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French agency for food, environmental and occupational health & safety
*Investigate, evaluate, protect*


# One step electrodeposition of multi-walled carbon nanotubes-chitosan for Quaternary Ammoniums Compounds biosensor

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# Outlines

- 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
- Detection of DDAC with MWCNT-Chi-AChE/SPE
- Conclusion and ongoing work

# Motivation

Biocidal disinfectants are used regularly in the food chain to limit the proliferation of unwanted microorganisms in the environment or on surfaces

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

# Motivation

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

Methods

**Physico-chemical**
- **pH**
- **Conductivity**
- **Specific test strips**

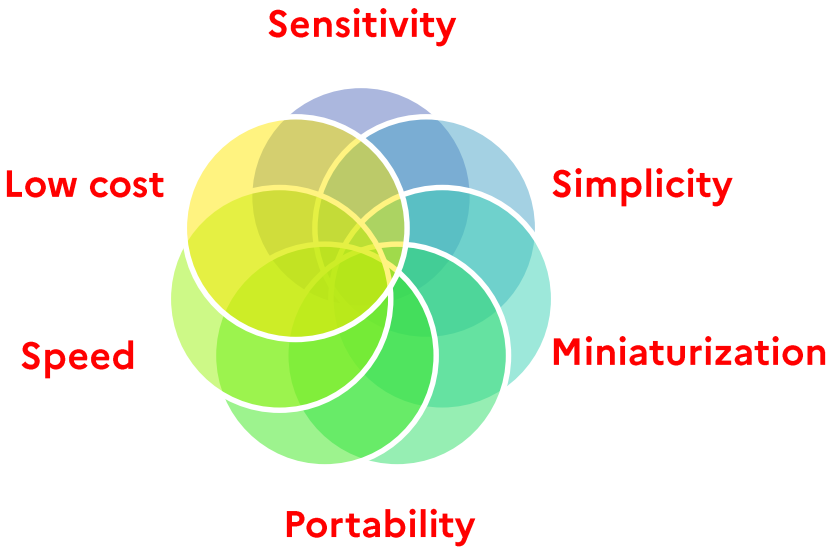
**Biological**
- **LUMIStox®**

**Immunochemical**
- **ELISA**

# Aim of work

**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)

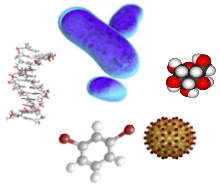


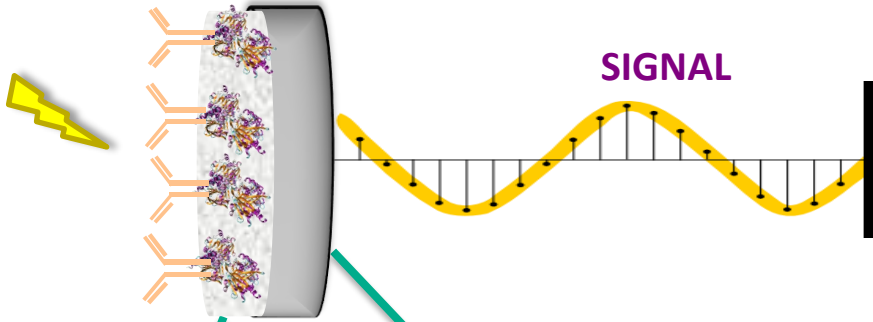
# Synthetic representation of a biosensor

*What is a biosensor?*

**Requirements**
- Sensitivity
- Biocompatibility
- Stability

**Immobilization / Surface modification**






**Analytes:**
QACs

**Biological receptor or bioreceptor:**
**Enzyme:** AChE

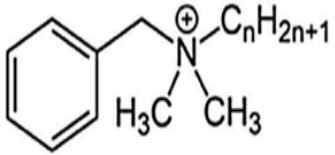
**Transducer**
**Screen printed electrode:** carbon

**Processor/Analysis**

# Analyte: Quaternary Ammoniums Compounds QACs

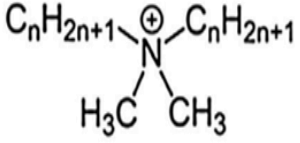
**Analytes:** QACs

- ✓ 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



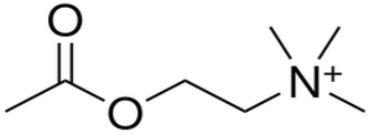

n = 8, 10, 12, 14, 16

Benzalkonium chloride (BAC)




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

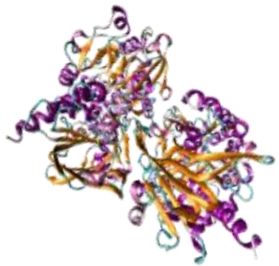
Dimethyldialkylammonium chloride (DDAC)




Acétylcholine

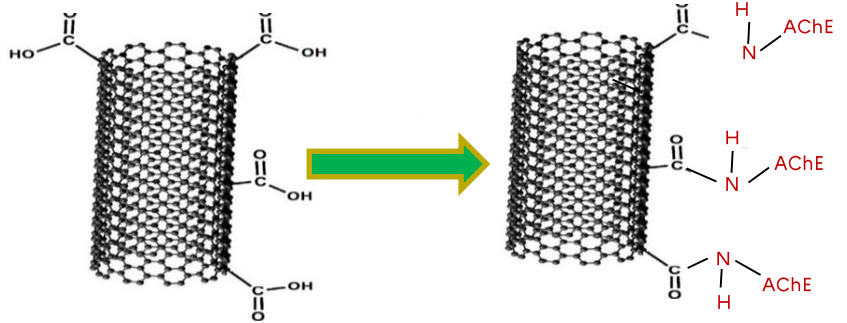
# Challenge for enzyme-based biosensor

- ✓ 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



# Choice of the nanomaterials

- *Multiwalled carbon nanotubes (c-MWCNT)*
  - ✓ Conductive matrix
  - ✓ Simple to functionalize
  - ✓ Large specific area
  - ✓ Mechanically stable
  - ✓ Maximum trapping of enzymes




MWCNT-COOH

MWCNT-COOH + AChE

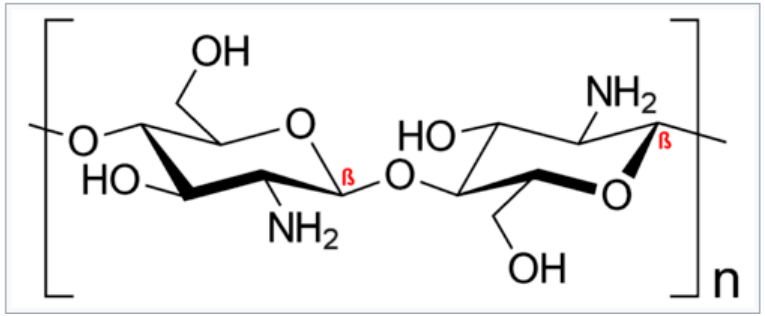
- The formation of amide bonds between c-MWCNT (modified with -COOH groups) and AChE molecules obviates the use of intermembranes, binder materials or cross-linking agents.

- This approach overcomes the impediment of electron transfer by membranes or crosslinking agents and improves detection sensitivity

# Choice of the Polysaccharide

- The native structure of the immobilized enzyme AChE was preserved on this composite film, because of the excellent biocompatibility and non-toxicity of chitosan.
- Incorporation of functionalized multiwalled carbon nanotubes (c-MWCNT) into a chitosan film promoted electron transfer reaction and enhanced the electrochemical response.
- Glutaraldehyde (GA) was used as cross-linker to covalently bind the AChE, and efficiently prevented leakage of the enzyme from the film.

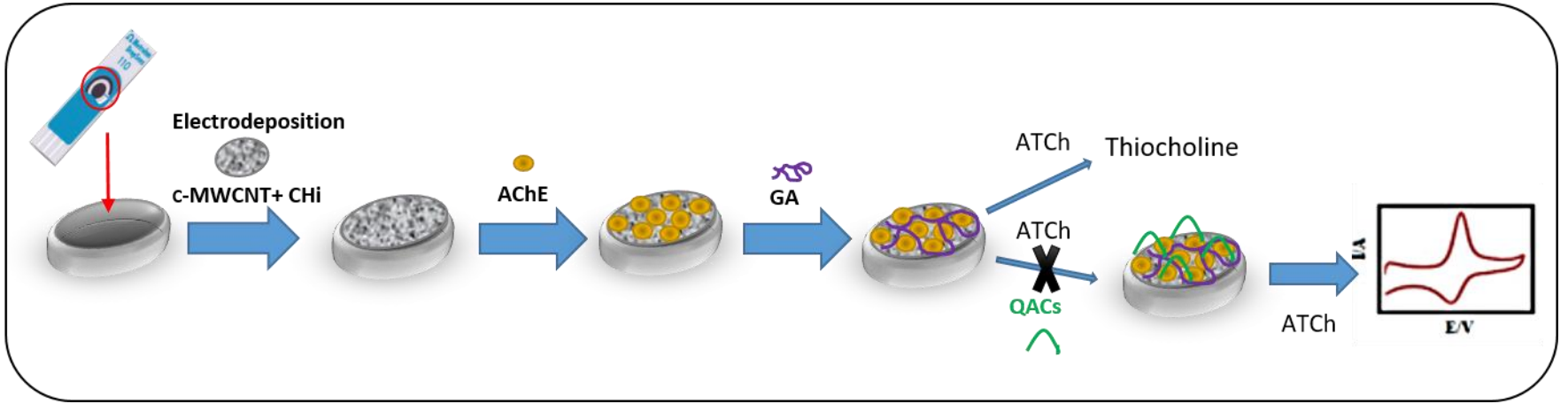
**Chitosan**



# Electrochemical biosensor for the detection of QACs

## Elaboration of c-MWCNT-Chi/AChE//SPE

This film was elaborated by one-step electrodeposition on a glassy carbon screen-printed electrode (GC).



# Characterization of modified carbon electrodes

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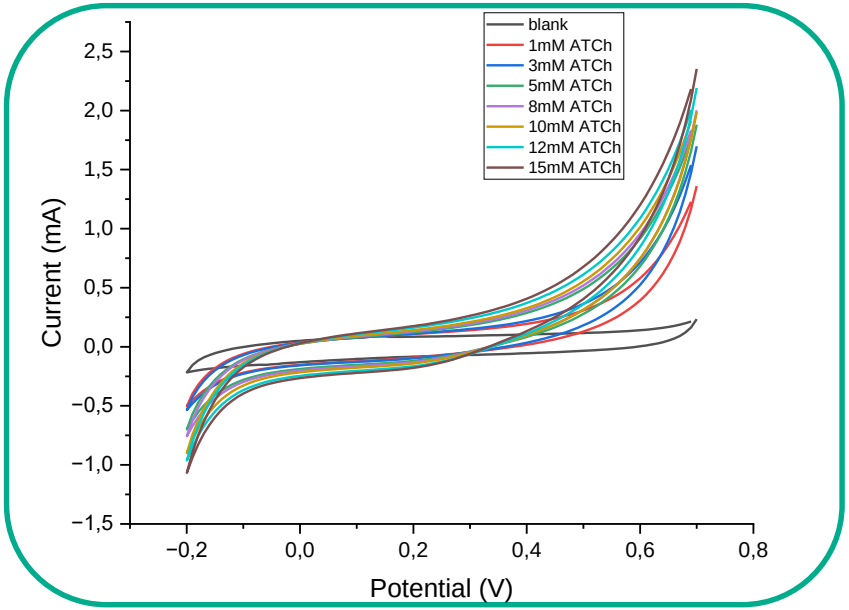
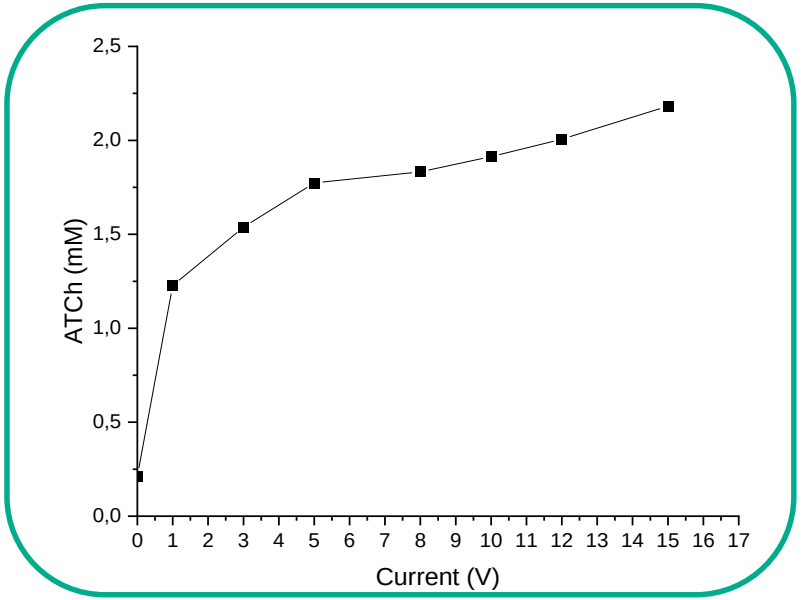
❑ Effect of ATCh amount



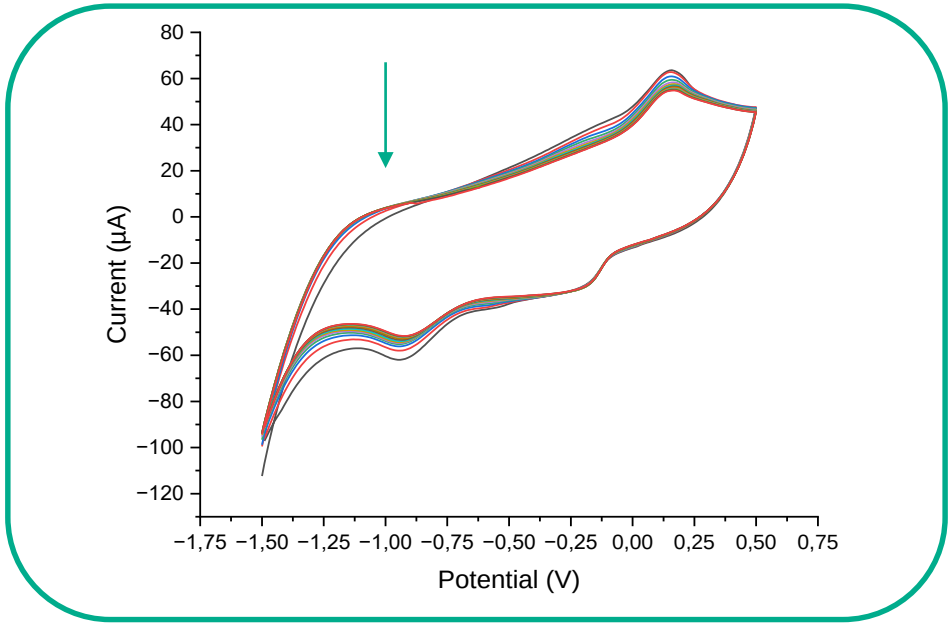

CV of **AChE-MWCNT/GC** recorded in PBS (pH 7.4)
With various concentrations of ATCh, 100 mV $s^{-1}$




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 15 mM, current dramatically increased up to 5 mM. Subsequently, no significant or no large change was found in the current with increase in ATCh concentration . For that reason, 5 mM of ATCh was used for further experiment

# Electrodeposition of MWCNT-CHi

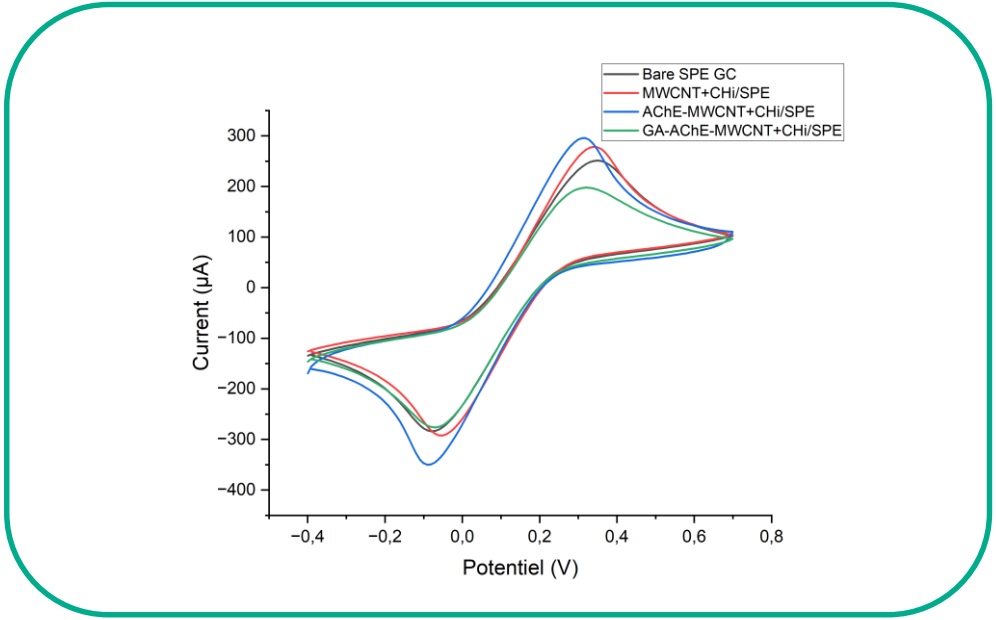



❖Cyclic Voltammogram of electrdodeposition of chitosan mixed with MWCNT on electrode SPE area

- The electrodeposition was followed by a cross-linking step, which consists of the use GA vapor for 20 min

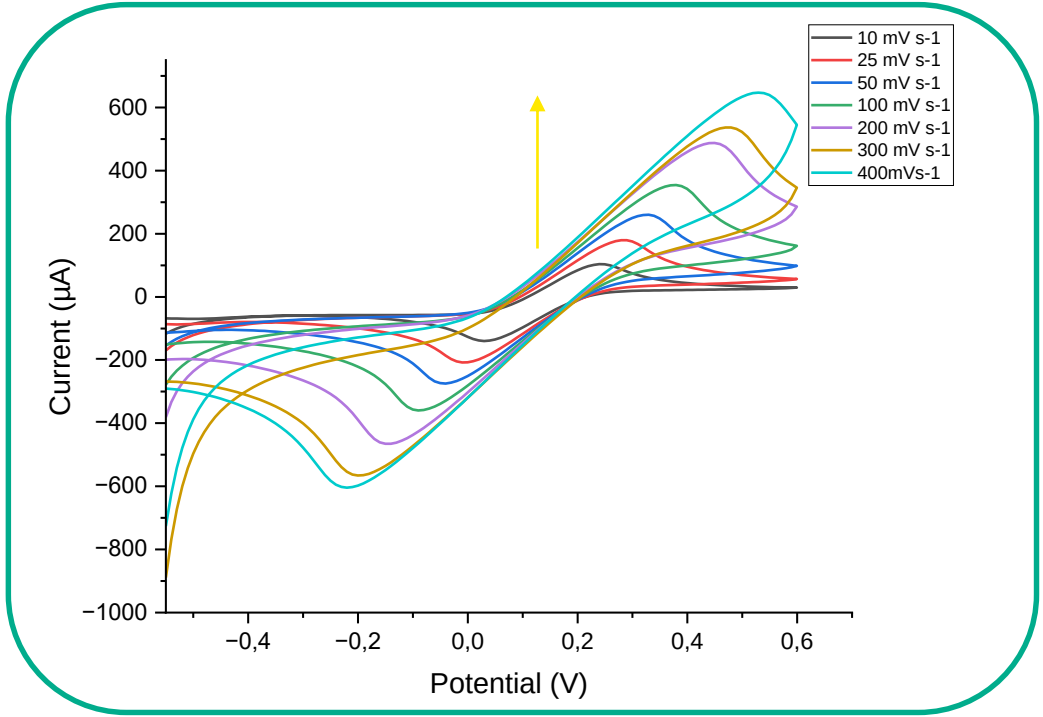
# Electrochemical characterization of the biosensor

❑ Characterization of the modified electrode: **AChE-AChE-MWCNT/SPE**




❖ CVs of different modified electrodes measured in 10 mM $[Fe(CN)_6]^{4-/3-}$ containing 0.1 M KCl (pH 7.4): **(black) Bare SPE GC**; **(red) MWCNT+CHi/SPE**; (blue)**AChE-MWCNT-CHi/SPE**; **(green)GA-AChE-MWCNT-CHi/SPE**.

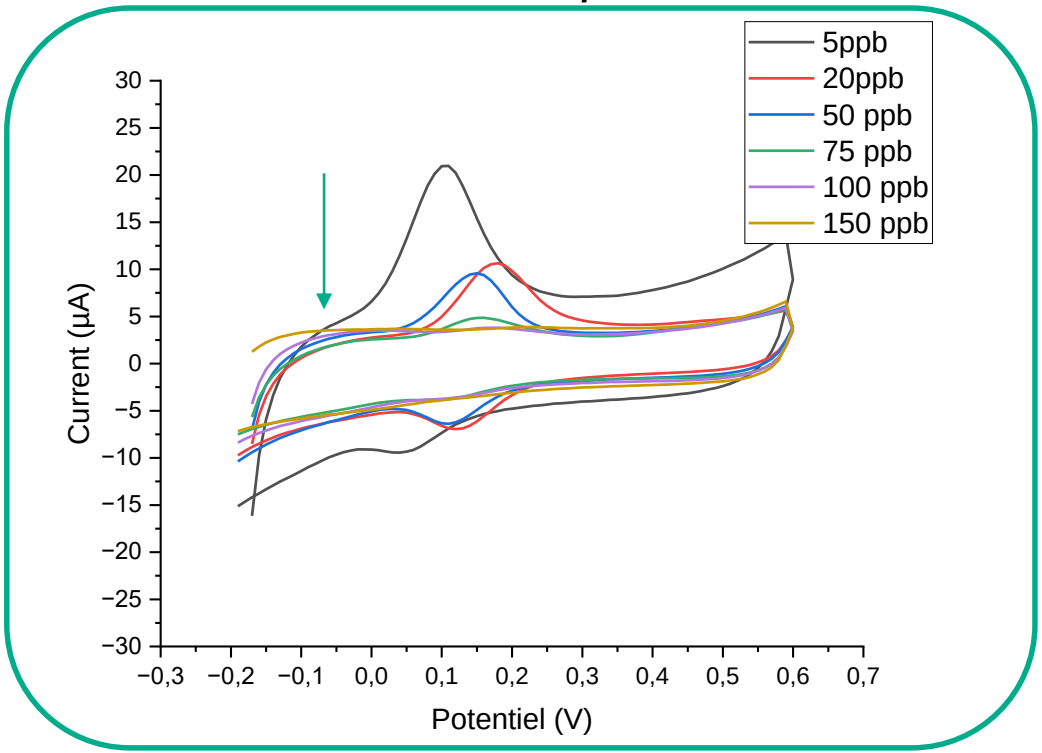
# Electrochemical characterization of the biosensor




❖ CVs of **MWCNT-Chi-AChE/SPE** in 100 mM PBS (pH 7.4) at different scan rates (from 10 to 400 mV $s^{-1}$ ).

# Detection of DDAC with MWCNT-Chi-AChE/SPE

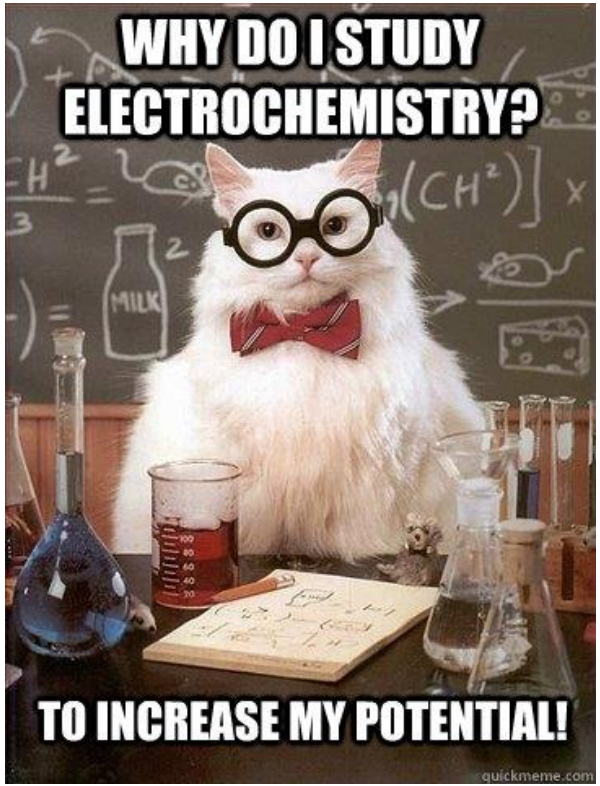
- Detection of DDAC with **AChE-AChE-MWCNT/SPE** electrode




❖ CV of **MWCNT-Chi-AChE/SPE** in 100 mM PBS (pH 7.4)containing 5mM ATCh with different concentration of DDAC from 5ppb to 150 ppb

# Conclusion and ongoing work

- 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




# THANK YOU for your ATTENTION!!!