

Protocol Sheet: OSP14_00_ElectroChem_Intro_CellSetup_Protocol_20190108

Introduction to electrochemistry and different electrochemical cell setups to be used in electrobiology experiments

Christian Zerfass, Kalesh Sasidharan, Orkun S Soyer
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Abstract: This document provides some practical considerations to electrochemical experiment design (in section 3), but it is more than a “protocol”. It also provides essential background and very basic introduction to electrochemistry and the key measurement techniques using a potentiostat. Interested readers are advised to complement this introduction with appropriate reading of related books (e.g. see text books in OSS lab library on introductory chemistry, physical chemistry for biologists, thermodynamics and analytical chemistry) and publications and technical notes, such as: application note from AutoLab (available as 3rd party protocol on OSS Lab NAS). Additional, relevant technical notes and web resources are listed in references section.

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1. Introduction to electrochemistry:

An electrochemical cell is a device, in which two half reactions (of a redox reaction couple) take place spontaneously to generate electricity, or are ‘forced’ to take place through application of an electrical current [1]. **While the phrase “electrochemical cell” refers to any system with redox reaction couples spatially separated, the case of spontaneously electricity generating cell is called as a “galvanic” (or “voltaic”) cell, and the case of electricity-consuming cell is called a “electrolytic cell”.** In either cell type, the redox half reactions (i.e. oxidation and reduction) are allowed to occur on electrically conducting surfaces (known as “electrodes”) that are submerged in the same conductive solution known as an “electrolyte” and connected through a wire. The electrodes themselves take part in the redox half reactions or not. Figure 1 demonstrates the first electrochemical cell design published by Alessandro Volta in 1799, where the metals (i.e. electrodes) themselves involve in the redox reactions, and their connection is achieved through cloths soaked in an electrolyte (e.g. salt water). Figure 1 also shows a more modern design where the two electrodes are physically separated in two different solutions, the connection of which is achieved by a so-called “salt bridge”.

The electrolyte-based connection between electrodes is sometimes said to “close the electrical circuit”, but it can be better understood as allowing “equilibration of charges”. As the half reactions occur on the electrodes (or as electrodes themselves undergo half reactions), they will involve generation or consumption of ions, and hence charge imbalances will be generated near the electrodes. These charge imbalances can be equilibrated by having other ions in the solution redistribute themselves (see Figure 2).

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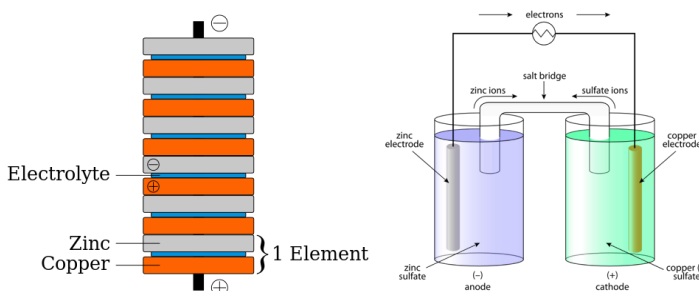


Figure 1. Left: The original design of an electrochemical cell, involving Zn^{2+}/Zn and Cu^{2+}/Cu half couples for oxidation and reduction respectively. The blue sections were cloth or paper soaked in salt water, acting as an electrolyte. Right: A modern design of a voltaic cell, of the same redox couple. Image Wikipedia.

Figure 2 also illustrates the overall redox reaction created by the two half reactions. Each half reaction is shown in the direction of the reduction (as is the custom), and their standard reduction potentials are listed. Note that “reduction potential” (or more generally electrochemical potential) is a state variable in thermodynamics, and as such it can only be defined as a difference between two reduction reactions (i.e. points). The “standard reduction potential” is defined as the reduction potential of a half reaction

under standard conditions (1M solution, 1atm, and 25°C) and in reference to the potential of a standard H^+/H_2 half reaction pair, which is taken as 0mV (at 1M $[H^+]$, at the biological conditions of $[H^+]=10^{-7}$, this half reaction has a reduction potential of 413mV). Note that this reference electrode is usually referred to as the “standard hydrogen electrode” (SHE), but there are some inconsistencies in the usage of the SHE (see discussion in [2]).

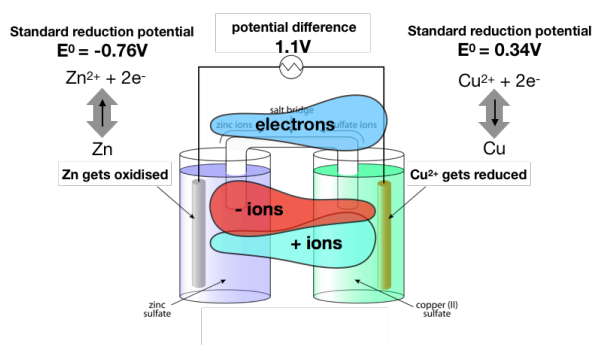


Figure 2. A cartoon representation of the distribution of ions and electrons in a galvanic cell. The standard reduction potentials of each half reaction are shown, along with the resulting potential difference. Note that in the presence of a reduction reaction with a more positive standard reduction potential (i.e. Cu^{2+}/Cu pair), the Zn^{2+}/Zn reduction reaction is reversed and runs in the oxidation direction (small arrows).

Note that as a thermodynamic variable, reduction potential has a direct relation to Gibbs free energy of reaction, given by;

$$E^{\circ} = -z \cdot F \cdot \Delta G^{\circ} \quad \text{Eq. 1,}$$

where z is the number of electrons involved in oxidizing or reducing one mole of substrate, and F is the Faraday constant, i.e. the charge of one mole of electrons: $F = 96485.3 \text{ coulomb/mole} = N_A \cdot e$, where N_A is Avogadro’s number and e is the elementary charge (charge of a proton; change sign for charge of electron) with $e = 1.60217662 \times 10^{-19} \text{ C}$.

The relation between reduction potential and Gibbs free energy of reaction is useful to better understand the concept of “galvanic” and “electrochemical” cells. To do so, recall that Gibbs free energy is used to decide on the spontaneity of a chemical reaction in a given direction. When a reaction is written in a certain direction to label participating molecules as “reactants” and “products” (e.g. $A+B \rightarrow C+D$), the Gibbs free energy of the reaction can be deduced as the sum of Gibbs free energies of formation for the products minus that for the reactants. If the Gibbs free energy of reaction is negative, it is concluded that the reaction would be spontaneous in the direction it is written. Since reduction potentials are the negative of Gibbs free energy of reaction (Eq. 1), a redox reaction in a given direction would only be spontaneous if total potential difference between the two half reactions is positive. Thus, we have a galvanic cell when the Zn^{2+}/Zn and

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Cu^{2+}/Cu half reactions are connected under standard conditions (i.e. 1M solutions of Zn^{2+} and Cu^{2+}), because the oxidation of Zn (i.e. Zn to Zn^{2+} ; $E^\circ = 0.76\text{V}$) combined with reduction of Cu^{2+} (i.e. Cu^{2+} to Cu ; $E^\circ = 0.34\text{V}$) results in a positive total potential difference ($E^\circ = 1.1\text{V}$), i.e. negative Gibbs free energy. Note that our consideration was based on the assumption of standard conditions. As such a cell “runs”, i.e. the redox reaction between Zn^{2+}/Zn and Cu^{2+}/Cu half reactions takes place in the direction we mentioned, the system will reach equilibrium, i.e. the Gibbs free energy of reaction (and the potential difference) will approach 0. Until that point, the electrochemical cell can be used as a source of electricity (i.e. current). If we had different concentrations for the Zn^{2+}/Zn and Cu^{2+}/Cu half systems, or if we provide external energy (e.g. in form of another electrical current or potential), we would then be able to make the reaction run in the opposite direction, reducing Zn^{2+} and oxidizing Cu. This would then become an “electrolytic cell”. In effect, this is the same as making a non-spontaneous reaction possible by coupling it to a spontaneous reaction.

We can thus consider redox reactions, as any other chemical reaction, as being directed by the level of Gibbs free energies of reaction educts and products. It is just that the “electrical” nature of redox reactions allows us to separate them spatially by means of wires and ionic salt bridges. This is highly convenient for measuring and monitoring reaction progression, but also allows us to reverse redox reactions through an electrical interface, i.e. by providing external energy as electricity.

2. Introduction to electrochemical cell measurements (Potentiostat/Galvanostat):

To achieve appropriate monitoring and control of redox reactions, engineers have constructed specialist equipment that allow different ways of (experimental) control over these reactions [3]. Potentiostat/Galvanostat is one such equipment, that allows control of either potential (potential mode) or current (galvanic mode) among electrodes within an electrochemical system.

The commonly used equipment design consist of three electrodes;

- A working electrode (WE), where the reaction of interest takes place;
- a reference electrode (RE), which features a known, user-defined redox chemistry and allows a “known” point of potential in the system; and
- a counter electrode (CE) that is used to “close the circuit” and supply/withdraw electrons from WE.

This common design is shown in Figures 3 and 4A, and its uses as a potentiostat is discussed in section 3a. Note that in practice, and to achieve appropriate electrical control, further electrodes can be utilized. A common electrode called S (abbreviation for “sense”) is shown in the implementation seen in Figure 3B, and is used for potential measurement. In this common design, **the potential is always measured between RE and S, and the current is always measured between CE and WE**. This setup, and its variants, can be used to achieve different electrochemical arrangements/measurements as discussed in the next section.

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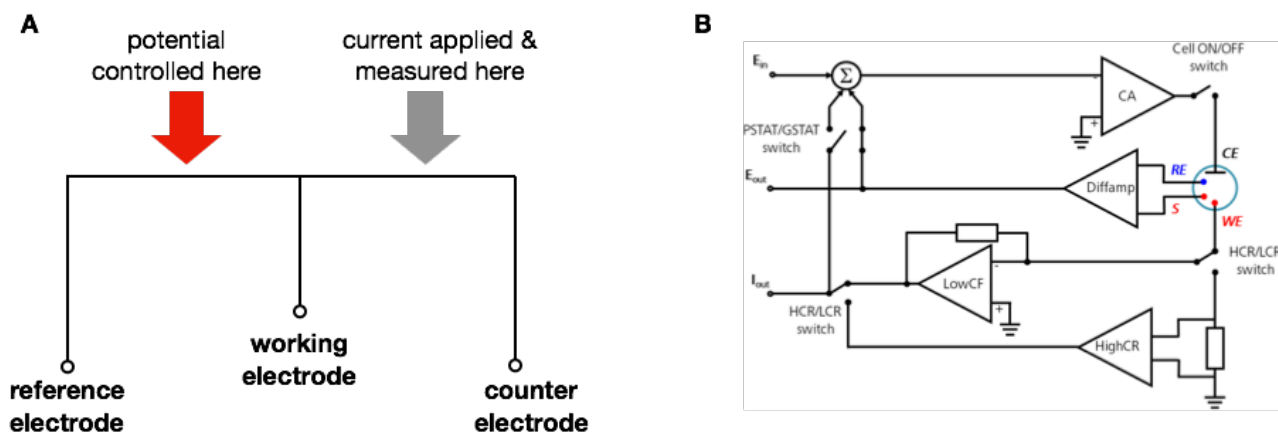


Figure 3. A. Schematic demonstration of the potentiostat working logic. The potential difference between the working and reference electrodes (RE) is controlled (fixed) by adjusting the potential between working and counter electrode (WE and CE), i.e. supplying/restricting to the required energy until the rate of reaction at the CE equals the input-current generated by a reaction at the surface of the WE. In the galvanostat, the current is controlled instead, and the potential between WE and CE is adjusted (and read by measuring against the WE potential against the RE). Note that in either mode, the process of interest is taking place at the WE. **B.** Electronic diagram of a common potentiostat circuit (from Metrohm, AutoLab technical note). Note particularly the “Diffamp” (differential, or operational amplifier) to which the RE and “S” electrodes are connected. “S” stands for “sense”, and this would in the 3-electrode setup be connected to the WE. S is exclusively used for potential measurement. As it is connected to an operational amplifier (a device through which no current flows) the potential of the WE (if S is connected to it) against the RE can be inferred without current flowing in or out of the RE. Further details of the circuit: See respective manuals.

3. Potentiostat experiments/setups:

The general design of the measurement unit allows for different types of experiments/measurements to be conducted, by setting the electrochemical system (i.e. the electrodes) in different ways as shown in Figure 4 (from [3], see also [4]).

3.a. Three-electrode (potentiostatic) setup (Figure 4A): In this setup, the S connection is short-circuited to WE (i.e. the potential is measured between RE and WE). This is a most commonly used setup, the working logic of which can be understood from the discussion given in the introduction. In essence, it utilizes the ability to reverse, or set the potential difference between two half reactions by linking it to a third reaction (or electrical source) as discussed above. The potential difference to be kept is the one considered between RE and WE, while the current can be introduced into the circuit between WE and CE (see Figure 3A). The desired potential difference between the half reactions at RE (which is known) and WE (which is the system under investigation) is maintained by providing additional electrons or withdrawing electrons from WE, through the use of current applied between WE and CE (i.e. by adjusting the redox chemistry on the CE, so to create sufficient push/pull of electrons to compensate for the electron donation / withdrawal at the WE).

The current applied is thus a measure of how far the actual potential of the half reaction at WE deviate from that at RE (i.e. the set reduction potential). Note that this setup is the one used for “cyclic voltammetry” (CV), in which one measures the current between WE and CE as the set potential difference between RE and WE is varied [5]. In a typical CV, the potential difference between RE and WE is step-by-step decreased from high to low. This sweeping is expected to convert the electrochemical cell between RE and WE from a galvanic to an electrolytic one (as discussed in section 1) or vice versa, depending on the nature of redox chemistry at WE. As

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this transition happens, the potentiostat would have to withdraw or provide electrons to WE, through the current applied between WE and CE, so to maintain the desired potential between RE and WE. For example, imagine the following scenario; RE is built to contain a redox reaction at 200mV (against SHE), and there is a redox reaction at WE that is at -100mV (against SHE). In this case, electrons would naturally want to move from WE to RE, with a current corresponding to 300mV potential difference (and circuit resistance). Thus, achieving a desired potential difference between RE and WE at for example 100mV, would require withdrawal of electrons from WE, while achieving a potential difference of 400mV would require provision of electrons into WE through CE. The CV experiment enables us to see these transitions as we move from withdrawal (through no current) to provision of electrons, by allowing us to monitor the current between WE and CE. Note that the withdrawal and provision of electrons known as “anodic” and “cathodic” current.

3.b. Two-electrode setup (Figure 4B): In this setup RE is short-circuited to CE, and S is short-circuited on WE. Since S is connected to WE, we get potential measured between RE and WE (remember that, by equipment design, potential is measured between RE and S). Similarly, since CE is connected to RE, we get current measured between RE and WE ((remember that, by equipment design, current is measured between CE and WE). **Thus, this setup allows simply measuring the potential/current arising between WE (system of interest) and RE (known system), providing a traditional electrochemical cell (electrolytic or galvanic) measurement.**

Note that when combined with CV, this setup can be used to infer the permitted current at a certain set potential difference. This provides a proximate measure of reaction rates permitted and indicates the feasibility of a redox reaction on a certain electrode material. This is a common setup in corrosion experiments. Here, it is not of interest where the potential of corrosion lies with respect to a known redox couple (i.e. in an RE). Instead, experiments aim to understand the rate of corrosion in a particular electrolyte compared to other electrolytes (i.e. to test the efficiency of anticorrosion additives compared to an electrolyte without this additive, or at different temperatures). In such experiments, WE is (or coated with) a material of interest, and the “corrosion potential” (also: open circuit potential) against the CE is measured. When no potential is set by the potentiostat, the net current will be zero. From here, a CV can be run to test for the current at a certain potential deviation from the corrosion potential. The same is done after e.g. spiking in a corrosion inhibitor or changing the temperature in order to infer the change in corrosion rate in the changed environment.

3.c. Four-electrode setup (Figure 4C): In this setup, S is used as an independent electrode. Thus, we are measuring the potential between RE and S, in the presence of CE and WE, where additional reactions can take place. Note that in this system, “RE” is also considered a “sense” connection and not necessarily a “traditional” reference electrode with known potential (e.g. both S and RE could be platinum wires). **The potential between RE and S, arising from current flowing between WE and CE (or alternatively, a set potential between WE and CE), is measured.** This setup can e.g. be used to measure the potential difference at a certain location of the system after setting a current / “global” potential. An example is to measure the potential in immediate proximity of an interface, e.g. a membrane or two-solvent interface. For instance, this potential will be different if the solvent itself has a resistance. This basically applies for all solvents, depending on their dielectric constant and the diffusability of electrolyte ions / molecules.

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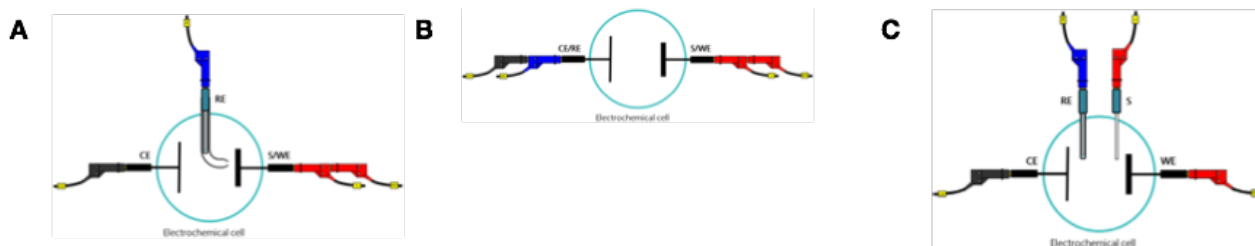


Figure 4. A cartoon representation of the different potentiostat setups, adapted from technical note of Metrohm, AutoLab. **A.** Three-electrode setup. **B.** Two-electrode setup. **C.** Four-electrode setup. In each diagram, the WE, CE, RE, and S electrodes are colored black/red, black/black, gray/blue, and gray/red respectively.

3.d. Zero resistance ammeter (ZRA) setup: This is a special setup not available on all potentiostats (but available on our Gamry unit). **In this setup, the WE and CE are “short-circuited” in a special manner, and are used to measure current between the WE and a secondary working electrode (e.g. WE’).** The special short-circuit mentioned allows to achieve a "zero - resistance - ammeter" (ZRA; see also [6] for details), which basically avoids any voltage drop that is normally associated with measuring current (i.e. ammeters). Because WE and CE are short-circuited, there can be no current applied into the system (i.e. no additional energy input), and therefore no control of potential between RE and S. This configuration allows the spontaneous complementary redox-reactions at WE and WE' to take place, and the spontaneous current (build up from the chemistry of the electrochemical cell itself) is measured without any errors introduced from measuring that current (due to ZRA configuration). Note that this application is also referred to as “galvanic current monitoring”.

4. Electrodes:

Electrodes can participate on redox reactions or provide conductive surfaces for them. There are certain considerations of their design depending on the setup to be used in an electrochemical experiment. The following are compiled and modified from Metrohm, AutoLab technical notes;

4.a. Counter electrode (a.k.a auxiliary electrode): It is usually made of an inert material (e.g. Pt, Au, graphite, glassy carbon) and usually it does not participate in the electrochemical reaction (i.e. the electrode material is catalytic, but not consumed itself). Because the current is flowing between the WE and the CE, the total surface area of the CE (source/sink of electrons) must be higher than the area of the WE so that it will not be a limiting factor in the kinetics of the electrochemical process under investigation. Note that this size-comparison assumes a similar rate of reaction per area of electrode; if for example electron-deposition is slow at the WE, but fast on CE (i.e. there is high concentration of redox-partner which withdraws electrons at the CE), this can counterbalance the size requirements.

4.b. Reference electrode: This should have a stable and well-known electrode potential, as the RE is used as a point of reference in the electrolytical cell for the control and measurement of potential. The high stability of the RE is usually reached by employing a redox system with constant (buffered or saturated) concentrations of each participants of the redox reaction. Moreover, the current flow through the RE is kept close to zero (ideally, zero), which is achieved by a very high input impedance ($> 100 \text{ G}\Omega$; which could be implemented also via an operational amplifier with virtually infinite resistance), and also by using the CE to close the current in the system circuit (i.e. so that the reference redox couple does technically not react itself). In a potentiostatic experiment the potential difference is set across RE and WE, it is also ideal to keep the RE very close to the WE for increasing the accuracy (note that also electrolyte solutions will exert some resistance,

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hence the distance between RE and WE will imply different potentials, the extent depending on the conductivity of the electrolyte). See separate **protocol XXX for** construction of a RE using the Ag^+/Ag half reaction. Note that in this Ag^+/Ag RE, fairly constant “concentrations” are achieved as Ag and AgCl will be deposited as insoluble metal/salt (which is technically not interpreted as concentration, but provides a fairly stable situation anyway by maintaining an activity of connected solids Ag_s and AgCl_s precipitates).

4.c. Working electrode: This is the electrode on which the reaction of interest is occurring. Common working electrodes can be made of inert materials such as Au, Ag, Pt, glassy carbon (GC) and Hg drop and film electrodes etc. The size and shape of the working electrode also varies and it depends on the application. For biological studies, as ours, a high surface area is preferred for increasing contact with microbial cells.

5. References:

1. <http://hyperphysics.phy-astr.gsu.edu/hbase/Chemical/electrochem.html>
- 2.
3. <https://www.gamry.com/application-notes/electrodes-cells/two-three-and-four-electrode-experiments/>
4. <http://compton.chem.ox.ac.uk/index.php?title=research&topic=fund>
5. https://chem.libretexts.org/Core/Analytical_Chemistry/Instrumental_Analysis/Cyclic_Voltammetry
6. http://www.bank-ic.de/encms/knowhow/1_praezisionsammeter.html