

# Supplementary Materials

Article

## Response Surface Methodology for the Optimisation of Electrochemical Biosensors for Heavy Metals Detection

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### 1. Table S1

**Table S1.** Variables and levels considered for the design of experiment (DOE).

| Name (Factor)             | Units  | Low | High |
|---------------------------|--------|-----|------|
| Enzyme concentration (X1) | U/mL   | 50  | 800  |
| Number of CV cycles (X2)  | -      | 10  | 30   |
| Flow rate (X3)            | mL/min | 0.3 | 1.0  |

### 2. Analysis of Variance of the Model for Al<sup>3+</sup> and Bi<sup>3+</sup>

The analysis of variance with the calculated F and p values were obtained for all the tested metal ions.

*Response Surface Regression: Sensitivity towards Al<sup>3+</sup> ions versus [Enzyme] (U/mL); Flow rate (mL/min); Number of cycles*

**Analysis of Variance.**

| Source                          | DF | Adj SS   | Adj MS   | F-Value | P-Value |
|---------------------------------|----|----------|----------|---------|---------|
| Model                           | 9  | 0.128467 | 0.014274 | 1.79    | 0.188   |
| Linear                          | 3  | 0.071472 | 0.023824 | 3.00    | 0.082   |
| [Enzyme] (U/mL)                 | 1  | 0.049279 | 0.049279 | 6.20    | 0.032   |
| Flow rate (mL/min)              | 1  | 0.000127 | 0.000127 | 0.02    | 0.902   |
| Number of cycles                | 1  | 0.022066 | 0.022066 | 2.77    | 0.127   |
| Square                          | 3  | 0.004253 | 0.001418 | 0.18    | 0.909   |
| [Enzyme] (U/mL)*[Enzyme] (U/mL) | 1  | 0.003239 | 0.003239 | 0.41    | 0.538   |

|                                       |    |          |          |      |       |
|---------------------------------------|----|----------|----------|------|-------|
| Flow rate (mL/min)*Flow rate (mL/min) | 1  | 0.000005 | 0.000005 | 0.00 | 0.981 |
| Number of cycles*Number of cycles     | 1  | 0.000657 | 0.000657 | 0.08 | 0.780 |
| 2-Way Interaction                     | 3  | 0.052743 | 0.017581 | 2.21 | 0.150 |
| [Enzyme] (U/mL)*Flow rate (mL/min)    | 1  | 0.006555 | 0.006555 | 0.82 | 0.385 |
| [Enzyme] (U/mL)*Number of cycles      | 1  | 0.045753 | 0.045753 | 5.75 | 0.037 |
| Flow rate (mL/min)*Number of cycles   | 1  | 0.000435 | 0.000435 | 0.05 | 0.820 |
| Error                                 | 10 | 0.079532 | 0.007953 |      |       |
| Lack-of-Fit                           | 5  | 0.056098 | 0.011220 | 2.39 | 0.180 |
| Pure Error                            | 5  | 0.023433 | 0.004687 |      |       |
| Total                                 | 19 | 0.207999 |          |      |       |

## Model Summary.

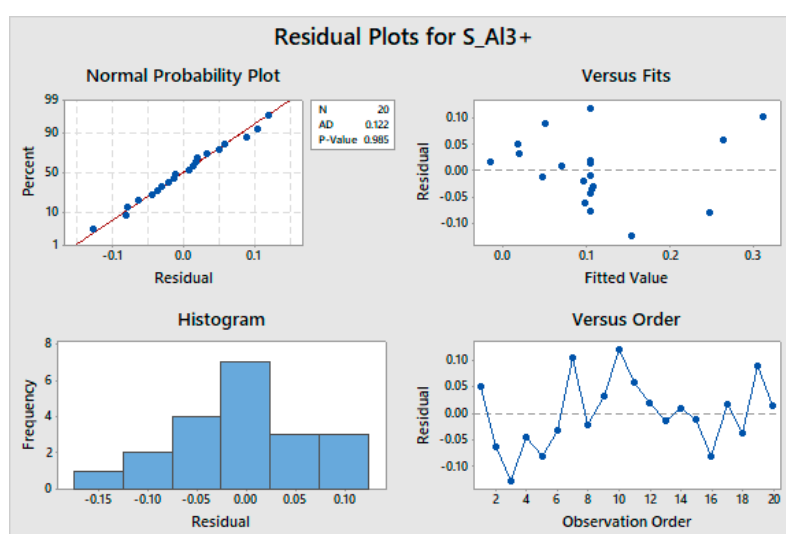
| S         | R-sq   | R-sq(adj) | R-sq(pred) |
|-----------|--------|-----------|------------|
| 0.0891806 | 61.76% | 27.35%    | 0.00%      |

## Fits and Diagnostics for Unusual Observations.

| Obs | S_Al3+ | Fit    | Resid   | Std   | Resid |
|-----|--------|--------|---------|-------|-------|
| 3   | 0.0260 | 0.1535 | -0.1275 | -2.28 | R     |
| 7   | 0.4170 | 0.3128 | 0.1042  | 2.03  | R     |

R: Large residual.

## Residual Plots for S\_Al3+



Response Surface Regression: Sensitivity towards Bi3+ versus [Enzyme] (U/mL); Flow rate (mL/min); Number of cycles.

## Analysis of Variance.

| Source                                | DF | Adj SS  | Adj MS  | F-Value | P-Value |
|---------------------------------------|----|---------|---------|---------|---------|
| Model                                 | 9  | 2.93908 | 0.32656 | 11.04   | 0.000   |
| Linear                                | 3  | 1.61191 | 0.53730 | 18.16   | 0.000   |
| [Enzyme] (U/mL)                       | 1  | 1.58134 | 1.58134 | 53.44   | 0.000   |
| Flow rate (mL/min)                    | 1  | 0.01054 | 0.01054 | 0.36    | 0.564   |
| Number of cycles                      | 1  | 0.02003 | 0.02003 | 0.68    | 0.430   |
| Square                                | 3  | 1.14477 | 0.38159 | 12.90   | 0.001   |
| [Enzyme] (U/mL)*[Enzyme] (U/mL)       | 1  | 1.12542 | 1.12542 | 38.03   | 0.000   |
| Flow rate (mL/min)*Flow rate (mL/min) | 1  | 0.05734 | 0.05734 | 1.94    | 0.194   |
| Number of cycles*Number of cycles     | 1  | 0.02296 | 0.02296 | 0.78    | 0.399   |
| 2-Way Interaction                     | 3  | 0.18240 | 0.06080 | 2.05    | 0.170   |
| [Enzyme] (U/mL)*Flow rate (mL/min)    | 1  | 0.00108 | 0.00108 | 0.04    | 0.852   |

|                                     |    |         |         |      |       |
|-------------------------------------|----|---------|---------|------|-------|
| [Enzyme] (U/mL)*Number of cycles    | 1  | 0.17731 | 0.17731 | 5.99 | 0.034 |
| Flow rate (mL/min)*Number of cycles | 1  | 0.00401 | 0.00401 | 0.14 | 0.721 |
| Error                               | 10 | 0.29591 | 0.02959 |      |       |
| Lack-of-Fit                         | 5  | 0.22835 | 0.04567 | 3.38 | 0.104 |
| Pure Error                          | 5  | 0.06757 | 0.01351 |      |       |
| Total                               | 19 | 3.23499 |         |      |       |

## Model Summary.

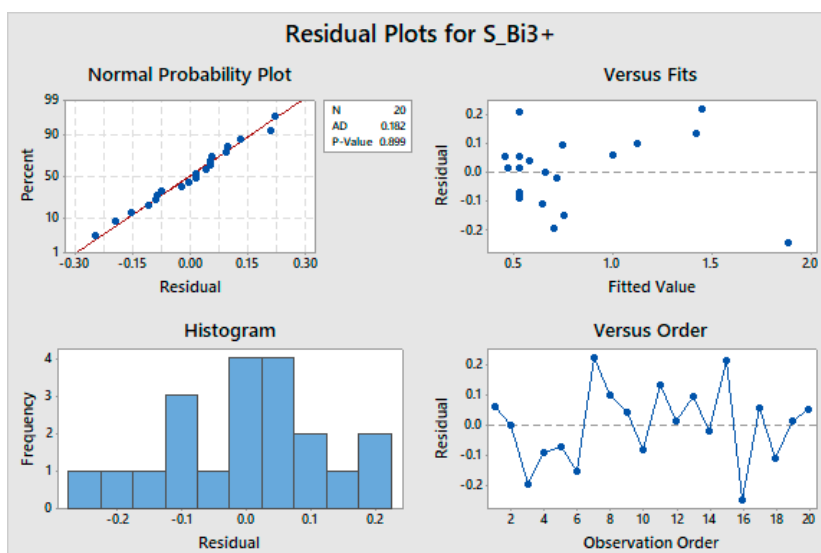
| S        | R-sq   | R-sq(adj) | R-sq(pred) |
|----------|--------|-----------|------------|
| 0.172022 | 90.85% | 82.62%    | 43.41%     |

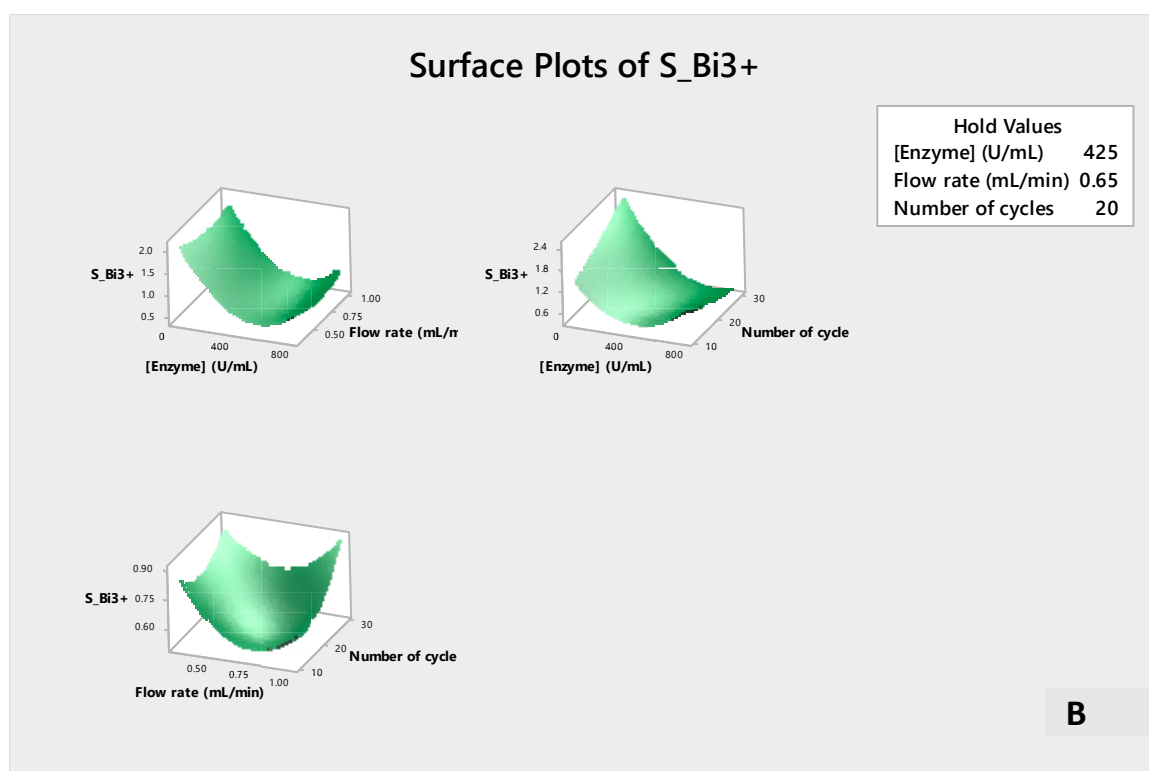
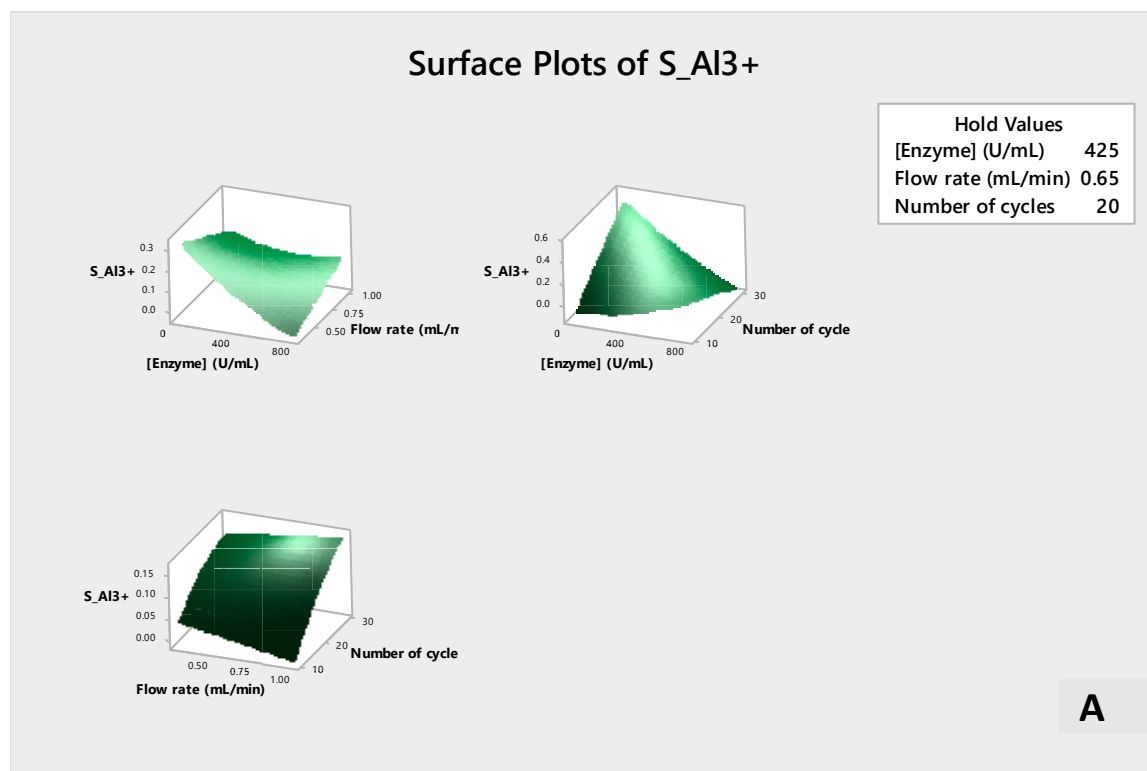
## Fits and Diagnostics for Unusual Observations.

| Obs | S_Bi3+ | Fit   | Resid  | Std   | Resid |
|-----|--------|-------|--------|-------|-------|
| 7   | 1.675  | 1.455 | 0.220  | -2.23 | R     |
| 16  | 1.642  | 1.890 | -0.248 | 2.30  | R     |

R: Large residual.

## Residual Plots for S\_Bi3+





**Figure S1.** Response Surface for Al<sup>3+</sup> (A), and Bi<sup>3+</sup> (B): the sensitivities were improved when low concentrations of enzyme and a high number of cycles were employed during the synthesis of biosensor.

### 3. Optimisation of the responses

Response Optimization: S\_Bi<sup>3+</sup>; S\_Al<sup>3+</sup>.

| Response           | Goal    | Lower | Target | Upper | Weight | Importance |
|--------------------|---------|-------|--------|-------|--------|------------|
| S_Bi <sup>3+</sup> | Maximum | 0.438 | 1.675  |       | 1      | 1          |
| S_Al <sup>3+</sup> | Maximum | 0.001 | 0.417  |       | 1      | 1          |

Optimization Plot

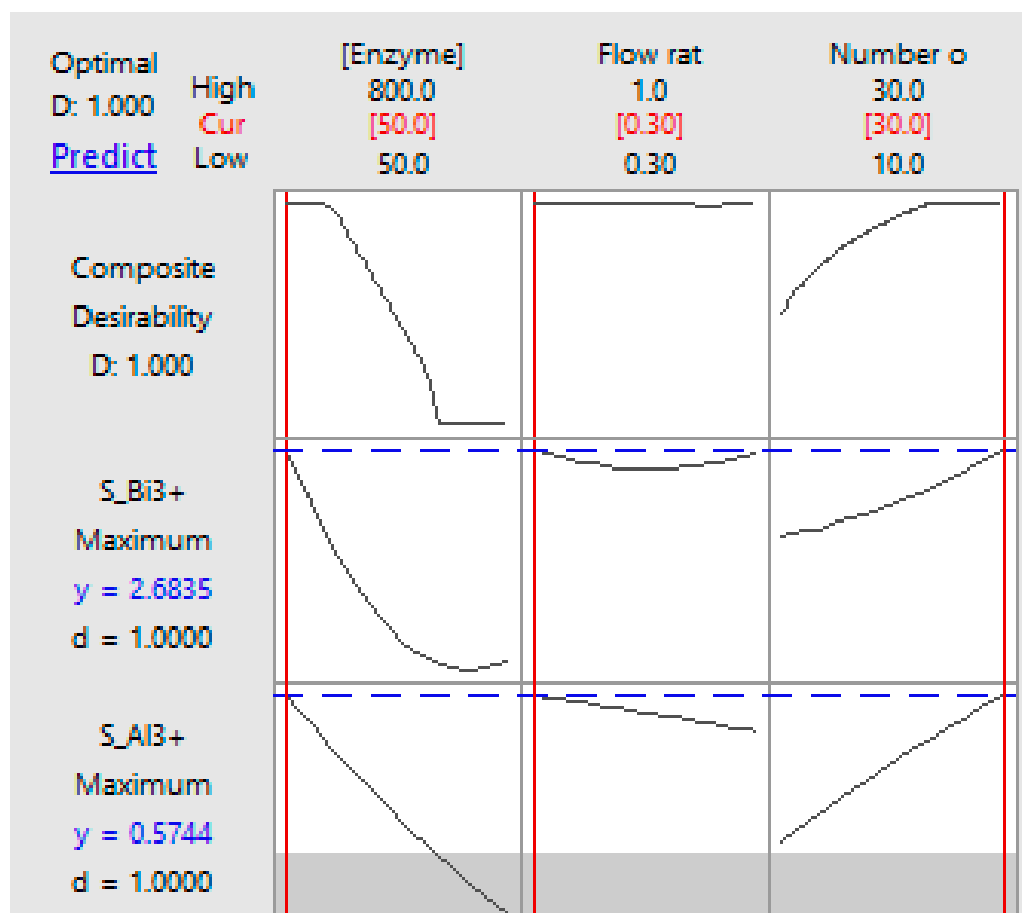
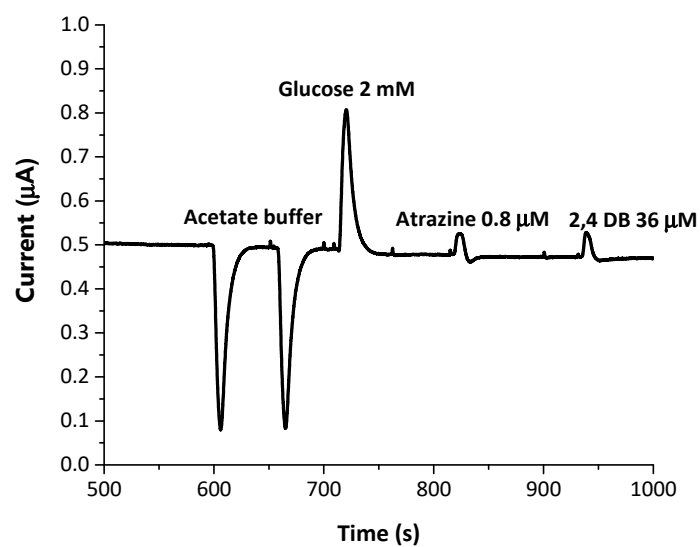


Figure S2. Numerical optimisation performed by the software.

Solution.

| Solution | [Enzyme](U/mL) | Flow rate (mL/min) | Number of cycles | S_Bi <sup>3+</sup> | S_Al <sup>3+</sup> | Composite Desirability |
|----------|----------------|--------------------|------------------|--------------------|--------------------|------------------------|
| 1        | 50             | 0.3                | 30               | 2.68352            | 0.574429           | 1                      |

#### 4. Selectivity of biosensor



**Figure S3.** Electrochemical responses of PPD/GOx biosensor after the injection of 0.8 μM of atrazine and 36.8 μM of 2,4-DB. Baseline: Glucose 1 mM.