Supporting Material for

AGNES (Absence of Gradients and Nernstian Equilibrium Stripping): an electroanalytical technique for chemical speciation. A tutorial review.

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General indications about the protocols in this file

Bold text is used in this document to highlight possible minimal changes of parameters to be provided by an end-user when using the existing scripts (procedures). See also sections whose title starts with "Use of the procedure." (e.g. F.2 or G.2) for a list of possible changes to existing protocols.

The specific examples gathered in this document can be combined to further obtain many other protocols. For instance, the procedures for the second stage AGNES-I, used in protocol B, can be combine with the procedures for the first stage 2P from protocol G.

The procedures shown here are written in NOVA program version 2.1.5. More details on the type of commands are given in NOVA manual: section 7.1.1 for "Message" type; section 7.1.3 for "Repeat" type; section 7.7.3 for "Calculate signal" type; section 7.5.1 for "Record signals" type; etc.

The labels of the commands (or parameters) from NOVA are indicated in between inverted commas. For instance, "Calculate signal" or "Message". To facilitate the interpretation of the commands some of them have been re-labelled with their specific function: "Epeak", "AGNES-I", "Record I2", etc.

In the snapshots of NOVA windows, purple arrows and underlines are added to highlight specific parts.

A.- Protocol to determine Epeak (to establish gains) with DPP

The NOVA script for DPP procedure can be found in the file DPP.nox

For the sake of specificity, this example for DPP assumes to work with a **solution consisting of 25 mL 0.1 mol L^{-1} KNO_3 + Zn^{2+} 1.0×10^{-5} mol L^{-1}, at a temperature of 25°C.**

A.1.- To the voltammetric cell, add 25 mL 0.1 mol L^{-1} KNO_3 and an adequate volume of Zn standard solution, so that $c_{T,Zn}$ is around 1.0×10^{-5} mol L^{-1}**. Fix pH around 4.0** with KOH 0.1 mol L^{-1} or HNO_3 0.1 mol L^{-1} (as needed). Close the cell, so that it becomes air-tight, and **purge it with N_2 for 20 min.**

A.2.- In the polarographic stand, turn the corresponding buttons to the option **SMDE** (Static Mercury Drop Electrode) with **drop size 3** (this size is relevant to use expressions for planar electrodes).

A.3.-In NOVA 2.1.5 software, in the list of default procedures, **select the Differential Pulse Voltammetry procedure** by double clicking on it. In the command "Differential Pulse", set the relevant parameters (or properties), like "Start potential", "Stop potential", "Step potential", "Modulation amplitude", "Modulation time" and "Interval time". For Zn, one can apply "Start potential" -0.85 V, "Stop potential" -1.1 V, "Step potential" -0.002 V, with "Modulation amplitude" 0.04995 V, "Modulation time" 0.05 s and "Interval time" 1 s. Once the parameters are fixed, the procedure can be executed by clicking on the option Start (►). **In Figure the DPP program is shown with the parameters for Zn ready to be executed.**

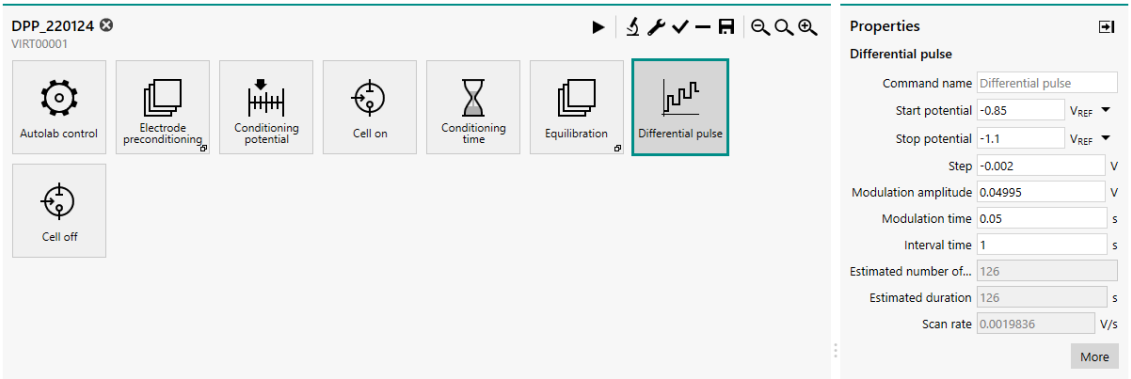


Figure S1. Snapshot of the DPP procedure. On the right-hand side there is the box with the parameters/properties to be adjusted to the current situation (e.g. if changing the analyte to Cd or Pb).

A.4.- The DPP answer is a difference of currents *versus* potential. When the procedure is run, a peaked-shape plot is obtained, see Figure S2. The sought DPP figure of merit is the peak potential, E_{peak}, which can be measured by double clicking on the plot. In the upper part of the window (see Figure S3), select "Add analysis command" ( ). From the pop-down options, select "Peak search". On the bottom of the right-hand side of the displayed window (see Figure S4), there are **several measured properties of the DPP peak. We are interested in the peak potential (E_{peak}),** which in this example turned out to be -0.9869 V. This value of E_{peak} can be used in the following equation in order to find the gain corresponding to a certain deposition potential E_1:

$$Y = \sqrt{\frac{D_{Zn^{2+}}}{D_{Zn^0}}} \exp\left[-\frac{2F}{RT}\left(E_1 - E_{peak} - \frac{\Delta E}{2}\right)\right] \quad (1)$$

(the values of the diffusion coefficients -at the working temperature- in previous equation have to be suitably changed for analytes other than Zn). By solving for E_1 in previous equation, one derives which deposition potential is needed for a desired gain Y. More details on how E_{peak} is used can be found in section B.1, below.

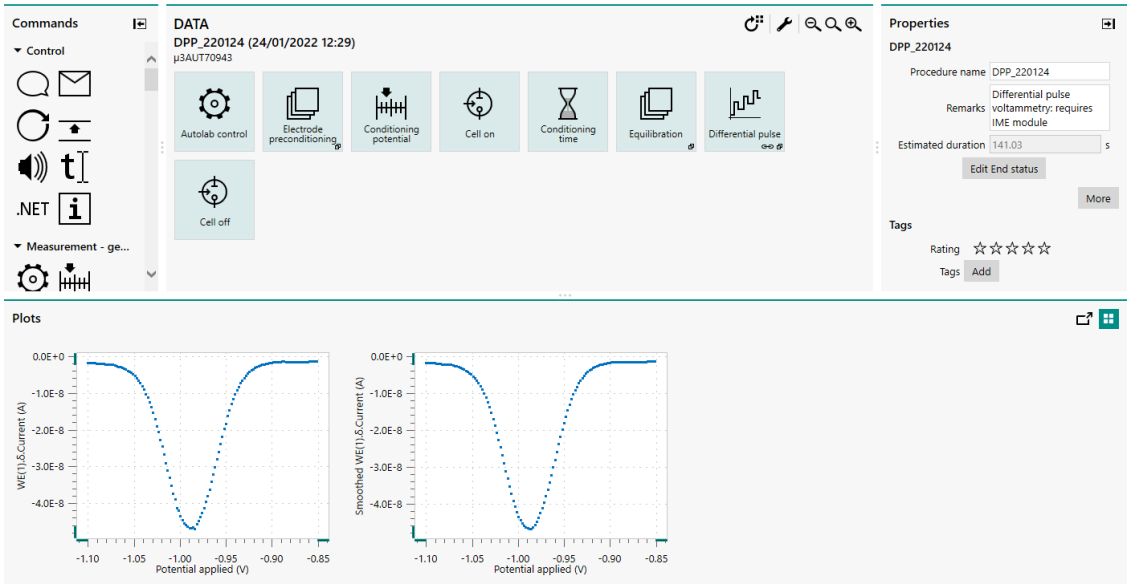


Figure S2. DPP polarogram in a solution with $c_{T,Zn}$ 1.0×10^{-5} mol L^{-1}.

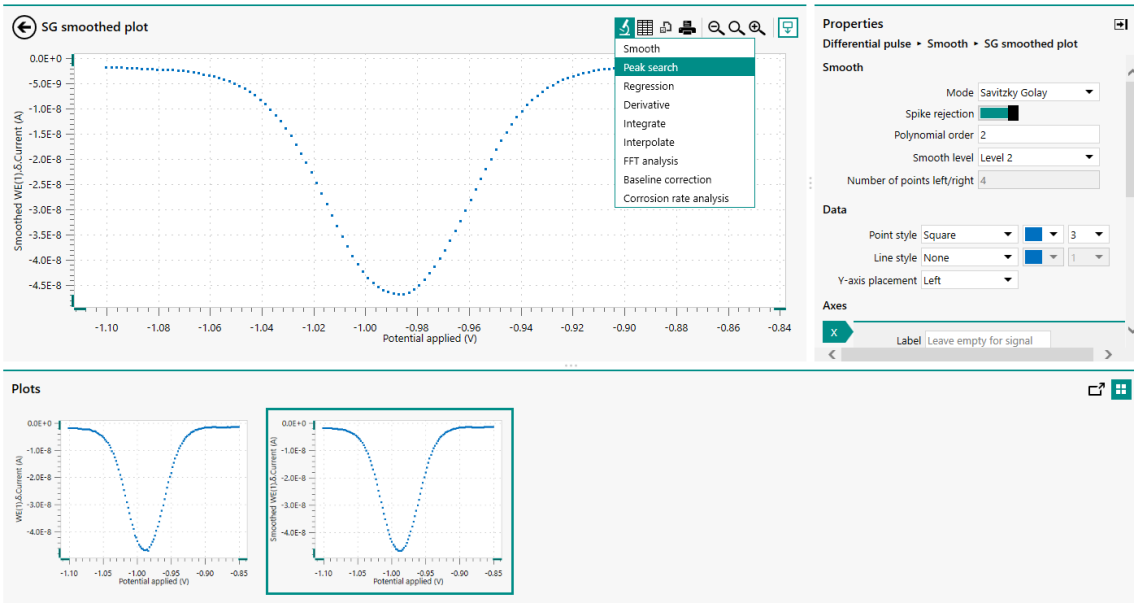


Figure S3. Selection of DPP polarogram and "Peak search" to find E_{peak}.

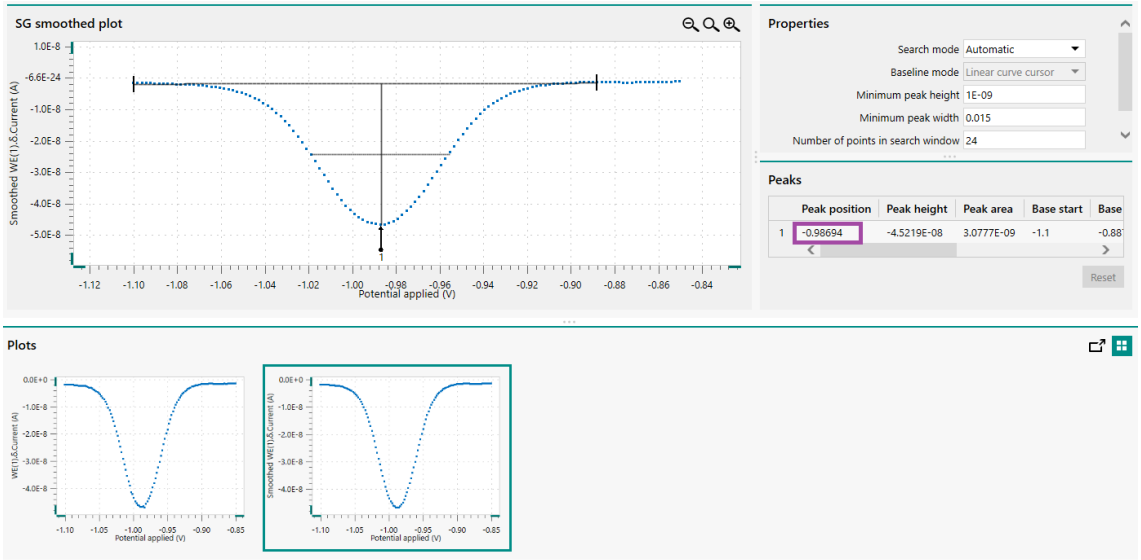


Figure S4. Determination of E_{peak} =-0.9869 V from the DPP polarogram.

B.-Protocol to obtain one AGNES point using 1 P in the first stage and AGNES-I in the second stage

The NOVA script with the procedure to run one AGNES point with AGNES-I (with 1 P variant in the first stage) can be found in the file AGNES-I_1P.nox. Typically, only ***E*peak, *t*1-*t*w and *Y* have to be adapted**, given that other parameters (t_w, t_2, etc.) are usually fixed.

For the sake of specificity, the example that follows assumes that one is working with the HMDE in a solution consisting of 25 mL 0.1 mol L^{-1} KNO_3 + $c_{T,Zn}$ 1.0×10^{-5} mol L^{-1} pH 4.0 in the solution. In this case, it is known that a gain Y=20 will yield currents sufficiently above the limit of quantification (see second paragraph in section B.1.2). Obviously, other gains are needed for other free concentrations.

B.1.- Establish parameters in the NOVA procedure.

B.1.1 The main commands in the AGNES-I_1P procedure are shown in Figure S5. In the **first command** of the list, of the type "Calculate signal" that we have re-labelled **"Epeak"**, the **value of the peak potential found with the DPP has to be set** (for this particular run it was -0.9869 V). Gains will be automatically computed by NOVA in further instructions based on this E_{peak} value.

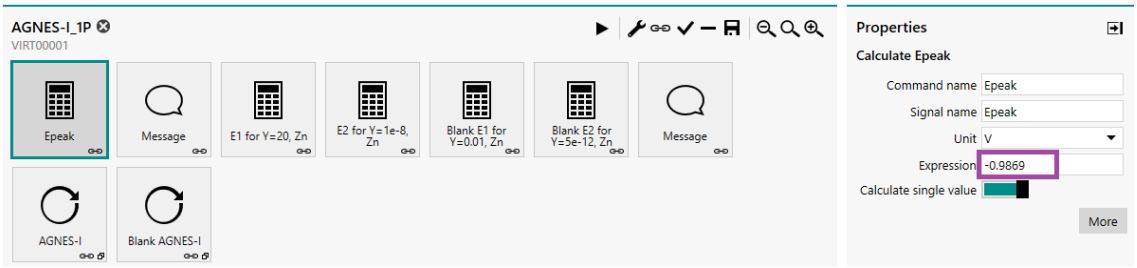


Figure S5. Command outlined in green (left) contains the function to specify which is the value of the peak potential. The box outlined in purple (right) contains the value (in this case, "Epeak" is set to -0.9869 V).

B.1.2. **The second command** of the type "Calculate signal" (which is the third command in the list of AGNES-I_1P procedure), labelled **"E1 for Y=20, Zn"** in Figure S6, computes the deposition potential E_1 associated to Y=20 for Zn. One can change the desired gain Y from 20 (the value set now) to any other value, both, in the label and within this "Calculate signal" command. For the computation of E_1, this command uses the value of E_{peak} introduced in the previous command "Epeak" as prescribed by an existing link. Notice that the icons of commands involved in links display a small chain in their right bottom corners (see details on links in subsection B.1.7. 9).

In the next paragraphs, we are going to justify why Y=20 was chosen for this exercise and how this Y value can be changed. Some readers might like to jump these details (in their first approach to AGNES) and move directly to the next subsection B.1.3.

When one can estimate a rough value for the expected free concentration, a suitable initial gain for an experiment can be computed by applying the basic AGNES equation

$$I = \eta Y \left[\mathrm{Zn}^{2+} \right] \tag{2}$$

while solving for *Y*. It is known that a typical η value for AGNES-I with HMDE (for Zn, Cd and Pb) is around 0.002 A L mol^{-1}. (Notice that an accurate value of η for the experiments is found from a calibration). It is also known that a current sufficiently above the limit of quantification with HMDE is 5.0×10^{-8} A. At pH 4 with no added ligand, most of Zn^{2+} is free in a solution, so that one can approximate $[Zn^{2+}] = c_{T,Zn}$. Introducing these estimations in eqn. (2) one obtains that a minimum suitable gain would be:

$$Y = \frac{I}{\eta \left[\mathrm{Zn}^{2+} \right]} = \frac{5\times10^{-8}}{0.002\times10^{-5}} = 2.5 \tag{3}$$

It is customary to use nominal (initially intended) gain values in the (euro) monetary scale (1, 2, 5, 10, 20, 50, 100…), so that, now we conclude that *Y*=5 would be enough to obtain a minimum charge. However, a larger gain might be safer. The "rule of thumb" in HMDE to estimate minimum deposition times (in solutions with just a divalent metal and no complexation) recommends

$$t_\mathrm{l} - t_\mathrm{w} = 7Y \tag{4}$$

where the result is in seconds. For instance, 35 s are needed for *Y*=5. To be on the safe side (with larger and more robust currents), here we decided **to use *Y*=20**, whose minimum (estimated) **deposition time of 140 s** is quite endurable. We recall here that the experimental minimum deposition time in more involved systems depends on more factors like the type of electrode and the matrix (especially if there is complexation).

To change the desired *Y* (from 20 to any other value), click on the **option "More"** seen in the right bottom corner of the upper part of Figure S6, pointed by the purple arrow. **In the pop-down window**, one can see a variant of formula (1) where the deposition potential E_1 has been solved in terms of the variable E_{peak} (previously fixed). **The number 20** (underlined in purple to be more clearly recognized) **can be changed with 50, 100, etc** as desired, to prescribe other gains. For a perfect book-keeping, we recommend adapting the label of the command which now is "E1 for Y=20 Zn" to indicate the corresponding gain. Notice that, when working with other analytes (such as Cd or Pb), the diffusion coefficients should also be changed.

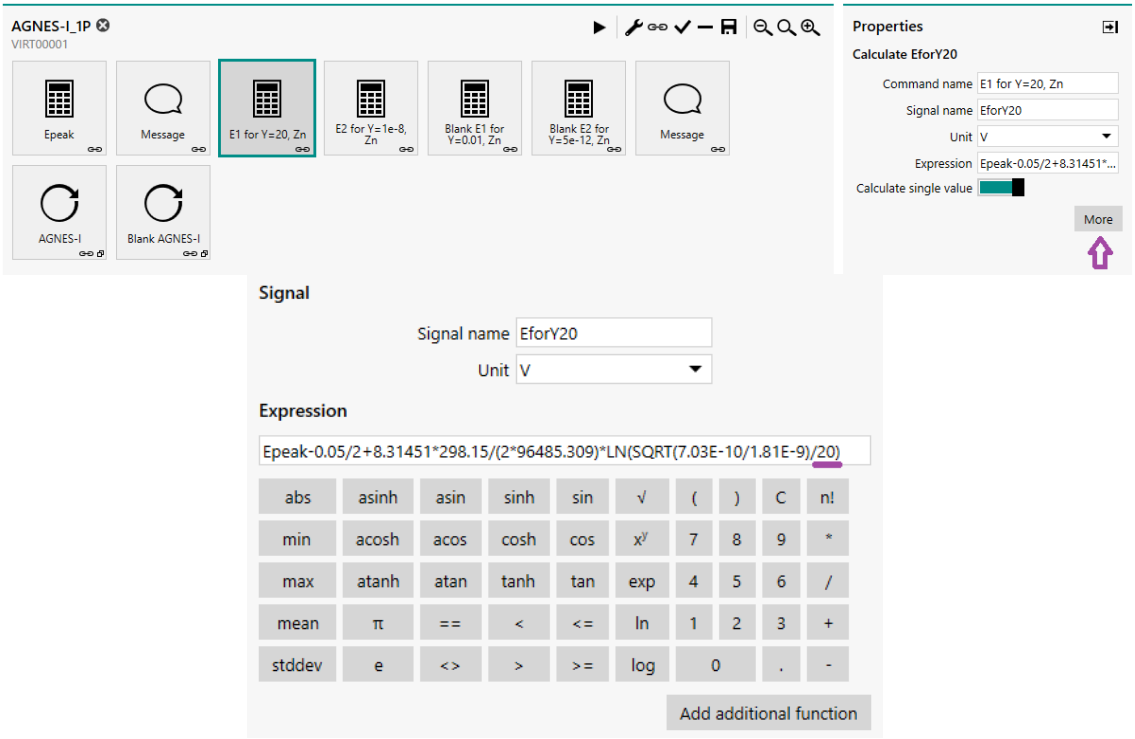


Figure S6. Third command of the type "Calculate signal", labelled "E1 for Y=20, Zn" that sets *Y*=20 (with the previously given E_{peak}) for Zn analysis at 25°C.

B.1.3. The third command of the type "Calculate signal", labelled "E2 for Y=1e-8, Zn", computes a sufficiently less negative potential E_2, so that reoxidation proceeds under diffusion limited conditions and AGNES-I quantification becomes possible. The

standard value corresponds to a gain (also called Y_2) of 1.0×10^{-8}, so that, typically, the user does not need to change anything here.

B.1.4. In the fourth command of the type "Calculate signal", labelled "Blank E1 for Y=0.01, Zn", the deposition potential for the shifted blank (accounting for currents other than the faradaic one of the analyte) is computed. This blank will be subtracted from the measurement current. The standard taken value is Y= 0.01, so that, typically, the user does not need to change anything here.

B.1.5. The fifth command of the type "Calculate signal" labelled "Blank E2 for Y=5e-12, Zn", computes the reoxidation potential for the blank. Typically, nothing needs to be changed.

For any command of the type "Calculate signal", changes can be done by clicking on the "More" button, as seen in Figure S6.

B.1.6. In between the first and second command of the type "Calculate signal" and after the fifth one, there is a command of the type "Message", so that the user can verify the gain and potential (E_1) being applied. See Figures S7 and S8. Typically, nothing needs to be changed.

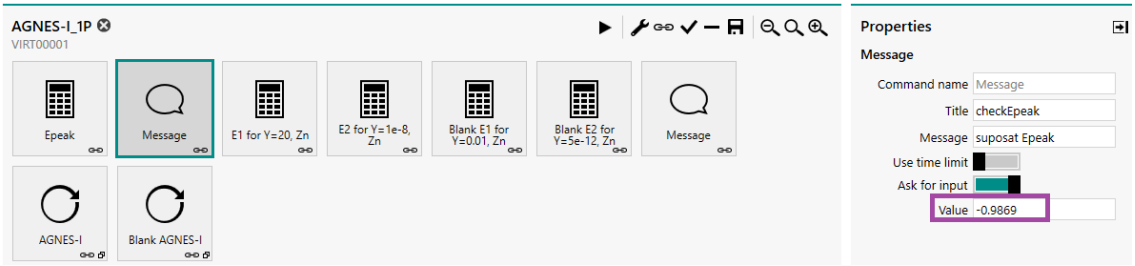


Figure S7.Command of "Message" type showing that the previously introduced E_{peak} value is -0.9869 V.

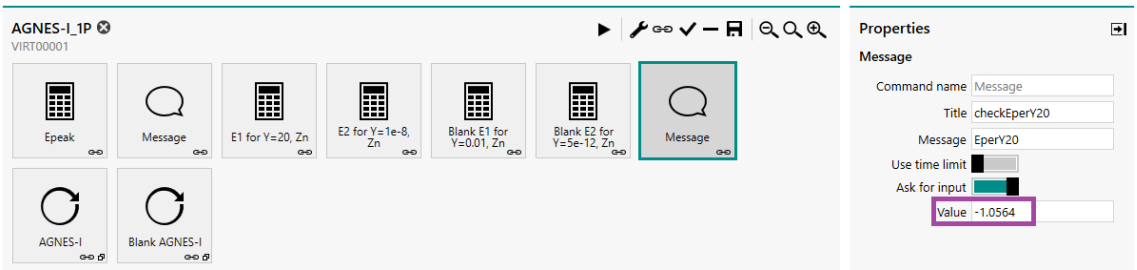


Figure S8.Command of the type "Message" detailing that the computed deposition potential (with the introduced gain and E_{peak}) is E_1 = -1.0564 V.

B.1.7. Then, the next two commands are of the type "Repeat" (see items in the second row of the procedure "AGNES-I_1P" in Figures S5 or S9). The first command has been labelled "AGNES-I" and provides the (main) measurement. The second one, "Blank AGNES-I", corresponds to the shifted blank. Each measurement is performed twice (see highlighted rightmost box in Figure S9). The response function (that will be processed with eqn. (2)) is the faradaic current I computed as the difference between the main measurement and the blank one.

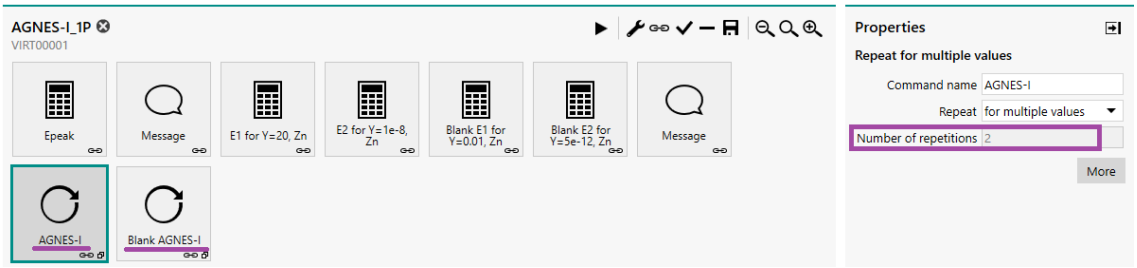


Figure S9. "Repeat" type commands: "AGNES-I" (for main measurement) and "Blank AGNES-I" (for shifted blank).

B.1.7.1 The command "AGNES-I" (of the type "Repeat") can be opened by clicking and then by accessing to the "Build text" type command (not re-labelled), see third line in Figure S10. In the right box, one can **check or modify the path where the files will be saved and the specific name of each generated file**. Next to the "Build text" command (see third line in Figure S10), there is a grouping of commands labelled "AGNES-I Procedure" with the set of instruction to run an AGNES measurement.

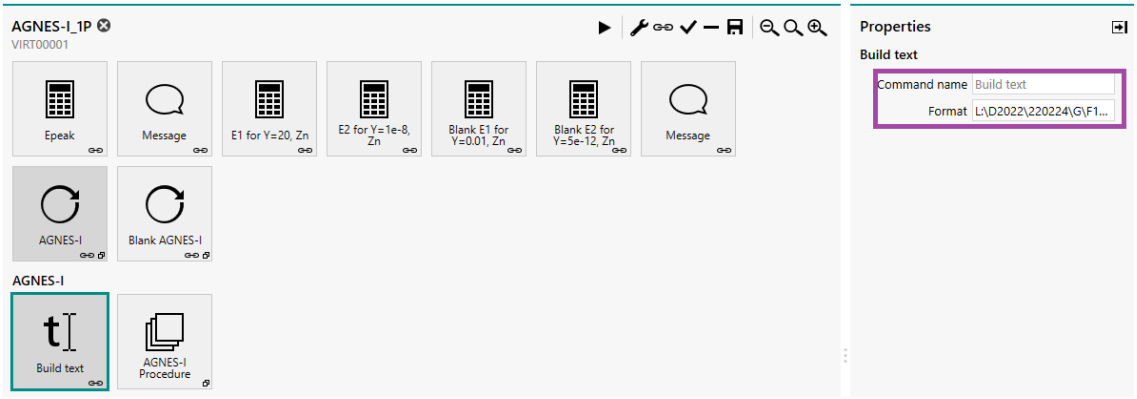


Figure S10. "Build text" command to set the path where files will be saved, followed by the grouping of commands to run and save the main AGNES-I measurements (see third row of commands).

B.1.7.2. Within "AGNES-I Procedure" (see fourth row in Figure S11), there are some commands of the type "Measurement-general", which allow various settings without performing (yet) any actual measurement. The command "Autolab control" specifies hardware adjustments, such as stirrer ON/OFF, drop formation, and waiting times in between processes. Typically, nothing needs to be changed.

B.1.7.3. The command (of the type "Apply") labelled "Apply E1" specifies E_1. There is no need to change here anything, as NOVA will use the value computed in the previous command "E1 for Y=20, Zn" as prescribed by the existing links. Recall that the icons of commands involved in links display a small chain in their right bottom corners. In this particular instance, E_1 is -1.0564 V (see highlighted box in Figure S11).

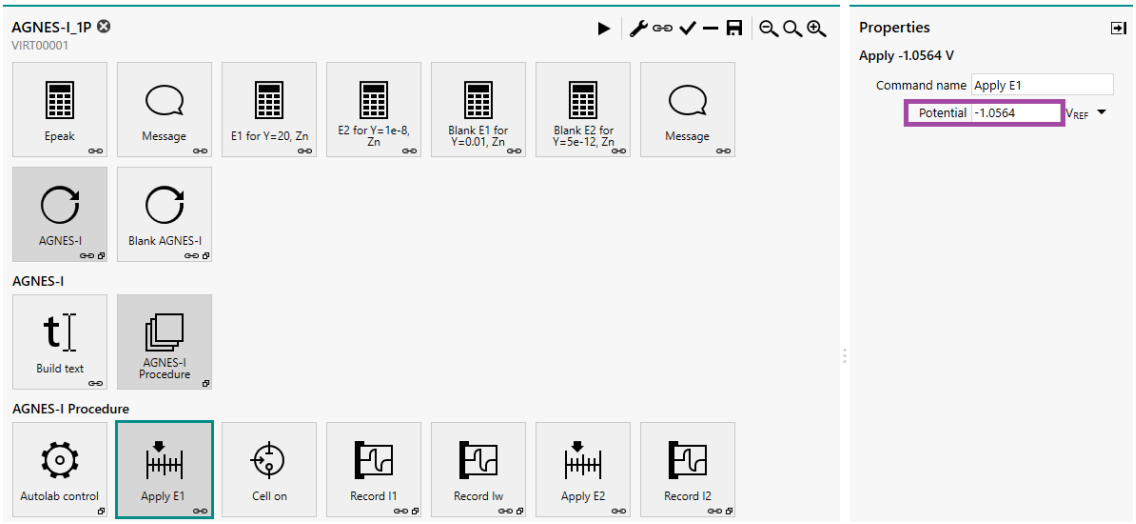


Figure S11. Command "Apply E1" where the potential E_1 to be applied (during the deposition stage of AGNES-I) is indicated for the recording instructions that follow after this command.

B.1.7.4. The command labelled "Record I1", of the type "Record signal" (see fourth row in Figure S11), measures the currents during the deposition time with stirring, denoted t_1-t_w. **This deposition time has to be indicated** in the right box (see purple arrow with the example of 100 s in Figure S12). By default, the interval time is 2 s.

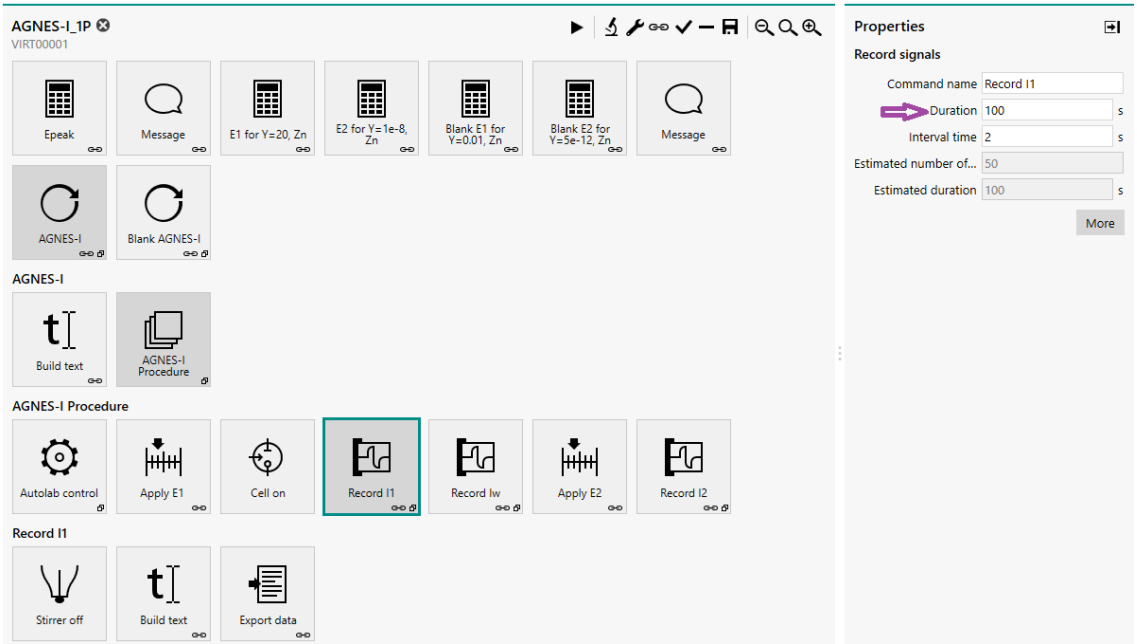


Figure S12. Details for the grouping of commands "Record I1" (fourth and fifth rows) to apply E_1 and record the generated currents. The purple arrow indicates the box where the crucial time t_1-t_w (i.e. the deposition time with stirring in AGNES-1P) has to be prescribed.

B.1.7.5. The following commands of type “Record signal” in the fourth row of Figure S11 is labelled “Record Iw”, to measure the currents during the resting or waiting time (without stirring), denoted t_w. A value of 50 s is sufficient in HMDE. By default, the interval time is 2 s.

B.1.7. 6. The following command, “Apply E2” (see Figure S13), sets that the next potential to be applied is the reoxidation potential E_2. This potential is automatically taken from previous calculations through a link. In this specific case, E_2 turns out to be -0.7813 V (corresponding to a reoxidation gain Y_2=10^{-8}).

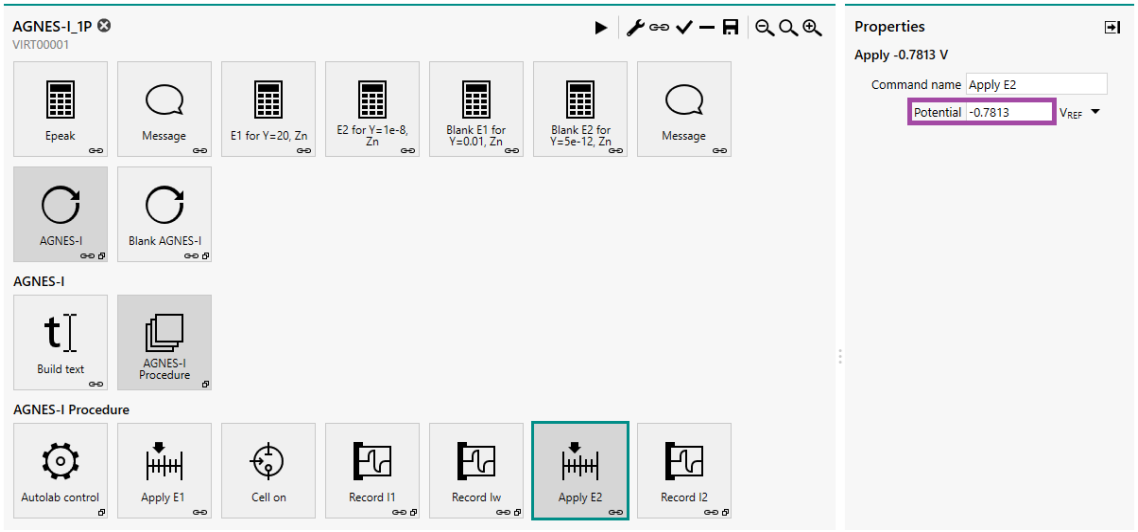


Figure S13. Command “Apply E2” to indicate the reoxidation potential in the second stage of AGNES-I.

B.1.7.7. The next command in the last row of Figure S13 is “Record I2”. Here, the total time of application of the reoxidation potential, t_2, is indicated. Typically, t_2 is 50 s. The main difference is that the frequency of data sampling is increased by indicating an interval time of 0.05 s, so that, in 50 s, 1000 intensity currents are taken (see Figure S14). So, there is usually no need to change anything in this command.

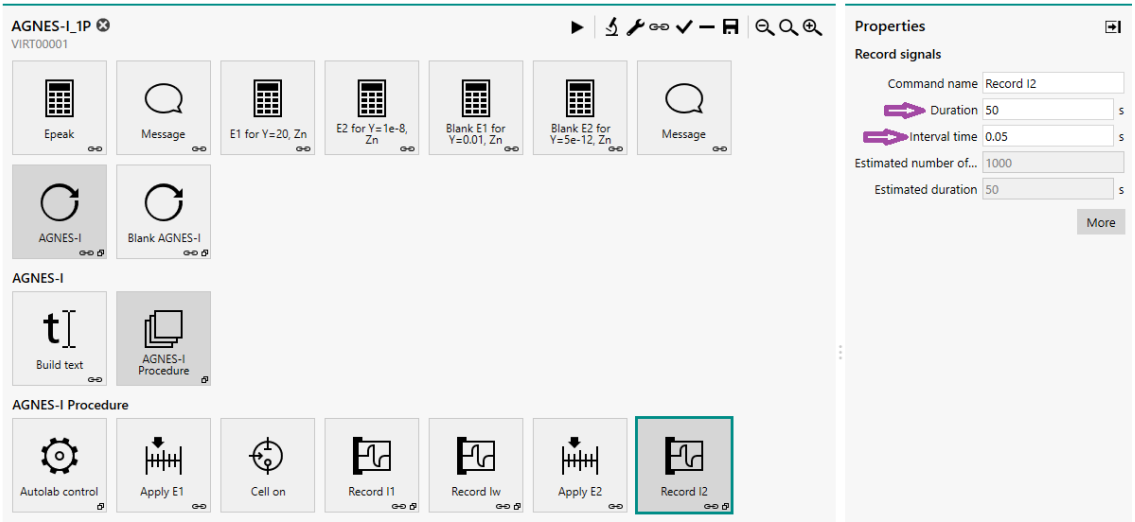


Figure S14. Command "Record I2" corresponding to the second or quantification stage in AGNES-I. The purple arrows indicate the typically used times.

B.1.7.8. The grouping of commands "Blank AGNES-I" (aimed at measuring the shifted blank current), in the second row of Figure S14, contains the same commands as the (previous) grouping "AGNES-I", but with "shifted" deposition and quantification potentials (automatically computed in previous commands such as "Blank E1 for Y=0.01, Zn"). In this specific case, the deposition potential is -0.9588 V (for Y=0.01) and the reoxidation potential is -0.6837 V (for Y_2=5.0×10^{-12}).

B.1.7. 9. Details on links

As already commented, the potentials to be applied are computed from the introduced E_{peak}-value and the indicated gains in commands of the type "Calculated signal". These values of the potentials are transferred to commands of the type "Apply" via links. See Figures S15 and S16. The link function is imposed by selecting the commands of interest and clicking the "Link" tool ( ) that is located at the upper right part of the procedure window in NOVA. For a standard use of this procedure, these links should not be changed.

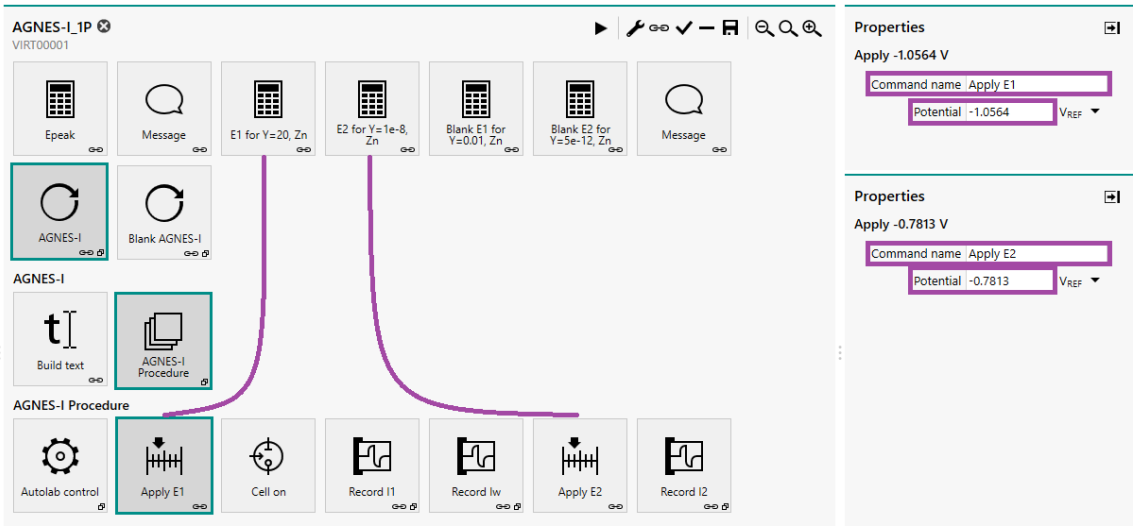


Figure S15. Schematic representation of the links between commands for the (main) measurement transferring E_1 and E_2 from "Calculate signal" commands to "Apply commands" (see specific values in the highlighted boxes).

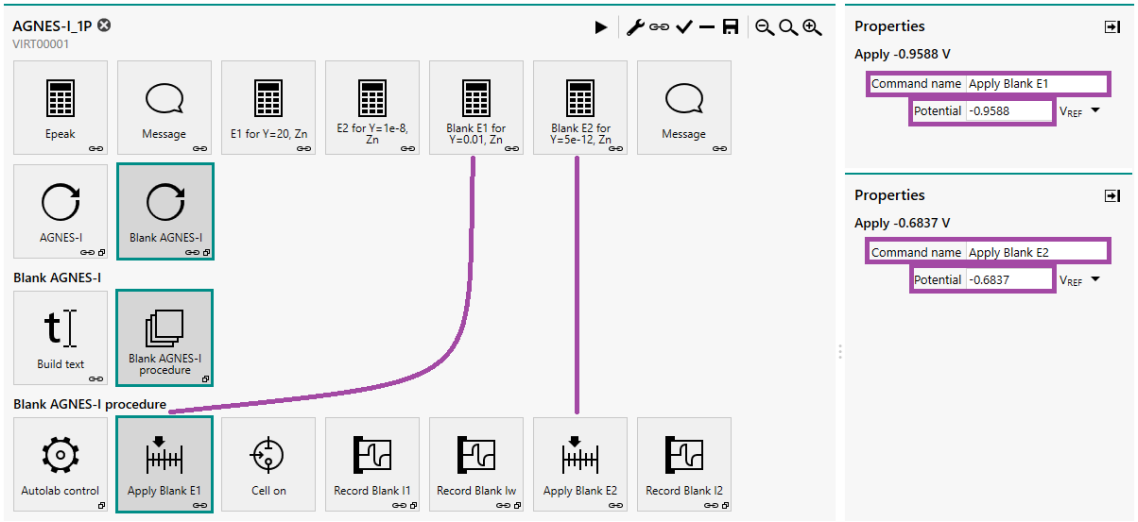


Figure S16. Schematic representation of the links between commands for the shifted blank measurement transferring E_1 and E_2 (see specific values in the highlighted boxes).

B.1.7.10. Auxiliary Excel file 210716_G_Trajectory.xls

One can use the sheet "Calculs Zn" in the file 210716_G_Trajectory.xls, to: 1) analyze possible values of potentials for each stage and/or 2) double-check that the values in NOVA and in the Excel file agree (i.e. so that there are no errors). Eqn. (1) has been introduced in the sheet, together with constants and other parameters. If working with analytes other than Zn, the values of the diffusion coefficients 1.81×10^{-9} and 7.03×10^{-10}

(cells B9 and B10) have to be changed (see Figure S17). If working with In or Sb, the number of exchanged electrons (cell B5) has to be changed to 3. Constants are gathered in column B from cells B4 to B10. The value of E_{peak} from the DPP is in cell B15. The values of possible prescribed gains are in the region A18:A22, while at their right (region B18:B22), their corresponding potentials are computed. Typically, one changes the value of the gain in cell A18 (now is Y=20) and obtains the associated potential computed in cell B18.

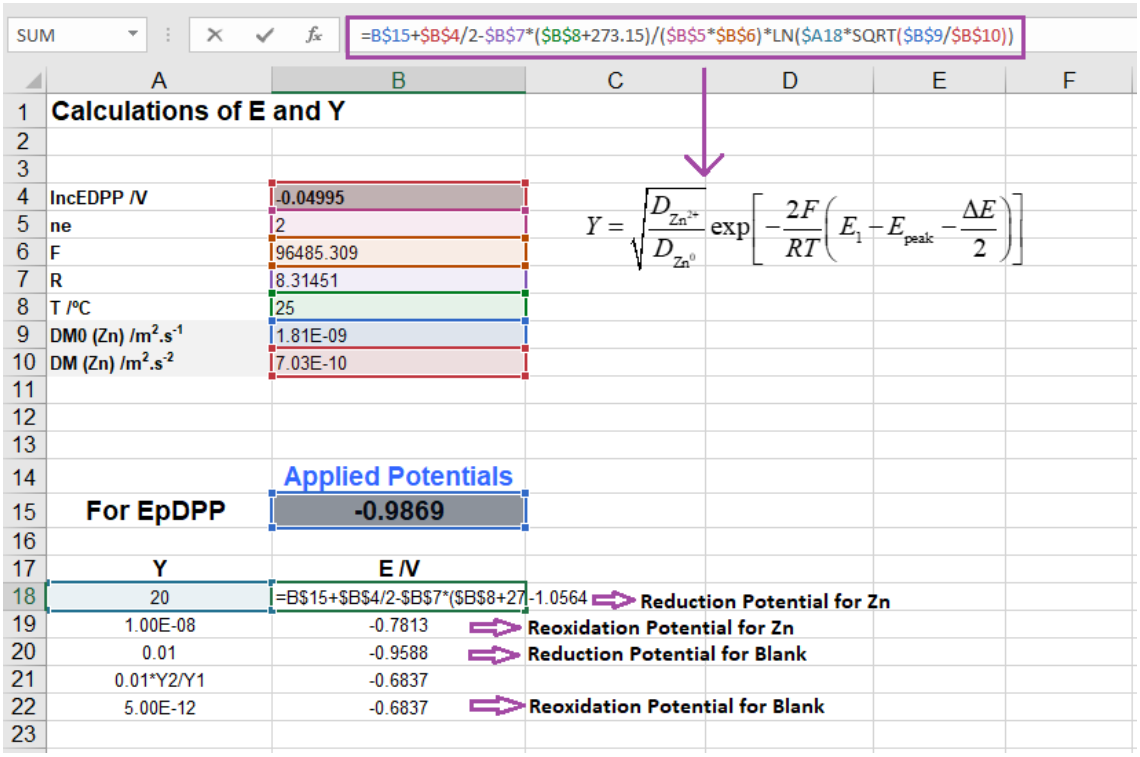


Figure S17. Screenshot of the auxiliary Excel file 210716_G_Trajectory.xls, sheet "Calculs_Zn" relating gains with potentials for any stage and any kind of measurement (main and blank).

B.2.- Run the procedure, save results and process them

B.2.1. Once the procedure has been updated with the desired parameters (see section B.1), the NOVA script to obtain one point of AGNES (two replicates of the main

measurement and two replicates of its shifted blank) can be run by **clicking on the Start button (►)**.

B.2.2. The currents measured along each of the "Record signal" type commands will automatically be saved in the indicated path with a coded name.

Table 1. Coded name for each file generated in each stage of AGNES-I.

"Record signal"	**Coded name (letter at the end of the compound name)**	**AGNES-I substage**
I1	P	Deposition with stirring, E_1 is applied during time t_1-t_w.
Iw	W	Deposition without stirring, E_1 is applied during time t_w.
I2	It does not have any letter	Quantification, E_2 is applied during time t_2.

So, a good book-keeping is to create (e.g. with Windows Explorer) a folder for some set of experiments (for instance, a trajectory, see next section below). The setting of the path has been detailed in section B.1.7.1

The names of the files are automatically generated (see Figure S18). The file name, for the shown case, starts with:

- the letter "Y" followed by the value of the gain (e.g. "20") + the letter "T" (for time) + the number of seconds of t_1-t_w, if it is from the main measurement

- the letter "B" followed by the value of the gain (e.g. "20"), if it is from a blank.

The central part of the names contains:

- the letter "M" that refers to metal + the letter "N" + the number of repetition (1 and 2 in this example).

Recall that this name is specified in the first command type "Build text" as mentioned in section B.1.7.1, at the end of the name of the save path. For details, see Figure S18.

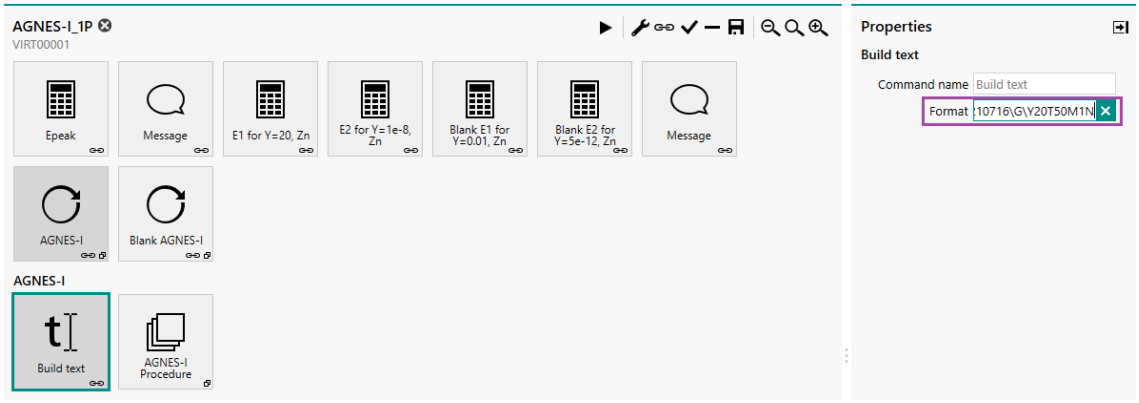


Figure S18. Command "Build text" to generate the name of the file (see right-hand-side highlighted box).

The overall name used to save the data recorded in a Record signal (see Figure S19) is defined in a "Build text" command that specifies the format items "{0}{1}" (for details, see page 230 in NOVA_2.1.5_User_Manual.pdf"), where {0} is linked to the name of the file, stated in the previous "Build text" command, {1} is linked to the repetition number, and afterwards, a letter is added according to the AGNES-I stage (P or W), as shown in Table 1.

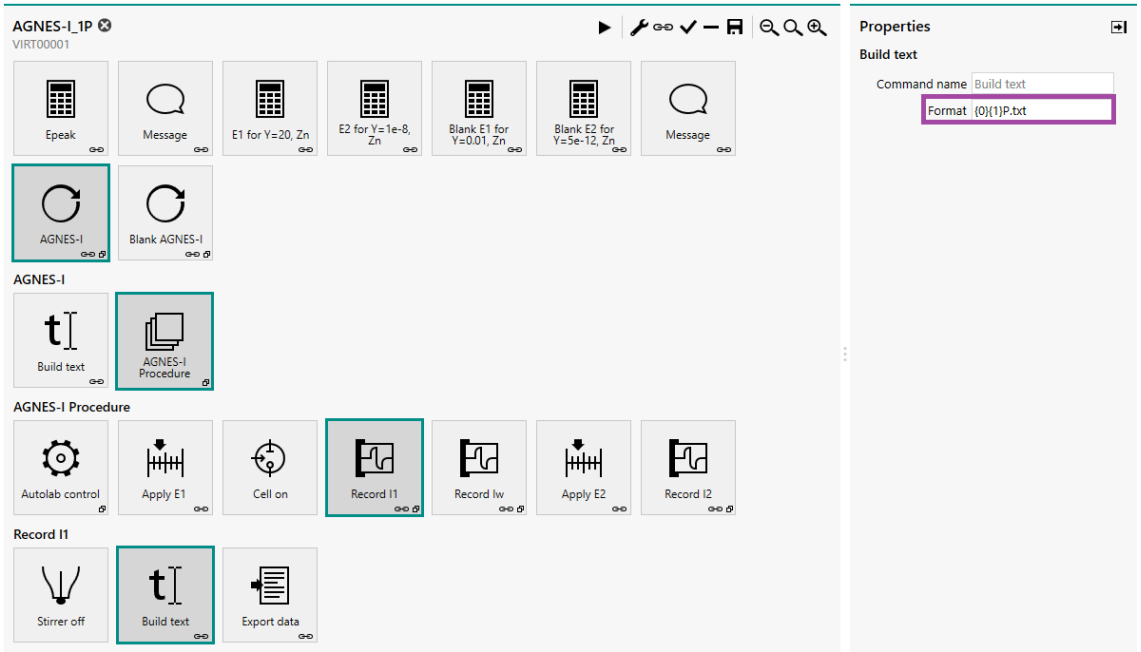


Figure S19. Command "Build text" to generate the name of the file (that is going to contain the results of the command "Record I1") by combining "{0}{1}P.txt" (see right-hand-side highlighted box)

The data are saved by NOVA with the command “Export data” (see Figure S20). No need to introduce any change here.

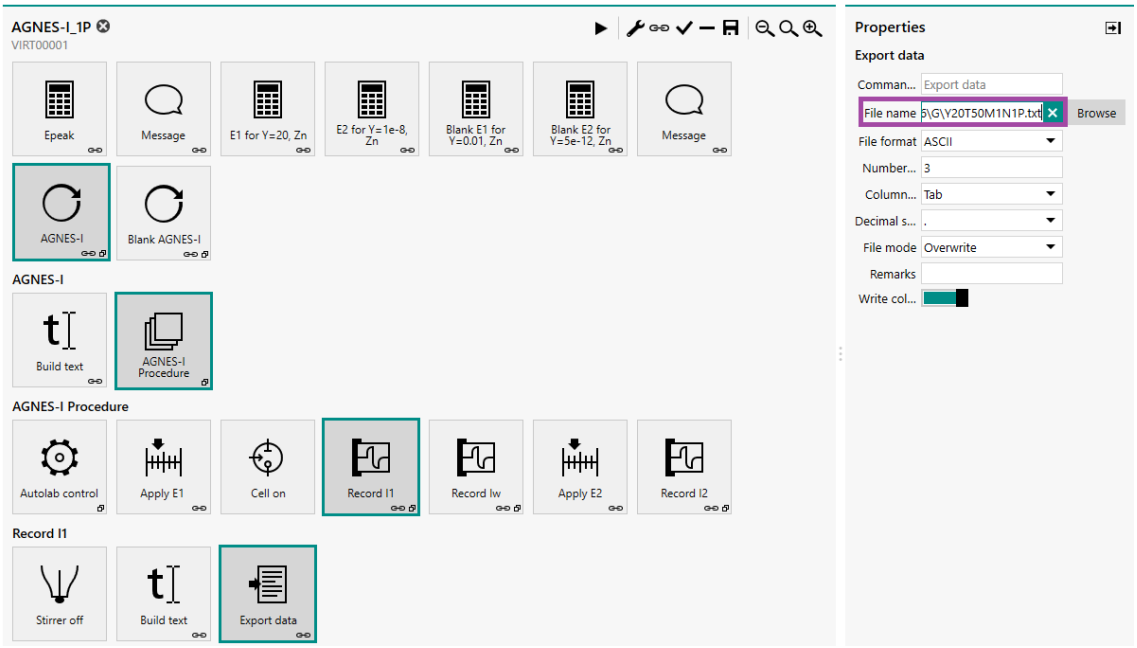
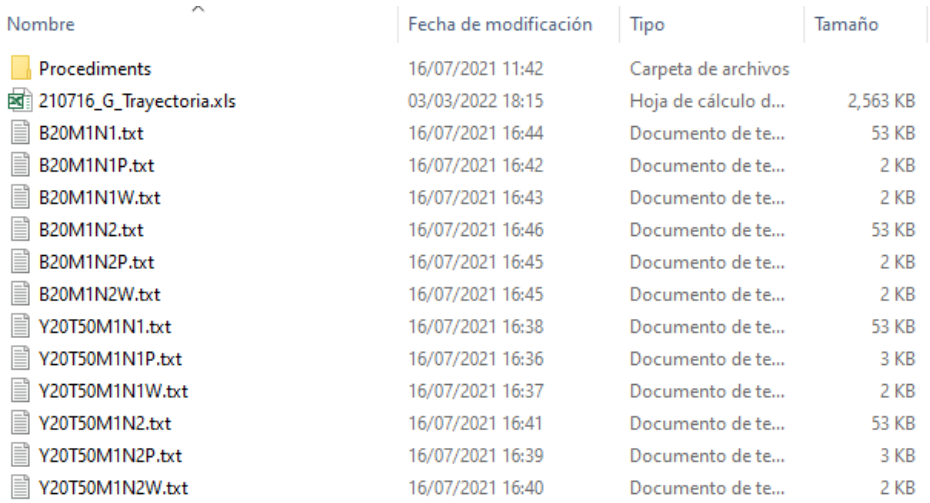


Figure S20. Command “Export data” of “Record I1” where one can see the name of the file with its path, “L:\D2021\210716\G\Y20T50M1N1P.txt” in this specific case.

In Figure S20, the file (ended in “P”, before the “.txt”) contains the data for the deposition substage with stirring. For the next files to be saved, the combination “{0}{1}W.txt” will yield “L:\D2022\210716\G\Y20T50M1N1W.txt” for the waiting substage filename, while “{0}{1}.txt” will yield “L:\D2021\210716\G\Y20T50M1N1. txt” for the reoxidation stage file name.

The number of files generated for one point is 12, resulting from two replicates for two measurements (main and shifted blank) and three (sub)stages (deposition without stirring, waiting and reoxidation). See Figure S21 with an example of the files for the point at t_1-t_w=50 s (and Y=20). This keeping of all the information about each run allows the user to easily access any specific data if needed (e.g. the analysis of the currents in the waiting step, “W”, can help in the diagnosis of a too high level of oxygen), although

-in a standard use- **only the data corresponding to the second stage (no added letter) are the ones used to compute the free concentrations.**



Nombre	Fecha de modificación	Tipo	Tamaño
Procediments	16/07/2021 11:42	Carpeta de archivos	
210716_G_Trayectoria.xls	03/03/2022 18:15	Hoja de cálculo d...	2,563 KB
B20M1N1.txt	16/07/2021 16:44	Documento de te...	53 KB
B20M1N1P.txt	16/07/2021 16:42	Documento de te...	2 KB
B20M1N1W.txt	16/07/2021 16:43	Documento de te...	2 KB
B20M1N2.txt	16/07/2021 16:46	Documento de te...	53 KB
B20M1N2P.txt	16/07/2021 16:45	Documento de te...	2 KB
B20M1N2W.txt	16/07/2021 16:45	Documento de te...	2 KB
Y20T50M1N1.txt	16/07/2021 16:38	Documento de te...	53 KB
Y20T50M1N1P.txt	16/07/2021 16:36	Documento de te...	3 KB
Y20T50M1N1W.txt	16/07/2021 16:37	Documento de te...	2 KB
Y20T50M1N2.txt	16/07/2021 16:41	Documento de te...	53 KB
Y20T50M1N2P.txt	16/07/2021 16:39	Documento de te...	3 KB
Y20T50M1N2W.txt	16/07/2021 16:40	Documento de te...	2 KB

Figure S21. Snapshot of the list of files generated when running the procedure AGNES-I_1P for Y=20. The letter "B" stands for blank. The letter "T" indicates the time t_1-t_w, the letters "M1" indicate that only one metal addition has been performed in the solution, the letter "N" is associated to the replicate 1 or 2. The letter "P" indicates first stage with stirring. The letter "W" indicates the waiting sub-stage (without stirring). The nox files for each procedure that is created in this case were stored in the "Procediments" folder.

B.2.3. Gathering of data.

The data (time and current) from the saved files can be gathered in an Excel file, with one specific tab for the data of the deposition with stirring (named "Proc1"), another for the data of the waiting sub-stage (named "Procw") and another one for the second stage (named "Proc2"). The currents coming from one file (whose label is in row 5 of the receiving Excel file, see Figure S22) are transferred in one column (of tabs Proc1, Procw or Proc2). The main data treatment is performed with the sheet "Proc2", so that we also include in this sheet other information, such as the value of the applied gain, the deposition time, etc. (see rows 7 and 8 in Figure S22).

To transfer the times or currents from one raw-data file to a column in one Excel sheet, one can open this file from Excel clicking on File, Open, in Browse choose the file of

interest saved in format txt, click on Open, mark the option Delimited, click on Next, mark the option delimited by Space, then Next and Finish.

In the just imported file, select the first column (to transfer times) or the second column (to copy currents). Paste these data into the suitable column of the receiving sheet (e.g. to "Proc2"): column A for the times or the column corresponding to the label indicated in row 5 (e.g. column C for the second replicate of the main measurement, coded "Y20T50M1N2" in cell C5). An Excel macro to automatically transfer these data is available from the authors upon request.

	A	B	C	D	E	F	G
1	L:\D2021\210716\G						
2	.txt						
3							
4							
5		Y20T50M1N1	Y20T50M1N2	B20M1N1	B20M1N2	Y20T100M1N2	Y20T100M1N3
6	cM /M	9.74E-6	9.74E-6	9.74E-6	9.74E-6	9.74E-6	9.74E-6
7	Y	20	20	20	20	20	20
8	t1-tw /s	50	50			100	100
9	I (t2=0.2) - Ilim /A	4.96E-7	5.05E-7	1.34E-9	1.38E-9	5.64E-7	5.63E-7
10	Ibshifted	1.36E-9	1.36E-9			1.36E-9	1.36E-9
11	I-Ibshifted	4.94E-7	5.04E-7			5.63E-7	5.62E-7
12	Q /C	4.25E-7	4.34E-7	1.25E-9	1.31E-9	4.99E-7	4.97E-7
13	Qb /C	1.28E-9	1.28E-9			1.28E-9	1.28E-9
14	Q-Qb	4.24E-7	4.33E-7			4.98E-7	4.95E-7
15	η	2.54E-3	2.59E-3			2.89E-3	2.88E-3
16	ηQ	2.18E-3	2.22E-3			2.56E-3	2.54E-3
17	I /µA	0.496	0.505	0.001	0.001	0.564	0.563
18							
19		Y20T50M1N1	Y20T50M1N2	B20M1N1	B20M1N2	Y20T100M1N2	Y20T100M1N3
20	0.05	1.00E-06	1.00E-06	4.59E-09	4.82E-09	1.00E-06	1.00E-06
21	0.10	8.20E-07	8.26E-07	1.74E-09	1.82E-09	9.11E-07	9.12E-07
22	0.15	6.16E-07	6.24E-07	1.15E-09	1.17E-09	6.89E-07	6.88E-07
23	0.20	4.95E-07	5.05E-07	8.73E-10	9.37E-10	5.64E-07	5.62E-07
24	0.25	4.13E-07	4.24E-07	6.53E-10	7.08E-10	4.80E-07	4.79E-07
25	0.30	3.52E-07	3.64E-07	4.97E-10	6.26E-10	4.20E-07	4.19E-07
26	0.35	3.06E-07	3.17E-07	3.88E-10	4.61E-10	3.74E-07	3.72E-07
27	0.40	2.71E-07	2.80E-07	3.36E-10	4.18E-10	3.37E-07	3.34E-07
28	0.45	2.45E-07	2.52E-07	2.50E-10	3.23E-10	3.05E-07	3.03E-07
29	0.50	2.24E-07	2.30E-07	1.98E-10	3.33E-10	2.79E-07	2.77E-07

Zn | G_Proc1 | Proc1 | G_Procw | Procw | G_Proc2 | Proc2 | G_I_vs_t1 | Calculs_Zn

Ready

Figure S22. Snapshot of the file 210716_G_Trajectory.xls, sheet "Proc2" where results from the second stage of various experiments are gathered. The folder (with path) of the recorded (raw data) files is in the cell A1. Purple vertical arrows indicate relevant sheets to compile data corresponding to a given (sub)stage. The green arrow and underlines indicate the deposition time without stirring t_1-t_w (e.g. what is changing, from one point to the other, in a trajectory). The blue arrow and underlines indicate the current at the selected time (usually 0.2 s) corrected (to account for a possibly-varying small oxygen interference) with the residual current I_{lim}. To obtain the faradaic current in row 11 (pink arrow and underlines), the current of the corrected shifted blank (in row 10) is subtracted from row 9.

B.2.4. Process the data.

The processing depends on the type of set of experiments performed. See section C for the specific case of a trajectory. **Typically, only data in the sheet "Proc2" is processed**, while sheets "Proc1" and "ProcW" are analysed only when specific problems call for additional checks.

The key information one is looking for is the faradaic current I (at a fixed time t_2) which will enable to apply eqn. (2), which we repeat here:

$$I = \eta Y \left[\mathrm{Zn}^{2+} \right] \tag{5}$$

Several t_2 could be taken, but, in HMDE, t_2=0.2 s has been shown to be optimal [1]. With the typical interval time of 0.05 s, this measurement time of 0.2 s corresponds to the fourth value in the file of the second stage (e.g. in "Y20T50M1N2.txt" or in "B20M1N2.txt") whose values have been copied in row 23 of the sheet "Proc2". Let's call $I(t_2=0.2)$ to the value of the (main or blank) current at t_2=0.2 s (e.g. cell C23 in Figure S22).

Let's call I_{lim} to the average residual ("limit") current for the longest times in the reoxidation step (e.g. around 50 s). This current is essentially due to traces of oxygen which might slightly change from one experiment to another. We consider this I_{lim} current as the baseline over which other currents are measured. In calculated cells in row 9, I_{lim} is computed as an average of the last 20 currents of the reoxidation stage. So, for instance, cell C9 shown in Figure S22 corresponds to

$$I\left(t_2 = 0.2\right) - I_{\mathrm{lim}} = 5.05 \times 10^{-7}\ \mathrm{A} \tag{6}$$

for the second replicate of the main measurement at t_1-t_w=50 s (and Y=20).

Analogously, one can see (cell D9) that

$$I_{sb}\left(t_2 = 0.2\right) - I_{lim,sb} = 1.34\times10^{-9}\ \text{A} \quad (7)$$

for the first blank (the subscript "sb" indicates that these are values for the shifted blank). The values of the two blanks (e.g. in cells D9 and E9) are averaged. For instance, their (common) average blank value is copied in cells B10 and C10.

To obtain the faradaic current, one has to subtract the (average) current of the blank from the main measurement:

$$I = I\left(t_2 = 0.2\right) - I_{lim} - \left\langle I_{sb}\left(t_2 = 0.2\right) - I_{lim,sb}\right\rangle \quad (8)$$

This *I* value (in row 11) is the sought faradaic current to be used in eqn. (2). If I_{lim} is essentially constant, one can simplify the faradaic current to

$$I = I\left(t_2 = 0.2\right) - \left\langle I_{sb}\left(t_2 = 0.2\right)\right\rangle \quad (9)$$

For instance, in cell C11 of Figure S22, expression (8) boils down to:

$$I = I\left(t_2 = 0.2\right) - I_{lim} - \left\langle I_{sb}\left(t_2 = 0.2\right) - I_{lim,sb}\right\rangle = 5.05\times10^{-7} - 1.36\times10^{-9} = 5.04\times10^{-7}\ \text{A} \quad (10)$$

C.-Protocol for a trajectory with 1 P in first stage and AGNES-I

As detailed in the main text of the article "AGNES (Absence of Gradients and Nernstian Equilibrium Stripping): an electroanalytical technique for chemical speciation. A tutorial review", a trajectory is a set of individual AGNES runs ("points") with a common gain and various deposition times over the same solution. The achievement of a plateau for sufficiently long deposition times is indicative of having reached the sought AGNES equilibrium.

One option to obtain a trajectory is to run the individual procedure AGNES-I_1P (or with other variants) for each of the desired t_1-t_w times. So, for each point of the trajectory, one must change the time t_1-t_w in the command labelled "Record I1". In this specific example, with the same conditions as in the previous protocol B (Y=20, $c_{T,Zn}$ 1.0×10^{-5} mol L^{-1}), we used times of 50, 100, 140, 200, 400 and 800 s. So, the original value 100 in **the "Duration" box, marked with a purple arrow, in Figure has to be changed**, in each run or point, with other values (50, 140, etc.).

After the **execution of two replicates of the (shifted) blank** at the fixed gain of the trajectory (Y=20 in this specific example) **and the six AGNES trajectory points** with two replicates of the main measurement (each with a different time t_1-t_w), 14 raw-data files will be generated.

For a trajectory, the (shifted) blank measurements can just be only executed after the first point of the trajectory (t_1-t_w = 50 s), because in the following points the system is not disturbed neither by opening the cell nor by changing the gain; therefore with a single measurement of the blank in duplicate is sufficient. Thus, in the procedure (see Figure S9 for the case of AGNES-I_1P), one can remove the grouping of commands "Blank AGNES-I" for the last 5 trajectory points.

To build a trajectory, one has to **plot the faradaic current** (see eqn. (8), data in row 11 of the Excel file shown in Figure S22) *versus* the deposition time t_1-t_w (which is changing, say from 50 s to 800 s), as done in Figure S23.

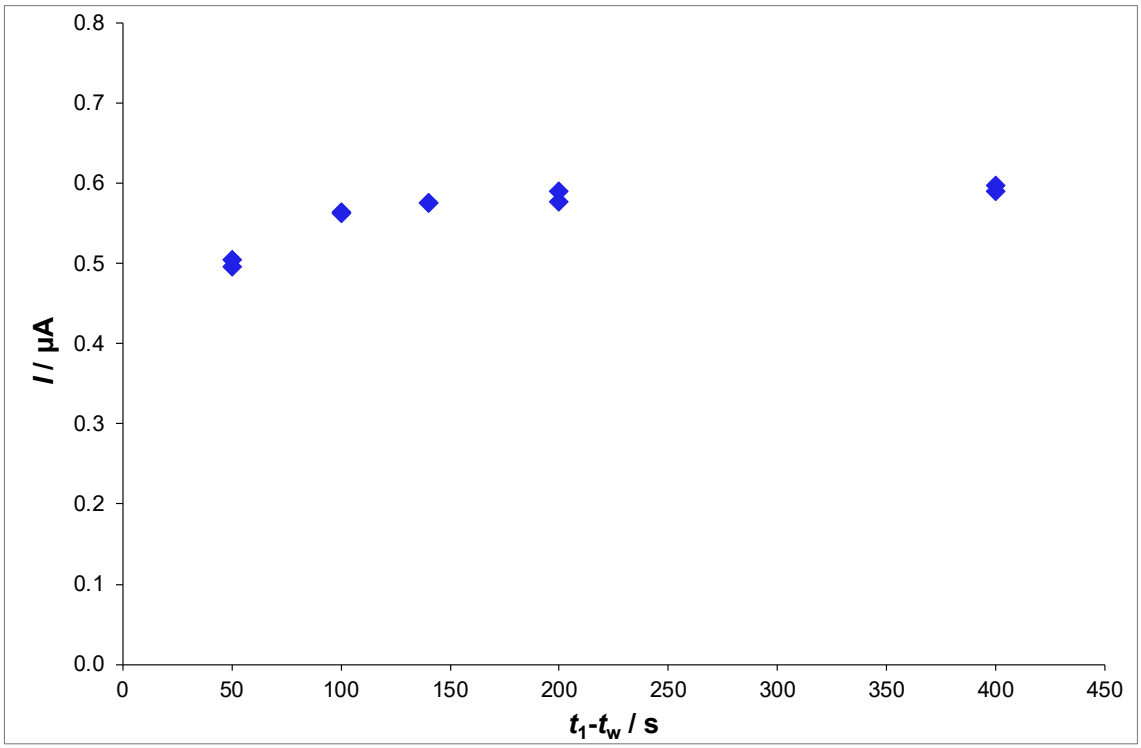


Figure S23. Trajectory for *Y*=20 (blue diamond markers) obtained in a solution with total Zn 1.0×10^{-5} mol L^{-1} in KNO_3 0.1 mol L^{-1}. Equilibrium is reached from 200 s onwards (see also tab G_I_vs_t1 in the Excel file 210716_G_Trajectory.xls).

D.-Protocol for a calibration with 1 P in the first stage and AGNES-I as variant for the second stage

D.1.- Description of the procedure

The calibration can be seen as the application of one-point experiments in each of the solutions prepared with known free Zn^{2+} concentrations. In NOVA version 2.1.5, if one wishes to use the variants 1P for the first stage and AGNES-I for the second stage, one can run the procedure detailed in section B (see Figure S12). Figure S24 shows the example of a calibration script with a safely higher gain (*Y*=20) with its standard deposition time (t_1-t_w=7×*Y*=140 s). In this procedure, the name identifying each Zn^{2+} addition (e.g. M1, M2…) should be changed according to the composition of the solution (see Figure S25).

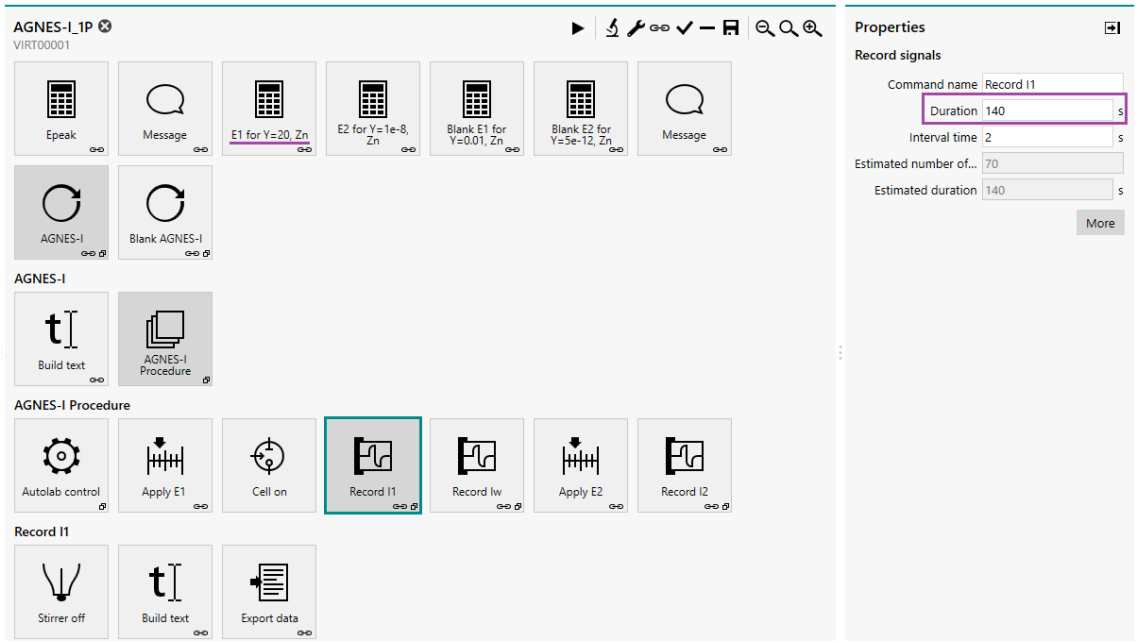


Figure S24. Details of a procedure in NOVA 2.1.5 to run a calibration. The purple box indicates the time t_1-t_w = 140 s.

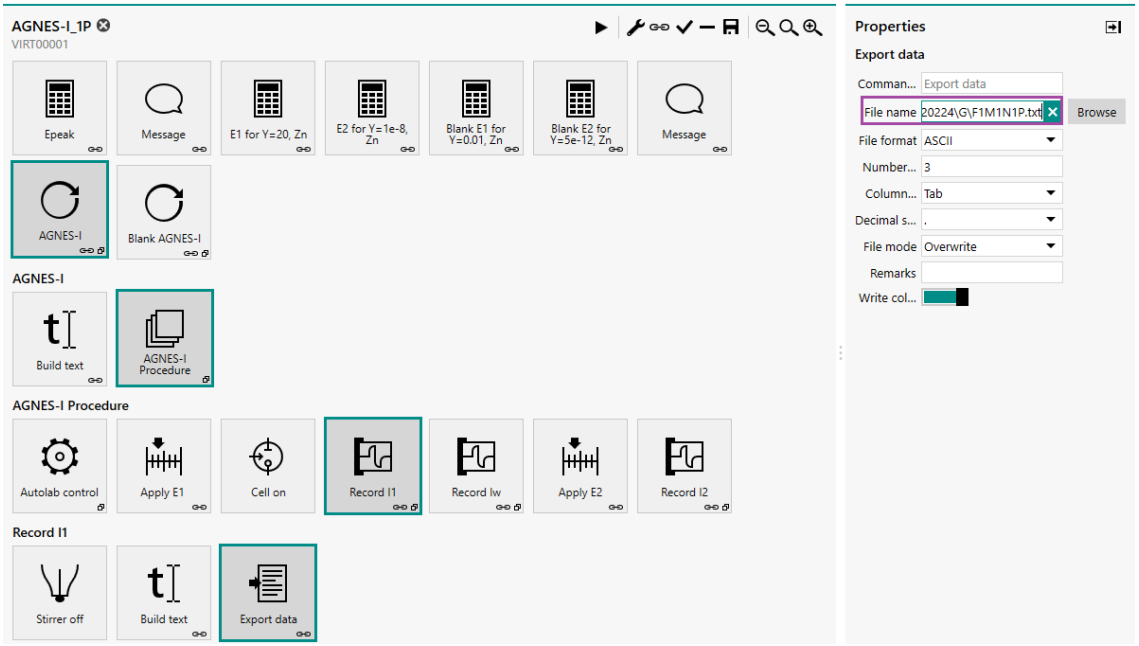


Figure S25. Details of a procedure in NOVA 2.1.5 to run a calibration. The purple box indicates the name of the file, where the number after M indicates the number of Zn^{2+} addition. In this example F1M1N1P results from F (a procedure of 1P in the first stage), M1 (for the first metal addition), N1 (for the first repetition) and P (first substage in the deposition process). It would change to F1M2N1P for the second addition, first replicate.

D.2.- Use of the procedure along the calibration.

D.2.1. Add 25 mL of 0.1 mol L^{-1} KNO_3 to the cell.

D.2.2. Do the first addition of Zn^{2+} (in this particular case 0.033 mL of a solution 1.53×10^{-3} mol L^{-1} in Zn^{2+}).

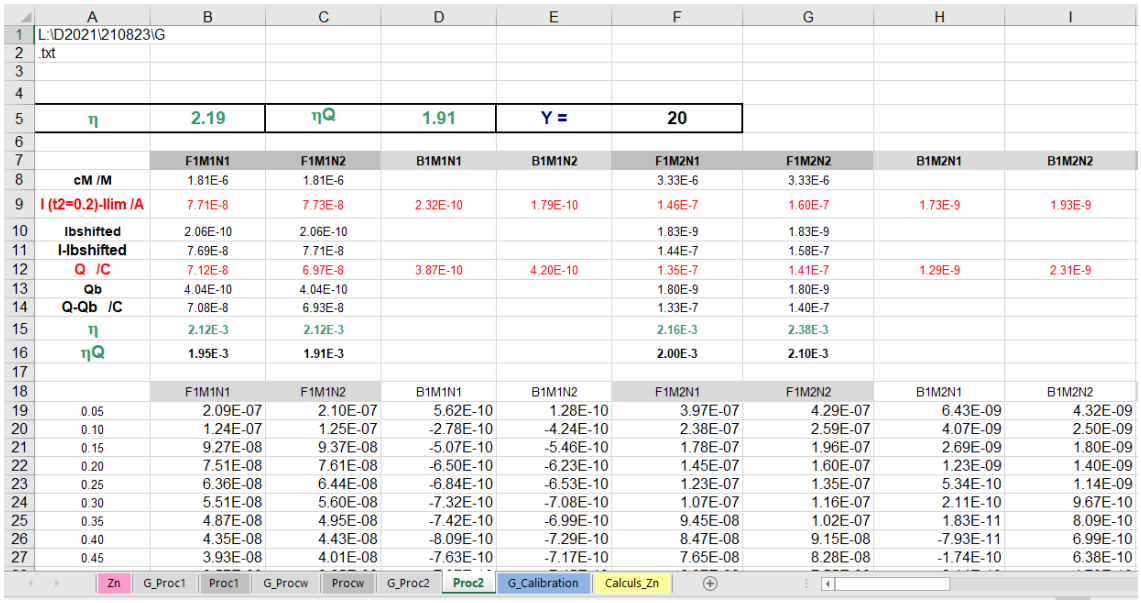
D.2.3. Adjust pH to 4.0 and purge with N_2 for 2 min.

D.2.4. Run each measurement and its blank in duplicate (using a procedure as the one shown in Figure S24).

D.2.5. For each Zn addition, compute the free Zn concentration, $[Zn^{2+}]$, with the speciation program Visual MINTEQ, taking into account also the added concentrations of HNO_3 or KOH used to control pH.

D.2.6. Transfer the data (from the files generated with NOVA) towards the Excel file as explained for the one AGNES point run in section B.2.3, taking into account that the files ending in P go to the Excel tab that we have called “Proc 1”, the files ending in W (for waiting time) go to the tab “ProcW” and the files for the quantification step go to the tab “Proc2”. The main difference is that, in row 8 of tab “Proc2”, data for the metal concentration c_M are now those provided for Zn^{2+} by Visual MINTEQ at each Zn^{2+} addition (see previous sub-section D.2.5 and Figure S26). The other rows are the same as before. For instance, in row 9, the average of the residual current (I_{lim}, measured at the last 20 points of the second stage) is subtracted from each measurement (columns B and C) and from the blanks (columns D and E). In row 10, the average of the blanks

(e.g. D9 and E9) is computed. In row 11, the average of the blanks is subtracted from the average of the measurements (e.g. B9-B10). In row 12, the value of the charge (*Q*, in case one wishes to use the variant AGNES-Q) is computed by integrating the current. In rows 13 and 14, data correspond to the average of the blanks and to the computation of the faradaic charge (see Figure S26).



	A	B	C	D	E	F	G	H	I
1	L:\D2021\210823\G								
2	.txt								
3									
4									
5	η	2.19	ηQ	1.91	Y =	20			
6									
7		F1M1N1	F1M1N2	B1M1N1	B1M1N2	F1M2N1	F1M2N2	B1M2N1	B1M2N2
8	cM /M	1.81E-6	1.81E-6			3.33E-6	3.33E-6		
9	I (t2=0.2)-Ilim /A	7.71E-8	7.73E-8	2.32E-10	1.79E-10	1.46E-7	1.60E-7	1.73E-9	1.93E-9
10	Ibshifted	2.06E-10	2.06E-10			1.83E-9	1.83E-9		
11	I-Ibshifted	7.69E-8	7.71E-8			1.44E-7	1.58E-7		
12	Q /C	7.12E-8	6.97E-8	3.87E-10	4.20E-10	1.35E-7	1.41E-7	1.29E-9	2.31E-9
13	Qb	4.04E-10	4.04E-10			1.80E-9	1.80E-9		
14	Q-Qb /C	7.08E-8	6.93E-8			1.33E-7	1.40E-7		
15	η	2.12E-3	2.12E-3			2.16E-3	2.38E-3		
16	ηQ	1.95E-3	1.91E-3			2.00E-3	2.10E-3		
17									
18		F1M1N1	F1M1N2	B1M1N1	B1M1N2	F1M2N1	F1M2N2	B1M2N1	B1M2N2
19	0.05	2.09E-07	2.10E-07	5.62E-10	1.28E-10	3.97E-07	4.29E-07	6.43E-09	4.32E-09
20	0.10	1.24E-07	1.25E-07	-2.78E-10	-4.24E-10	2.38E-07	2.59E-07	4.07E-09	2.50E-09
21	0.15	9.27E-08	9.37E-08	-5.07E-10	-5.46E-10	1.78E-07	1.96E-07	2.69E-09	1.80E-09
22	0.20	7.51E-08	7.61E-08	-6.50E-10	-6.23E-10	1.45E-07	1.60E-07	1.23E-09	1.40E-09
23	0.25	6.36E-08	6.44E-08	-6.84E-10	-6.53E-10	1.23E-07	1.35E-07	5.34E-10	1.14E-09
24	0.30	5.51E-08	5.60E-08	-7.32E-10	-7.08E-10	1.07E-07	1.16E-07	2.11E-10	9.67E-10
25	0.35	4.87E-08	4.95E-08	-7.42E-10	-6.99E-10	9.45E-08	1.02E-07	1.83E-11	8.09E-10
26	0.40	4.35E-08	4.43E-08	-8.09E-10	-7.29E-10	8.47E-08	9.15E-08	-7.93E-11	6.99E-10
27	0.45	3.93E-08	4.01E-08	-7.63E-10	-7.17E-10	7.65E-08	8.28E-08	-1.74E-10	6.38E-10

Zn | G_Proc1 | Proc1 | G_Procw | Procw | G_Proc2 | Proc2 | G_Calibration | Calculs_Zn

Figure S26. Excel sheet "Proc2" in file 210823_G_Cal_Zn.xls, where some calibration data were collected. For each calibration point (i.e. Zn^{2+} addition), there are four columns (e.g. F1M1N1, F1M1N2, B1M1N1, B1M1N2 for the first addition).

D.2.7. Move to the next Zn^{2+} addition and repeat steps from D.3 to D.6.

D.2.8. After the last addition, one can build the calibration curve (Figure 4 of the main text of the article).

E.-Protocol for a titration using AGNES-I_1P

E.1. Add 25 mL 0.1 mol L^{-1} KNO_3. Add 0.163 mL of Zn^{2+} solution (in this case of concentration 1.53×10^{-2} mol L^{-1}) to have in the cell the initial concentration $[Zn^{2+}]$ = 9.64×10^{-5} mol L^{-1}. Adjust the pH to 6.0 with KOH 0.1 mol L^{-1} and HNO_3 mol L^{-1} as necessary (volumes must be taken into account). Obtain the measurement (in this solution, with no oxalate) and blank in duplicate by running the procedure created in section B.1 corresponding to AGNES-I_1P (similar to Figure S24).

E.2. Add, to the cell, the first volume of ligand (in this particular case 0.005 mL of a solution of 0.5 mol L^{-1} potassium oxalate), close the cell and purge with N_2 for 2 min.

E.3. Run the procedure AGNES-I_1P.nox (with blanks). After running each point of the titration (including the case of Zn^{2+} before the first addition of ligand), export the data in the same way as detailed in section B.2.3 to an Excel file like the one shown in Figure S27.

E.4. Add the next volume of ligand, fix pH, purge and repeat previous step until completing the desired additions (six, in this example).

E.5. Then construct the graph with the free concentration of Zn versus the concentration of ligand (see Figure 5 of the main text of the main article).

Data generated by NOVA are exported into Excel with a similar layout as that shown in Figure S22. The main difference is that now the value of the proportionality factor (η=

0.0022 A mol^{-1} L found in the associated calibration) is fixed in the cell B4 (tab Proc2 in the file 210824_G_Tit_Zn+Oxal.xls). The values in row 9 are linked to the values in the tab Zn-OXAL (in the file 210824_G_Tit_Zn+Oxal.xls), where ancillary computations are kept together with the output of the free concentrations estimated by Visual MINTEQ. Rows 15 and 16 contain the decimal logarithm of the concentrations measured by AGNES and predicted by Visual MINTEQ (see Figure S27).

	A	B	C	D	E	F	G	H	I
1	L:\D2021\210824\G								
2	.txt								
3									
4	η	0.0022	ηQ	0.0019	Date:	23/08/2021			
5									
6		F1M1L0N1	F1M1L0N2	B1M1L0N1	B1M1L0N2	F1M1L1N1	F1M1L1N2	B1M1L1N1	B1M1L1N2
7	Y aprox	2	2	2	2	2	2	2	2
8	t1 - tw /s	50	50	50	50	50	50	50	50
9	cM (VMIN) /M	8.85E-5	8.85E-5	8.85E-5	8.85E-5	5.89E-5	5.89E-5	5.89E-5	5.89E-5
10	cTL / mM	0.0E+0	0.0E+0	0.0E+0	0.0E+0	9.6E-2	9.6E-2	9.6E-2	9.6E-2
11	I (t2=0.2)-Ilim /A	3.61E-7	3.65E-7	2.59E-9	2.62E-9	2.54E-7	2.68E-7	1.57E-9	1.65E-9
12	Iblanc shift	2.61E-9	2.61E-9			1.61E-9	1.61E-9		
13	Q	3.25E-7	3.30E-7	2.24E-9	2.20E-9	2.26E-7	2.43E-7	1.35E-9	1.44E-9
14	Q-Qb	3.23E-7	3.27E-7			2.24E-7	2.42E-7		
15	log cM (AGNES) /M	-4.089	-4.084	-6.230	-6.224	-4.241	-4.217	-6.448	-6.426
16	log cM (VMIN) /M	-4.053	-4.053			-4.230	-4.230		
17									
18	cM (AGNES) /M	8.14E-05	8.24E-05			5.74E-05	6.06E-05		
19									
20									
21		F1M1L0N1	F1M1L0N2	B1M1L0N1	B1M1L0N2	F1M1L1N1	F1M1L1N2	B1M1L1N1	B1M1L1N2
22	0.05	9.54E-07	9.61E-07	7.86E-09	7.44E-09	6.94E-07	7.28E-07	4.16E-09	4.34E-09
23	0.1	5.89E-07	5.95E-07	4.30E-09	4.20E-09	4.15E-07	4.36E-07	2.26E-09	2.39E-09
24	0.15	4.43E-07	4.47E-07	2.94E-09	2.96E-09	3.11E-07	3.28E-07	1.61E-09	1.68E-09
25	0.2	3.61E-07	3.65E-07	2.29E-09	2.28E-09	2.54E-07	2.68E-07	1.24E-09	1.31E-09
26	0.25	3.06E-07	3.10E-07	1.80E-09	1.79E-09	2.15E-07	2.28E-07	1.04E-09	1.05E-09
27	0.3	2.67E-07	2.70E-07	1.50E-09	1.46E-09	1.87E-07	1.99E-07	8.24E-10	8.54E-10
28	0.35	2.37E-07	2.40E-07	1.22E-09	1.21E-09	1.66E-07	1.76E-07	7.42E-10	7.14E-10
29	0.4	2.13E-07	2.16E-07	1.08E-09	1.04E-09	1.49E-07	1.58E-07	5.98E-10	6.26E-10
30	0.45	1.94E-07	1.96E-07	8.70E-10	8.73E-10	1.35E-07	1.44E-07	5.49E-10	5.10E-10
31	0.5	1.77E-07	1.79E-07	8.00E-10	7.57E-10	1.23E-07	1.31E-07	4.33E-10	4.49E-10

Zn-OXAL | G_Proc1 | Proc1 | G_ProcW | Procw | G_Proc2 | Proc2 | G_log cM | Calculs_Zn

Figure S27. Snapshot of the tab "Proc2", in the file 210824_G_Tit_Zn+Oxal.xls, with the titration data of a fixed amount of Zn with increasing amounts of oxalate.

F.- Protocol for measurements of free metal concentrations with AGNES-SCP_1P

One can use the procedure AGNES-SCP_1P (see file AGNES-SCP_1P.nox) for a calibration, for a trajectory or for an individual measurement.

F.1.- Description of the procedure AGNES-SCP_1P

The procedure for AGNES-SCP_1P is similar to the one for AGNES-I_1P (see Figure S20), but with no need of blanks. On the other hand, AGNES-SCP_1P uses Potentiometric stripping analysis (PSA) with constant current in the second stage (see Figure S28). So, the changes with respect to the procedure AGNES-I_1P are:

- As seen in the first row, one keeps the commands "Calculate Signal" and "Message" that correspond to the introduction of "Epeak" and to the computation of E_1 for a given gain (corresponding, in the example of Figure S28, to Y= 10000). In contrast with AGNES-I variant, now there is no need of computing a potential for the second stage (E_2). We have added a "Repeat" command called "AGNES-SCP-1P".

- As seen in the second row of Figure S28, one keeps the "Build text" command with the file name and path as previously explained in section B.1.7.1 (see also Figures S10 or S18). The group "AGNES-SCP Procedure" (detailed in the third row) follows.

- As seen in the third row of Figure S28, in the "Apply" type command that we have called "Apply E1", we set the potential E_1 (defined in the first row for Y=10000). Keep in mind that this command called "Apply E1" is linked to the "E1 for Y=10000,Zn" command on the first row. "Record signal" commands called "Record I" and "Record Iw" follow for the deposition periods with and without stirring (for details of these commands see sections B.1.7.4 and B.1.7.5 or Figure S12). Then, the command "PSA Constant current", already pre-defined in NOVA, is adapted as

shown in the upper right region of Figure with the value of the prescribed stripping current (e.g. 3 µA). After the command "PSA Constant current", the commands "Autolab control", "Apply -0.7 V" and "Wait 5s" which are required to keep the conditions constant for PSA between experiments.

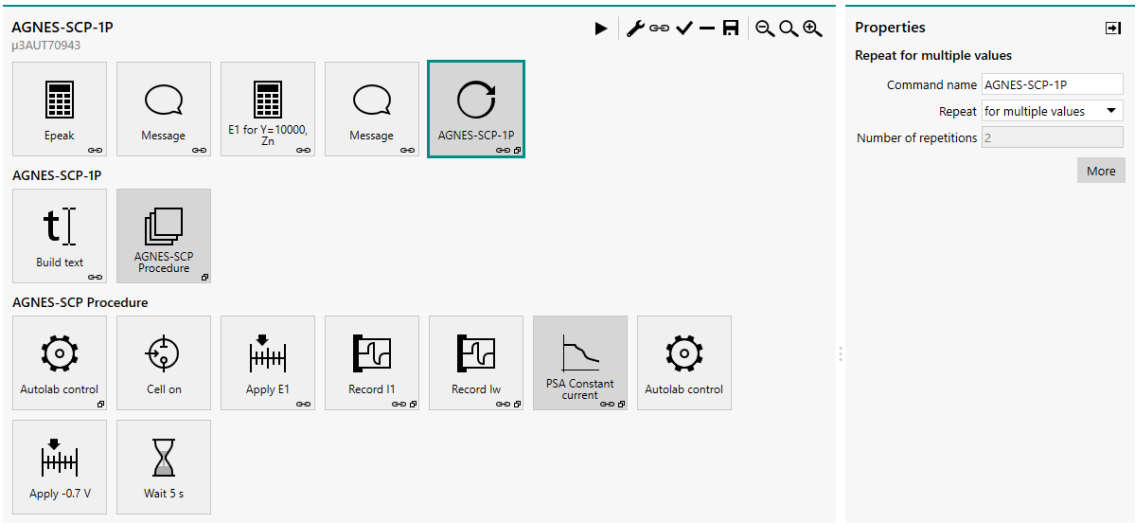


Figure S28. Screenshot of a procedure created in NOVA 2.1.5 for AGNES-SCP_1P.

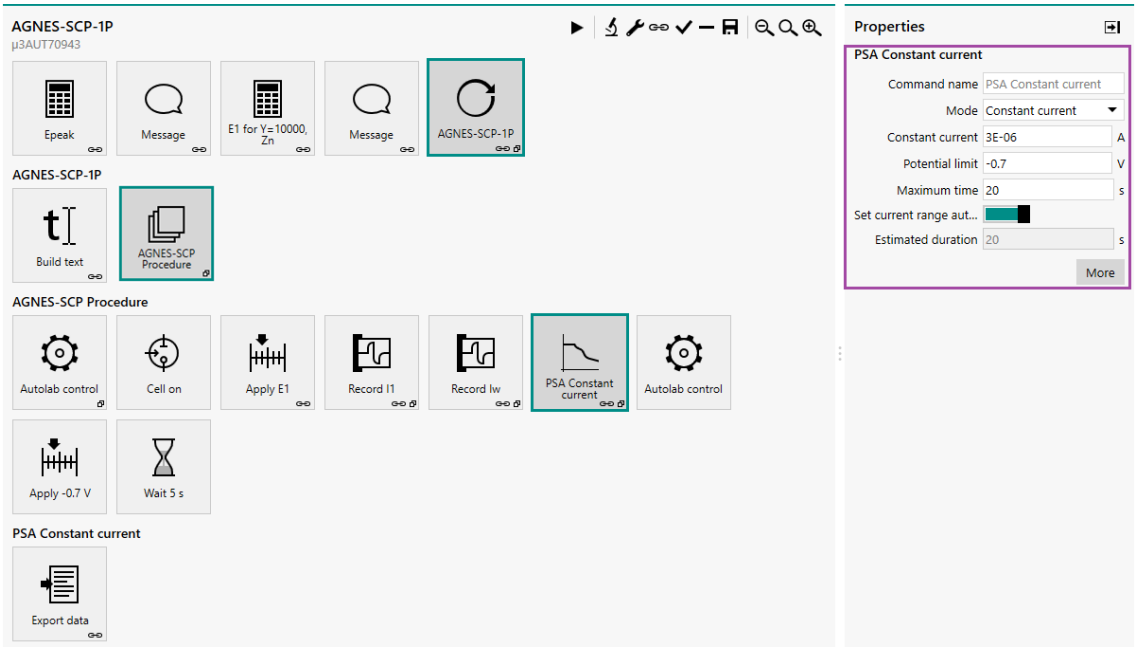


Figure S29. Screenshot with details of the command "PSA Constant current". In the box highlighted in purple on the right, the settings for PSA are prescribed: Mode, Constant current with the desired value (I_s=3×10^{-6} A in the example), with a Potential limit of -0.7 V (suitable for Zn) and a Maximum time of 20 s. These parameters do not need to be changed unless one changes I_s or the analysed metal ion.

F.2.- Use of the procedure AGNES-SCP_1P

In order to adapt AGNES-SCP_1P.nox provided here to other conditions, one must change:

- If working with an **element other than Zn or another temperature**, change Epeak calculations in command "Epeak" (see section B.1.1, Figure S5).

- To **modify the desired gain**, change the value in command "E1 for Y=20, Zn" (see section B.1.2, Figure S6). Typically, **deposition times** should also be changed (see section B.1.7.4, Figure S12) when the desired gain is modified.

- If the stripping current needs to be changed, modify the command "PSA Constant current" (see section F.1, Figure S29).

G.- Protocol for measurements of free metal concentrations with AGNES-SCP_2P.

G.1.- Description of the procedure AGNES-SCP_2P

The Procedure AGNES-SCP_2P can be seen as a modification of AGNES-SCP_1P.nox where two substages ("a" and "b")[2] in the deposition stage with stirring are distinguished. Figure S30 shows an example of AGNES-SCP_2P

- In the first row, in the command of type "Calculate signal" that we labelled "E1,a for Y1,a=1E10,Zn", the potential $E_{1,a}$ (for the first substage) corresponding to a gain $Y_{1,a}$ of 10^{10} is computed for Zn, so that diffusion limited conditions for deposition are prescribed.

- In the third row, the command “Apply E1,a” sets the potential computed in the first row (in this example the one corresponding to $Y_{1,a}=10^{10}$). Notice that the value of $E_{1,a}$ from the command “E1,a for Y1,a=1E10,Zn” in the first row is passed to the command “Apply E1,a” via a link (see left purple line in Figure S30) command. Also in the third row, there are “Record signal” commands to keep track of the currents in the first substage (“Record I1,a”), in the second substage (“Record I1,b”), and in the waiting period (“Record Iw”).

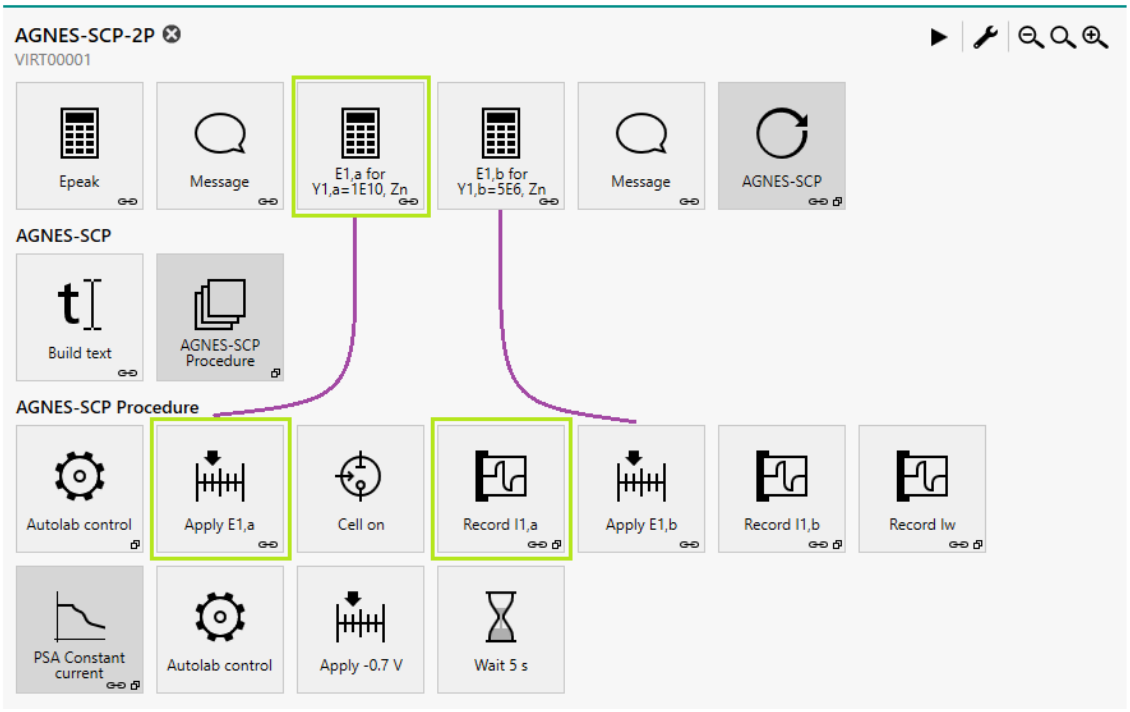


Figure S30. Screenshot of the Procedure AGNES-SCP_2P. Green boxes highlight commands added with respect to AGNES-SCP_1P. The purple lines indicate the links between calculation of the potentials (in the first row) and setting (with “Apply” type commands in the third row).

G.2.- Use of the procedure AGNES-SCP_2P

In order to adapt AGNES-SCP_2P.nox provided here to other conditions, one must follow the same changes as described in section F.2

H.- Index of NOVA procedures, raw data and Excel files included in the Supporting Information

File name	Purpose
210716_G_Trajectory.xls	Gathering and processing of results for a trajectory, to check the special equilibrium situation required in AGNES.
210823_G_Cal_Zn.xls	Gathering and processing of results for a calibration using AGNES-I with HMDE, to find the value of η.
210824_G_Tit_Zn+Oxal.xls	Gathering and processing of results for a titration, to validate the results obtained with AGNES by comparing them with data predicted with Visual MINTEQ.
220210_G_Cal_2_Zn.xls	Gathering and processing of results for a calibration using AGNES-SCP with RDE, to find the value of η_Q.
220215_G_Segre_2P.xls	Gathering and processing of results to quantify [Zn2+] of Zn in a water sample of the Segre river. The sheet with the key computations is Proc_w_2.
AGNES-I_1P.nox	Procedure in NOVA 2.1.5 to run AGNES with the 1pulse variant for the first stage and the I variant for the second stage.
AGNES-I_2P.nox	Procedure in NOVA 2.1.5 to run AGNES with the 2 pulse variant for the first stage and the I variant for the second stage.
AGNES-SCP_1P.nox	Procedure in NOVA 2.1.5 to run AGNES with the 1pulse variant for the first stage and the SCP variant for the second stage.
AGNES-SCP_2P.nox	Procedure in NOVA 2.1.5 to run AGNES with the 2 pulse variant for the first stage and the SCP variant for the second stage.
DPP.nox	Procedure in NOVA 2.1.5 for the determination of DPP E_{peak}.
Y20T50M1N1.txt **Y20T50M1N1P.txt** **Y20T50M1N1W.txt**	AGNES-I_1P data files obtained with NOVA 2.1.5 corresponding to one point of the trajectory (t_1-t_w = 50 s).

X1M1N1.txt **X1M1N1PA.txt** **X1M1N1PB.txt** **X1M1N1PW.txt**	AGNES-SCP_2P data files obtained with NOVA 2.1.5 corresponding to one point of the Segre sample ($Y = 1.0\times10^{10}$; $t_{1,a} = 50$ s; $t_{1,b} = 1$ s).

I.- References

1. Galceran, J.; Companys, E.; Puy, J.; Cecília, J.; Garcés, J.L. AGNES: A New Electroanalytical Technique for Measuring Free Metal Ion Concentration. *J. Electroanal. Chem.* **2004**, *566*, 95–109.
2. Companys, E.; Cecília, J.; Codina, G.; Puy, J.; Galceran, J. Determination of the Concentration of Free Zn^{2+} with AGNES Using Different Strategies to Reduce the Deposition Time. *J. Electroanal. Chem.* **2005**, *576*, 21–32.

J.- List of symbols and abbreviations.

A. Latin Symbols

Symbol	Description	Units
$c_{T,Zn}$	Total concentration of Zn in the solution	mol L^{-1}
$c_{T,Oxalate}$	Total concentration of Oxalate in the solution	mol L^{-1}
$D_{Zn^{2+}}$	Diffusion coefficient of Zn^{2+} in the solution	m^2 s^{-1}
D_{Zn^o}	Diffusion coefficient of M^o in the amalgam	m^2 s^{-1}
$E^{0'}$	Formal standard potential of the redox couple	V
E_1	Deposition potential (associated to the gain Y)	V
$E_{1,a}$	Deposition potential in diffusion-limited conditions (in the first sub-stage of AGNES-2P)	V
E_2	Stripping potential (in AGNES-I or AGNES-Q)	V
EC	Electrical conductivity	µS cm^{-1}
E_{peak}	DPP Peak potential	V
F	Faraday constant	C mol^{-1}
I	Faradaic intensity current	A
I_{lim}	Average limiting current for the longest times in the reoxidation step	A
I_{Ox}	Oxidants current (in the resting period at the end of the deposition stage)	A
I_s	Stripping current in AGNES-SCP	A
L	Free macromolecular sites, ligand	none
Q	Faradaic charge	C
R	Gas constant	J K^{-1} mol^{-1}
T	Temperature	K
t_1	Deposition time in AGNES-1P	s
$t_{1,a}$	Time of the first sub-stage of AGNES-2P deposition	s
$t_{1,b}$	Time of the second sub-stage of AGNES 2P deposition	s
t_2	Time of the second stage or reoxidation time	s
t_w	Waiting time (with no stirring) at the end of the first stage	s
V_{Hg}	Volume of the mercury electrode	L
Y	Gain or preconcentration factor achieved by the end of the first stage (also Y_1 in some literature)	none
$Y_{1,a}$	Gain or preconcentration corresponding to $E_{1,a}$	none
$Y_{1,b}$	Gain or preconcentration corresponding to $E_{1,b}$	none
Y_2	Gain corresponding to E_2 (in AGNES-I)	none
Zn^{2+}	Free zinc	none
Zn^0	Reduced zinc	none
$[Zn^{2+}]$	Free zinc concentration	mol L^{-1}
$[Zn^o]$	Concentration of reduced zinc inside the amalgam	mol L^{-1}

B. Greek Symbols

Symbol	Description	Units
γ_X	Activity coefficient of species X	none
ΔE	Modulation amplitude	V
η	Proportionality factor between the faradaic stripped current (I) and the reduced metal concentration	A L mol^{-1}
η_Q	Proportionality factor between the faradaic stripped charge and the reduced metal concentration	C L mol^{-1}
μ	Ionic strength	mol L^{-1}
τ	Transition time (in the SCP measurement)	s

C. Abbreviations.

Abbreviations	Signification
AGNES	Absence of Gradients and Nerstian Equilibrium Stripping
AGNES-1P	AGNES one pulse
AGNES-2P	AGNES two pulses
DPP	Differential Pulse Polarography
GPES	General Purpose Electrochemical System Software
HMDE	Hanging Mercury Drop Electrode
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
NOVA	Software package to control the Autolab instrument with USB interface
PSA	Potentiometric Stripping Analysis
SCP	Stripping Chronopotentiometry
SMDE	Static Mercury Drop Electrode
TFM-RDE	Thin Film Mercury – Rotating Disc Electrode
TC	Total Carbon
TIC	Total Inorganic Carbon
TN	Total Nitrogen
TOC	Total Organic Carbon
VM	Visual MINTEQ