IMMOBILIZATION OF ELECTROACTIVE MOLECULES IN ORGANIZED THIN FILMS

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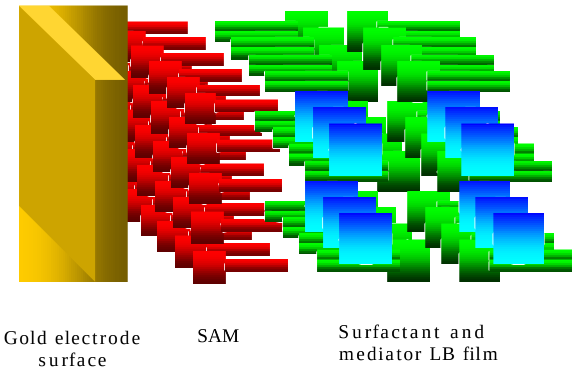
INTRODUCTION

Electrode modification by means of
Self-Assembling **(SAM)**,Langmuir-Blodgett **(LB)** and Hybrid **(HF)** Technologies for biosensor application

Immobilization of redox mediator in the ultra-thin film

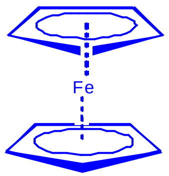


Characterization (electrochemical and spectroscopical) of the film in order to:

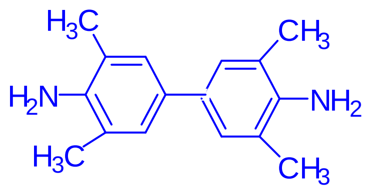
- Verify the presence of the mediator
- Investigate its electroactive behaviour

MATERIALS

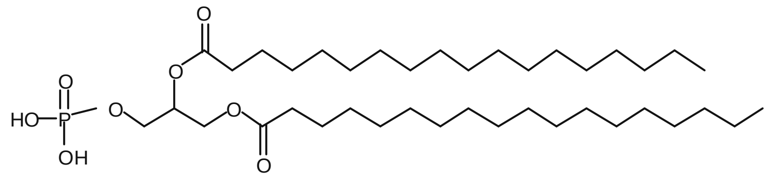
REDOX MEDIATORS



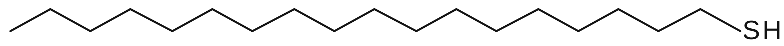
Ferrocene ≡ Fc



3,3’-5,5’ Tetramethylbenzidine ≡ TMB



Dipalmitoylphosphatidic acid ≡ DPPA



Octadecylthiol ≡ ODM

AMPHIPHILIC MATRICES

METHODS

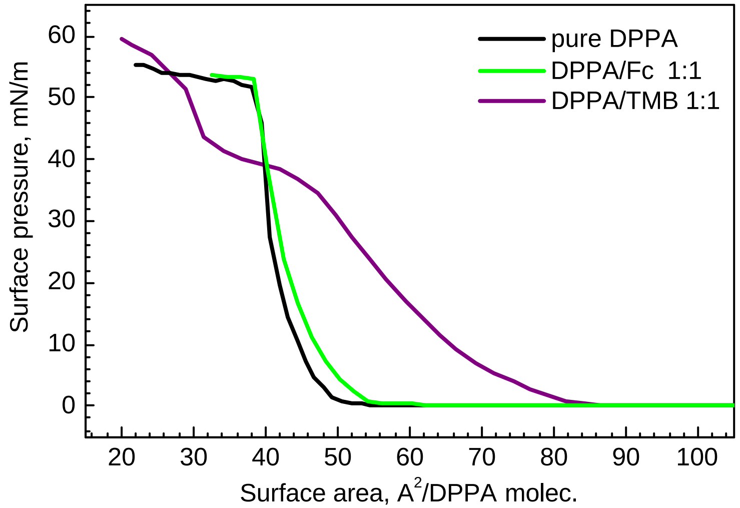
- **Surface pressure measurements**
  KSV5000 LB System
- **Ellipsometry**
  Rudolph Research Ellipsometer (Mod. 437-02)
- **Contact Angle measurements**
  Ramé-Hart Automated Contact Angle Goniometer
- **Cyclic Voltammetry**
  Ecochemie Autolab (Mod. PG STAT 10)
- **UV-Vis Spectroscopy**
  Perkin Elmer Lambda 900 Spectrometer

MONOLAYERS AT THE WATER-AIR INTERFACE

LANGMUIR-BLODGETT FILMS **(LB)** AND HYBRID [SAM/LB] FILMS **(HF)**

WATER-AIR INTERFACE:
cospreading of redox mediators with DPPA matrix

Sperading isotherms of DPPA/Fc and DPPA/TMB as a function of **mediator molar ratio**



Fc: irrelevant displacement of π-A isotherm

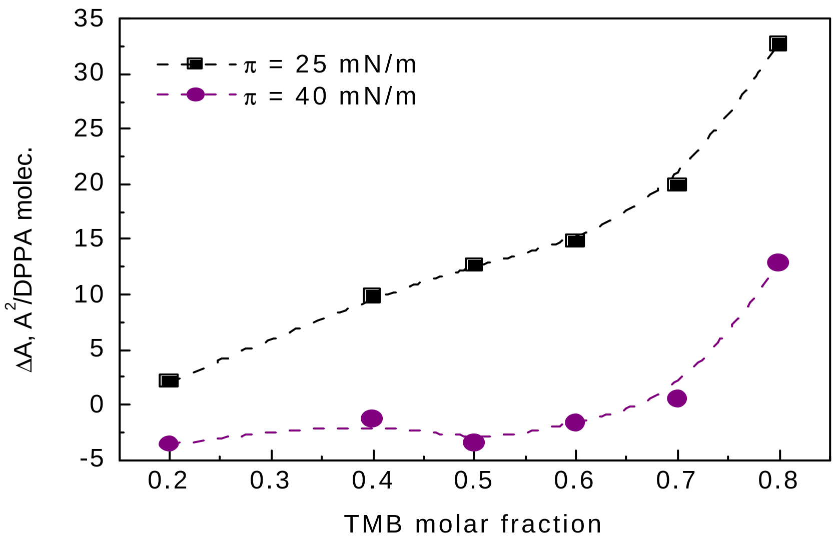
TMB: significant displacement of π-A isotherm up to π_{tr}=36 mN/m

TMB IMMOBILIZED IN THE DPPA MONOLAYER

Variations in DPPA/TMB molecular areas with respect to pure DPPA

before p_{tr} (π=25mN/m) and

after p_{tr} (π=40 mN/m)



AFTER p_{tr} TMB IS SQUEZEED OUT THE MONOLAYER (except DPPA/TMB 1:4)

HYBRID FILMS (SAM/LB)

Preparation of hybrid films
(SAM and LB Technologies)
with and without **redox mediator**

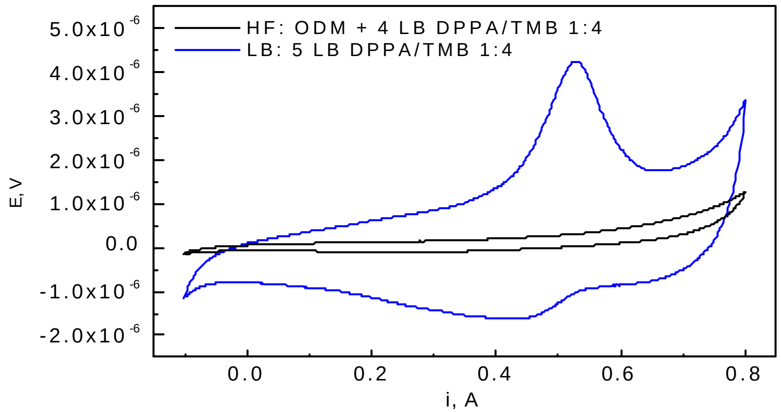
Characterization by means of:

- Ellipsometric thickness
- Contact angle
- Transfer ratio
- Cyclic Voltammetry

	SAM (ODM)	HF (ODM+2 LB pure DPPA)	HF (ODM+2 LB DPPA/Fc)	HF (ODM+2 LB DPPA/TMB)
s	10-13 Å	42-58 Å	48-60 Å	31-43 Å
q	100-106°	105-107°	97-101°	101-107°
TR	-	1.000	0.809	1.058

s = thickness; q = contact angle of water; **TR** = transfer ratio

- *HFs are compact and hydrophobic structures*
- *The presence of a redox mediator slightly modifies the features of the film (**TMB** in particular)*



HF → *NO ELECTROCHEMICAL SIGNAL*

LB → *ELECTROCHEMICAL SIGNAL*

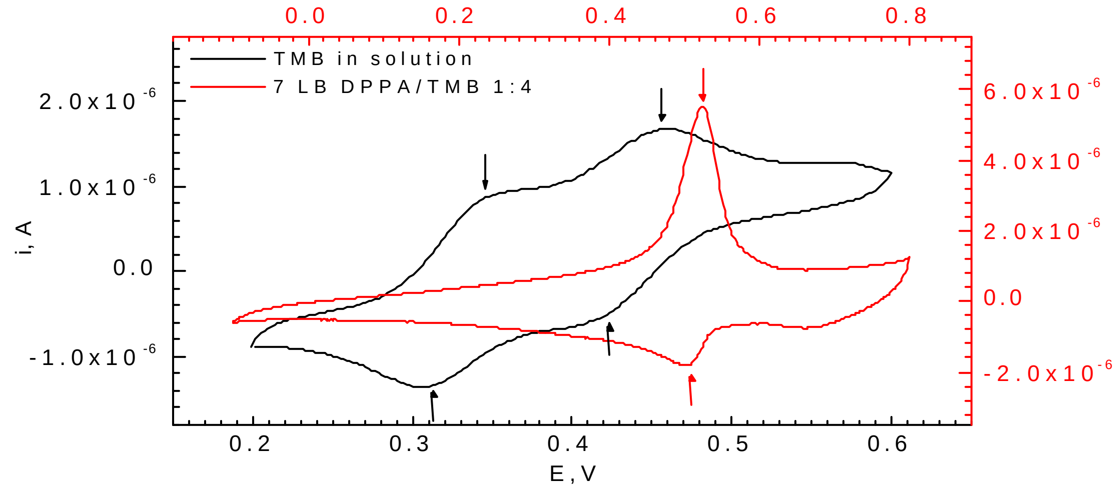
TMB, even though it moves through LB layers, does not penetrate the SAM and cannot approach the electrode

CYCLIC VOLTAMMETRY

Preparation of LB films
of DPPA/TMB 1:4 on gold
(**co-spreading**)

Characterization recording
cyclic voltammograms as a function of:

- layers number
- π transfer
  - π_{tr} = 25 mN/m — before the TMB transition
  - π_{tr} = 40 mN/m — after the TMB transition

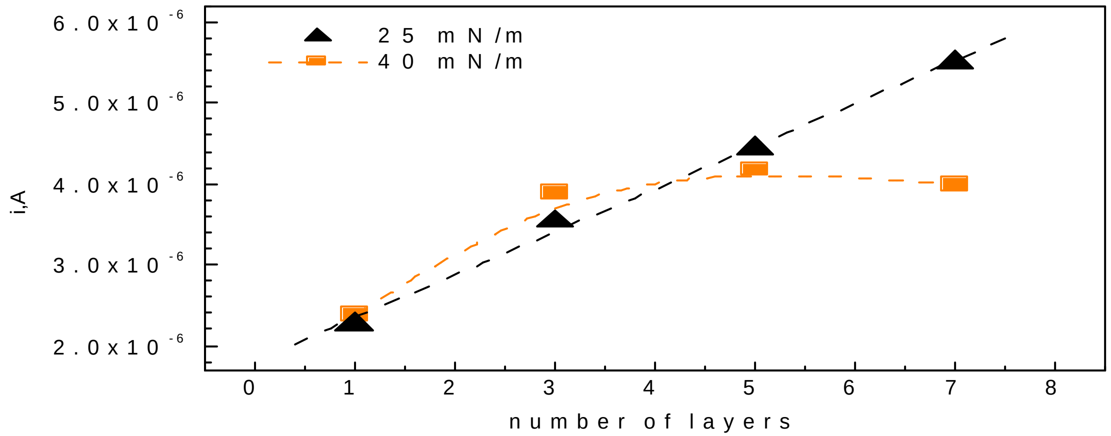


TMB immobilized in LB films mantains its electroactive behaviour even if:

- In solution: **two-step** oxidation
- In LB films: **one-step** oxidation

Reduced mobility *of TMB in LB films with respect to the solution*

OXIDATION PEAK INTENSITY
E = 0.525 V



π_{tr} = **25** mN/m
Less compact LB film

Linear relationship
between
current intensity (**i**)
and layer number

π_{tr} = **40** mN/m
More compact LB film

Saturation threshold
of **i** values
vs layer number

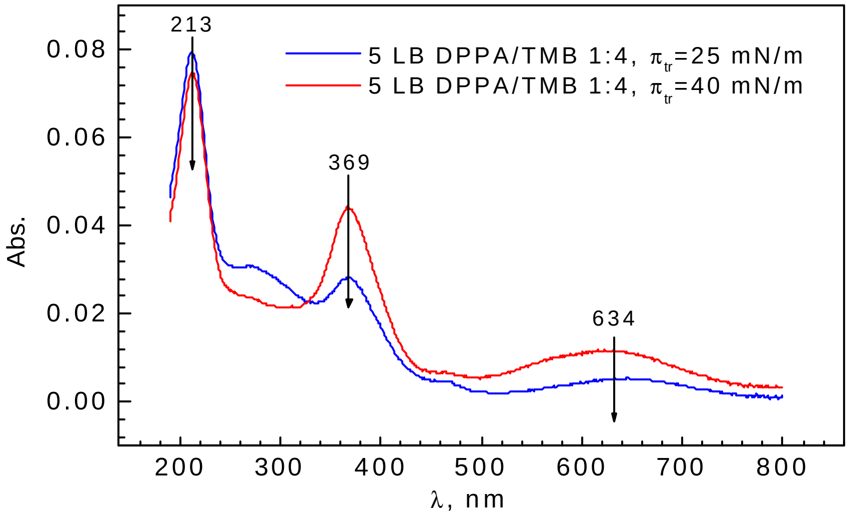
UV-Vis SPECTROSCOPY

Preparation of LB films
of DPPA/TMB 1:4 on quartz
(**co-spreading**)

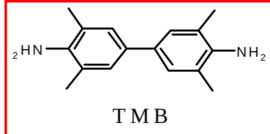
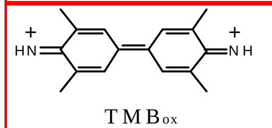
Characterization recording
UV-vis spectra as a function of:

- layers number
- π transfer
  - π_{tr} = 25 mN/m — before the TMB transition
  - π_{tr} = 40 mN/m — after the TMB transition

TMB in LB films



TMB in solution

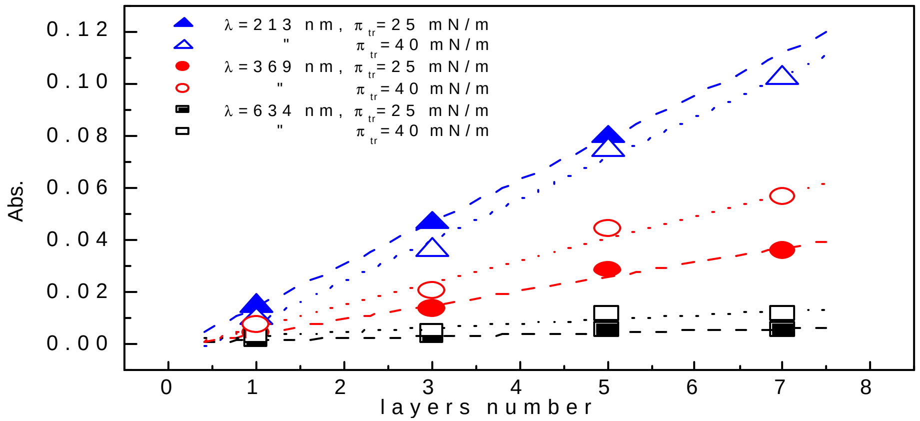
	λ_1 (nm)	λ_2 (nm)	λ_3 (nm)	λ_4 (nm)
 TMB	211	292	370	-
	ε_1 (mM)	ε_2 (mM)	ε_3 (mM)	ε_4 (mM)
	≈ 20	≈ 22	≈	
 TMB$_{ox}$	λ_1 (nm)	λ_2 (nm)	λ_3 (nm)	λ_4 (nm)
	211	294	370	644
	ε_1 (mM)	ε_2 (mM)	ε_3 (mM)	ε_4 (mM)
	≈ 20	≈ 25	≈ 57	≈35

M.Vernois, G.Friedmann, M.Brini et P.Federlin, *Bull. Soc. Chim.*, 5, **1973**, 1793

*LB films of DPPA/**TMB** contain simultaneously **TMB** and **TMBox***

Photochemical oxidation of TMB

INTENSITY OF ABSORPTION BANDS



Linear relationship between
Abs. and **layer number**

QUANTITATIVE TRANFER OF TMB IMMOBILIZED IN THE DPPA MONOLAYER

CONCLUSIONS

- **Hybrid films** (HF), built-up combining SAM and LB Technologies, are *compact and robust structures*, which allow to **immobilize electroactive molecules** (TMB)

  → **but** TMB cannot penetrate The SAM: loss of electrochemical signal

- **TMB**, immobilized in LB films by co-spreading with a DPPA matrix, mantains its **elecroactive behaviour**

  → electrochemical signal in CV is correlated with the number of LB layers and strongly dependent by the **mobility** of TMB in the film

- Independent spectroscopical investigation of TMB immobilized in LB films confirms the **presence** of the mediator in LB layer

  → Intensity of absorption bands is **proportional** to the number of LB layers