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ORIGINAL ARTICLE

Automated detection of organophosphate warfare gases (nerve agents) in air based on micro-SIA – lab-on-valve system

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Summary
Equipment for fast and accurate detection of organophosphate nerve agents is developed and tested. The method is based on the spectrophotometric monitoring of the enzyme activity of butyrylcholinesterase after its contact with air in a special absorption unit (a "scrubber") developed for the purpose. The scrubber was made from a glass tube filled with glass beads (diam. 3 mm) and filled with approx. 5 ml of butyrylcholinesterase in a phosphate buffer of pH 7.4. The air sample was bubbled through this solution for 20 s at a flow rate of 80 l hour^{-1}. Thereafter 8 µl of the enzyme solution were aspirated into the micro-SIA-LOV analyzer and the activity of the enzymes were evaluated by using Ellman's reagent, i.e. 2.5 mmol l^{-1} butyrylthiocholine iodide and 0.25 mmol 5,5'-dithiobis (2-nitrobenzoic acid). The absorbance of the coloured reaction product was measured at 412 nm after the reaction time of 60 s. The residue of the absorption liquid was washed away from the absorber and the system was washed with the enzyme solution prior to next analysis. The contaminated air caused partial inhibition of the enzyme activity of the absorption liquid. The activity of the contaminated sample was compared with the activity of the unaffected enzyme (blank measurement). The analysis was controlled by two PCs. The effect of the concentration of analyte in the absorption liquid on the enzyme activity was tested for 10^{-5}–10^{-9} mol l^{-1} sarin. A single analysis (including the absorption step) took <130 s.

Key words: sequential injection analysis; lab-on-valve; organophosphate; cholinesterase; nerve agent; sarin

INTRODUCTION

Organophosphate-based warfare gases (nerve agents such as sarin, soman, tabun and VX that interfere with the central nervous system through inhibition of cholinesterases) are potentially fatal chemicals that are banned by the Chemical Weapons Convention (CWC) (OPCW 1994). The relative simplicity of their illegal production at minimal expense, release of sarin vapours in the Tokyo subway system in 1995 and several other events in the past decade have raised concerns that they could possibly be used for large-scale terrorist attacks against civilians. Therefore relatively inexpensive automated analytical devices capable of rapid on-spot sensing of nerve agents (NAGs) in air and giving early warning to civilian responders or military personnel are of interest. A number of sophisticated but costly analytical methods (such as ion-mobility MS, GC-MS, Surface Acoustic Wave Sensing and FT-IR) have been officially approved by the CWC for routine

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detection and monitoring of CWC chemicals (Mesillakso 2005).

In the present paper we attempt to utilize the inhibitory effect of the NAGs on butyryl-cholinesterase (BuChE) for their quantitative assay through spectrophotometric measurement of the decreased activity of BuChE (exposed to an NAG) by Ellman's reaction (Ellman et al. 1961). This indicator reaction involves interaction of the BuChE with butyrylthiocholine (BTCh) that is hydrolysed to thiocholine; thiocholine reacts with Ellman's reagent [5,5′-dithio-bis(2-nitrobenzoic) acid] (DTNBA) to produce yellow thionitrobenzoate exhibiting maximum absorption at 412 nm (proportionally related to the activity of the BuChE). The concept of sequential injection analysis (SIA) in the lab-on-valve (LOV) format developed by Ruzicka (2000) was employed to automate the manipulation of reactant solutions at µl levels.

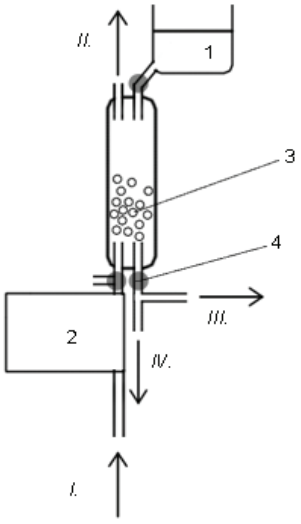

Fig. 1. **Scrubber module.**
1, fresh enzyme solution container; 2, air pump; 3, glass beads and absorbing liquid; 4, PC-controlled pinch valves; I, air inlet; II, air outlet; III, absorbing liquid to SIA; IV, waste.

MATERIAL AND METHODS

Chemicals

Lyophilized BuChE from equine serum was purchased from SEVAC (Prague); BTCh, DTNBA, insecticide tetraethyl pyrophosphate (TEPP), insecticide 1-naphthyl-N-methylcarbamate (Sevin) and anhydrous ethanol were obtained from Aldrich; KH_2PO_4 and $Na_2HPO_4.12H_2O$ were obtained from Lachema (Brno), sarin was obtained from the NAGs collection of the Faculty of Military Health Sciences, Hradec Králové.

Apparatus

A PC-controlled micro-SIA apparatus equipped with a lab-on-valve platform (FIAlab Instruments Inc., Bellevue, USA) comprising a 6-port selection valve, a 1.0 ml syringe pump and USB2000 Ocean Optics spectrophotometer was used as the basis of the NAG detection setup. A glass scrubber packed with glass beads (see Fig. 1) included three PC-controlled valves; it was manufactured in the lab workshop. A miniature air pump was used to bubble the sample air through the scrubber. Air samples with defined sarin concentrations were prepared by using a dynamic evaporation chamber (Ševelová et al. 2003). For a scheme of the measurement protocol see Table 1.

Optimization and procedures

Initially the SIA conditions for Ellman's reaction were optimized away from the scrubber by using less hazardous BuChE inhibitor solutions, namely Sevin and TEPP, with respect to concentrations and volumes of reactants aspirated, time of BuChE – inhibitor interaction (20–600 s) and inhibited BuChE (Ellman's reagent + BTCh) incubation time (15–120 s). Inhibition curves relating the percentage of BuChE retained activity (E_A%) to the concentration of the inhibitors (10^{-9}–10^{-4} mol l^{-1}) were measured; the E_A% was calculated as $(h_x/h_0).100$ where the h_x and h_0 are the peak heights of the thionitrobenzoate at 412 nm obtained with the inhibited and native uninhibited enzyme injections respectively. After this, the inhibition curve of BuChE with sarin solutions was measured. Consequently the dependence of the level of inhibition of enzyme on the time of BuChE – sarin interaction (300–700 s) was also examined.

To study the effect of the air contaminated with sarin on the E_A%, the air sample containing 1 µg/l of sarin was pumped through the scrubber (packed with glass beads) containing 5 ml of absorbing liquid (0.12 mg ml^{-1} of BuChE in phosphate buffer, pH 7.40) at the flow rate of 80 $l.h^{-1}$ for 20 s. Thereafter 8 µl of the absorbing liquid and 2 µl of the reagent (2.5 mmol BTCh + 0.25 mmol DTNBA) were aspirated, allowed to react for 60 s and sent to the detector at a flow rate of 4 µl s^{-1} (peak height h_x); the h_0 was obtained by aspirating 8 µl of uninhibited enzyme solution (0.12 mg ml^{-1} of BuChE in phosphate buffer of pH 7.4) instead of the absorbing liquid. Also in this measurement the dependence of the level of inhibition of enzyme on the duration of BuChE – sarin interaction (300–700 s) was evaluated.

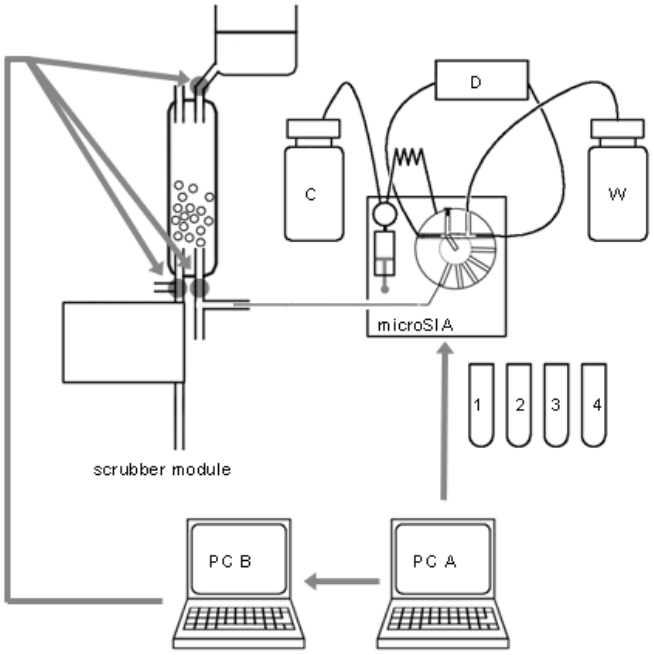

Fig. 2. **Scheme of the system, connection of the absorber and SIA units.**
PC A, controlling micro-SIA-LOV; PC B, controlling the scrubber valves; C, carrier solution; D, spectrophotometric detector; W, waste. In the given part of the program, PC A acts as an actuator giving a signal to PC B to execute a new absorption cycle. 1, 2, 3, 4 – vials containing reagents and substrate solution.

RESULTS AND DISCUSSION

Inhibition curves

Optimum SIA conditions for the measurement of the inhibition curves of BuChE with Sevin as a relatively weak inhibitor (carbamate) and TEPP as much stronger inhibitor (organophosphate) were the following: carrier water, order of the aspirated zones: 4 µl of the inhibitor solution, 8 µl of 0.12 mg ml^{-1} BuChE in phosphate buffer pH 7.4, another 4 µl of the same inhibitor solution (sandwich mode) and inhibition time 60 s; thereafter 8 µl of reagent solution (2.5 mmol l^{-1} BTCh + 0.25 mmol l^{-1} DTNBA) aspirated and after the incubation time of 60 s the zones with the yellow reaction product thionitrobenzoate were pushed at 4 µl s^{-1} into the detector channel of the LOV module. The inhibition curves of Sevin (curve 1 and 2) and TEPP (curves 3 and 4) for inhibition times 60 s and 600 s are shown in Fig. 3. It can be clearly seen that even at 60 s inhibition time TEPP can be reliably detected (causing 10% BuChE inhibition) at 0.1 µmol l^{-1} concentration levels with repeatability characterized by RSD 3.6% (n=5).

A sarin inhibition curve was obtained with aqueous sarin solutions. Consequently, 0.2 ml of sarin solution was added to 1.8 ml of the enzyme solution (0.012 mg ml^{-1} BuChE in phosphate buffer, pH 7.4) to obtain concentration scale 10^{-5} to 10^{-9} mol l^{-1} of sarin. After 300 s inhibition, enzyme activity was determined by SIA. 8 µl of this enzyme solution and

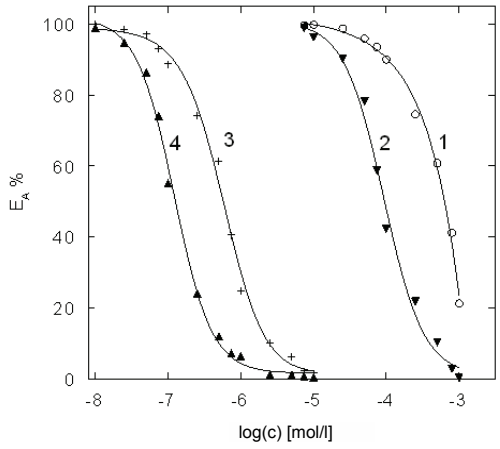

Fig. 3. **Inhibition curves (dependence of E_A% on the concentration of inhibitor) of BuChE** as measured by the spectrophotometric SIA-LOV technique with inhibitor solutions containing Sevin (1, 2) or TEPP (3, 4) at inhibition times 60 s (1, 3) and 600 s (2, 4).

Table 1. **Measurement protocol.**

Step	SIA measurement protocol
1	SIA washes the detection cell, aspirates and flushes 500 µl of water
2	spectrometer performs a reference scan
3	SIA aspirates 300 µl water + 8 µl of blank enzyme solution + 2 µl of substrate – reagent solution
4	enzyme – substrate interaction, delay 60 s
5	SIA delivers 75 µl to spectrophotometer
6	reaction product measurement at 412 nm
7	SIA flushes the holding coil and empty the detection cell
8	SIA aspirates 600 µl of enzyme solution from absorber and flushes it to prevent sample contamination
9	SIA aspirates 300 µl water + 8 µl of enzyme from absorber
10	PC A, controlling SIA sends information to PC B, controlling absorber, to wash the scrubber from the inhibited enzyme solution and to start a new cycle of absorption of sarin from the air
11	SIA aspirates 2 µl of substrate – reagent solution
12	enzyme – substrate interaction, delay 60 s
13	SIA delivers 75 µl to spectrophotometer
14	reaction product measurement at 412 nm
15	SIA flushes the holding coil and empty the detection cell

Step	Absorber protocol
1	filling absorption space with approx. 5 ml of enzyme solution
2	air sample bubbles through for 20 s
3	enzyme solution is flushed to collection point, where is consequently aspirated by SIA
4	PC B is waiting for impulse from PC A
5	the residual enzyme solution is flushed into waste, the scrubber is washed by filling and emptying with the enzyme solution into absorption space
6	new absorption process starts

2 µl of reagent solution were aspirated, allowed to react for 60 s and sent to detector at the flow rate of 4 µl s^{-1}. The enzyme activity was then determined with the same samples in the same way at the times of inhibition 400, 500, 600 and 700 s.

This observation of the dependence of inhibition on the time for each concentration of sarin showed that at a time of inhibition under 400 s it is not possible to reach 0% enzyme activity. See Fig. 4.

The inhibition curve resulting from the SIA-LOV detection of sarin in liquid samples under optimum conditions indicated in the experimental section is shown in Fig. 5.

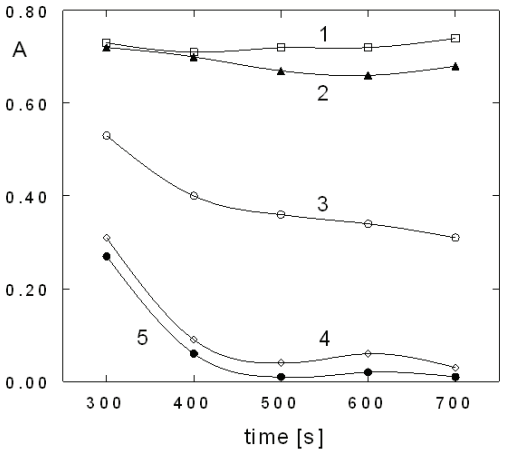

Fig. 4. **Dependece of inhibition on the time of interaction between the enzyme and sarin.**
A, absorbance of the reaction product at 412 nm; concentrations of sarin [mol/l]: 1, 10^{-9}; 2, 10^{-8}; 3, 10^{-7}; 4, 10^{-6} and 5, 10^{-5}.

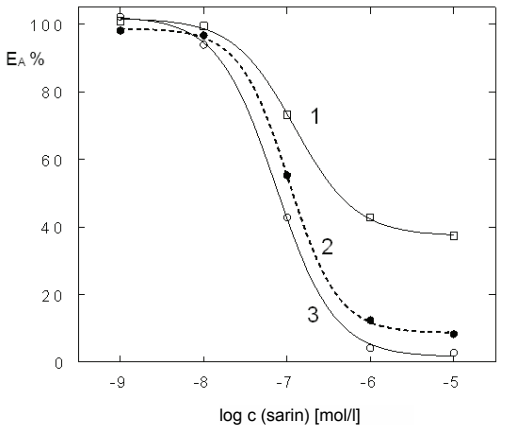

Fig. 5. **The inhibition curves of BuChE with sarin in liquid samples at different times of inhibition.**
Inhibition time: 1, 300 s; 2, 400 s and 3, 700 s.

Detection of sarin in air

In order to verify the ability to convert sarin from the air sample (containing 1 μg l^{-1} of sarin) into a liquid medium, solution of BuChE in phosphate buffer was used as the liquid phase. The enzyme solution (placed in the scrubber) was purged with the sarin-spiked air sample for 20 s and consequently the enzyme activity was determined in the way described above by the reaction of 8 μl of enzyme solution and 2 μl of reagent-substrate solution.

For 300–700 s time of inhibition 65–45% of the original enzyme activity was retained. This is equivalent to 0.1 μmol l^{-1} of sarin in the enzyme solution. This fact shows that only approx. 3% of sarin has been recovered from the air sample. Decreasing the air flow rate would be desirable for improving the conversion efficiency but this was not possible because of the technical limitations of the absorber unit.

CONCLUSIONS

It can be concluded that under these conditions the detectable threshold concentration of sarin in liquid medium causing 10% inhibition of BuChE is approximately 1.4 mg m^{-3} of sarin. Considering approx. 3% efficiency of 20 s recovery of sarin from air samples into liquid, the threshold concentration corresponds 0.2 mg m^{-3} of sarin in the air. Effective dose of sarin vapours causing miosis, the very first recognizable effect of it, is EC_{t50} = 2 mg.min/m^3, while the lethal dose is LC_{t50} = 70 mg.min/m^3 (Riegle et al. 1994). Using proposed instrument, sarin vapours can be safely detected. Our data compare well with data appearing recently for a commercial acoustic wave sensor (30 mg m^{-3}) (Matsushita et al. 2005) or portable ion mobility mass spectrometer (below 0.1 mg/m^{-3}) (Maruko et al. 2006). Selectivity of the proposed micro-SIA-LOV device to NAGs is limited by the fact that any other strong BuChE inhibitors (e.g., insecticides) will also give a response. On the other hand, this fact can be considered an advantage since any dangerous BuChE inhibitors can be detected in this way. While the tested SIA-LOV method using cholinesterase activity detection according to Ellman's reaction is fast and sensitive and the the scrubber – SIA-LOV enables fully automated collection and testing of the air samples for the presence of sarin, the consumption of enzyme solution in the absorber unit and power consumption are excessive. The low efficiency of the scrubber decreases the sensitivity of the system.

ACKNOWLEDGEMENTS

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