Supporting Information

SERS substrates fabricated with star-like gold nanoparticles for Zeptomol detection of Analytes

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I. Calculation of total error for the parameters in table 1 (Column H).

The balance Sartorius, the Poseidon beta micropipette and the Labmate Soft LM100 micropipette were used to weigh and to prepare our solutions with Rhodamine B (RhB), the specifications for those instruments are:

Poseidon beta micropipette: volume range from 100µl to 1000µl, ISO 8655 validation and controls; V (100µL) accuracy (%): +-0.152, precision (%): 0.01; V (1000µL) accuracy (%): +-0.319, precision (%):+- 0.031.

Labmate Soft LM100 micropipette: volume range from 10µl to 100µl, ISO 8655 validation and controls; V (10µl) accuracy (%): -+1.600, precision (%): +-0.8; V (100uL) accuracy (%): -+0.800, precision (%): +-0.2.

Balance Sartorius CPA224S: Readability 0.1 mg, maximum weigh 200 gr.

In order to determine the error for the amount of molecules and moles distributed on our SERS substrates, the following procedure was achieved:

1. **Calculation of the error for weighing:** We weighed 0.9876 gr (0.002 moles) of RhB 10 times and we calculated the standard deviation (SD), the value for SD was ±0.0185%.

2. **Calculation of the error for volume:** We measured 10 times 1000 µl, 500 µl, 100 µl and 50 µl of tri-distilled water utilizing these micropipettes, and we weighed 10 times each one of these volumes of liquids. The values of SD were obtained using the differences of weight for these volumes of liquids taking into account that 1000 µl should weight 1 gr, 500 µl should weigh 0.5 gr, 100 µl should weight 0.1 gr and 50 µl should weigh 0.05 gr. Thus, the SD were ±0.012%, ±0.0008%, ±0.014% and ±0.0278% for the volumes of 1000 µl, 500 µl, 100 µl and 50 µl respectively.

3. The 0.002 moles of RhB were dissolved in 2 ml. of ethanol (this is named “main solution”) and we proceeded to dilute that solution in ethanol sucessively in order to get another solution from which we took 10 µl, and this last volume contained the amount moles of RhB shown in the column C of table 1:

Table 1. Errors for the preparation of solutions of RhB.

A	B	C	D
Sample	**Number of dilutions**	**Number of moles on the substrate**	**Error for preparation of solutions (±%)**
R1	**0**	**$1.031x10^{-05}$**	**0.0425**
R2	**3**	**$1.031x10^{-11}$**	**0.798**
R3	**4**	**$1.031x10^{-12}$**	**1.038**
R4	**6**	**$1.031x10^{-14}$**	**1.518**
R5	**8**	**$1.031x10^{-16}$**	**1.998**
R6	**10**	**$1.031x10^{-18}$**	**2.478**

4. The error for the main solution (sample R1 with 0 dilutions) was obtained with the operation: (2*0.012)+0.0185= 0.0425, where 2 corresponds to the number of times for which we used the micropipette to get 2 ml. for the “main solution”, 0.012 is the error corresponding to the volume of 1000 µl and 0.0185 is the error for weighing RhB. The solution used for the sample R2 was prepared by taking 200 µl of the main solution and it was diluted in 19.8 ml of ethanol.We carried out this procedure 3 times (3 dilutions) and the error to prepare the solution used in sample R2 was calculated as follows: 0.024+3(21*.012)+.0185=0.798, where 0.024 is the total error corresponding to the volume of the main solution (2*0.012), 3(21*.012) corresponds to the number of dilutions (3) multiplied by the number of times for which the micropipettes were used (21) and this is multiplied by the error of the micropipette used to measure 1000 µl (0.012) and 0.0185 is the error for weighing RhB. The rest of errors for the solutions used to make samples R3-R6 were calculated using a similar calculation than that for R2, those errors are presented in column D of table 1.

5. The total error was obtained by adding the error corresponding to the volume of 10 µl (±0.0278%) and the error for the preparation of dilutions. The total error corresponding to each number of moles on the substrate is summarized in column D of table 2:

Table 2: Total error for each sample used for Raman detection

A	B	C	D
Sample	Error for preparation of solutions (±%)	Error for volume of 10 µl (±%)	Total Error (±%)
R1	0.0425	0.0278	0.0703
R2	0.798	0.0278	0.826
R3	1.038	0.0278	1.066
R4	1.518	0.0278	1.546
R5	1.998	0.0278	2.026
R6	2.478	0.0278	2.506