red} = - J_{Ag+} \cdot A \cdot F$

$[A] = [mol/sm^2] \cdot [m^2] \cdot [C/mol] = [C/s] = [A]$

Slide prepared by W. Olthuis, University of Twente

How does potentiometry works?

Potentiometry is the measurement of a working electrode potential with respect to a reference electrode as a function of the concentration of some chemical species in the electrolyte at (almost) zero current



Following transparancies: derivation of **Nernst Law**, relating the concentration of chemical species to an electrode potential

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Ion Selective Field Effect Transistor

- The Ion Sensitive Field-Effect Transistor (ISFET) is a special potentiometric sensor.
- Why special?
  - Unlike other potentiometric sensors, absolutely no galvanic contact exists between the solution and the silicon sensor chip
- The ISFET potentiometric sensor functions essentially different:
  - Information is transferred via an electric field (FET)
  - Charge is the source of any (static) electric field
  - The nature of this charge is ions (IS)

charge from ions in the chemical world	predictable behaviour of charge in the electrical world

mirror

Topics in this section

Monitoring demands
Quality and reproducibility.

Multiplate® *www1.qiagen.com*

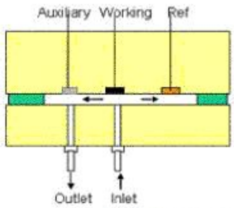
Technology
Integration and yield.

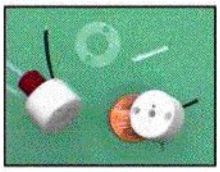
Source: R. Schasfoort, University of Twente

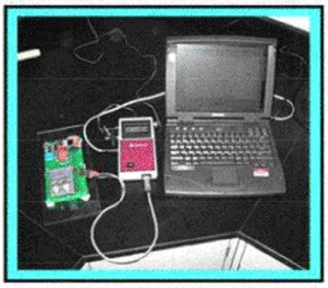
Strategic developments

- Step-by-step increase of integration complexity.
- Understanding different sensor principles and expanding their field of applications:
  - Impedance spectroscopy,
  - Ion-selective measurements (ISFET),
  - Electrophysiology (Living organisms).

4.2. Strategic developments







http://www.technet.pnl.gov/sensors/chemical/projects/ES4EC-SnsrCell.html

Systems have to be smaller

- Early electrochemical detection cells are mounted as an end-column detector.
  - Dead volume and detector line width too large for integration into flow lines and small volume applications (drug screening).
  - Sensors can respond faster if diffusion length is decreased.
  - Sensor design for integrated microfluidics need to adapt to manufacturing strategy of microelectronics techniques, i.e. planar processing.
  - Due to integration, systems can be made compact and cheap enough for in-line monitoring.

Microelectrodes

- Microelectrodes are understood as electrodes whose characteristic dimensions are comparable to or smaller than the diffusion layer thickness in a specific experimental design.
- Early systems are millimeter sized, e.g., glass pipettes filled with a "dropping mercury" electrode.
- For electrodes with diameters < 1μm...100μm the term "ultramicroelectrode" was coined.
- When smaller and smaller electrode area is approached the characteristics for microelectrodes, e.g. enhanced mass transport and rapid attainment of steady state, are established gradually.
- Arrays of sensing elements thus provide enhancement in detection sensitivity and permit multichannel detection (electronic nose, electronic tongue).
- Small electrode size permits measurements and stimulation in single cells (in vivo and in vitro)
- Passivation of metal electrode surface with a monomolecular, non-conductive film and formation of conductive channels in the film by uniformly dispersing in it molecules of a suitable substance allow to obtain single-molecule electron and ion gate sites.

Membrane sensors

- Coated wire electrodes led to standard ion-selective electrodes. Coating acts as a membrane passing only specific ions.
- Systematic investigations of charge transfer across interfaces between two immiscible electrolyte solutions and conclusions from biological membranes open potential analytical uses primarily in designing selective voltametric detection cells for flow-through measurements (e.g., chromatographic separation).
- Membrane sensors suitably complement common semiconductor devices when operation under ambient temperature is required
- Advantages are also given as a detection princple for gas since it can be selectively accumulated in the liquid phase through the membrane (gas sampling).
- Drawback is the rather slow response caused by limited velocities of mass transport.

Electrophysiology

- Single-use sensor strip
  - Semi-quantitative.
  - Cheaply mass produced (microfabrication!).
  - Poor stability is avoided (no reconditioning required!).
  - Routine monitoring (e.g., blood sugar in diabetics).
- Biosensors
  - Processes occurring in living organism are often involving electroactive substances, such as oxygen, hydrogen peroxide or NAD^+.
  - Poor stability of organisms restrict practicality and demands on experimental conditions are high (pH, ionic strength, temperature).
  - Single-use devices are favored.

Renaissance of conductometry in microfluidic systems (μTAS)

- In ion chromatography and capillary electrophoresis for small, primarily inorganic, ions low frequency conductometry became popular.
  - Extreme simplicity, cheapness, universal applicability to charged analytes.
- High-frequency measurement of impedance
  - Universal and more sensitive then low-frequency measurements though sensitivity to experimental conditions is higher than for low-frequency in capillary electrophoresis, (CE often carried out under constant conditions).
  - Technique has the advantage of placing the electrodes outside the measuring liquid avoiding direct contact between sensor and test medium (non-contact measurement).
- Both techniques are important for routine separations but probably will be restricted to special applications in further methodological development of detection principles.

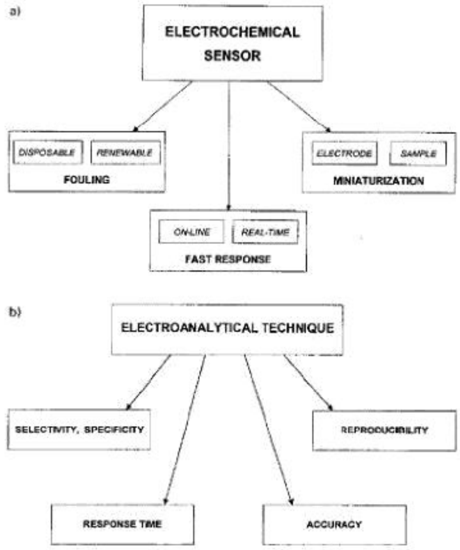
Topics in this section

Proof of concept

Research & Development

Tackling integration

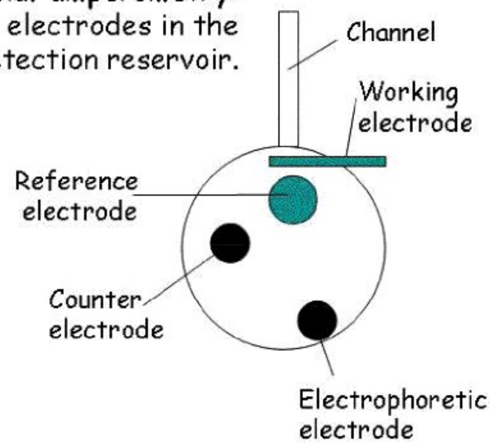
- Establishing miniaturized sensing techniques.



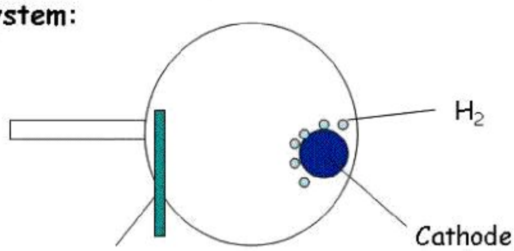
C.M.A. Brett, Electroanalysis 1999, 11, 4, 1013- 1016

Simplified electrode configuration

Conventional amperometry:
Three-electrode system, there are four electrodes in the detection reservoir.



Simplified two-electrode system:

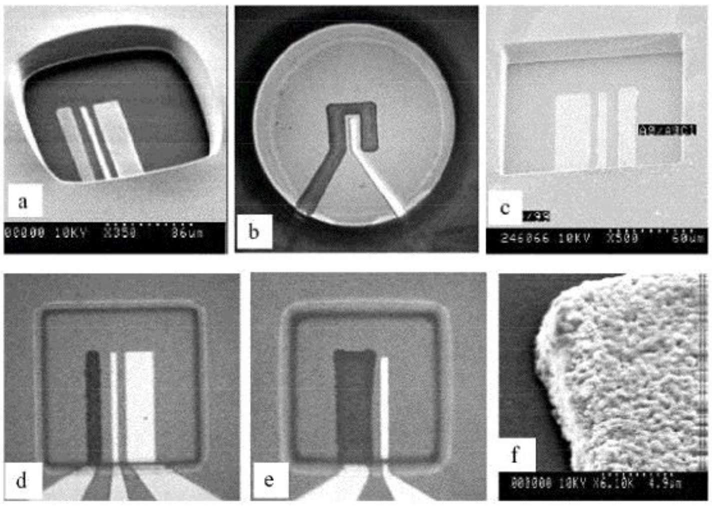


Working electrode H_2 (cathode) or O_2 (anode) electrode is formed during electrophoresis

P. Hauser et al., University of Basel

Miniaturized electroanalytical sensors in well plate format

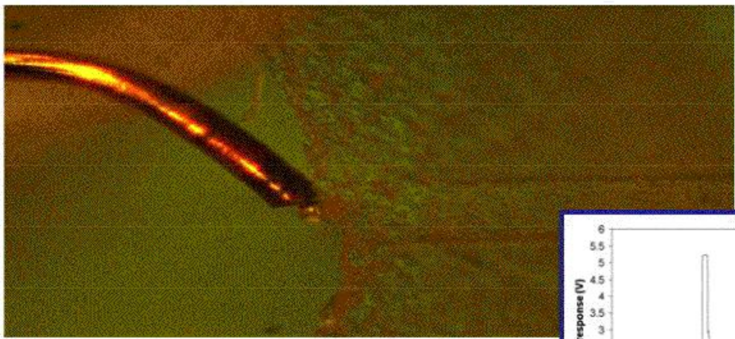
- Small volume applications

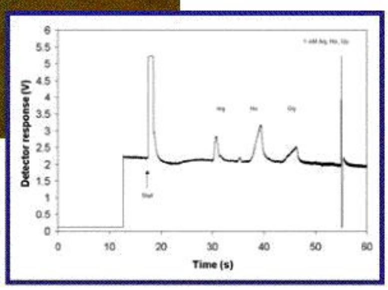


X. Cai et al., Electroanalysis 2000, 12, 9, 631-639

Electrode material

- Material studies vs. higher integration density.



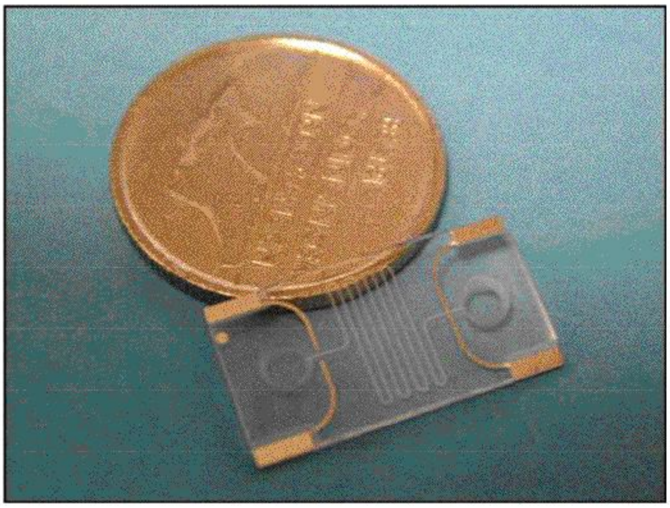


Electropherogram of
1mM Arg, His, Gly at Cu-wire.
2 cm separation length on chip.

E. Vrouwe and R. Luttge, University of Twente

Thin-film electrodes in glass

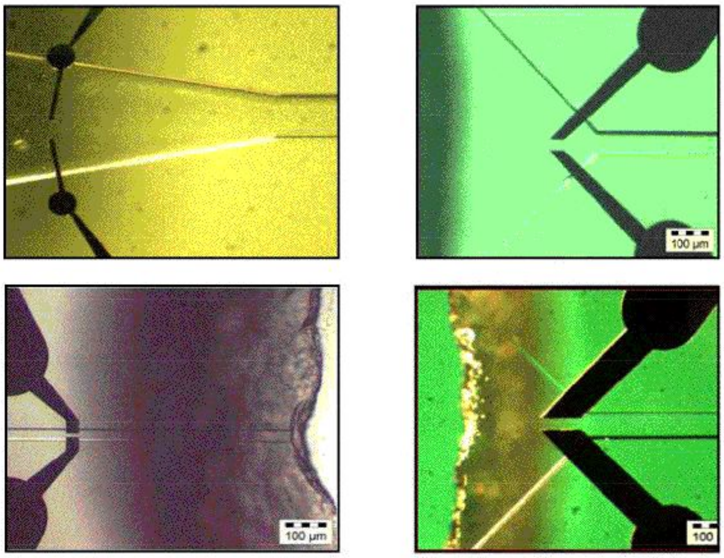
- Process development for leakage-free thin film electrodes



S. Schlautmann, University of Twente

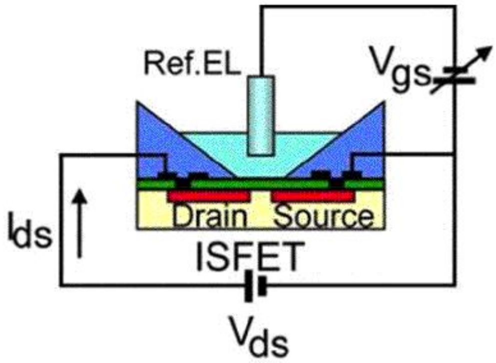
Electrode configurations

- Positioning of electrodes inside of microfluidic channel



Integrated electrical pH measurement

A special potentiometric sensor: the ISFET

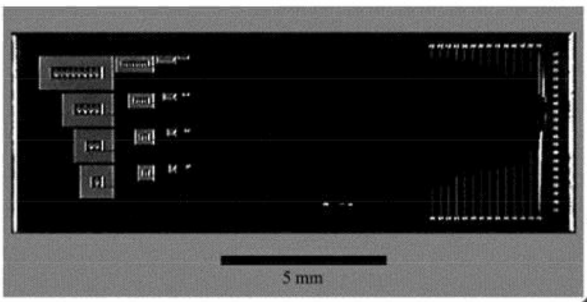


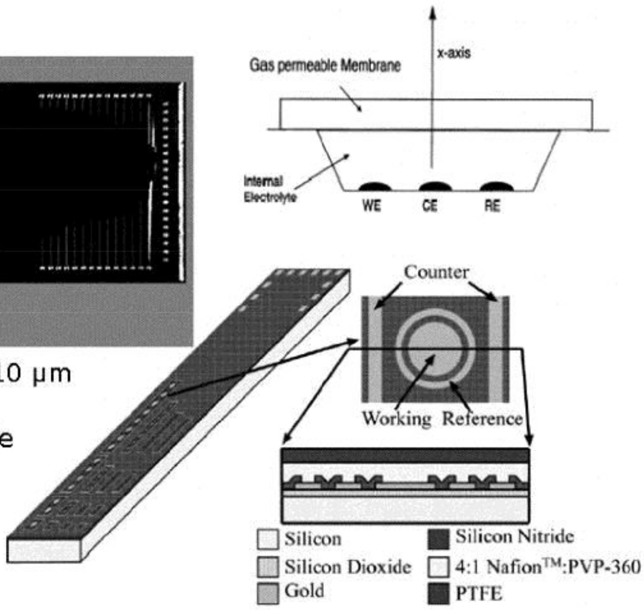
Ion sensitive field effect transistor (ISFET) can be used as pH sensor

P. Bergveld, University of Twente

Clark-type micro oxygen sensors

- Membrane sensor





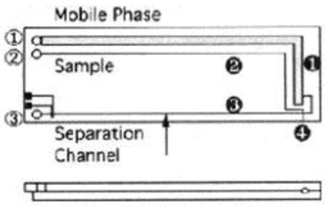
Smallest sensor: 10 µm x 10 µm

Amperometric principle

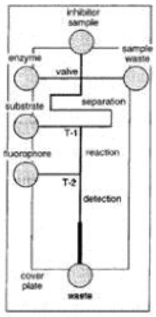
G.W. McLaughlin e.a., Sens. Act. B 83, 2002, 138-148

Miniaturized analytical systems

- Original focus: miniaturization of the analytical methods towards micro Total Analysis Systems (μTAS), e.g., capillary electrophoresis for in-line process monitoring
  - Flow injection analysis
  - Biochemical screening
- Today, the development is driven by stand alone or parallel working fully integrated "Lab-on-a-Chip" systems.



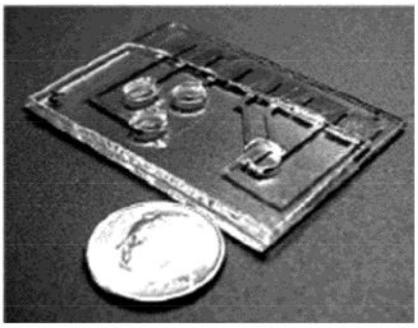
Harrison and Manz et al. / Anal. Chem. (1992), 64, 1926-1932

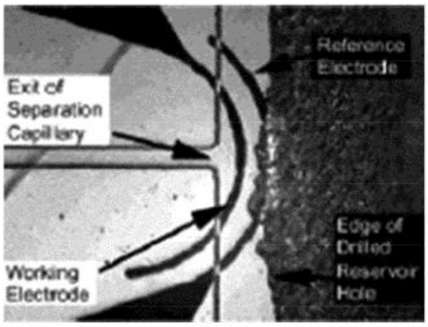


Hadd et al. /, Anal. Chem., 1999, 71, 5206-5212

End-column integration

- Little knowledge on systematic electrode configuration.
- Interference of electrical field due to operational principle of the separation method.
- Characteristics of thin-films for electrochemical detection may differ quite substantial from established bulk electrodes.

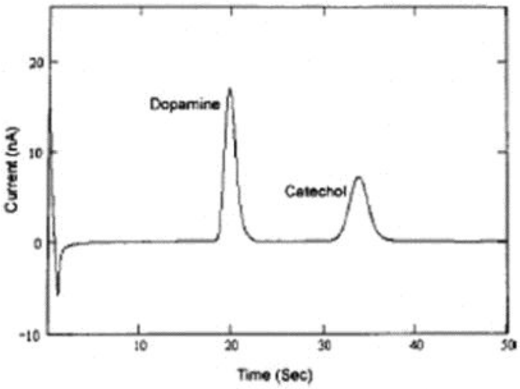




Baldwin et al. / Anal. Chem.2002, 74, 3690-3697

Restricted electroactivity

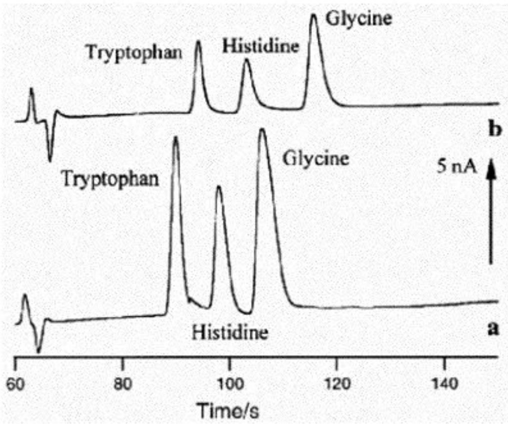
- Established material for glass integration:
  - Platinum (Pt)
- Limited number of constitutes are electroactive at Pt-electrode.
- Many materials in microelectronics but little standardization in glass micro-fluidics.



Baldwin et al. / Anal. Chem.2002, 74, 3690-3697

Back-end processing electrode modification

- Detection of amino acids (all 1 mM) at a copper-coated Pt-electrode without (a) and with (b) the addition of Cu(n) ions to the buffer.
  - Potential: 600 mV.
  - Buffer: 50 mM CAPS-NaOH with and without 0.1 mM copper(n) sulfate, pH 10.
  - Separation voltage: 2 kV



P. Hauser et al., University of Basel

Examples of sensing devices

We run a kinetic experiment in a batch reactor

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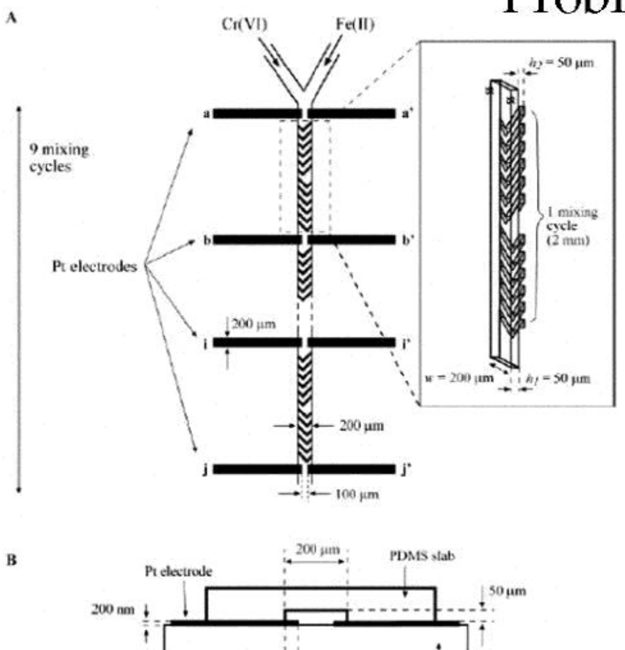
MESA+

Probing mixing efficiency



- Schematic representation of the device used to demonstrate that complete mixing is achieved in the potentio-metric titration device.
- Ten pairs of 200-nm-thick platinum electrodes were evaporated on a glass support.
- Channels embedded in PDMS
- Each pair of dual-facing electrodes located between two consecutive mixing cycles.

Ferrigno et al., Anal. Chem.2004, 76,2273-2280

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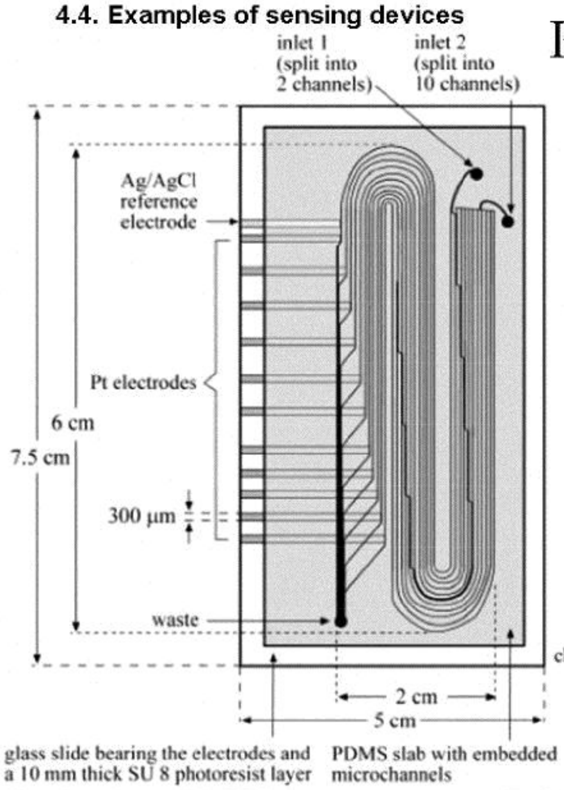
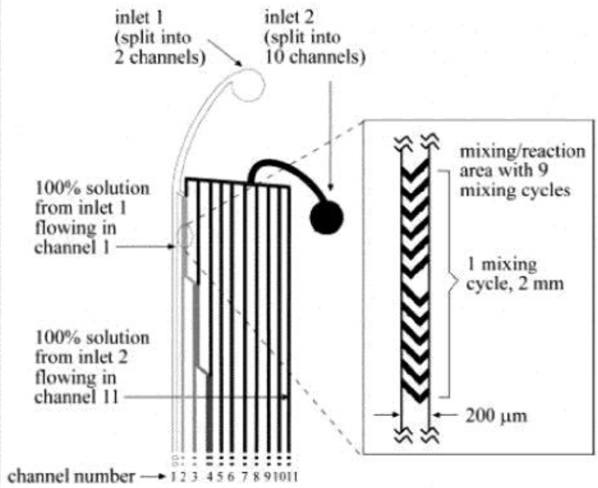
4.4. Examples of sensing devices

Potentiometric titration device

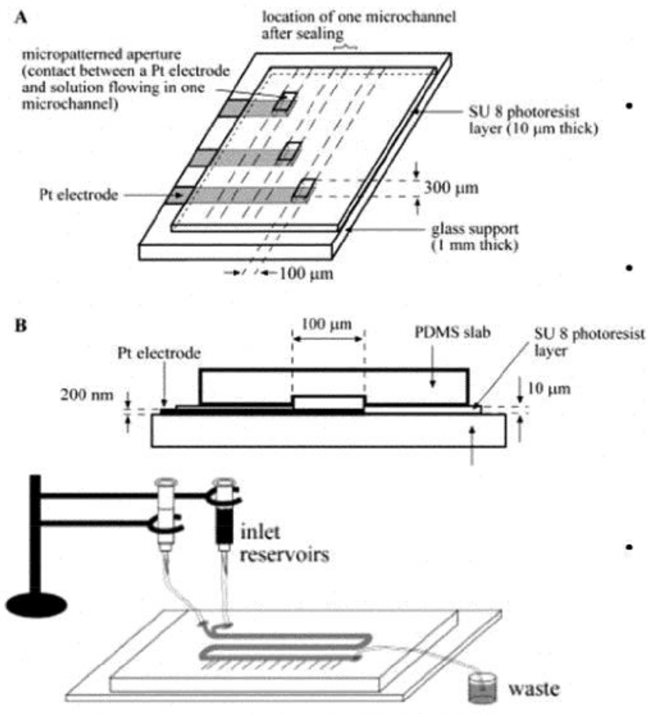




Ferrigno et al., Anal. Chem.2004, 76,2273-2280

4.4. Examples of sensing devices

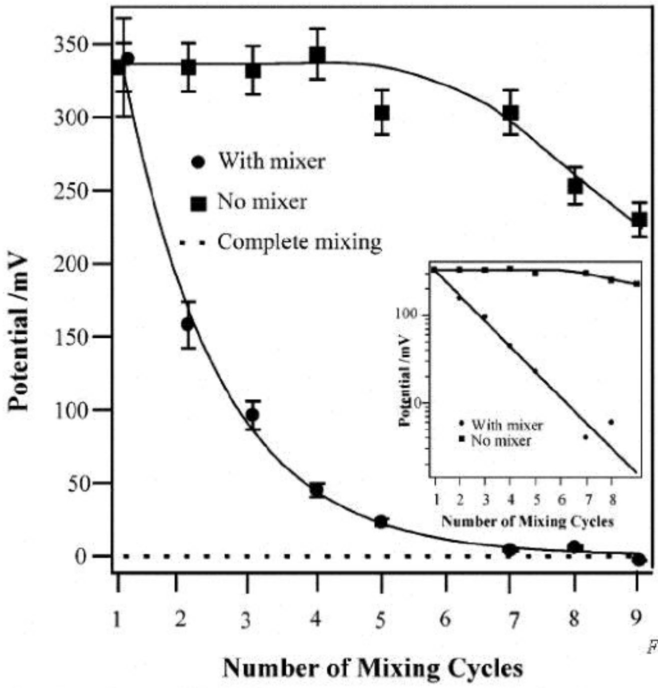
Experimental set-up and operation



Ferrigno et al., Anal. Chem.2004, 76,2273-2280

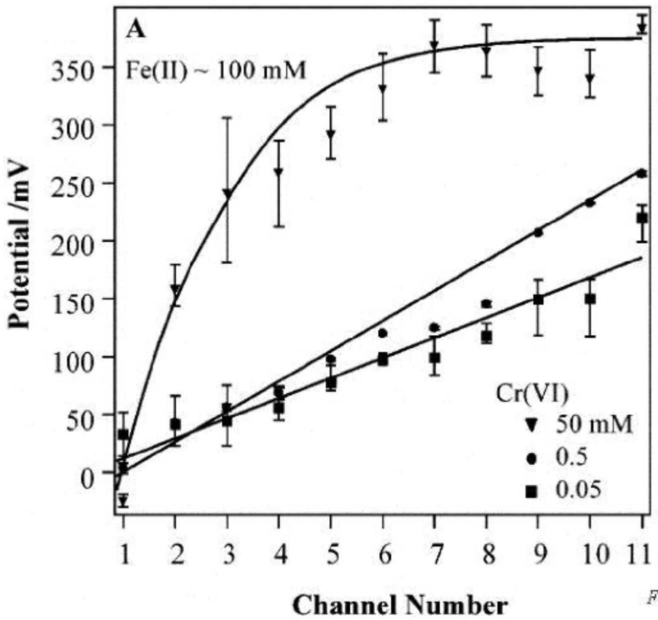
- Potentials between pairs of dual-facing electrodes were measured and plotted as a function of the channel length.
- Converting Pt-microelectrode to a reference Ag/AgCl electrode in a solution of commercial silver bath and subsequent anodic polarization. AgCl layer is ca. 100 nm in thickness.
- Potential between Pt-electrode located in one of the channels and the Ag/AgCl reference measured by using a Fluke 75 Multimeter.

Characterization results (1)



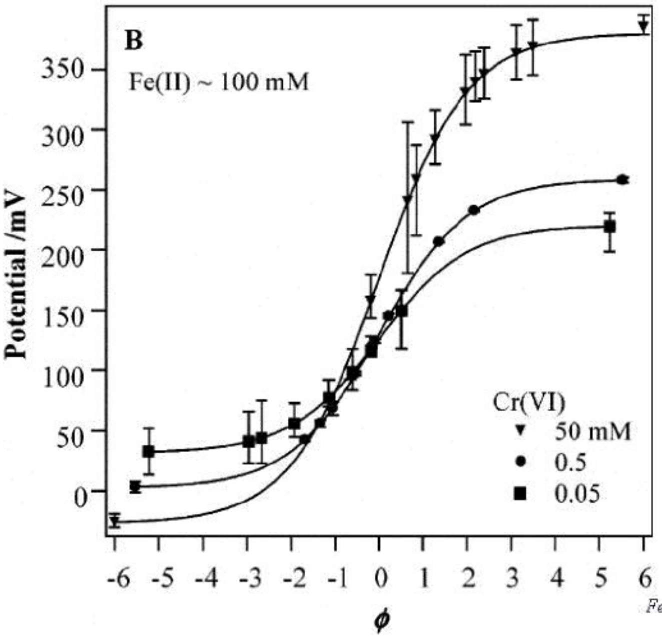
Ferrigno et al., Anal. Chem.2004, 76,2273-2280

Characterization results (2)



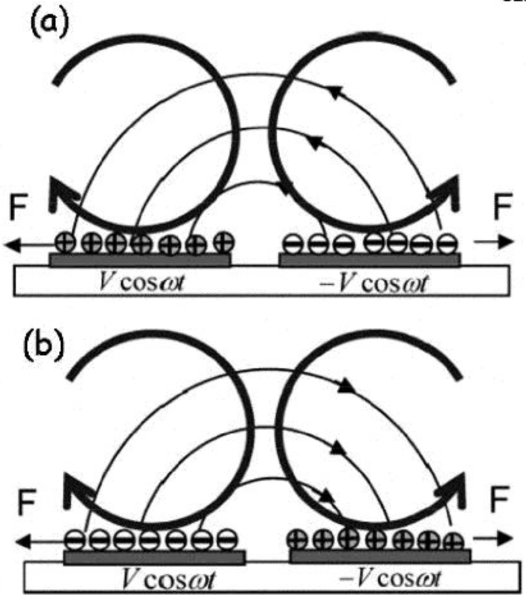
Ferrigno et al., Anal. Chem.2004, 76,2273-2280

Characterization results (3)



Ferrigno et al., Anal. Chem.2004, 76,2273-2280

Long-range AC electroosmotic trapping and detection of bioparticles



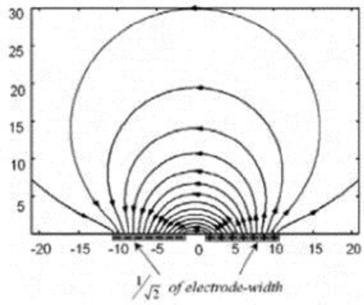
- AC electroosmosis by capacitive charging of a planar electrode pair.

(a) during the half cycle when the left electrode has a negative polarity,

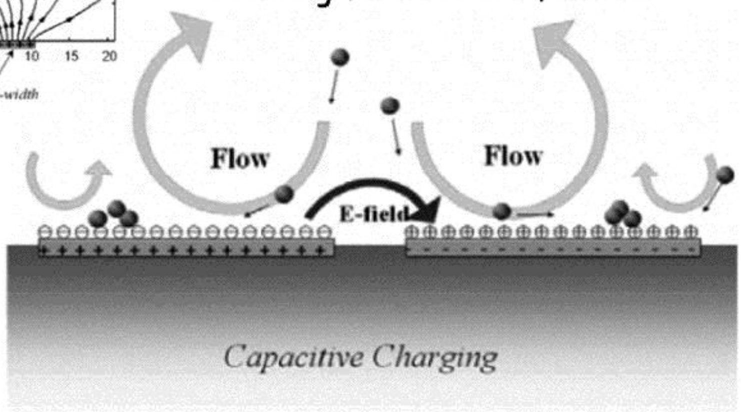
(b) during the next half cycle with opposite electrical polarity.

J. Wu et al., Ind. Eng. Chem. Res. 2005, 44, 2815-2822

Principle of particle trapping

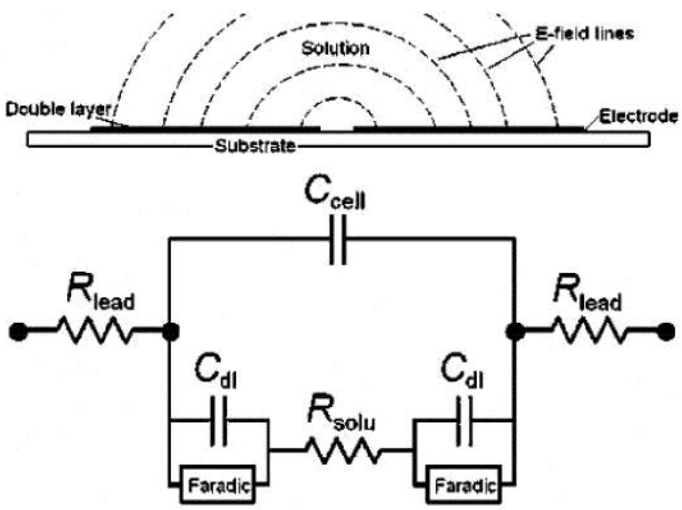


- Electric field distribution and induced flow motion above a pair of capacitively charged electrodes in an electrolyte.
- With capacitive charging, four counter-rotating vortices are formed.



J. Wu et al., Ind. Eng. Chem. Res. 2005, 44, 2815-2822

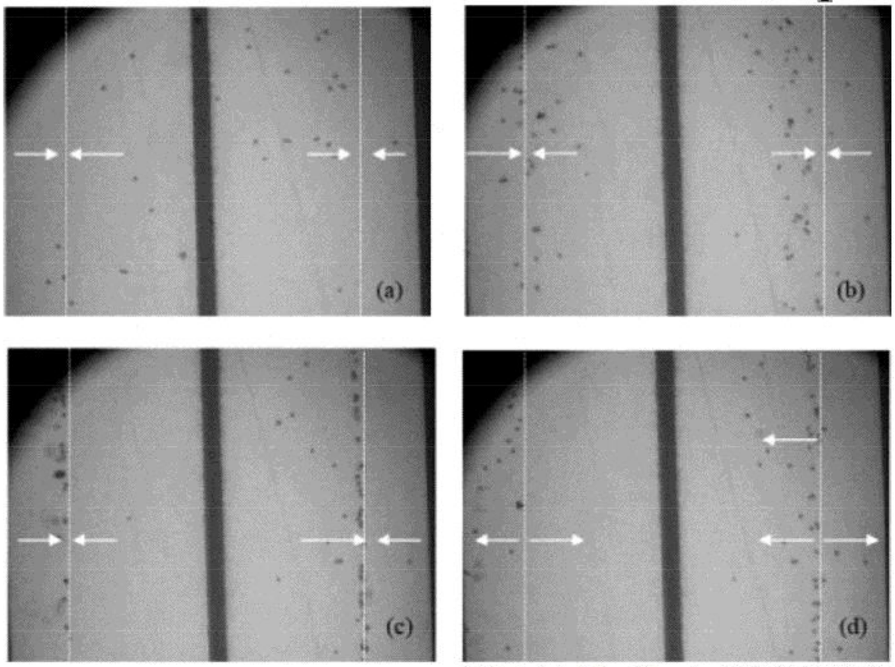
Sensor model



- Equivalent circuit for the planar parallel detection electrodes

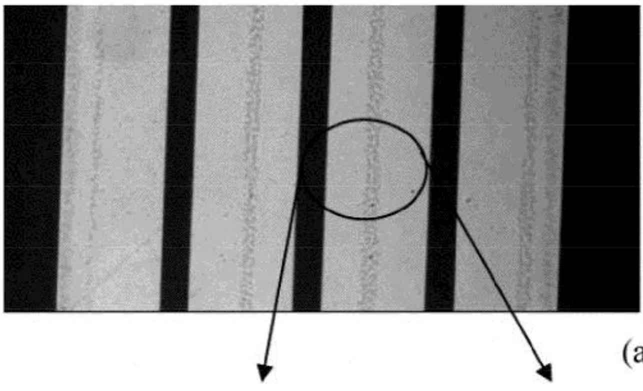
J. Wu et al., Ind. Eng. Chem. Res. 2005, 44, 2815-2822

Particle behaviour in operation

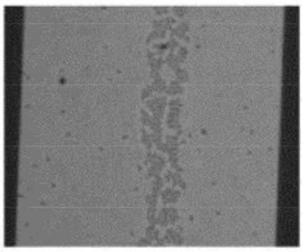


J. Wu et al., Ind. Eng. Chem. Res. 2005, 44, 2815-2822

E. coli collection



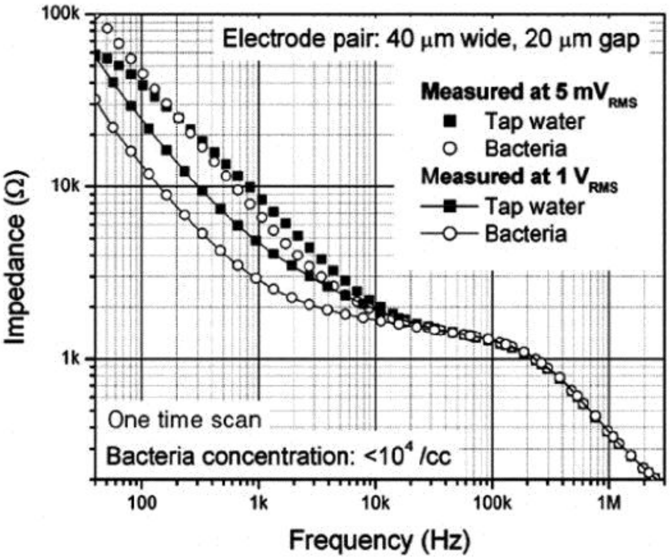
(a)



(b)

J. Wu et al., Ind. Eng. Chem. Res. 2005, 44, 2815-2822

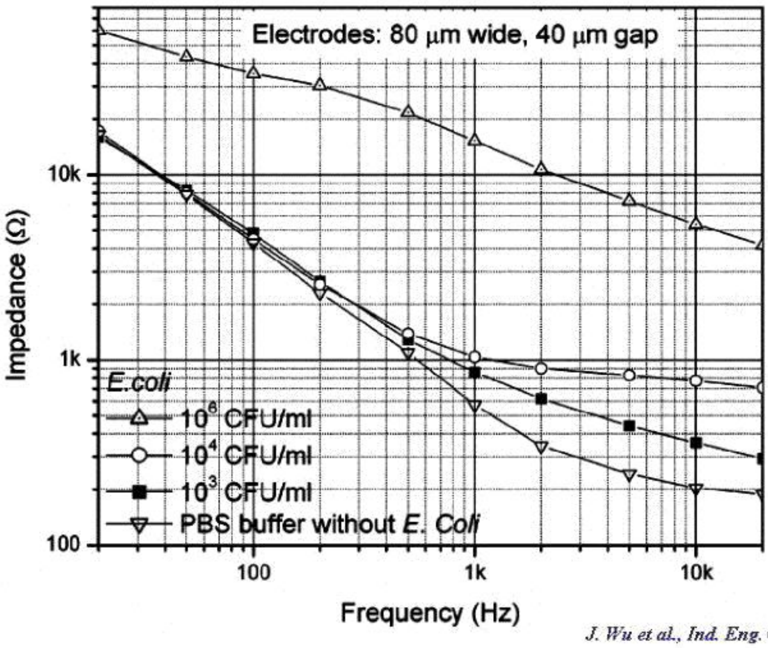
Results of water sample



J. Wu et al., Ind. Eng. Chem. Res. 2005, 44, 2815-2822

- Impedance spectra of *E. coli* in tap water at 1 Vrms

Results in Phophate Buffered Saline (PBS)



J. Wu et al., Ind. Eng. Chem. Res. 2005, 44, 2815-2822

- Impedance spectra of *E. coli* in PBS at 1 Vrms.

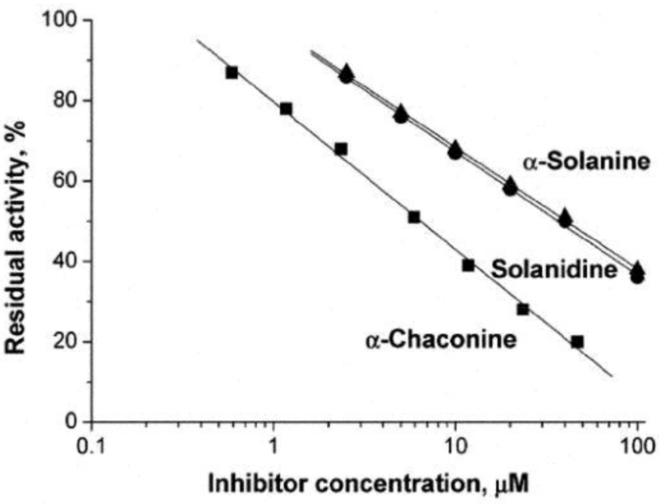
Field-effect transistor based biosensor system

- Every year, consumption of potatoes containing higher than normal levels of steroidal glycoalkaloids (a-solanine, a-chaconine and solanidine) is associated with human deaths and poisonings and a lot of livestock deaths.
- Innovative process for the detection of steroidal glycoalkaloids based on the use of pH-sensitive field effect transistors coupled to butyryl cholinesterase.
- In comparison to the methods routinely used, the biosensor method proposed is simple, inexpensive, fast (the overall time for one analysis is less than 10 min) and reliable.
- The biosensor developed could find successful application fields, especially in agriculture, food quality control and health care.

Y.I. Korpan et al, Bioelectrochemistry 55 (2002) 9 –11

4.4. Examples of sensing devices

Enzyme biosensor for steroidal glycoalkaloids detection based on pH-sensitive FET



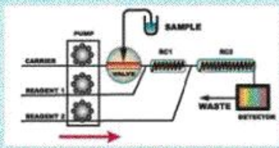
- Calibration curves for the detection of glycoalkaloids by pH-sensitive FETbased biosensor.
- Measurement conditions: 10 mM K,Na-phosphate buffer, pH 7.5, room temperature.

Y.I. Korpan et al, Bioelectrochemistry 55 (2002) 9 –11

Systems at-line

www. eng. bham. ac. uk

Systems in-line



www. flowinjection. com

www. nanotream. com

Outlook: Future developments

- Detection and quantification of more and more constituents in complex fluids using electrical means.
- Further reduction of operator influence and skills e.g., in-line process monitoring.
- Integration of the information obtained to optimize the process parameters/conditions. Since information is available in quasi-real-time utilization for process control loops becomes feasible.

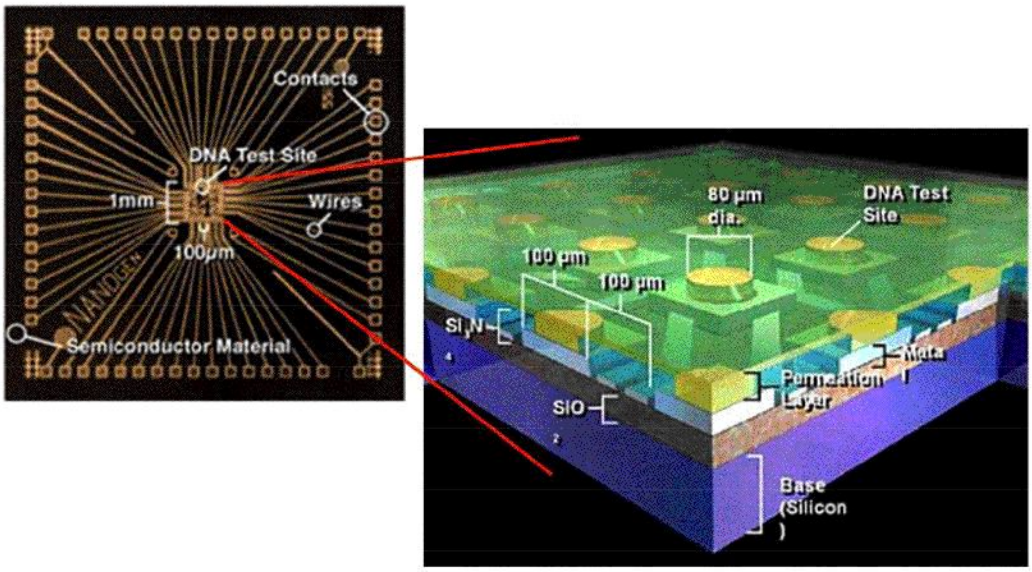
5.1. Outlook: Future developments

Industrial demands are getting more and more visible

- Integrated sensors in batch processes (including well-plates and point-of-care).
- Flow-injection analysis (semi-continuous).
- Continuous flow-through monitoring (downstream).
- Transferring traditional laboratory tools into quicker, potentially in-line process tools by means of moving to multi- and short column designs as offered by microfluidic integration.

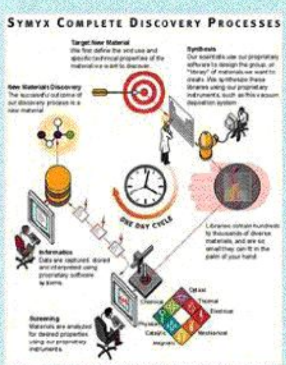
5.2. Outlook: Future developments

CMOS integrated array device for advance electrochemical assays



5.3. Outlook: Future developments

Summary



- Challenging working principles. Not simple.
- Great many number of sensors. Selection of suitable sensor type for microfluidics still driven by manufacturing feasibility instead of desired function within process monitoring.
- Product control widely established but feed-back to process line requires still major developments, though some good working principles of miniaturized systems have been introduced scientifically.