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BIOSENSOR BASED ON COVALENTLY IMMOBILIZED ENZYMES ON POLYMERS FOR THE NITRITE AND NITRATE MONITORING IN WATERS

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Introduction

The purpose of the work was to obtain an electrochemical screen printed biosensor for the monitoring of nitrite and nitrate in waters for human consumption.

Experimental section

In order to prepare such biosensors it was researched the possibility of immobilization of an enzyme on electro conductive polymer. Table 1 contains a centralization of the substances which were used.

The polymer used was polypyrrole. Polypyrrole was obtained from the polymerization of pyrrole, using ammonium persulfate (APS) as polymerization initiator.

Recipe for polymerization of pyrrole

For the polymerization of pyrrole it was necessary to prepare 2 solutions, according to table 2.
For polymerizing pyrrole solution 2 was dripped on solution 1 during 2 hours, while temperature was controlled not to exceed 5 ◦C.

Checking the reproductibility of polymerization recipe

For checking the reproductibility of the polymerization recipe, pyrrole was polymerized twice according to the same recipe. For the two polymers in this way it was made a Differential Scanning Calorimetric analysis (DSC).

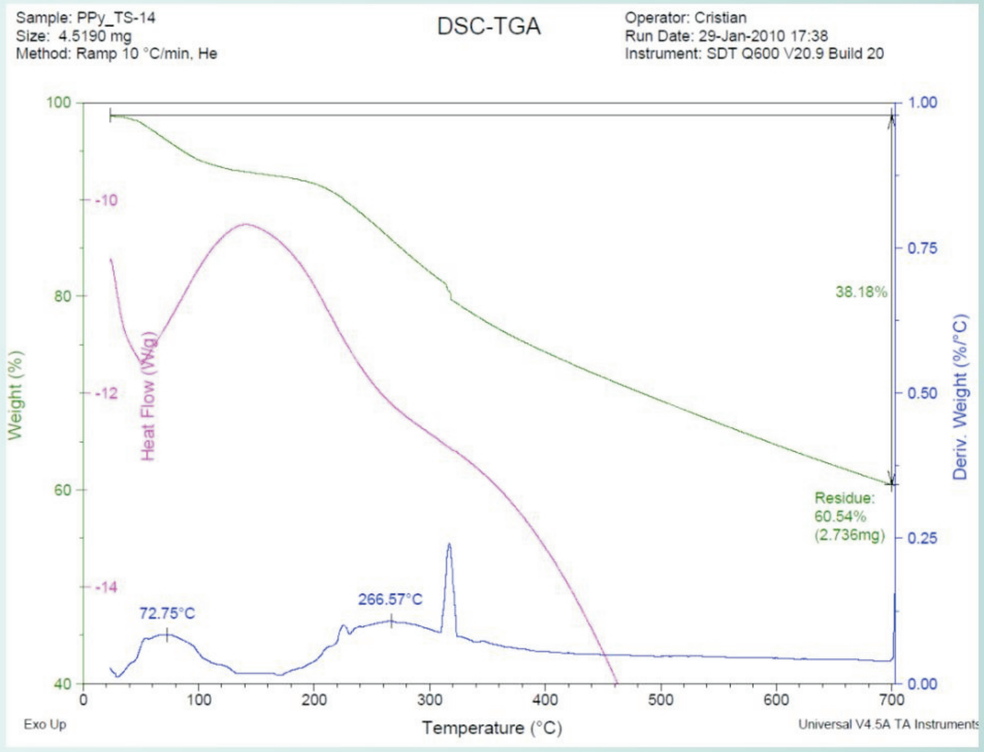

Figure 2: DSC for polypyrrole obtained second, according to the recipe

As it can be noticed from the DSC analysis the recipe is reproductible because the temperatures corresponding to the weight loss are appropriate as value.

The obtained polymer was functionalized at nanoscale by reaction with glutardialdehyde in order to insert on the polymer surface of binding sites for covalent immobilization of the enzyme, a study dedicated to this issue being performed. In order to establish the best quantities of compounds for creating binding sites 3 experiments were performed at 70 ◦C for 30 minutes.

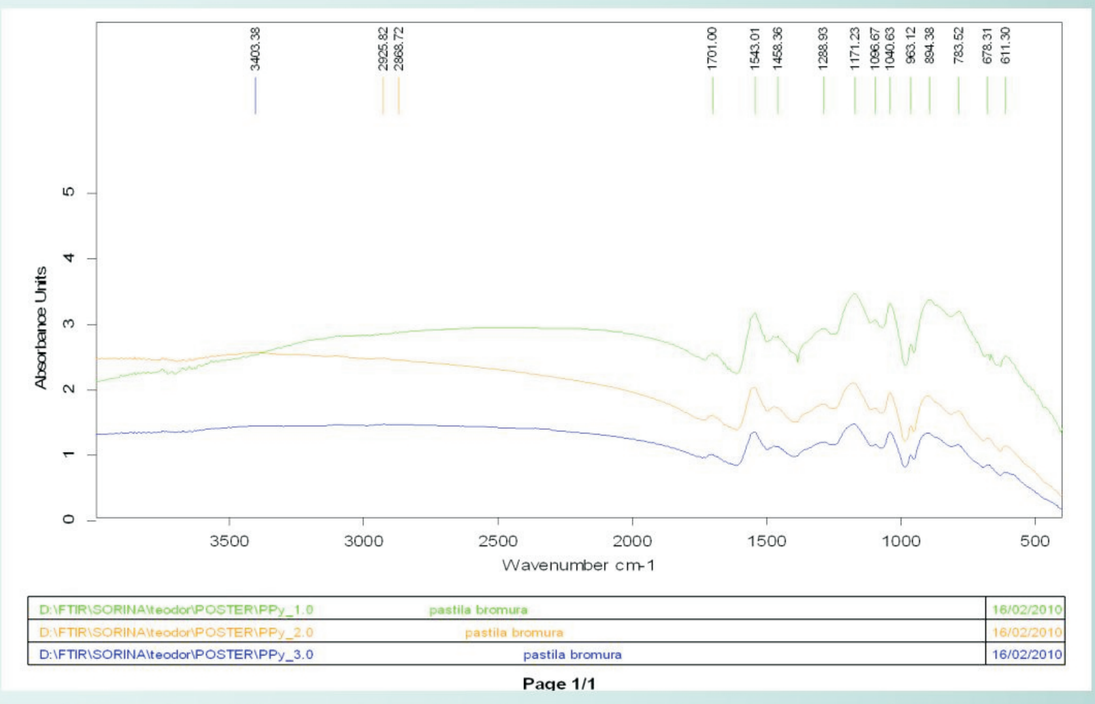

Figure 3: FTIR for confirming that binding sites were created

Table 1: *Substances which were used:*

Substance	Function
Pyrrole	Monomer
Glutardialdehyde (GA)	Compound used to create binding sites
Water	Reaction medium
H_2SO_4	Catalyst

Table 2: *Solutions used for pyrrole polymerization:*

Number of solution	Py, mL	APS, g	H_2O, mL
1.	4	-	100
2.	-	13.144	100

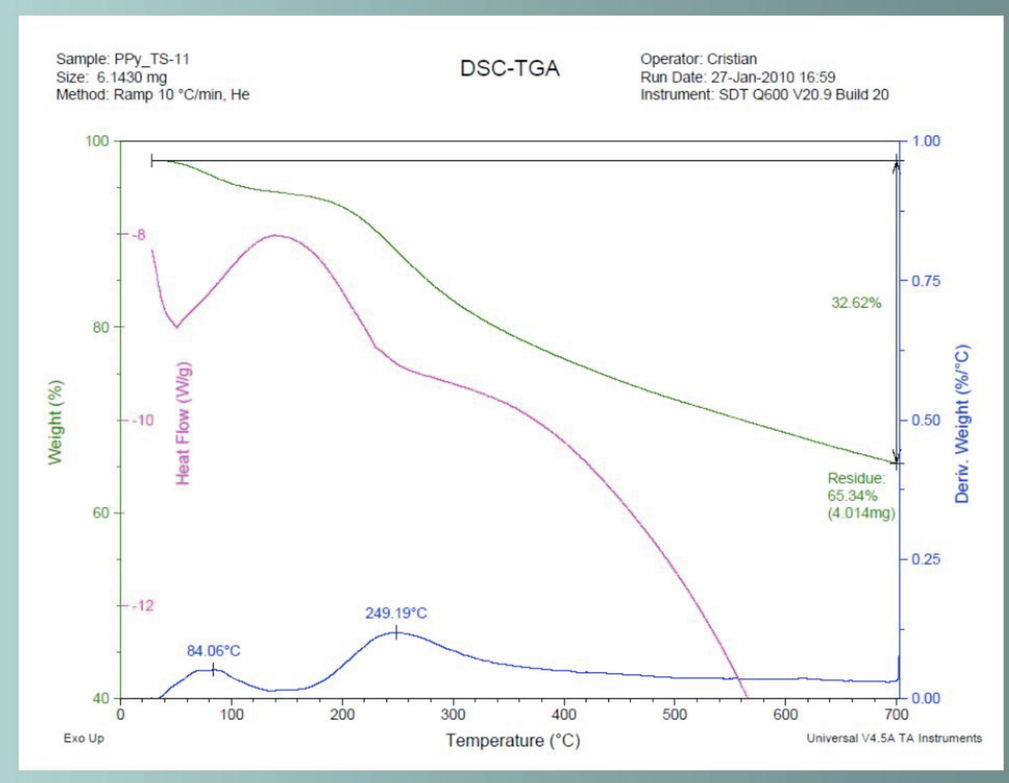

Figure 1: DSC for polypyrrole obtained first, according to the recipe

Table 3: Recipes for creating binding sites for covalent immobilization:

Nr. crt	PPy, g	H_2O, mL	GA, mL	H_2SO_4 conc., mL
1.	0.1	0	0	0
2.	0.1	9	1	0.136
3.	0.1	8	2	0.136

The last step was the immobilization of horseradish peroxidase on the nanofunctionalized polypyrrole.

In figure 4 it is presented an image of a screen printed sensor on which it is intented to immobilize horseradish peroxidase:

Figure 4: Image of a screen printed sensor

Conclusions

- Ammonium persulfate is a good initiator for pyrrole polymerization;
- The recipe for pyrrole polymerization using ammonium persulfate as initiator is reproductible;
- From Fourrier Transform Infrared Spectroscopy it can be concluded that the change of glutardialdehyde quantity don't give rise to important changes.