

# Electrochemical evaluation of acetylcholinesterase activity and construction of biosensor suitable for fast detection of nerve agents

Miroslav Pohanka, Oto Drobik, Zuzana Krenkova, Jana Zdarova-  
Karasova, Ondrej Holas, Kamil Kuca, Irena Kasalova, Jiri Kassa

*Faculty of Military Health Sciences  
University of Defense  
Trebesska 1575, 50001 Hradec Kralove  
Czech Republic  
e-mail: [rau@atlas.cz](mailto:rau@atlas.cz)*



# Topics

- *Nerve agents*
- *AChE and biochemistry of it assays*
- *Electrochemical biosensors based on AChE*
- *Assay of nerve agents as well as organophosphate pesticides*
- *Proposition of automatic detector and future trends*

# Nerve agents

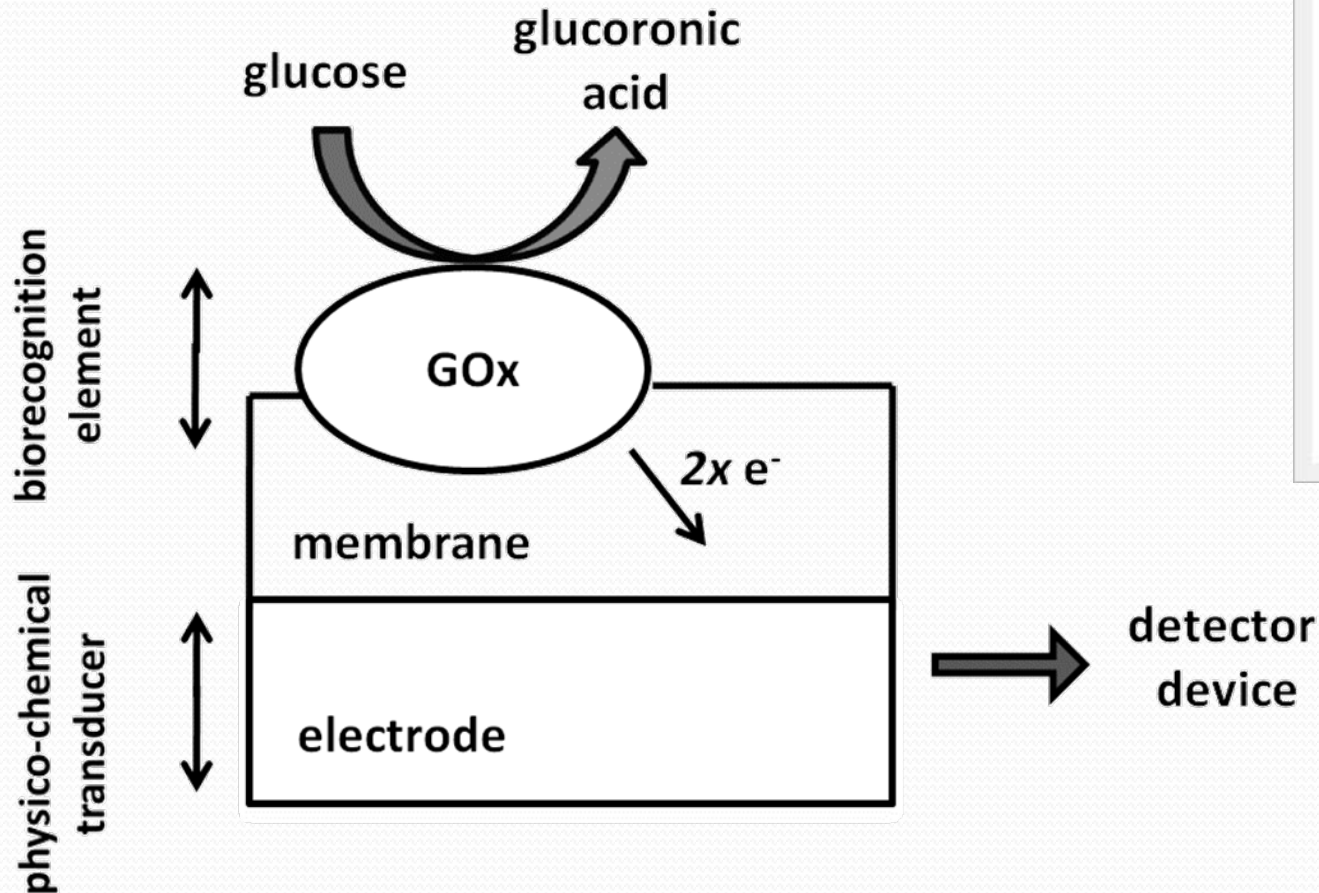
- *Nerve agents are able to esterify hydroxyl of serine in active site of acetylcholinesterase (AChE) respectively serine in active site of other serine hydrolases (butyrylcholinesterase, trypsin, acetylCoA hydrolase, succinylCoA hydrolase, phospholipase A2 ....)*
- *AChE is an important enzyme in body participating in termination of signal transmission through neurosynaptic cleft*
- *Nerve agents are divided into two basic groups: G („Germany“) found in 1930s by group of prof. Gerhard Schrader and V („Victory“ or Venomous“?) extensively researched after World War II.*
- *The first symptomatic manifestation of intoxication is accompanied by miosis. The further symptoms are salivation, seizures, and neuromuscular dysfunctions. Symptoms arising fast; above all, when NA penetrates by lungs. Death occurs due to paralysis of respiratory musculature.*



# AChE as a recognition element

- *Nerve agents (NA) and organophosphate pesticides have common action: inhibition of acetylcholinesterase (AChE).*
- *AChE would be simply applied as a recognition element for assay of anticholinergic compounds including NA and OP.*
- *Presence of analyte is proved by decrease of enzyme activity.*
- *Enzyme activity would be followed by multiple methods based on electrochemical or optical principle.*
- *When AChE is intercepted on sensor surface, device called „biosensor“ is constructed.*
- *AChE can be used also in a simple dipstick*

# How does biosensor work? Personal glucometer as typical example

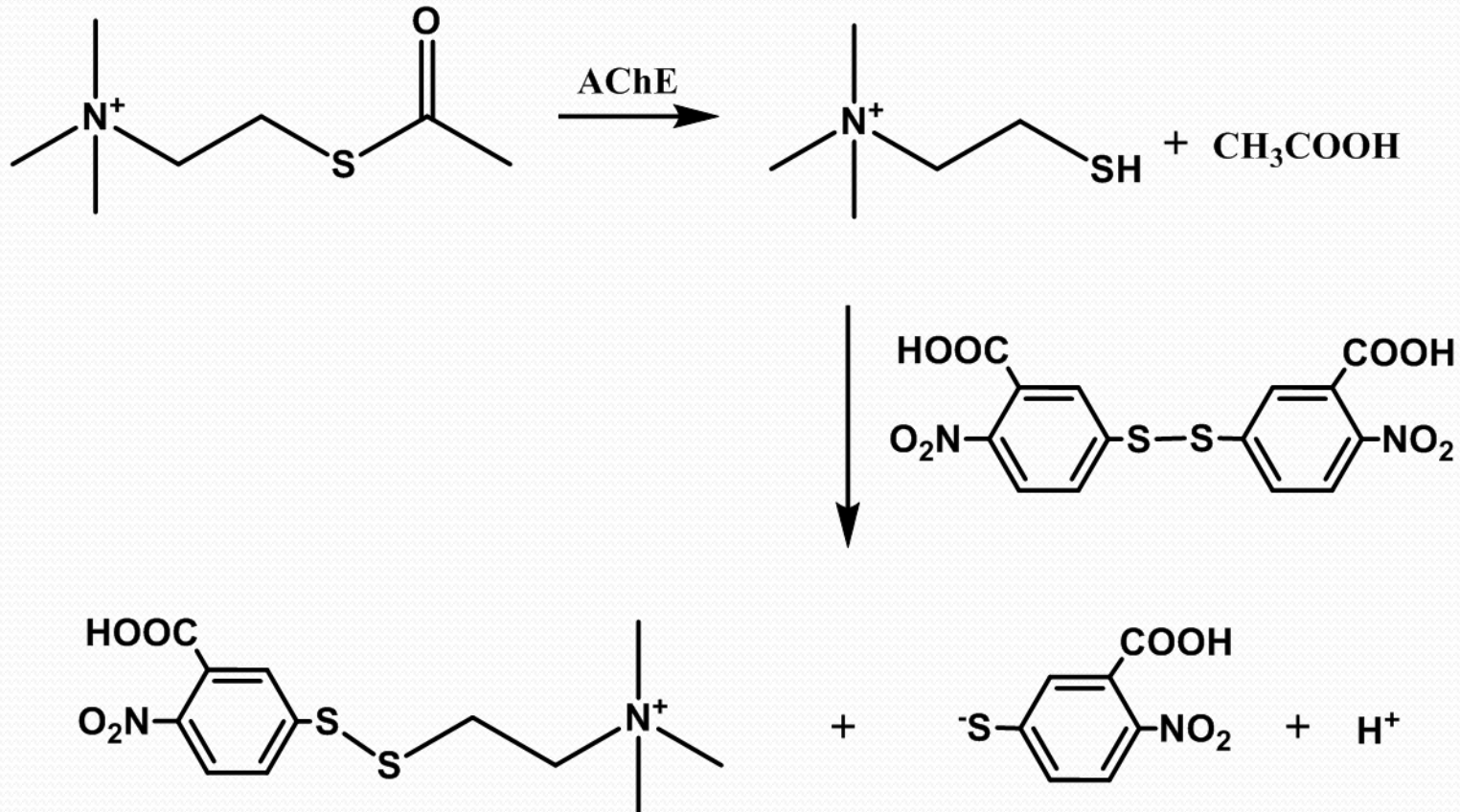


*Typical electrochemical biosensor used in personal glucometers.*

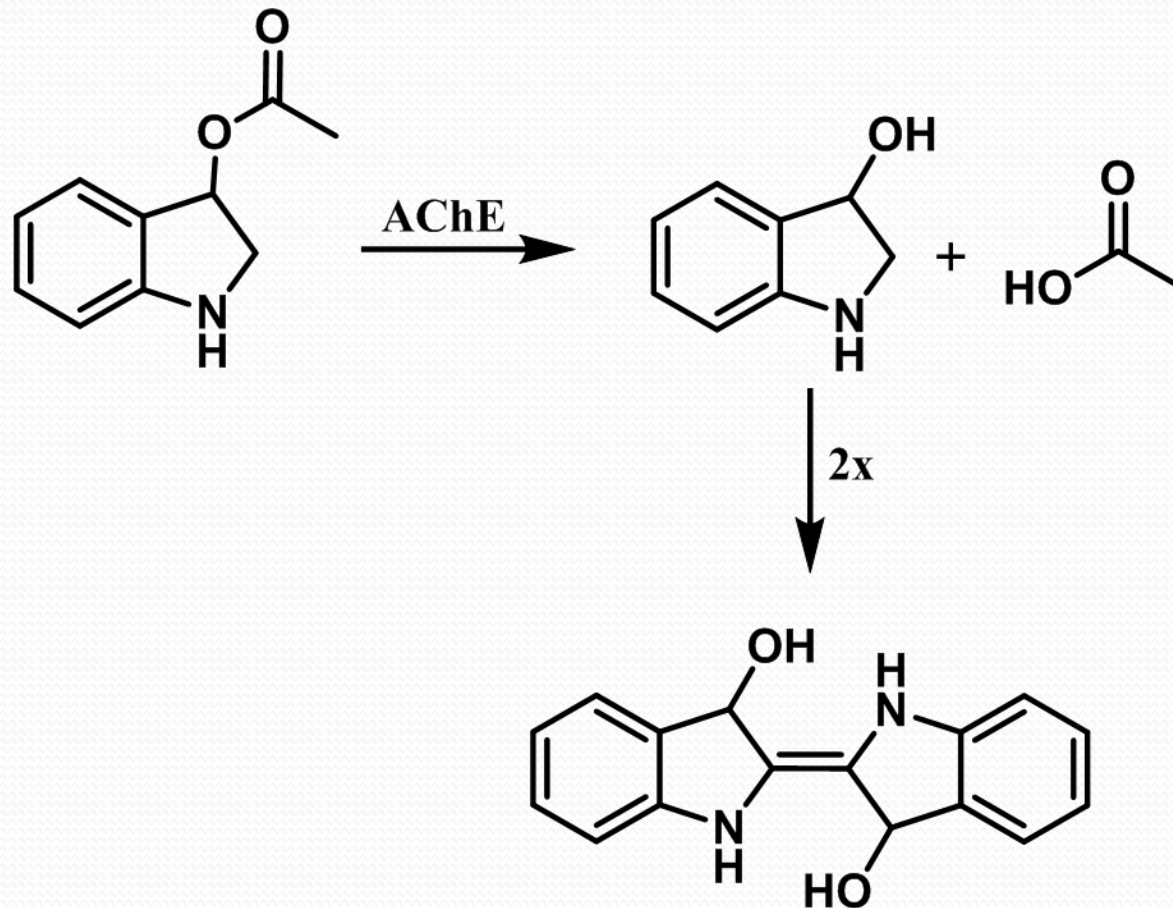
# Biochemical methods for AChE activity estimation

- *Ellman method*
- *Fluorogens such as indoxyl acetate*
- *Electrochemical oxidation of thiocholine*
- *Estimation of acidification by acetic acid (pH sensitive assay)*
- *Oxidation of choline into betaine*

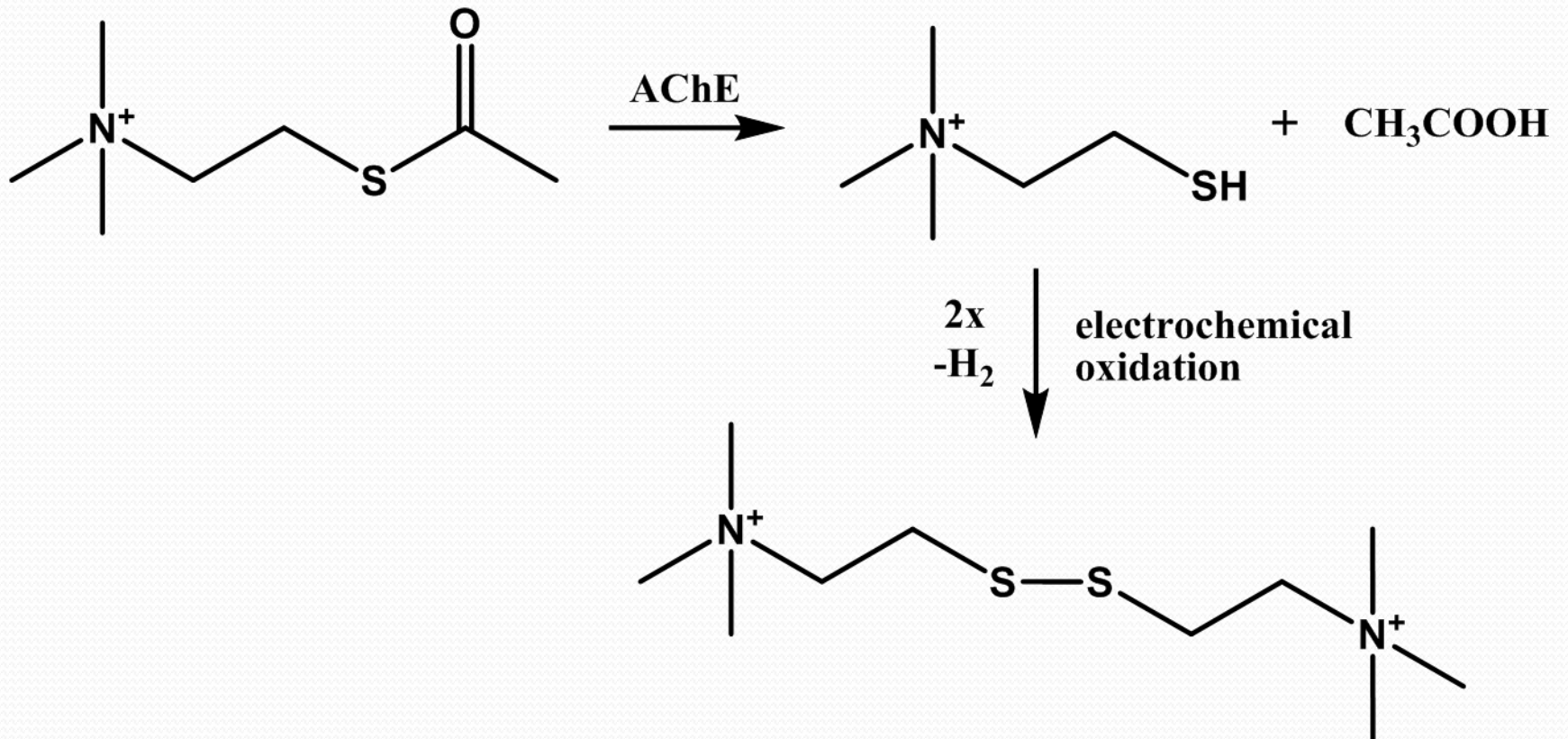
# Photometrical principle of AChE activity evaluation – Ellman reagent *5,5'-dithiobis-(2-nitrobenzoic) acid*



# Photometrical principle of AChE activity evaluation – indoxyl acetate



# Electrochemical principle of AChE activity evaluation

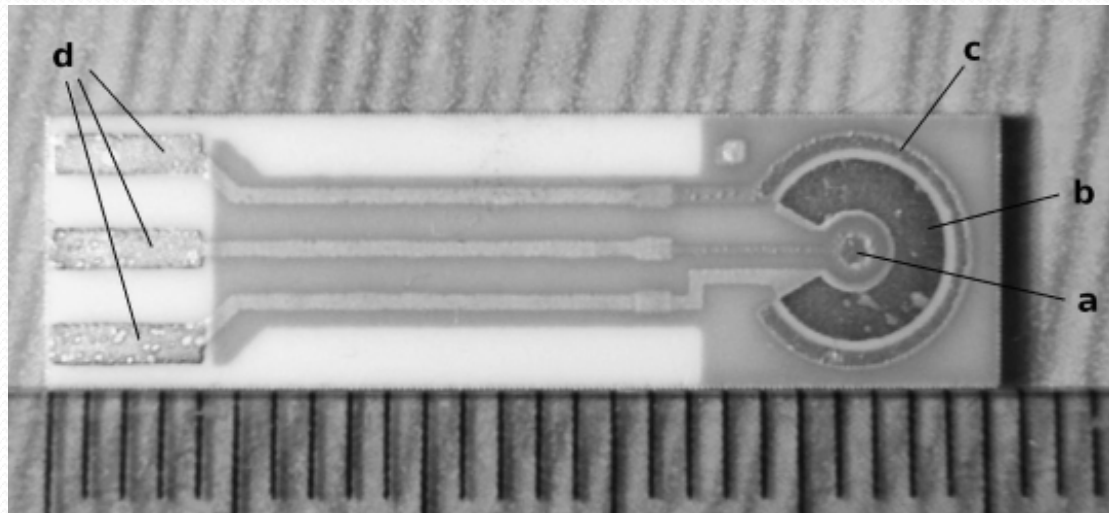


# Principles of electrochemical assay

- **Potentiometry**: performance of pH sensitive electrodes (ISFET, glass pH potentiometric electrodes, LAPS ....). The current going through system is near zero.
- **Chronoamperometry**: fixed potential – typically 450 – 500 mV vs Ag/AgCl or less when used voltage modificans. Chronoamperometry is the less fastidious in way of used device.
- **Voltammetry**: cyclic voltammetry, square wave voltammetry, differential pulse voltammetry. Voltammetry is more precision than chronoamperometry, there is also lower interference of oxidizable compounds.

# Construction of biosensor – immobilization of AChE

*Different physico-chemical transducers are approachable for AChE immobilization. The most common ones are optical fibers, potentiometric, and amperometric electrodes.*

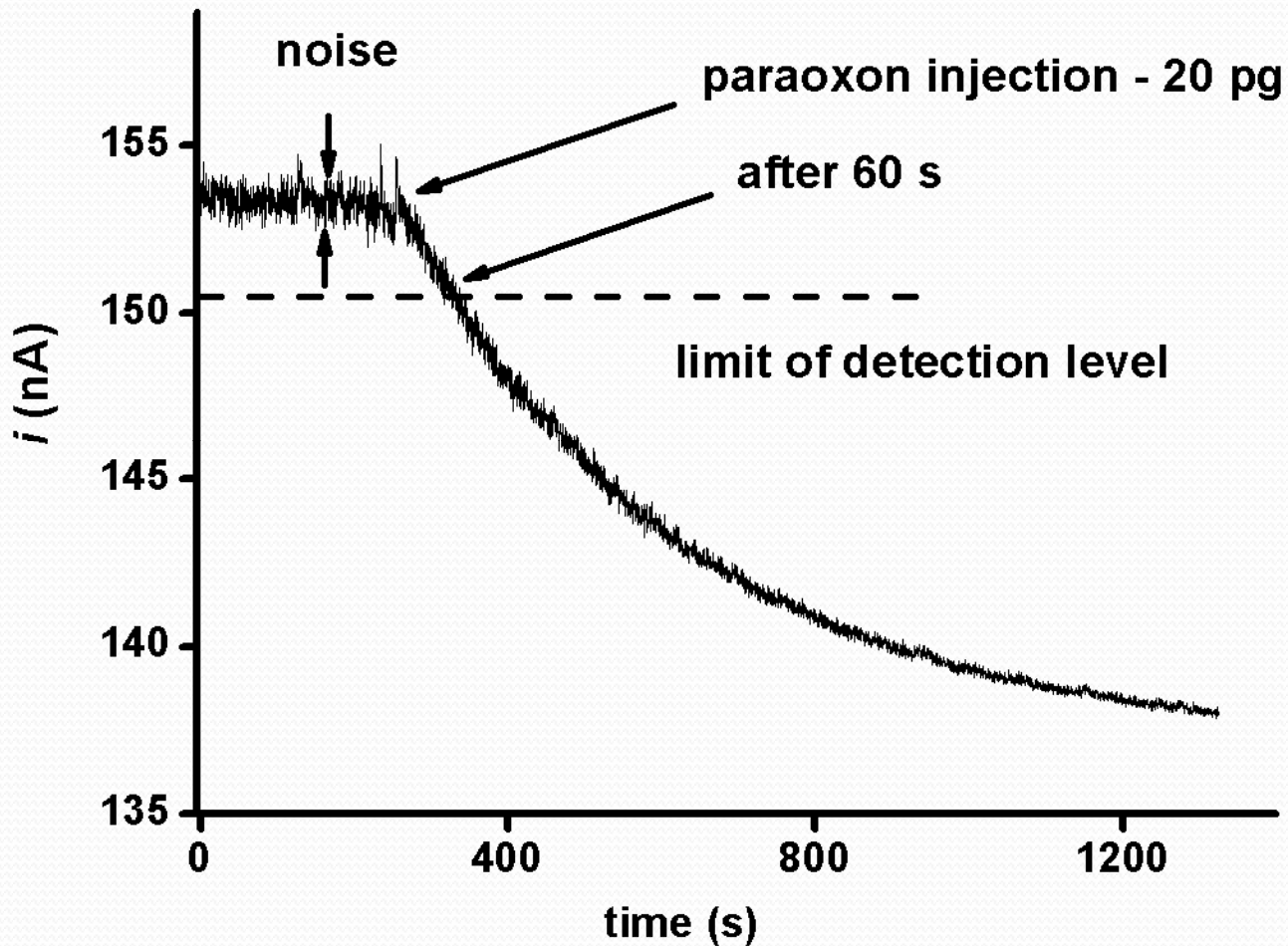


*Photograph of used biosensor. A millimeter ruler is beside for size estimation. The significance of letters: a – working electrode with immobilized AChE; b – Ag/AgCl reference electrode; c – auxiliary electrode; d – outputting contacts.*

# Immobilization procedures

- *AChE can be intercepted by many ways.*
- *Here, some basic immobilization procedures were tested.*
- *The simple methods were preferable researched regarding to disposable application of AChE based biosensors.*
- *The interception based on simple sorption, glutaraldehyde precipitation, gelatin precipitation, acetylcellulose and sol-gel with reagents 3-aminopropyl triethoxysilane were examined.*
- *Precipitation by glutaraldehyde was chosen as the most convenient when residual AChE activity considered.*

# AChE based biosensor – a fast assay of AChE inhibitors



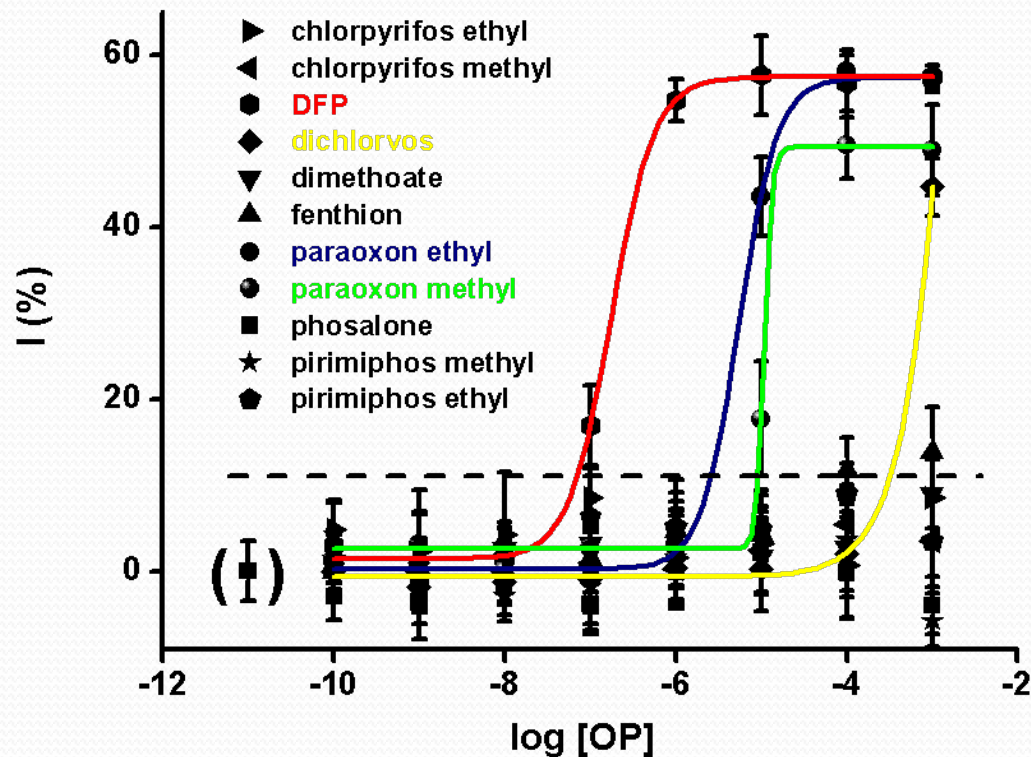
# Percent of inhibition

*The outputting signal should be recalculated in order to remove error due to AChE activity changes and differences between individual biosensors. The achieved parameter is called as **percent of inhibition**:*

$$I = \left(1 - \frac{i_i}{i_0}\right) \times 100$$

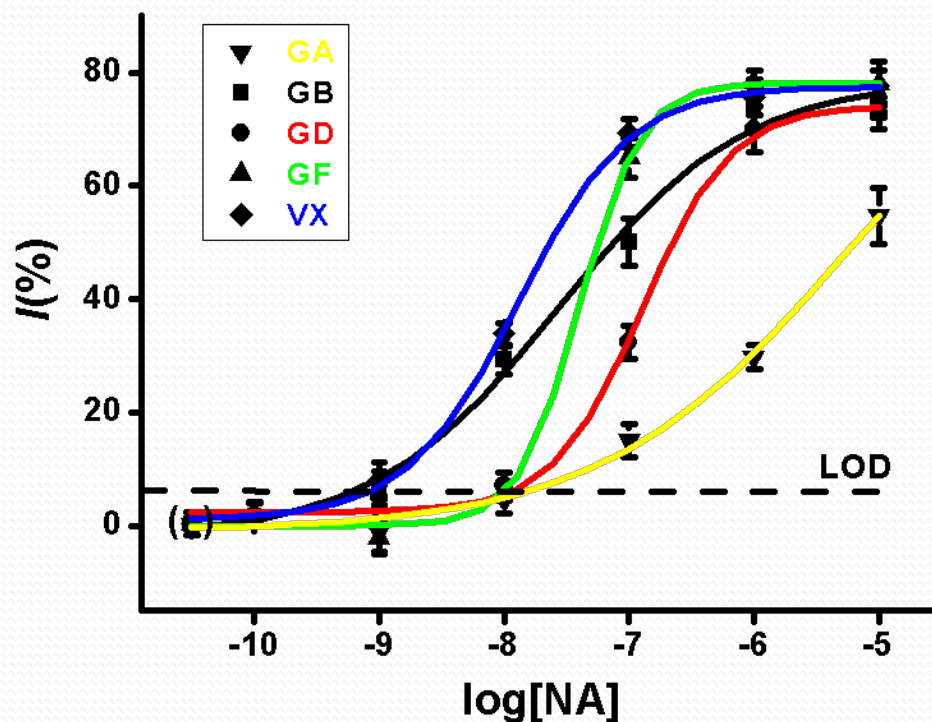
*The percent of inhibition could be easily compared with the other method such as photometrical assay. It also brings better reproducibility.*

# Examples of pesticides assay by cholinesterase based biosensor



	DFP	Paraoxon ethyl	Paraoxon methyl	Dichlorvos
LOD (mol/l)	$6.83 \times 10^{-8}$	$2.51 \times 10^{-6}$	$7.76 \times 10^{-6}$	$2.63 \times 10^{-4}$
IC50 (mol/l)	$1.70 \times 10^{-6}$	$5.62 \times 10^{-6}$	$1.15 \times 10^{-5}$	$1.41 \times 10^{-3}$

# Examples of nerve agents assay by cholinesterase based biosensor

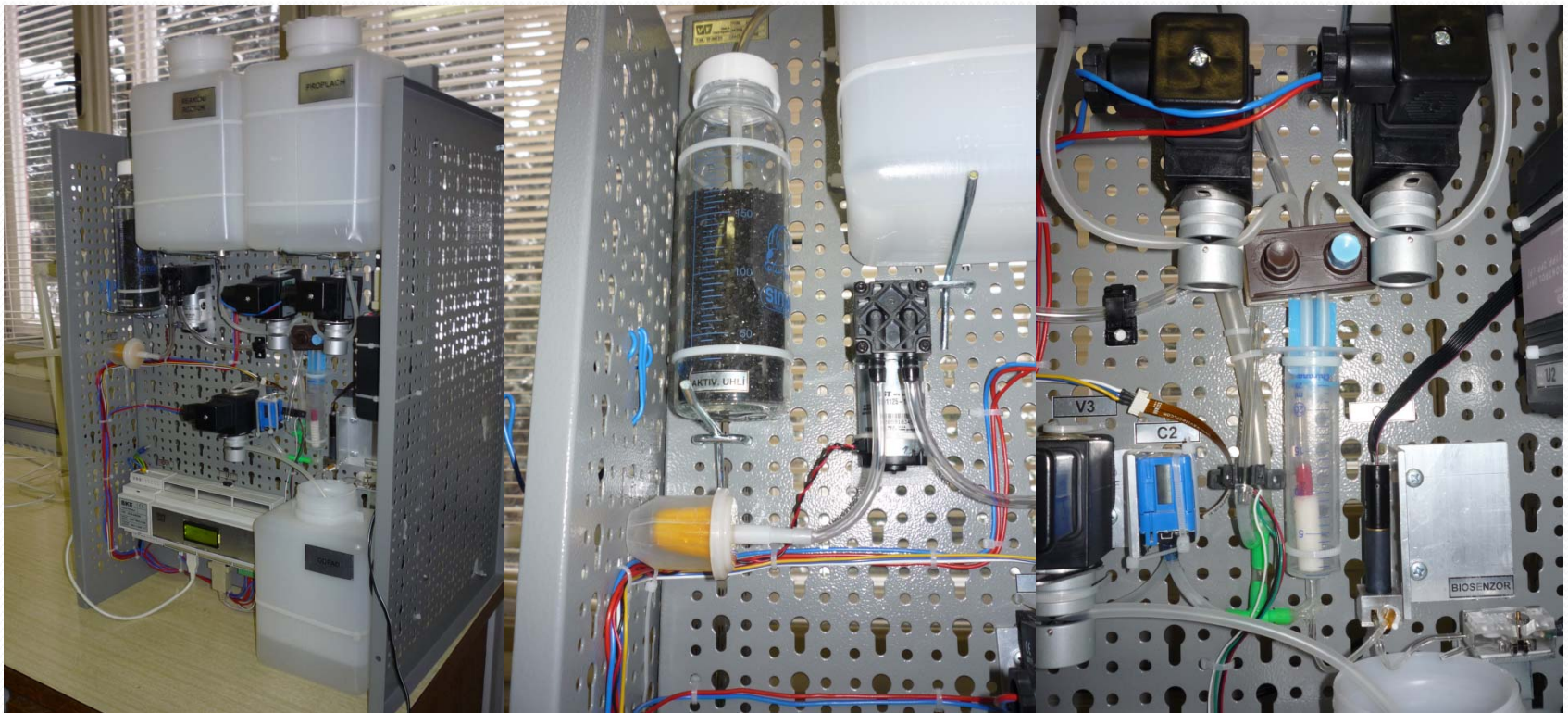


	Tabun (GA)	Sarin (GB)	Soman (GD)	Cyclosarin (GF)	VX
LOD (mol/l)	$1.48 \times 10^{-8}$	$5.88 \times 10^{-10}$	$1.07 \times 10^{-8}$	$9.12 \times 10^{-9}$	$8.51 \times 10^{-10}$
IC50 (mol/l)	$6.01 \times 10^{-6}$	$2.77 \times 10^{-8}$	$1.28 \times 10^{-7}$	$4.08 \times 10^{-8}$	$1.29 \times 10^{-8}$

# Effect of organic solvents

<i>Solvent concentration</i>	20%	10%	5%
<i>methanol</i>	<b>48±3</b>	<b>33±4</b>	<b>24±2</b>
<i>ethanol</i>	<b>56±4</b>	<b>47±3</b>	<b>21±1</b>
<i>propanol</i>	<b>77±3</b>	<b>70±5</b>	<b>41±4</b>
<i>butanol</i>	<b>75±5</b>	<b>79±5</b>	<b>61±5</b>
<i>pentanol</i>	<b>64±6</b>	<b>63±6</b>	<b>57±3</b>
<i>propan-2-ol</i>	<b>58±3</b>	<b>24±2</b>	<b>5±1</b>
<i>PEG (500)</i>	<b>44±4</b>	<b>20±4</b>	<b>13±2</b>
<i>DMF</i>	<b>80±4</b>	<b>77±3</b>	<b>62±5</b>
<i>DMSO</i>	<b>64±5</b>	<b>43±1</b>	<b>10±1</b>
<i>THF</i>	<b>72±3</b>	<b>68±5</b>	<b>48±4</b>
<i>acetonitrile</i>	<b>79±7</b>	<b>57±6</b>	<b>37±5</b>

# Proposal of detector with air sampler



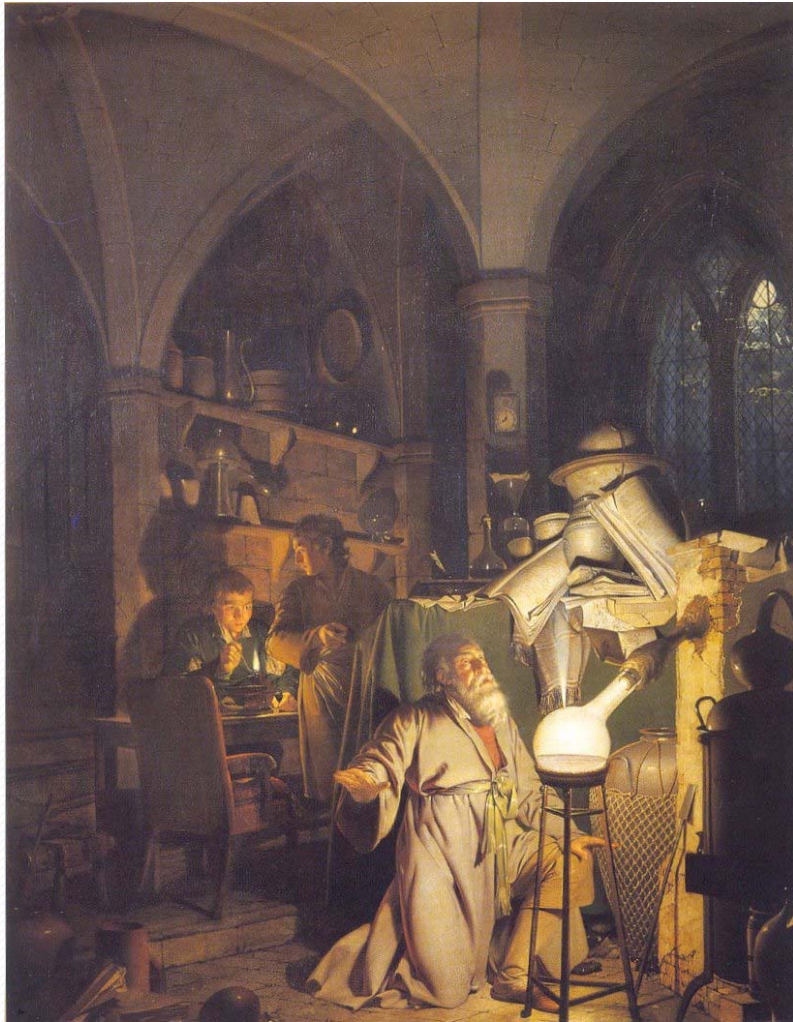
# Conclusions & Next effort

- *AChE is suitable recognition element for construction of devices for fast assay of nerve agents and other inhibitors such as pesticides and some natural toxins.*
- *Optimization of immobilization procedures, testing of AChE capturing on carbon nanotubes, carbon paste electrodes, magnetic microparticles etc.*
- *Construction of colorimetric dipsticks for fast assay of nerve agents.*
- *Improvement of sensitivity as well as limits of detection by differential pulse voltammetry or square wave voltammetry instead of simple chronoamperometry.*
- *Fast evaluation of drugs targeted on AChE.*

# Acknowledgements

*Ministry of Defense, Czech Republic for support through project no. OVUOFVZ200807*

**..... and you for your attention**



*The alchemist discovering phosphorus (1771)*

*by Joseph Wright*