

Elaboration and characterization of enzymatic biosensor for quaternary ammonium compounds

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- **Motivation**
- **Aim of the work**
- **Challenge for enzyme-based biosensor**
- **Choice of the nanomaterials**
- **Electrochemical biosensor for the detection of QACs**
- **Characterization of modified electrodes**
- **Conclusion and ongoing work**



Biocides



That come into contact with food or animal feed



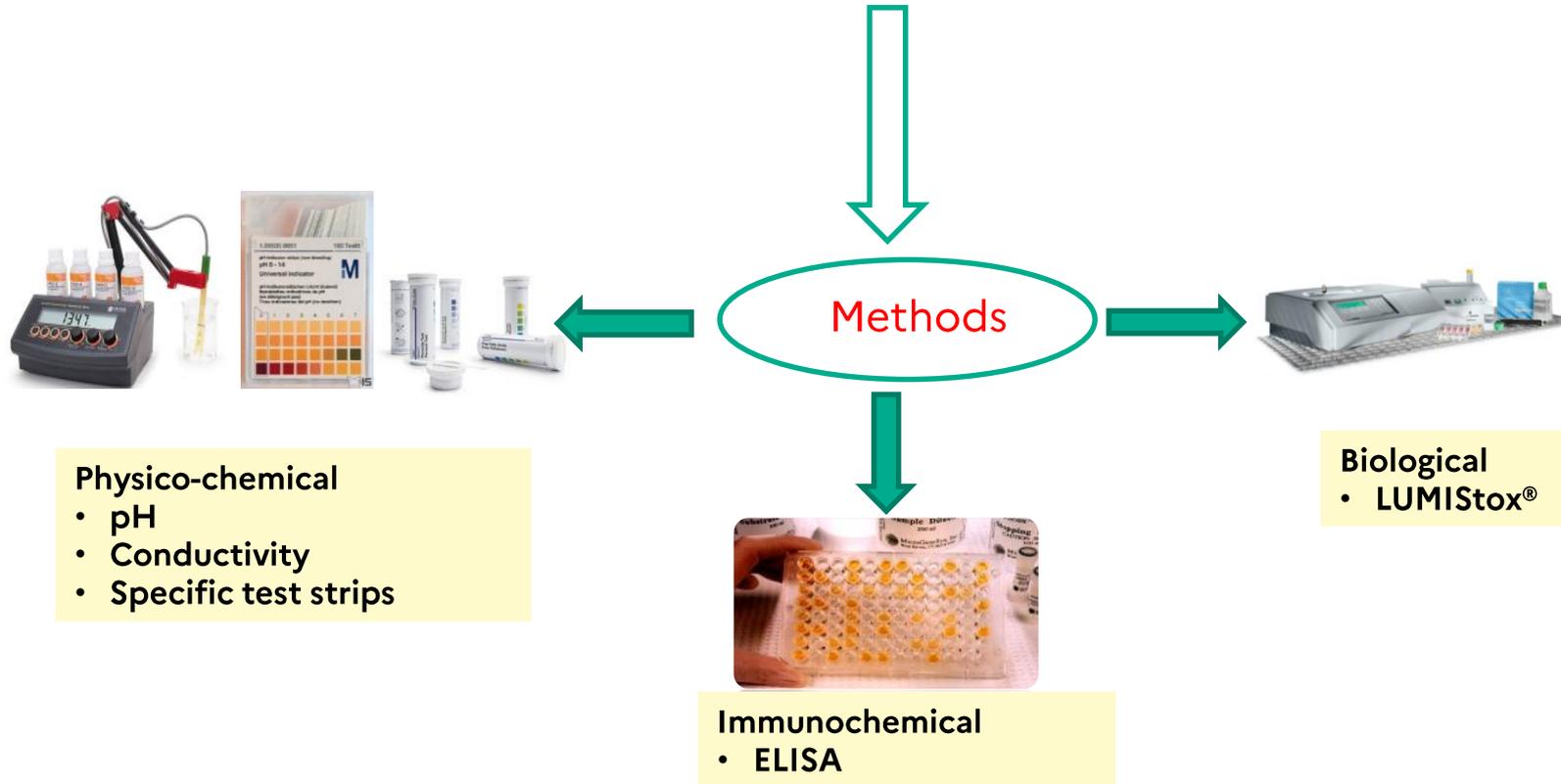
The presence of these biocide residues is a human health concern

- i) Evolution of biocide resistance and cross-resistance to antibiotics,
- ii) Toxicological danger, notably if they are not completely removed during rinsing process.



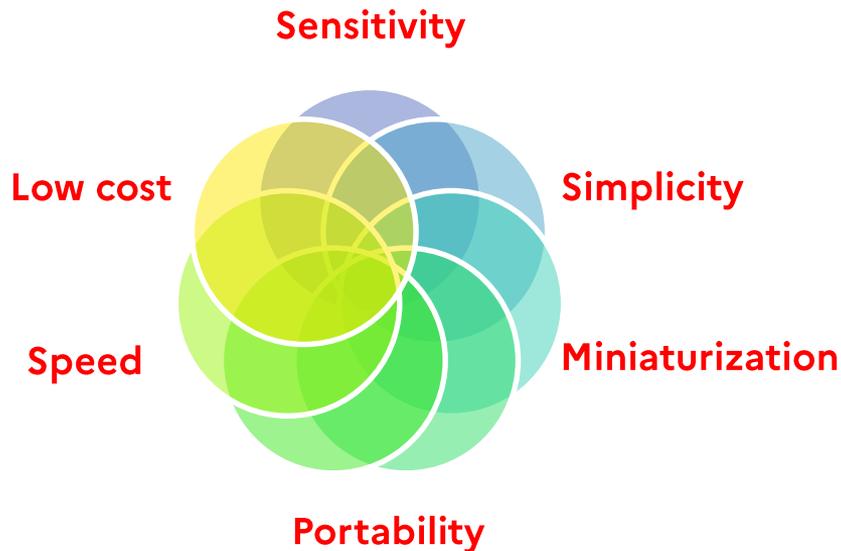
Development of innovative methods based on biosensors for the detection of biocidal residues

Prospective study of the performance of self-monitoring methods for biocide residues



Objective: Detect and quantify disinfectant biocide residues on food contact surfaces and in food

Approach: Develop a miniaturized electrochemical enzymatic sensor based on modified carbon nanotubes for detecting QACs, below the MRL in milk (0.1 mg/kg)



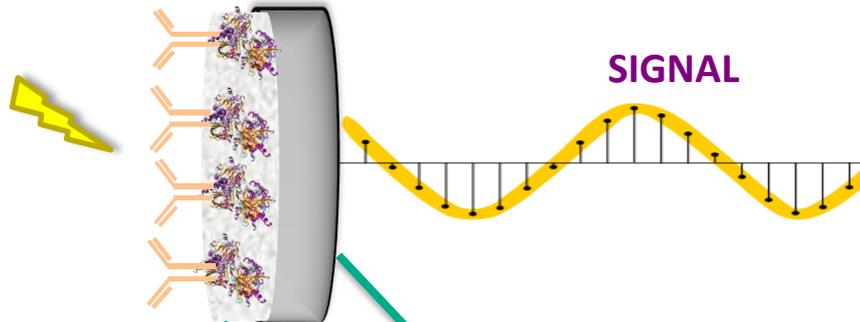
Synthetic representation of a biosensor

What is a biosensor?

Requirements

- Sensitivity
- Biocompatibility
- Stability

Immobilization / Surface modification



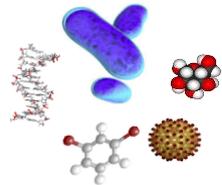
Processor/Analysis

Biological receptor
or bioreceptor:

Enzyme: **AChE**

Transducer

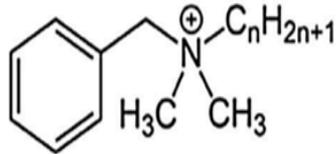
Screen printed electrode: **carbon**



Analytes:
QACs

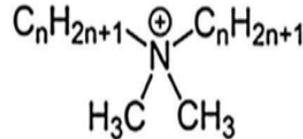
Analytes: QACs

- ✓ Cationic disinfectants are used in the food industry to decontaminate and prevent the spread of infection
- ✓ The EU regulation 1119/2014 has set Maximum Residue Limits (MRL) at 0.1 mg/kg for BAC and DDAC residue in milk that should not be exceeded
- ✓ QACs have a chemical structure close to that of acetylcholine (substrate of acetylcholinesterase (AChE))
- ✓ The inhibitory effect of some QACs (tetraethylammonium ion) on the activity of AChE (such as the electric eel) was demonstrated in 1952 using manometric methods



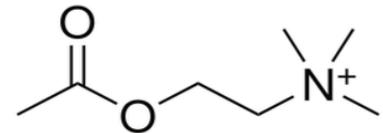
$n = 8, 10, 12, 14, 16$

Benzalkonium chloride (BAC)



$n = 10, 12, 14, 16, 18,$

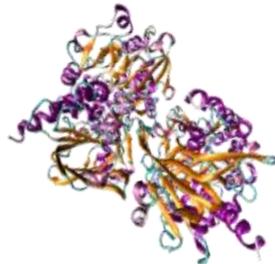
Dimethyldialkylammonium chloride (DDAC)



Acétylcholine

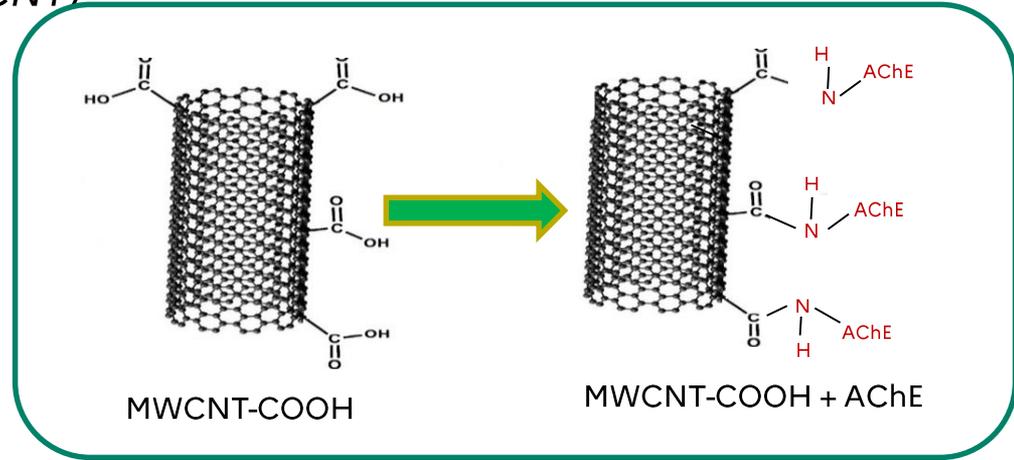
Challenge for enzyme-based biosensor

- ✓ AChE: Rapid detection of neurotoxic insecticides (organophosphates or carbamates), nerve agents and natural toxins (aflatoxin, glycoalkaloids, etc.).
- ✓ A quantitative measurement of the enzyme activity before and after exposure to a target analyte
- ✓ Typically the percentage of inhibited enzymatic activity (I%) that results after exposure to the inhibitor is quantitatively related to the inhibitor (i.e. analyte) concentration and to the incubation time
- ✓ The AChE activity in amperometric biosensors based on the use of a pseudosubstrate (acetylthiocholine) and the oxidation of the produced



❖ *Multiwalled carbon nanotubes (c-MWCNT)*

- ✓ Conductive matrix
- ✓ Simple to functionalize
- ✓ Large specific area
- ✓ Mechanically stable
- ✓ Maximum trapping of enzymes

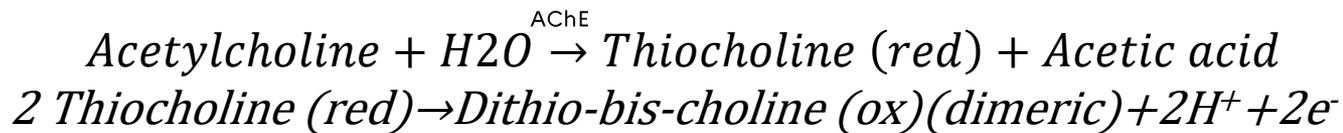
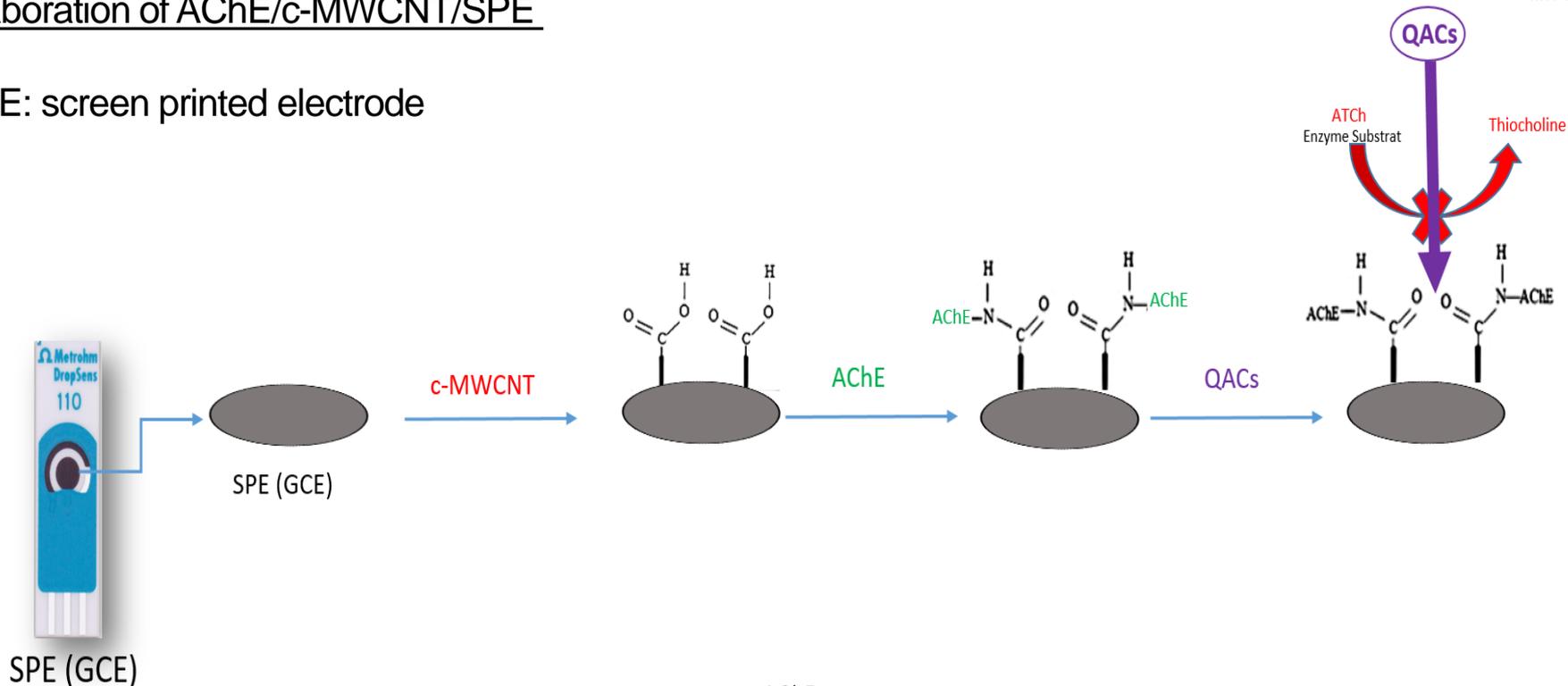


- ❖ The formation of amide bonds between c-MWCNT (modified with -COOH groups) and AChE molecules obviates the use of intermembranes, binder materials or cross-linking agents.
- ❖ This approach overcomes the impediment of electron transfer by membranes or crosslinking agents and improves detection sensitivity

Electrochemical biosensor for the detection of QACs

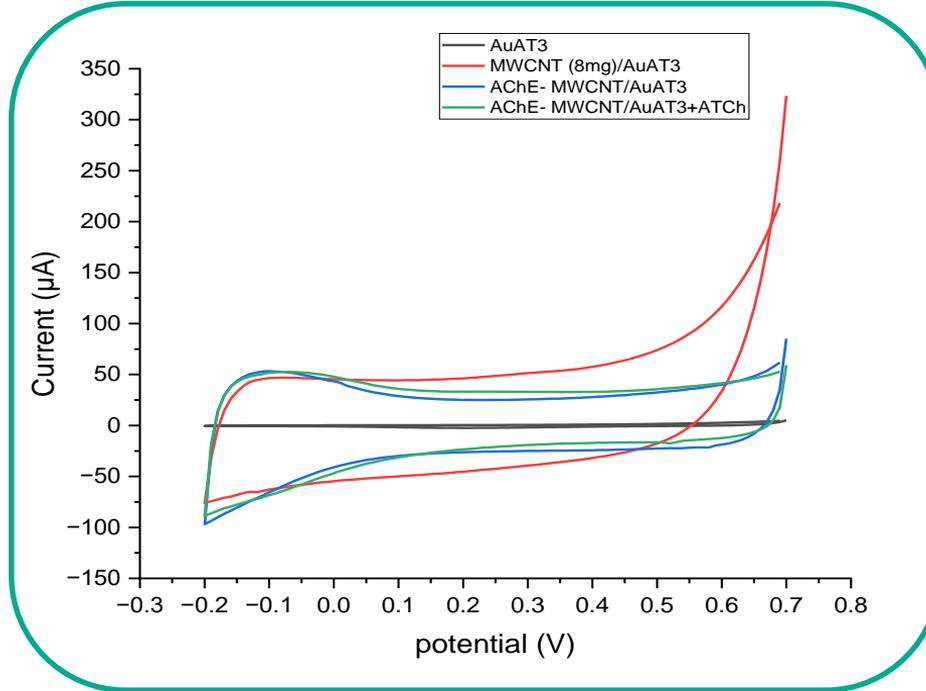
Elaboration of AChE/c-MWCNT/SPE

SPE: screen printed electrode



Characterization of modified gold electrodes

Characterization of MWCNTs/Au and AChE/MWCNTs/Au electrode



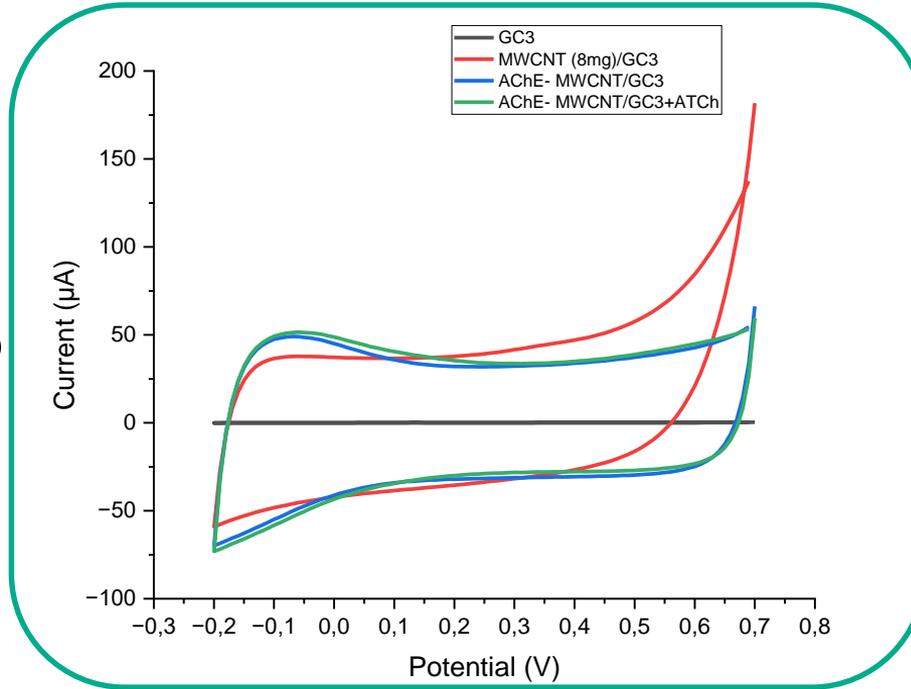
CV of (a) bare Au, (b) Au + MWCNTs and (c) Au + MWCNTs + AChE and (d) Au + MWCNTs + AChE + ATCh 1mM in solution, recorded in PBS (pH 7.4)

- ✓ The current increase after coating Au electrode with MWCNTs because of increased surface area and higher electric conductance property of CNTs
- ✓ The current decreased after the enzyme was coated on MWCNTs. It may be a consequence of increased interface after enzyme coating causing deterrence to electron transfer. This also ensures that the enzyme itself is not getting oxidized or reduced during the voltage cycling.

Characterization of modified carbon electrodes

Characterization of MWCNTs/GCE and AChE/MWCNTs/GCE electrode

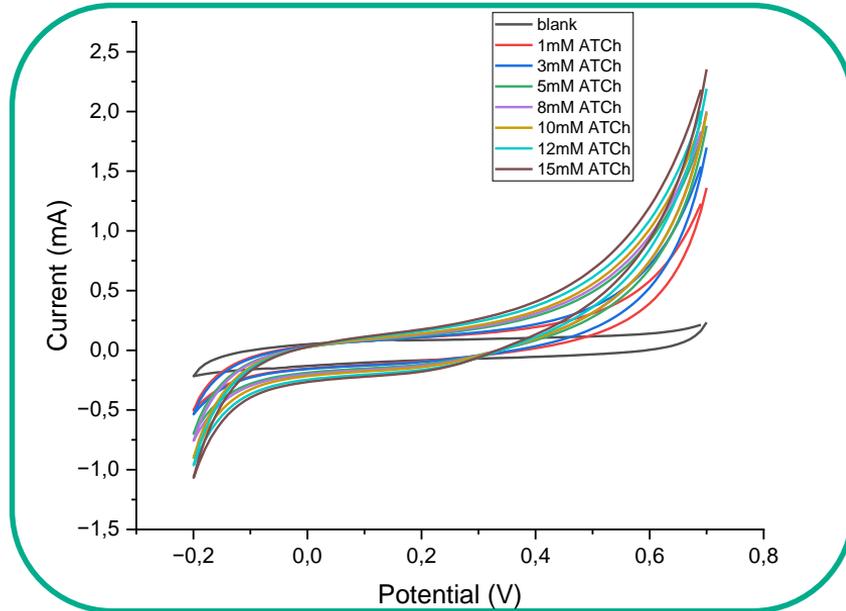
CV of (a) bare GC, (b) GC + MWCNTs (8mg-ml) and (c) GC + MWCNTs + AChE and (d) GC + MWCNTs + AChE + ATCh 1mM in solution, recorded in PBS (pH 7.4)



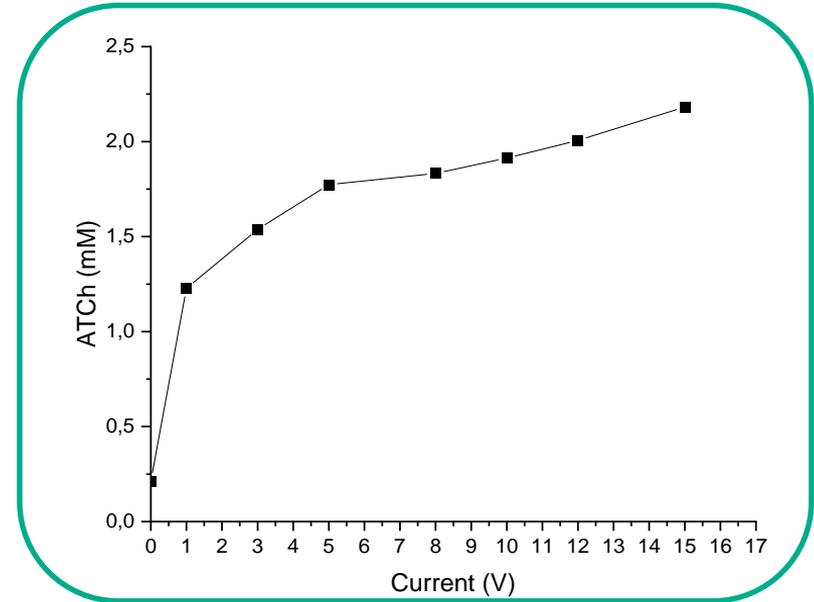
Selection of GC electrode (more stable than Au)

Same observations and conclusions on MWCNTs than on Au SPE:

Effect of ATCh amount



CV of **AChE-MWCNT/GC** recorded in PBS (pH 7.4)
With various concentrations of ATCh, 100 mV s^{-1}



Effect of various concentrations of ATCh on the sensor recorded in
PBS (pH 7.4)

✓ When the concentration of ATCh was varied from 0 to 16 mM, current dramatically increased up to 1 mM. Subsequently, no significant or no large change was found in the current with increase in ATCh concentration. For that reason, **1 mM** of ATCh was used for further experiment

- ❖ This presentation presents only preliminary results because the research is in progress.
- ❖ The developed method will be used to analyze milk and rinsing water samples collected during cleaning and disinfection operations to determine whether residual concentrations of QACs are detectable.
- ❖ Given the low sensitivity of the tests currently used in the dairy industry, a more efficient method provided by the drop down work.
- ❖ Optimisation of the effect of of enzyme AChE loading and the effect of inhibition time.
- ❖ Development of this biosensor with other nanomaterials (es. Pt, etc.);
- ❖ Further improve the performances of this biosensor in real sample;
- ❖ Further biosensor testing in rinsing water to improve the specificity.

Acknowledgment



Team Antibiotics, Biocides, Residues and Resistance (AB2R)

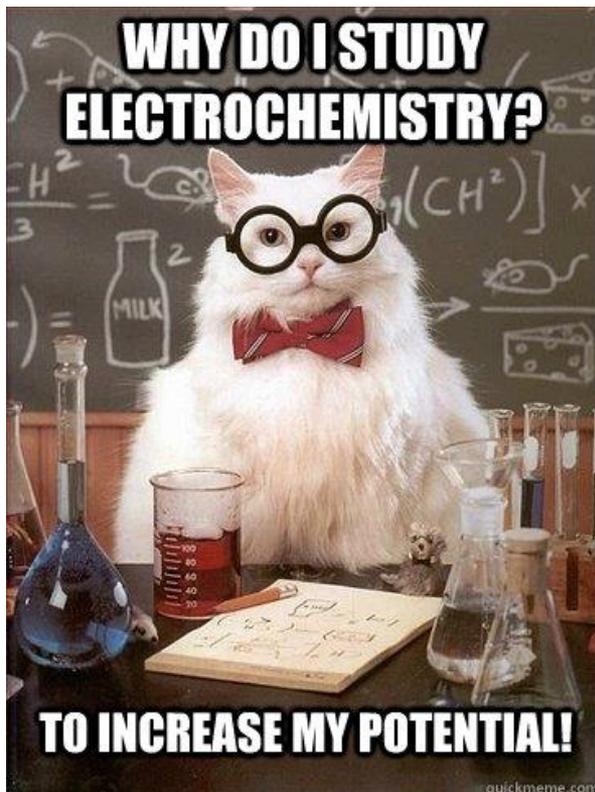


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THANK YOU
for your
ATTENTION!!!