

GENERAL CONCLUSIONS

This thesis has been focused on the development of biosensors and biosensing devices based on cholinesterase inhibition for environmental and food analysis.

In the first part of the work the biosensing and biosensor devices for pesticides detection to avoid the interferences such as heavy metals were proposed.

In the first case, a novel method for the detection of pesticides by inhibition of free acetylcholinesterase and spectrophotometric detection has been developed. It makes use of two immiscible phases in which the pesticide (organic phase) and the enzyme (aqueous phase) are solubilised. In this way the pesticide analyte is introduced into the assay system in an organic phase like (or compatible) that resulting from the standard extraction methods and so, the presence of heavy metals, or other water soluble enzyme inhibitors, that could interfere with the inhibition-based assay is largely avoided. This method seems very promising as a biosensing test for screening pesticides in environmental samples with good sensitivity (an inhibition of 50% has been observed with $9 \cdot 10^{-7}$ M of paraoxon).

An alternative method to measure pesticides avoiding interferences is the use of the immobilised enzymes as for the biosensors by using the “medium exchange method”.

To develop a ChE biosensor screen-printed electrodes (SPEs) modified with Prussian Blue were chosen as probe. This mediator was selected due to its good sensitivity toward thiocholine (enzymatic product) together with high stability in operative and storage conditions. The modified SPEs were then used as substrate for the successive immobilisation of two different ChE enzymes to obtain a biosensor for pesticides detection. The method of analysis is achieved via a two steps (“medium exchange” method) avoiding the effect of any interfering compound eventually present in real samples, such as SDS (sodium dodecyl sulfate) for example. Different pesticides were tested: AChE based biosensors have demonstrated a higher sensitivity towards aldicarb (50% inhibition with 50 ppb) and carbaryl

(50% inhibition with 85 ppb) while BChE biosensors have demonstrated a higher affinity towards paraoxon (50% inhibition with 4 ppb) and chlorpyrifos-methyl oxon (50% inhibition with 1 ppb) using 30 minutes as incubation time. Real water samples were then analysed demonstrating the suitability of the method.

The biosensor previously reported was also optimised in order to have a faster measurement and more stable biosensor for nerve agents detection. The Sarin and VX solution were tested. We have challenged our biosensor against the Sarin gas obtaining a degree of inhibition higher than 20% with Sarin gas at 0.05 mg/m^3 in 30 s as incubation time. The results obtained demonstrated that using this developed system it is possible to detect low concentration of Sarin in a few minutes. Furthermore, the biosensor showed a good storage stability at room temperature in dry conditions.

In the last chapter the biosensing for aflatoxin B₁ (AFB₁) detection has been proposed for the first time. A preliminary investigation of the kinetics and inhibitory mechanism of AFB₁ on acetylcholinesterase indicated a reversibility type and mixed mechanism. The reversibility of the inhibition permits a rapid analysis of AFB₁ (3 minutes) with a detection limit of 10 ppb. To evaluate the selectivity of this method, the cross reactivity was investigated showing that the assay detected AFB₁ and AFB₂ with similar sensitivity. The suitability of the assay for AFB₁ quantification in barley was also evaluated with good recovery. Further studies are in progress to detect lower concentration of aflatoxin according to the legal limits.

ARTICLES

Francesco Ricci, **Fabiana Arduini**, Aziz Amine, Danila Moscone, Giuseppe Palleschi
Characterisation of Prussian Blue modified screen printed electrodes for thiol
detection. *J. Electroanal. Chem.* (2004), 563(2), 229-237

Fabiana Arduini, Francesco Ricci, Ilhame Bourais, Aziz Amine, Danila Moscone,
Giuseppe Palleschi.
Extraction and Detection of Pesticides by Cholinesterase Inhibition in a Two-phase
System: a Strategy to Avoid Heavy Metal Interference.
Anal. Letters (2005), 38(11), 1703-1719.

Francesco Ricci, **Fabiana Arduini**, Catalin S. Tuta, Ugo Sozzo, Danila Moscone,
Aziz Amine, Giuseppe Palleschi.
Glutathione amperometric detection based on a thiol-disulfide exchange reaction.
Anal. Chim. Acta (2006), 558 (1-2), 164-170.

Fabiana Arduini, Francesco Ricci, Danila Moscone, Aziz Amine, Giuseppe Palleschi
New electrochemical method for non protein thiols detection
Biochimica clinica (2003), 27 (3), 53

Fabiana Arduini, Francesco Ricci, Catalin S.Tuta, Danila Moscone, Aziz Amine,
Giuseppe Palleschi
Detection of carbamic and organophosphorous pesticides in water samples using
cholinesterase biosensor based on Prussian Blue modified screen printed electrode.
Anal. Chim. Acta (2006), 580 (2), 155-162.

Fabiana Arduini, Ilenia Errico, Aziz Amine, Laura Micheli, Giuseppe Palleschi,
Danila Moscone
Rapid enzymatic method for AFB₁ detection
Anal. Chem. (2006) submitted.

D.G. Mita, A. Attanasio, **F. Arduini**, N. Diano, V. Grano, U. Bencivenga, S. Rossi,
A. Amine, D. Moscone
Enzymatic determination of BPA by means of tyrosinase immobilized on different
carbon carriers
Biosens. Bioelectron. (2006) submitted.

CHAPTERS in books

Fabiana Arduini, Francesco Ricci, Giuseppe Palleschi, Danila Moscone.
Sensors and Microsystems
(Di Natale, D'Amico, Martinelli, Carotta, Guidi Eds) World Scientific, Singapore,
2005

A.F. Danet, M.C. Cheregi, J. Martinez-Calatayud, M. Catala-Icardo, F. Arduini, G.
Papeschi, L.Cruceru, R.Debiasi, I. Niculecu, E. Bucur, M. Badea, C.Bratu, J. Emneus
Environmental Pollution Monitoring, laboratory guide

(Danet, Cheregi, Badea Eds), Bucarest 2005.

CONFERENCE COMMUNICATIONS AT INTERNATIONAL CONGRESS

International Workshop on "Biosensors for food safety and environmental monitoring", Marrakech, Morocco, October 9-11, 2003.

Thiocholine mediated oxidation at Prussian Blue modified electrodes for pesticide and heavy metals detection.

F. Ricci, **F. Arduini**, D. Moscone, G. Palleschi, A. Amine.

Euroanalysis XIII, "European conference on Analytical Chemistry", Salamanca, Spain, 5-10 September 2004.

Modified Screen Printed Electrodes for glutathione detection.

F. Ricci, **F. Arduini**, A. Amine, U. Sozzo, D. Moscone, G. Palleschi.

Euroanalysis XIII, "European conference on Analytical Chemistry", Salamanca, Spain, 5-10 September 2004.

Extraction and detection of pesticides in organic solvent by cholinesterase inhibition.

F. Arduini, F. Ricci, I. Bourais, A. Amine, D. Moscone, G. Palleschi.

8th World Congress on Biosensors, Granada, Spain, 24-26 May 2004.

Extraction of enzyme inhibitors using a mixture of organic solvent and aqueous solution and their detection with electrochemical biosensors.

A. Amine, H. Mohammadi, **F. Arduini**, F. Ricci, D. Moscone, G. Palleschi.

8th International Symposium on Kinetics in Analytical Chemistry, Rome, Italy, 8-10 July 2004.

Kinetic aspect of the disulfide interchange reaction for glutathione amperometric detection.

F. Ricci, **F. Arduini**, U. Sozzo, D. Moscone, A. Amine, G. Palleschi.

8th International Symposium on Kinetics in Analytical Chemistry, Rome, Italy, 8-10 July 2004.

Enzymatic kinetics in a biosensing for pesticide detection.

F. Arduini, F. Ricci, A. Amine, D. Moscone, G. Palleschi.

XVII International Symposium on "Bioelectrochemistry and Bioenergetics", Florence, Italy, June 19-24, 2003.

Thiols mediated oxidation at Prussian Blue modified screen printed electrodes.

F. Ricci, **F. Arduini**, A. Amine, G. Palleschi, D. Moscone.

Second International Workshop on "Biosensors for Food Safety and Environmental Monitoring", Agadir, Morocco, 10-12 November, 2005

A disposable biosensor for pesticides detection: development and application in waste water samples.

F. Arduini, F. Ricci, A. Amine, D. Moscone, G. Palleschi

SPQ-Analitica 05 Divisao de Quimica Analitica ,Sociedade Portuguesa de Quimica
Chemical sensor, biosensor and immunosenosrs for clinical, food, and environmental control.

G.Palleschi, D.Moscone, L.Micheli, G.Volpe, F.Ricci, **F.Arduini**, A.Radoi

International Congress on Analytical Sciences ICAS-2006 25-30 June, Moscow, Russia

Analytical application of chemical sensors, biosensors and immunosensors.

G.Palleschi, D.Moscone, L.Micheli, S.Piermarini, G.Volpe, F.Ricci, **F.Arduini**, A.Radoi

9th International Symposium on Kinetics in Analytical Chemistry, Marrakech, Marocco, 2-4 November 2006.

Analytical Kinetic aspects of nerve agent detection based on cholinesterase inhibition.

F.Arduini, F.Ricci, A.Amine, D.Moscone, G.Palleschi

9th International Symposium on Kinetics in Analytical Chemistry, Marrakech, Marocco, 2-4 November 2006.

Electrokinetic investigation of cobalt hexacyanoferrate screen printed electrode as new probe for pesticides detection.

F.Arduini, A.Cassisi, A.Amine, D.Moscone, G.Palleschi

CONFERENCE COMMUNICATIONS AT NATIONAL CONGRESS

II Simposio sulle Tecnologie Avanzate,
Rome, 23-24 June 2005.

Verso la costruzione di uno strumento portatile per la misura amperometrica di agenti neurotossici.

F. Arduini, F. Ricci, F. Cataldo, G. Palleschi

XVIII Congresso Nazionale di Chimica Analitica, Parma, Italy, 19-23 September 2004.

Detection of pesticide in organic solvent by cholinesterase inhibition to avoid heavy metals interference.

F. Arduini, F. Ricci, I. Bourais, A. Amine, D. Moscone, G. Palleschi.

VI Convegno nazionale Istituto Nazionale Biostrutture e Biosistemi Napoli, 4-6 Novembre 2004.

Determinazione di pesticidi organofosforici e carbammici mediante un biosensore monouso.

F.Arduini, F.Ricci, A.Amine, D.Moscone, G.Palleschi

XIX Congresso nazionale di Chimica Analitica Pula (Ca), 11-15 Settembre 2005.

Determination of Lead in Milk by Anodic Stripping Voltammetry with Mercury thin film: Assessment of the influence of sample pretreatment.

H. Mohammadi, A. Amine, **F. Arduini**, D. Moscone, G. Palleschi

XIX Congresso nazionale di Chimica Analitica Pula (Ca), 11-15 Settembre 2005.
Sviluppo di un nuovo metodo di screening per l'aflatossina B1 basato sull'inibizione enzimatica.

I. Errico, **F. Arduini**, A. Amine, L. Micheli, G. Palleschi, D. Moscone

AISEM - Associazione Italiana Sensori e Microsistemi X Conferenza Annuale
Firenze, 15-17 Febbraio 2005

Amperometric cholinesterase biosensor for pesticide detection

F. Arduini, F. Ricci, D. Moscone, G. Palleschi.

XXII Congresso Nazionale della Società Chimica Italiana Firenze, Italy, 10-15
September 2006.

Sviluppo di un biosensore elettrochimico per la misura del BPA.

A. Attanasio, **F. Arduini**, A. Amine, G. Mita, D. Moscone.

XXII Congresso Nazionale della Società Chimica Italiana Firenze, Italy, 10-15
September 2006

Misure di Sarin in fase gassosa con biosensori stabili e monouso

F. Arduini, F. Ricci, A. Amine, D. Moscone, G. Palleschi

XXII Congresso Nazionale della Società Chimica Italiana Firenze, Italy, 10-15
September 2006.

Caratterizzazione di elettrodi screen-printed modificati con esacianoferrato di cobalto per la misura della tiocolina

F. Arduini, A. Cassisi, F. Ricci, A. Amine, D. Moscone, G. Palleschi

XXII Congresso Nazionale della Società Chimica Italiana Firenze, Italy, 10-15
September 2006.

Sviluppo di un biosensore per la determinazione dell'AFB₁

F. Arduini, L. Micheli, A. Amine, J.L. Marty, D. Moscone, G. Palleschi

XIX Congresso nazionale di Chimica Analitica
Pula (Ca), 11-15 Settembre 2005.

Determinazione di pesticidi organofosforici e carbammici in campioni reali mediante un biosensore monouso

F. Arduini, F. Ricci, C.S. Tuta, A. Amine, D. Moscone, G. Palleschi.