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Automated flow based biosensor for quantification of binary organophosphates mixture in milk using artificial neural network

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ABSTRACT

This work presents an application of automatic flow based biosensor to detect binary (chlorpyriphos-oxon (CPO) and malaoxon (MO)) organophosphate (OP) mixtures in milk, based on artificial neural network (ANN). Genetically modified acetylcholinesterase (AChE) B394 and B4 were used as a biological recognition element for sensor development. AChE binds with OPs irreversibly, creating an anionic phosphonyl species. The enzymes were coupled on screen printed electrodes (SPEs) and inserted in a flow cell connected to the potentiostat and syringe pump. In order to model the combined response of CPO and MO, a total set of 19 mixtures were prepared using ANN. The modeling was validated with an external test of 6 milk samples spiked with CPO and MO mixtures. The spiked concentrations of CPO and MO were ranged from 5×10^{-10} to 5×10^{-12} M and 1.01×10^{-10} to 9.17×10^{-11} M, respectively. These concentrations were determined using factorial designing (FD) method and the obtained and expected recovery values in milk showed good co-relation. The average % recovery yields for CPO and MO are 109.53 and 100.66, respectively.

1. Introduction

Excessive contamination of various environmental component leads to pollution of the biosphere and eventually reaches humans via the food chain. The ecological balance thus gets disturbed primarily due to the accumulation of toxic chemicals and carcinogenic compounds. The main class of pesticides that poses a serious problem are OPs and carbamate [1,2]. While pesticides are associated with many adverse health effects, there is lack of monitoring data on these contaminants. OPs are neurotoxic substances which are used as pesticides, insecticides and chemical warfare agents [3]. In view of the major concerns regarding the toxicity of these compounds, early and simple detection of OP compounds is very important for protecting water resources and food supplies [4] such as milk. However, overuse of these pesticides result in pesticide residues in food [4,5], water [6,7] and environment [8], and leads to a severe threat to human health due to their high toxicity to AChE, which is essential for the functioning of the central nervous system in humans [9].

Due to their high acute toxicity and risk toward the population, some directives have been established to limit the presence of pesticides in water and food resources. Concerning the quality of water for human consumption, the European Council Directive 98/83/EC [10] has set a maximum admissible concentration of 0.1 $\mu g\,L^{-1}$ per pesticide and 0.5 $\mu g\,L^{-1}$ for the total amount of pesticides. Food Safety and Standards Authority of India (FSSAI) set the limits for milk and milk products such as the tolerance level is 0.01 mg/kg ppm for chlorpyrifos and 0.001 mg/kg ppm for Malathion and MO [11].

Numerous analysis methods including gas chromatography [12], liquid chromatography [13], ultraviolet spectroscopy [14], gas-mass spectroscopy [15], fluorimetry [16] and surface plasmon resonance (SPR) [17] have been developed to assess OPs exposures. Although these methods could quantitatively provide an accurate evaluation of the health risk of integrated OPs exposure, but they still suffer from some intrinsic disadvantages of either low

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Table 1
A list of some previous works in biosensor for organophosphates detection in liquid phase based on amperometric sensing using acetylcholinesterase.

SN	Pesticides	Detection limits	Matrix	Analysis	References
1.	Chlorpyriphos-oxon Ethyl paraoxon Malaoxon	5×10^{-12} M 5×10^{-9} M 5×10^{-10} M	Milk	Individual	[21]
2.	Chlorpyriphos-oxon Chlorfenvinphos Azinphos-methyl oxon	1.1137×10^{-10} M 8.595×10^{-10} M 2.806×10^{-10} M	Water	Mixture	[28]
3.	Dichlorvos Carbofuran	0.79 nM 4.1 nM	Water	Mixture	[30]
4.	Methyl Paraoxon Dichlorvos	0.001–2.5 μM 0.1 μM	Water	Mixture	[31]
5.	Paraoxon	0.4 pM	Water	Individual	[33]
6.	Dichlorvos, Monocrotophs Parathion	4–7 μg L^{-1}	Water	Individual	[34]
7.	Dichlorvos	10 nM	Sea water	Individual	[35]
8.	Methyl parathion	0.6 ng/mL	Water	Individual	[36]
9.	Paraoxon Dichlorvos Carbofuran	3.91×10^{-8} M 6.30×10^{-11} M 5.84×10^{-10} M	Water	Individual	[37]
10.	Paraoxon Monochrotophos Dichlorvos	0.87×10^{-11} M 1.08×10^{-11} M 1.22×10^{-10} M	Water	Mixture	[38]
11	Chlorpyriphos-oxon Malaoxon	5×10^{-12} M 2.17×10^{-10} M	Milk	Mixture	This work

detection specificity and sensitivity, or expensive analysis settings entailing well-trained personnel and inconvenience for online analysis. Hence, simple, sensitive, and automated deployable tools are still highly desired for determination of OP exposures.

Milk is an essential nutritional food and widely consumed by infants and the young population [18,19]. OPs can be reached throughout the food chain by various sources such as when dairy cows are fed with OPs polluted forages or drink polluted water, bovine milk and dairy product could be tainted with pesticide residues [20–22]. Detection of OPs in milk has been reported by researchers using different techniques [19,21,23]. Co-existence of OPs is commonly encountered in contaminated water and other parts of the environment [24,25] and possibility to present in milk. Pesticide mixtures are widely used to deal with the array of arthropod pests encountered in greenhouse and nursery production systems due to the savings in labor costs [26]. Furthermore, the use of pesticide mixtures may result in synergism or potentiation (enhanced efficacy) and the mitigation of resistance [27]. Although the effects of individual OPs on activity of cholinesterases (ChEs) activity have been studied for decades but the neuro toxicity of mixtures is still poorly understood. In order to understand the toxic effect of these chemicals in totality, it is essential to study the inhibition at various concentration levels. Of course, a few efforts have been made to study the effect of pesticide mixtures [25,28–31] but they have not been extensively exploited to study the pesticide synergism in a matrix such as milk. Evaluation of combine toxic action of pesticide mixtures is one of the priority research areas due to the simultaneous occurrence of pesticides in the environment and the health risk they posed to humans and the environment as a mixture.

A trend in biosensors is the flow-bioelectronics tongue which combines high sensitivity array of biosensors in flow systems, and chemometric tools with added advantages of possibility to miniaturize the required instrumentation with compact and portable analysis devices. In order to improve experimental parameters, current research efforts focus on the use of genetically modified enzymes with tailor-designed properties [32]. The combination of biosensors with flow based technique offers the possibility to control the whole procedure, simplifying the sequence of steps, high sensitivity and allowing an easier optimization of the reaction conditions [21,28]. Recently, flow based systems have been used for automated detection of pesticides in water and milk [21,28,33–38]. A list of some previous works in biosensor for organophosphates detection in liquid phase based on amperometric sensing using acetylcholinesterase is presented in Table 1.

The innovation of the present work is to use the ANN for analysis of highly toxic OPs mixture in milk samples using automated flow based biosensor at trace level. Previous attempts were mainly focused on single pesticides detection in water matrix. This work describes the construction of two different biosensors (selected based on previous results) using genetically modified enzymes B4 and B394. The constructed biosensors were trained as neural network and have been employed to detect the inhibition sum of binary pesticides, e.g. MO and CPO, in milk samples. The pesticide concentrations were determined using factorial design methodology. The developed automated flow based biosensor system is very rigid, practical and easy to use without human intervention for long time. The results obtained by the system are highly reproducible and can be reused. The use of SPE makes the analysis cost effective. The results presented in the manuscript are original and meets the standards set by regulatory agencies.

2. Materials and methods

2.1. Chemicals, biochemicals and stock solutions

Genetically modified enzymes AChE B394 and B4 from *Drosophila melanogaster* were produced by our laboratory (IMAGES, Perpignan, France). Acetylthiocholine iodide (ATChI), acetylthiocholine chloride (ATChCl), 5,5′-dithio bis (2-nitrobenzoic acid) (DTNB), and phosphate buffer (PBS, 0.1 mol L^{-1} K_2HPO_4/KH_2PO_4 pH 7 containing 0.1 mol L^{-1} KCl) were obtained from Sigma–Aldrich (Germany). A 0.1 M ATChCl stock solution was prepared daily and stored at 4 °C. HPLC-grade acetonitrile was supplied by Carlo Erba (Italy) and photocrosslinkable poly (vinyl alcohol) (azide unit pendant water-soluble photopolymer, PVA-AWP) was purchased from Toyo Gosei (Japan). Malaoxon was obtained from Fluka, Sigma–Aldrich (Germany) and chlorpyriphos-oxon was procured from Dr. Ehrenstorfer (Augsburg, Germany). Stock solutions of