ELECTROCHEMICAL IMMUNOSENSORS BASED ON ELECTROPOLYMERISED FILMS FOR THE DETECTION OF ANTIBIOTICS, PESTICIDES

Département de Chimie Moléculaire Grenoble
UMR 5250

CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE

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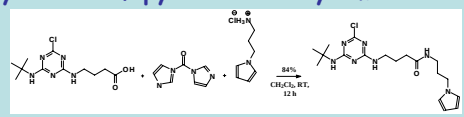
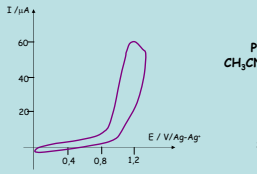
In analytical chemistry, the interest in development of new devices which are easy to use, highly selective and sensitive is constantly growing. For the detection and the quantification of pollutants the emergence of immunosensors exploiting the specificity of the processes of catalysis and biological recognition has risen a great interest. In particular, functionalized polypyrrole films offer very interesting features such as entrapment, affinity and grafting properties with biological macromolecules and their redox properties can be exploited for the reagentless transduction of hybridization and immunoreaction event. Here, immunosensors for the detection of pollutants as atrazine and fluoroquinolones, using electogenerated polymers, are presented.

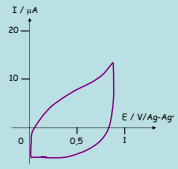
Atrazine detection: a powerful pesticide

Synthesis of pyrrol-terbutryn model





Pyrrole terbutryn 2 mmoL^{-1}
CH_3CN, $LiClO_4$ 0,1 molL^{-1} on CV , Ø 5mm , 100mV/s.

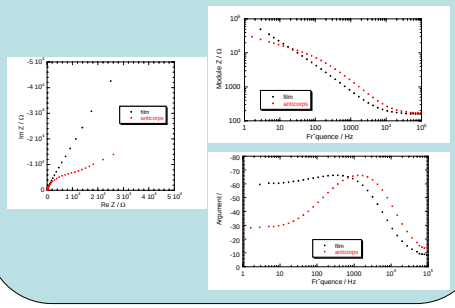


Modified electrode obtained at a fixed potential (900mV)
CH_3CN . 0.1 molL^{-1} $LiClO_4$ on CV, Ø 5mm 100mV/s.

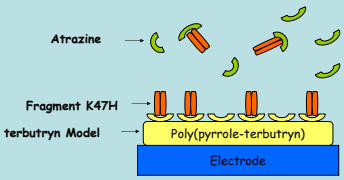
Yield of polymerisation: 30 -40 %

Impedance spectrocopy

Of the film and after immobilisation of the specific antibody

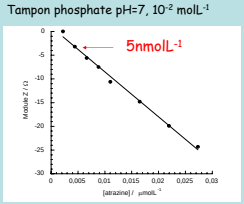


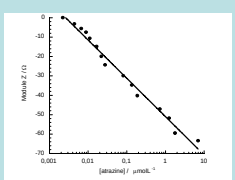
Pesticide detection

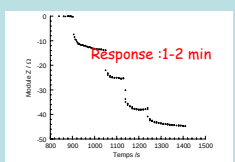


Atrazine solubility = 30mgL^{-1}

Tampon phosphate pH=7, 10^{-2} molL^{-1}







Competitive detection limit : 5nmolL^{-1}

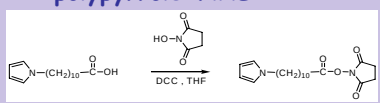
Tatsuma T. et al, Anal. Chim. Acta, 530, 2005, 191-197 13 nmolL^{-1}

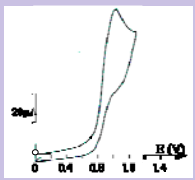
Jaffrezic N. et al, Talanta, 63, 2004, 365-370 5 nmolL^{-1}

Giardi M. T. et al, Biosensors Bioelectronics, 20, 2005, 1984-1992 50 nmolL^{-1}

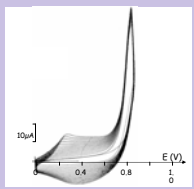
fluoroquinolone detection : a chemical synthetic antibiotic

Synthesis of the polymer : polypyrrole-NHS

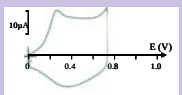




Pyr-NHS (2mM) in CH_3CN+TBAP 0,1M .Cyclic voltammetry : 100mv/s, sur CV (3mm). Reference : Ag/Ag$^+$ 10mM in CH_3CN+TBAP 0.1M.



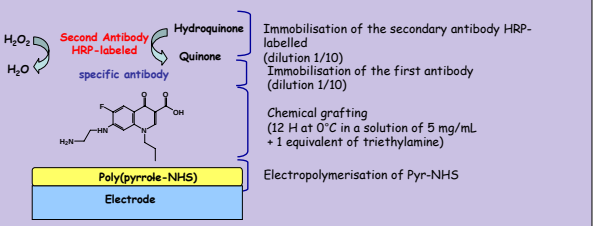
Electropolymerisation in CH_3CN + TBAP 0.1 M between 0.00 V and 0.85 V.



Cyclic voltammetry of the modified electrode Pyr-NHS (2 mM) in CH_3CN + TBAP 0.1M (Q = 0.5 mC) on carbon vitreous diameter 5 mm

Yield of polymerisation : 65 %

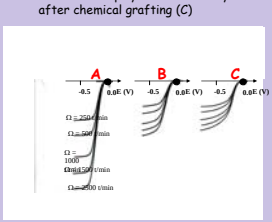
Elaboration and optimisation of the biological material : 4 steps

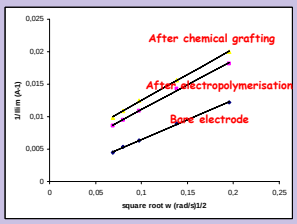


Immobilisation of the secondary antibody HRP-labelled (dilution 1/10)
Immobilisation of the first antibody (dilution 1/10)
Chemical grafting (12 H at 0°C in a solution of 5 mg/mL + 1 equivalent of triethylamine)
Electropolymerisation of Pyr-NHS

Permeability curves after the different steps of elaboration of the immunosensor

Experiments of rotating electrode of $Ru^{III}(NH_3)_6$ (2 mM) on vitreous electrode diameter 5 mm (A) after electropolymerisation of Pyr-NHS (B) (Q = 0.5 mC) after chemical grafting (C)

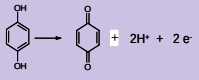


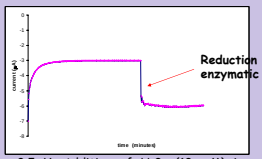


Koutecky-Levich curves

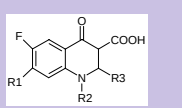
Decrease of the permeability value from 1.6x10^{-2} cm^2/s to 1.1x10^{-2} cm/s

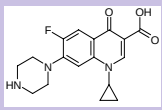
Enzymatic reaction

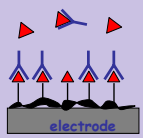


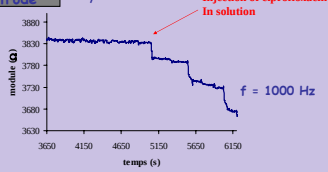


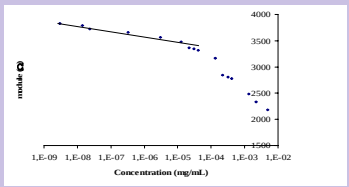
E = 0.5 V. Addition of H_2O_2 (10 mM) in presence of hydroquinone (2 mM) in PBS 10 mM pH = 7











Limit detection = 3 pg/mL

Linear range: between 3 pg/ mL and 12,5 ng/mL

Navalon et al, 2002, Anal. Chim.Acta, 454, 83-91

Idowu et al, 2004, J.Pharm. Biomed. Anal.35, 143-163

Limit detection = 10 pg/mL

CONCLUSION

These new immunosensors are useful for atrazine and fluoroquinolone quantification, by the way of specific antibodies immobilisation

ELISHA

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