Development of a sensor for the determination of tetracycline residues in milk using a Quartz Crystal Microbalance (QCM) and UV-Vis Spectroscopy as detectors

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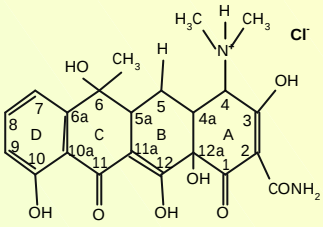
Introduction

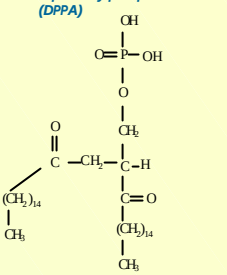
Residues originating from the administration of veterinary drugs to animals can survive the manufacturing cycle and contaminate animal-derived food such as meat and dairy products. Tetracyclines are a subclass of the polyketide antibiotics, widely used to treat bovine mastitis and to prevent and control diseases. Unknowing consumption through milk or meat contaminated with tetracycline residues results in the development and spreading of antibiotics resistance. Due to the lack of screening methods for the determination of antibiotics residues, the aim of this project is to develop a new sensor device able to detect these type of molecules using an innovative capture and analysis method based on thin film technology. The detection systems used are UV-Vis Spectroscopy and Quartz Crystal Microbalance (QCM)

Materials

Tetracycline hydrochloride (TC•HCl)

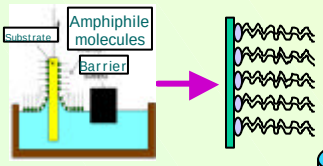


Dipalmitoylphosphatidic acid (DPPA)



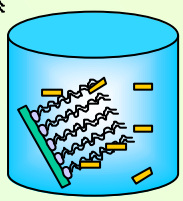
Methods

LB (Langmuir-Blodgett) films:



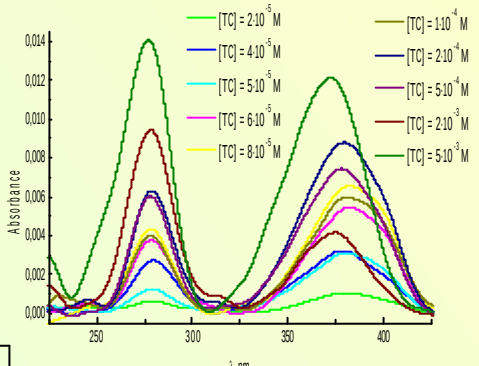
Molecular ordered systems mechanically assembled building-up oriented monolayers on a solid support by means of repeated loops of extraction-immersion at controlled speed

Preparation of LB-TC multilayers

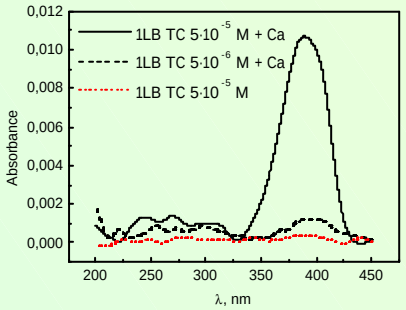


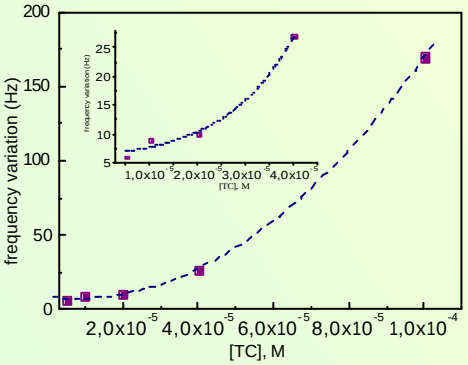
Immersion of the quartz slide covered with layers of DPPA in a water solution containing TC

UV-Vis spectra of3 LB layer of DPPA after immersion in TC solution at different concentration

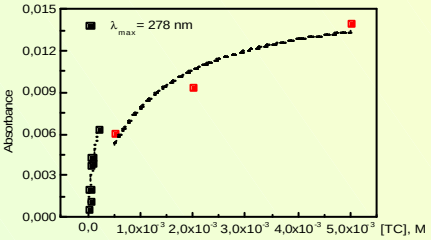


UV-Vis spectra of a DPPA monolayer after immersion in a solution of TC in TRIS buffer at pH =8.2 (red line) and in presence of Ca^{2+} ions at two different concentration of TC (black lines)

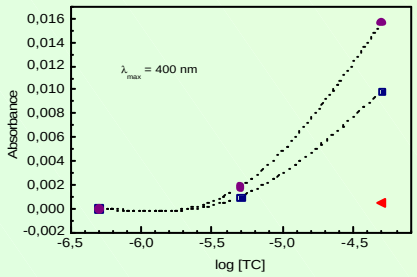




Calibration Curve obtained with QCM for Quartz covered with Au and modified with 3 LB of DPPA in a solution of TRIS pH=8.2 and $NiCl_2$ 0.2mM, containing different concentration of TC. In the insert are reported the lower concentrations



Absorption of the band at 278 nm as function of TC concentration in two different range of concentration (■ 2·10-5 M - 2·10-4 M ; ■ 5·10-4 M - 5·10-3 M)



Absorption of the band at 400 nm as function of TC concentration in TRIS buffer at pH =8.2:
■ 1 LB layer after immersion in TC/Ca
● 3 LB layer after immersion in TC/Ca
◀ 1 LB layer after immersion in TC

Conclusion

The presence of TC should be detected in solution in a range of concentration between $2 \cdot 10^{-5}$ M and $5 \cdot 10^{-3}$ M by UV-Vis detection. The detection limit is lower ten times in presence of Ca^{2+} ions at pH =8.2. By using a QCM (Quartz Crystal Microbalance) as detector, the better detection limit is found in presence of Ni2+ ion in solution at pH =8.2. The lower concentration of TC detected with this system is $5 \cdot 10^{-6}$ M The results should probably be improved to match the MRL corrisponding to TC's family which is equal to $2.25 \cdot 10^{-7}$ M