A printed and disposable electrochemical biosensor based on cholinesterase inhibition for nerve agent detection

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Abstract

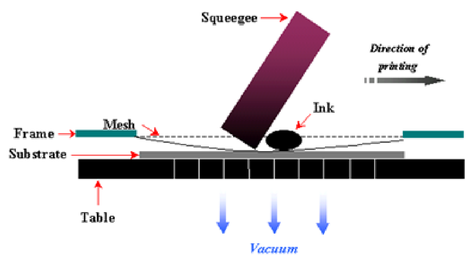
The nerve agents are chemical warfare agents known to be used during terrorist attacks, potent nerve agents are Sarin (GB), Soman (GD), Tabun (GA), and VX. The extreme toxicity of these compounds is due to their ability to irreversible inhibit Acetylcholinesterase (AChE) enzyme in the neuromuscular junction of the central nervous system. The nerve agents also have the ability to irreversible inhibit Butyrylcholinesterase (BChE) in blood. The vapor pressures of these agents (especially in case of Sarin) and their rapid effect on the central nervous system (CNS), combined with the low cost and unsophisticated technology required for production, make these compounds or agents among the preferred choices for terrorists. For this reason an inexpensive, sensitive, miniaturized, and portable system to be used by first responder and military personnel is of interest owing to the continuing threat of possible terrorist attacks. Amperometric biosensors based on cholinesterase inhibition shows such potentialities. In this work butyrylcholinesterase was immobilized onto screen-printed electrodes modified with Prussian blue and the nerve agent detection was performed by measuring the degree of enzyme inhibition.

Keywords: Cholinesterase biosensor, Nerve agents, Screen-printed electrodes.

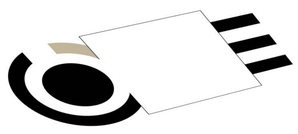
Materials and Methods

1. Screen-Printed Electrodes

DEK 248 Screen-Printer machine.

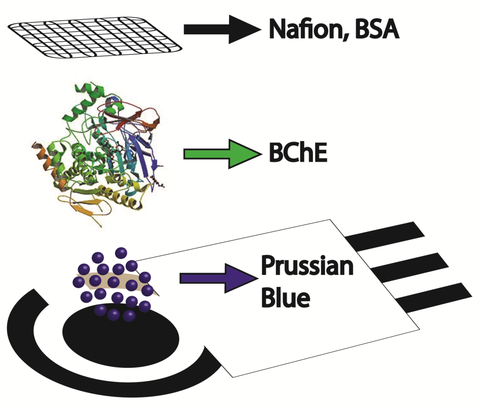


Screen-Printing process.



Screen-Printed electrode.

2. Biosensor Fabrication



Steps involved in biosensor fabrication.

3. Electrochemical Measurements

PalmSens portable potentiostat.

Reactions Involved

Butyrylthiocholine + BChE → **Thiocholine** + Butyryc acid

Thiocholine (RSH) +PBox → PBred + ½ Thiocholine-Thiocholine(RS-SR)

PBred → PBox + e^-

Oxidation current proportional to thiocholine concentration.

If inhibitor (I) is present … Butyrylthiocholine + BChE + I → **Thiocholine**

Current (i_i) decreases respect to the current in absence of inhibitor (i_0)

$$I(\%) = (i_0 - i_i/i_0) \times 100$$

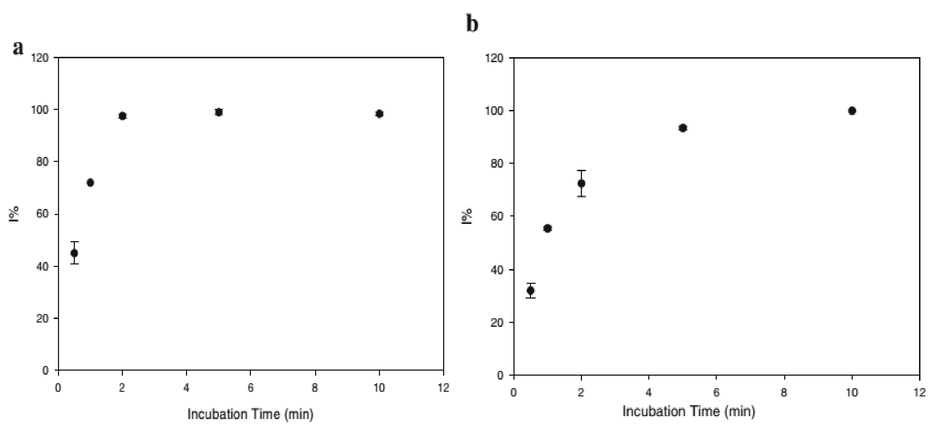


Fig. 4 The relative inhibition of BChE activity as a function of incubation time using Sarin gas at 0.5 mg m^{-3}, a) and 0.1 mg m^{-3}, b) Measurement conditions 0.05 M phosphate buffer + 0.1 M KCl, pH 7.4; applied potential + 200 mv vs Ag/AgCl; 5 mM BTChCl.

Results

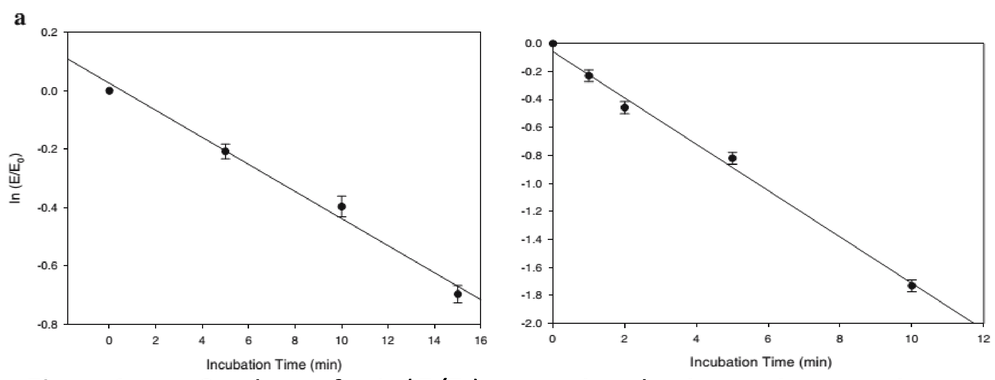


Fig. 1 Study of ln(E/E_0) vs incubation time using a. Acetylcholinestease (AChE) or b. butyrylcholinesterase (BChE) biosensors. Paraoxon concentration 200 ppb. Applied potential +200 mV vs Ag/AgCl; 0.01 U AChE, 0.05 M phosphate buffer +0.1 M KCl, pH 7.4. in the case of the AChE biosensor 1 mM acetylthiocholine chloride (ATChCl) was used, while for the BChE biosensor 5 mM butyrylthiocholine chloride (BTChCl) was used. All the values are the average of triplicate measurements.

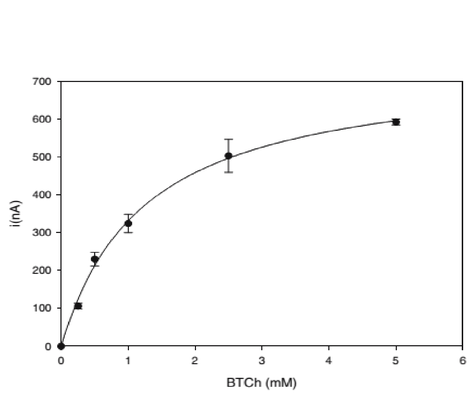


Fig. 2 Calibration plot of butyrylthiocholine using the BChE biosensor. Applied potential + 200 mV vs Ag/AgCl; 0.01 U BChE, 0.05 M phosphate buffer + 0.1 M KCl, pH 7.4. All the values are the average of triplicate measurements.

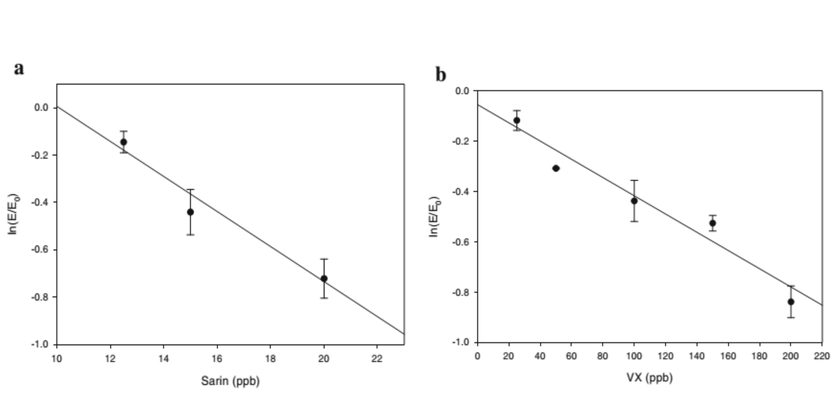


Fig. 3 Study of ln (E/E_0) vs inhibitor concentration. a) Sarin and b) VX using the BChE biosensor. Incubation time 10 min; applied potential + 200 mV vs Ag/AgCl; 0.01 U BChE, 5 mM BTChCl, 0.05 M phosphate buffer + 0.1 M KCl, pH 7.4. All the values are the average of triplicate measurements.

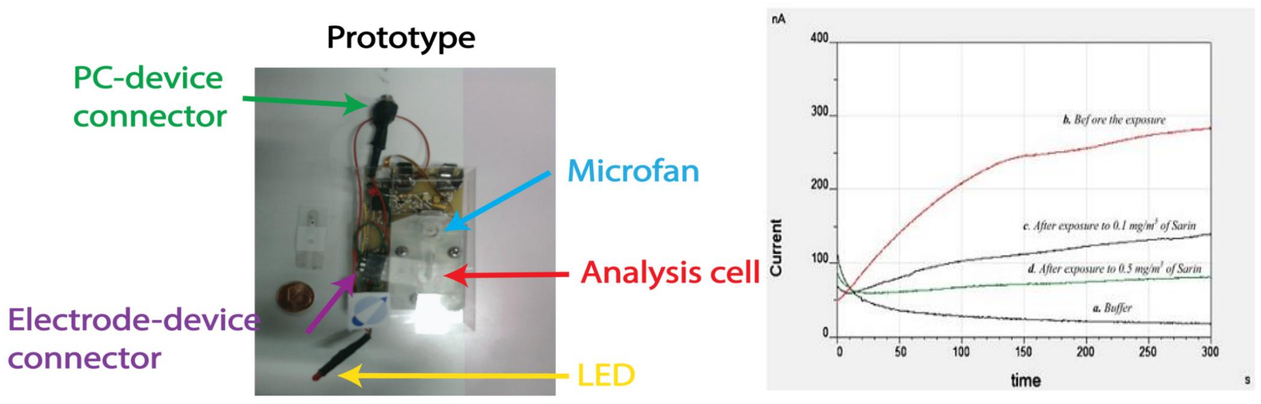


Fig. 5 (Left) image of the prototype, (right) original recording obtained using a biosensor in 0.05 M phosphate buffer solution + 0.1 M KCl, pH 7.4. Applied potential + 200 mV vs Ag/AgCl. Signal recorded in phosphate buffer (a) and in a solution of butyrylthiocholine (5 mM) before the exposure of the biosensor to sarin gas (b) and after 1 min exposure to 0,1 mg m^{-3} (c) and to 0.5 mg m-3 (d) of Sarin gas.

Conclusion

In this work the results obtained using a biosensor based on cholinesterase inhibition were reported. The biosensor was firstly tested toward Sarin gas detection using a portable instrument, demonstrating its suitability for a fast analysis. Successively, the biosensor was embedded in a prototype and tested with nerve agent simulant solution (paraoxon) obtaining satisfactory results in terms of reproducibility; in fact, a solution of 1 ppm tested 12 times provoked always the switch-on of the alarm. On the contrary, a blank solution did not cause any alarm. Taking in consideration that i) the system of sampling of the prototype is able to preconcentrate the sample; ii) nerve agents such as Sarin are stronger inhibitors than paraoxon, it can be concluded that the system could be suitable to detect lower concentration of nerve agents. Further studies will be focused to demonstrate the suitability of this prototype with nerve agents in gas phase.