Electronic Supplementary Information (ESI1)

PVA-AWP/Tyrosinase functionalized screen-printed electrodes for dopamine determination

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Mathematical model for biofunctionalization conditions optimization.

In this paper, a heuristic way to select the best optimization parameters is applied, using the following Algorithm 1:

Step 1: ∀ Diffusion coefficient (*D*) and Enzyme Activity (*E*) values, re-scale to its MAX value:

$$sD^{(i)} = \frac{Diffusion\ coefficient^{(i)}}{max\left[Diffusion\ coefficient^{(1)} \quad \cdots \quad Diffusion\ coefficient^{(n)}\right]}$$

$$sE^{(i)} = \frac{Enzyme\ activity^{(i)}}{max\left[Enzyme\ activity^{(1)} \quad \cdots \quad Enzyme\ activity^{(n)}\right]}$$

where i is each individual value and n is Total of Diffusion coefficient/Enzyme activity values. sD and sE are the scaled Diffusion coefficient and the scaled Enzyme activity.

Step 2: ∀ sD and sE values compute the angle between them, having sD as y-axis, and sE as x-axis:

$$\theta^{(i)} = Tan^{-1}\left[\frac{sD^{(i)}}{sE^{(i)}}\right]$$

Step 3: Select the best Curing Time(i), PVA-AWP concentration(i) tuple whose θ^((i)) asociated is max.

Preprocessing data is a fundamental step in any numeric analysis. Combination of two or more variables of different scales must entail the use of any data normalization technique, i.e. removal of scale. Given that the two parameters (Diffusion coefficient/Enzyme activity) possess different scales, the relative-to-MAX strategy [1] was selected. Traditionally [0,1] normalization has been a highly solicited technique, but angle calculation requires non-zero values.

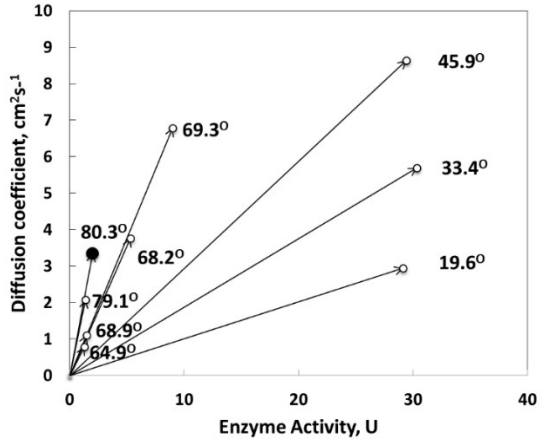
ESI Table 1 shows the computed values. The best combination Diffusion coefficient/Enzyme activity is boldfaced as selected by Step 2 rule. Fig. 3 shows a graphical representation of the parameter selection. x-y axis are depicted in their original scales for consistency purposes. The max angle, which represents the best tuple (Curing time, PVA-AWP concentration), is indicated as a black dot.

Therefore, the optimum condition for electrode biofunctionalization were found to be: PVA-AWP 3 wt.% and 60 min UV light exposure.

ESI1 Table 1 Parameters optimization for electrodes biofunctionalization. The values of the input parameters are the average of five measurements with RSD<3%.

Biofunctionalizing conditions		**Input parameters**	
Curing time, min	**PVA-AWP concentration, %**	**Diffusion coefficient, *D*x10^{-7}, cm^2 s^{-1}**	**Enzyme activity, *E*, U**
15	1.5	8.64	29.4
30	1.5	5.69	30.3
60	1.5	2.95	29.1
15	3	6.79	9.0
30	3	3.77	5.3
60	**3**	**3.35**	**2.0**
15	6	2.08	1.4
30	6	1.11	1.5
60	6	0.79	1.3

Scaled Diffusion coefficient, sD	**Scaled Enzyme Activity, sE**	$\theta = Tan^{-1}\left[\frac{sD}{sE}\right]$
1.000	0.970	45.9
0.659	1.000	33.4
0.341	0.960	19.6
0.786	0.297	69.3
0.436	0.175	68.2
0.388	**0.066**	**80.3**
0.241	0.046	79.1
0.128	0.050	68.9
0.091	0.043	64.9



ESI1 Fig. 1 Parameters selection by Diffusion coefficient/Enzyme Activity analysis

References

1. G. Gan, C. Ma and J. Wu. Data Clustering-Theory, Algorithms, and Applications. SIAM, 2007.

Electronic Supplementary Information (ESI2)

PVA-AWP/Tyrosinase functionalized screen-printed electrodes for dopamine determination

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Mathematical model for experimental conditions optimization. A mathematical modelling was applied for the optimization of the working conditions to achieve a maximum sensitivity of the DA determination. The input parameters are shown in ESI2 Table1.

ESI2 Table 1 Input parameters for working conditions optimization. The values of the input parameters are the average of five measurements with RSD<3%.

	Sensitivity, μA L^{-1} mmol				
pH	**20°C**	**25°C**	**30°C**	**35°C**	**40°C**
6.0	10.26	12.63	14.03	8.38	6.56
6.5	12.59	18.79	18.14	14.36	11.14
7.0	7.02	7.43	7.86	3.27	2.07
7.5	5.27	4.49	3.80	2.18	1.34
8.0	3.26	2.89	2.03	1.18	1.20

The sensitivity model was generated by Multi-variable Polynomial Regression Analysis. Polynomial regression was used to fit nonlinear data into a least squares linear regression model. Second and Third order polynomial function of two-variables has the following form:

$$f(x,y) = p_1 + p_2x + p_3y + p_4x^2 + p_5xy + p_6y^2$$

$$f(x,y) = p_1 + p_2x + p_3y + p_4x^2 + p_5xy + \\ + p_6y^2 + p_7x^3 + p_8x^2y + p_9xy^2 + p_{10}y^3$$

Let consider *pH* and *T* the input variables, and Sensitivity the response variable. Two polynomial models were assembled as follows:

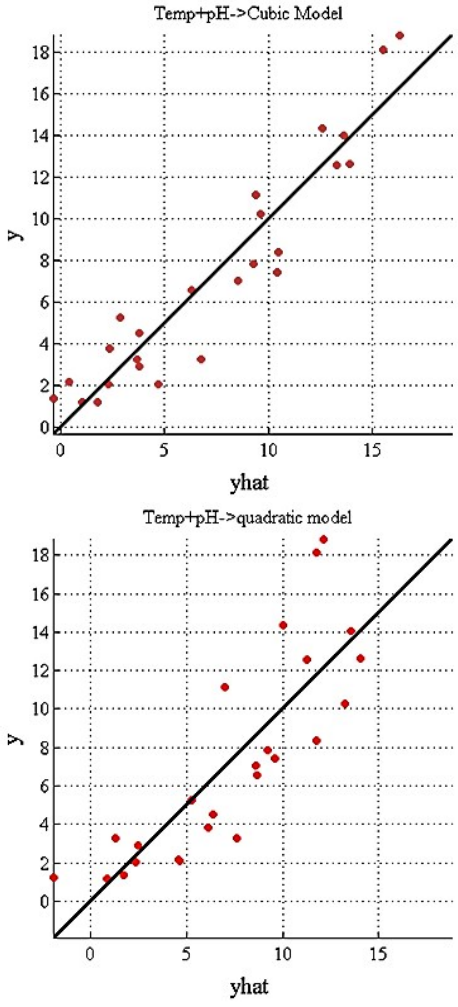
$$S(pH,T) = -24.910 + 11.372\,pH + 1.131T - 1.290\,pH^2 + \\ + 0.035\,pH \cdot T - 0.026T^2$$

$$S(pH,T) = -3703.937 + 1516.130\,pH + 23.040T - \\ - 211.931\,pH^2 - 2.647\,pH \cdot \mathrm{T} - 0.451T^2 + 9.943\,pH^3 + \\ + 0.061\,pH^2 \cdot \mathrm{T} + 0.030\,pH \cdot T^2 + 0.002T^3$$

ESI2 Table 2 shows main Regression Analysis readings. It can be seen that Cubic model renders the best Response values. ESI2 Fig. 1 shows Predicted vs. Observed analysis of two Polynomial models. Red dots indicates samples or data points; the solid line is a reference about "what is the desirable or ideal" i.e. the prediction is the same as the observed data, so the model achieves zero-error in the modeling. It is part of "classical" regression analysis.

ESI2 Table 2 Regression Analysis main statistics

Model	R^2	Mean Absolute Error
Cubic	0.892	0.337
Quadratic	0.693	0.481



ESI2 Fig. 1 Predicted vs. Observed biosensor response analysis. x-axis shows experimental biosensor response and y-axis shows the predicted biosensor response for each polynomial function.

To find the optimum *pH* and *T* values i.e. the ones that maximize sensitivity response, two well-known optimization

algorithms were used: the Simulated Annealing (SA) applying the Metropolis et al. [1] stochastic relaxation method and the SA algorithm of Kirkpatrick [2], as well as the Genetic Algorithm (GA) used for solving optimization problems based on natural selection process [3]. In GA scenario, individuals and their features are represented by a sequence of 0's and 1's. For a 5-bit schema, a basic binary coding is given by the following expression:

$$Fitness\_pop(i) = \frac{\sum\left[\left[16 \quad 8 \quad 4 \quad 2 \quad 1\right]*\left[1 \quad 0 \quad 0 \quad 1 \quad 0\right]\right]*(b-a)}{2^{stringlength}-1} + a$$

where [a, b] is the i-feature interval, and string length is the number of bits used to code-decode the real-valued features or variables.

ESI2 Table 3 shows parameter optimization results. Cubic Model yields the Maximum Sensitivity response with 16.931 µA L^{-1} mmol at *pH*=6.33 and *T*=26.40°C.

ESI2 Table 3 Parameters (*pH* and temperature) optimization results

Model	**Max Sensitivity, µA L^{-1} mmol**	**(*pH*, *T*)**
SA + Cubic	16.931	(6.335, 26.40)
SA + Quadratic	14.084	(6, 25.43)
GA + Cubic	16.919	(6.330, 26)
GA + Quadratic	14.080	(6, 26)

References

1 N. Metropolis, A. Rosenbluth, M. Rosenbluth, A. Teller and E. Teller, *J. Chem. Phys*.,1953, **21**, 1087-1092.

2 S. Kirkpatrick, *J. Stat. Phys*., 1984, **34**, 975-986.

3 M. Mitchell, An Introduction to Genetic Algorithms. MIT Press, Cambridge, MA, USA, 1996.