



# RAPID AND HIGHLY SENSITIVE ELECTROCHEMICAL DETERMINATION OF ALKALINE PHOSPHATASE USING A COMPOSITE TYROSINASE BIOSENSOR

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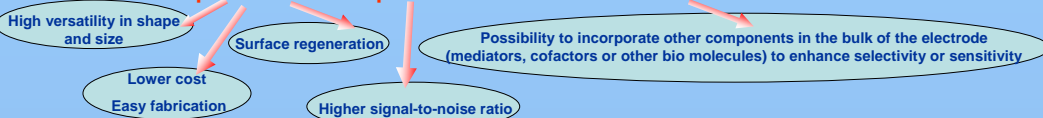
## INTRODUCTION

The determination of alkaline phosphatase (ALP) activity is widely used for establishing an adequate pasteurization of milk, as inactivation of ALP takes place at temperatures slightly higher than necessary to kill *M. Tuberculosis*, *S. senftenberg* or *L. monocytogenes*. Remaining ALP activity after pasteurization points to improperly operating pasteurization units or possible contamination by raw milk.

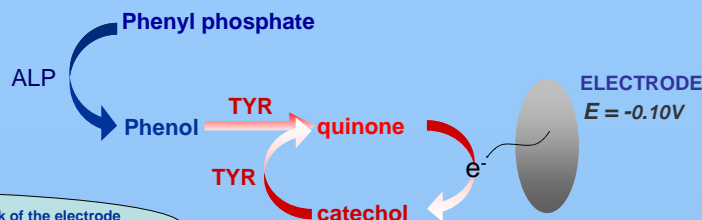
## OBJECTIVE

Development of an amperometric composite tyrosinase biosensor for the rapid monitoring of ALP (6 min of assay time), with no need of an incubation step and using phenyl phosphate as the substrate.

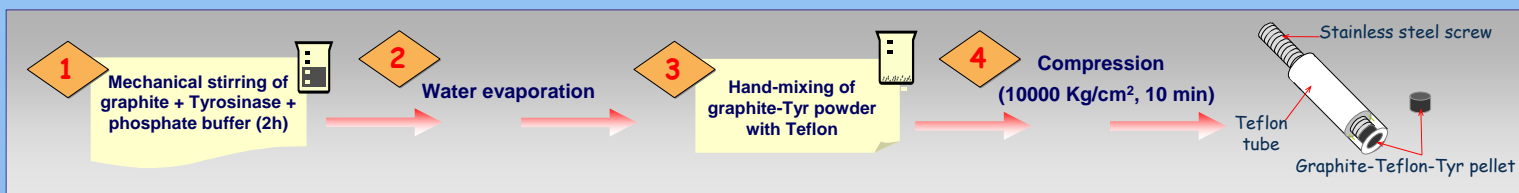
### Graphite-Teflon composite electrodes as biosensors



### Schematic representation of alkaline phosphatase detection at the graphite-Teflon-tyrosinase electrode

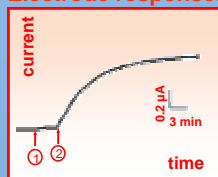


## FABRICATION PROCEDURE OF GRAPHITE-TEFLON-TYROSINASE ELECTRODE



## RESULTS AND DISCUSSIONS

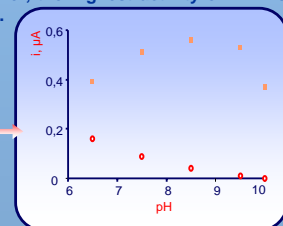
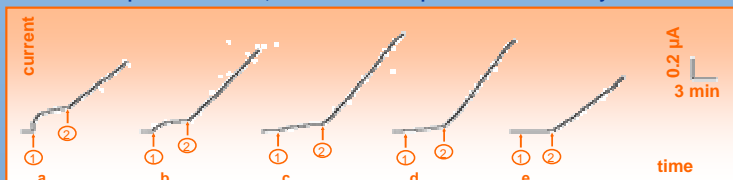
### Electrode response:



The initial changes in current after the addition of ALP have a linear dependence with the enzyme concentration, thus it is possible to obtain a calibration plot within the first minutes of reaction, although 25 minutes are needed for steady-state to be reached.

### pH optimization:

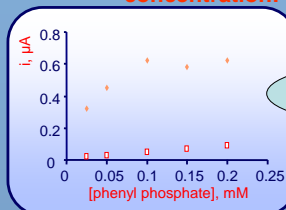
Spontaneous hydrolysis of phenyl phosphate is observed for pH values lower than 8.0, as the graphite-Teflon-tyrosinase electrode shows its higher steady-state current for phenol at more acidic values. However, the highest activity of ALP is found around pH 10. Therefore, the biosensor response is influenced by these two effects.



Current-time recordings obtained at a graphite-Teflon-tyrosinase electrode after the addition of phenyl phosphate to reach a concentration of  $5.0 \times 10^{-5}$  M (1), and of ALP to obtain a concentration in the cell of  $2.5 \times 10^{-11}$  M. 5 mL of Tris buffer of pH: 6.5 (a), 7.5 (b), 8.5 (c), 9.5 (d) and 10.0 (e). Eapp = -0.10 V.

• Background response for phenol from phenyl phosphate spontaneous decomposition  
• Analytical response for ALP activity

### Optimization of phenyl phosphate concentration:



Chosen phenyl phosphate concentration:  $1.0 \times 10^{-4}$  M

Amperometric responses obtained at the graphite-Teflon-tyrosinase electrode for different phenyl phosphate concentrations (○), and when  $2.5 \times 10^{-11}$  M of ALP is present in the cell (●), 5 mL of Tris buffer of pH 8.5. Eapp = -0.10 V.

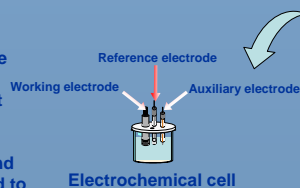
### Analytical characteristics

Linear range (ALP):	r:	Slope:	Intercept:	Limit of detection :	R.S.D. (n=10), ALP $2.0 \times 10^{-11}$ :	Biosensor lifetime :
$2.0 \times 10^{-13}$ - $2.5 \times 10^{-11}$ M	0.996	$8.0 \times 10^{10} \mu\text{A M}^{-1}$	0.0077 $\mu\text{A}$	$6.7 \times 10^{-14}$ M	5.6 %	30 days

### Determination of ALP in milk

1 Electrochemical cell: 5.0 mL of the Tris buffer solution (pH 8.5) mechanically stirred at a constant rate.

A potential of -0.10V is applied and the background current is allowed to stabilize (1 min).



2 An aliquot of milk without any pretreatment is added to the electrochemical cell.

The current measured after 5 minutes of the milk addition is interpolated into the ALP calibration plot.

	Pasteurized whole milk	Skimmed UHT milk	Raw milk
Expected ALP activity (1)	0	0	500 U L <sup>-1</sup>
Biosensor analysis (n=3)	0	0	540±30 U L <sup>-1</sup>

## CONCLUSIONS

- The great amplification of the electrochemical signal for phenol obtained at the graphite-Teflon-tyrosinase biosensor permits the development of a simple, rapid and highly sensitive method for the determination of ALP with a very low detection limit ( $6.7 \times 10^{-14}$  M) and very short time of analysis (6 min) with no incubation step.
- This method is applied to rapid control of the pasteurization process in milk.

### REFERENCE

(1) W. L. Claeys, A.M. Van Loey and M.E. Hendrickx; Trends in Food Science & Technology 13 (2002) 293-311