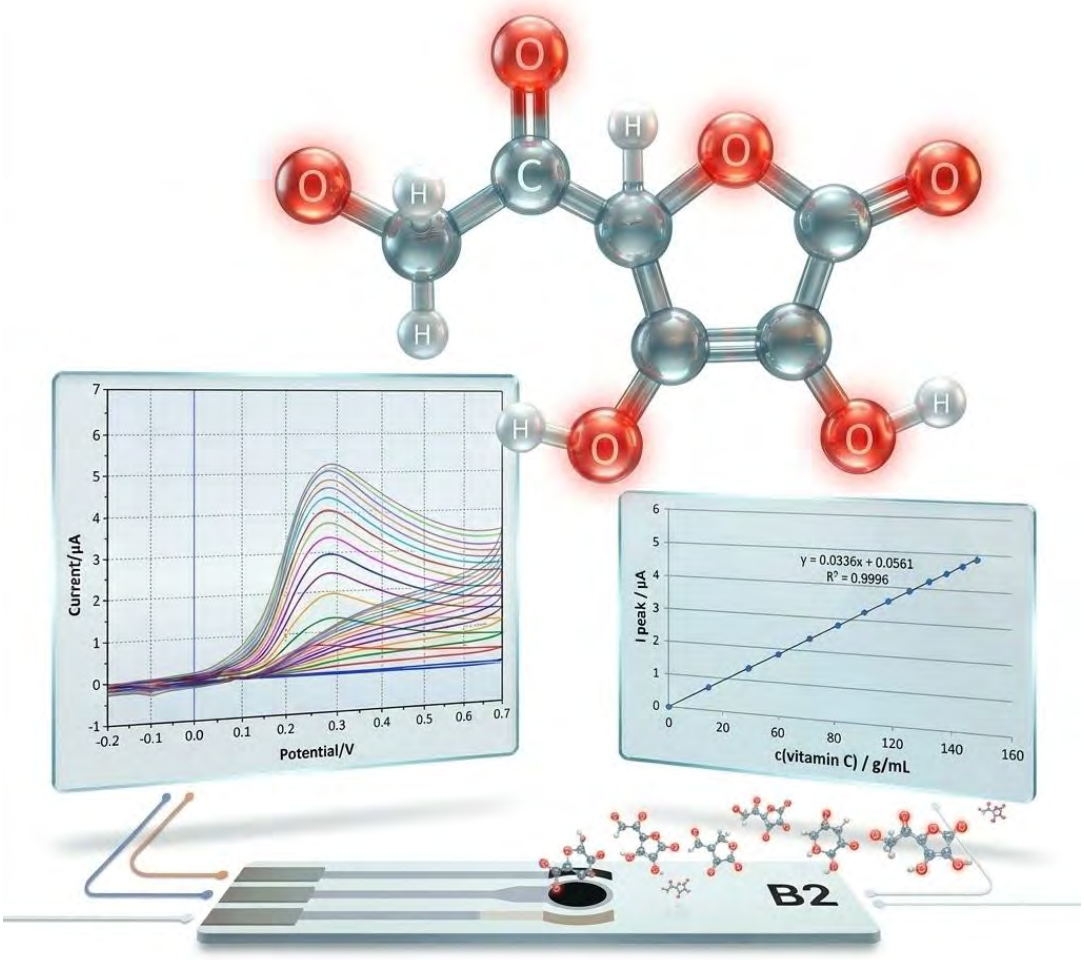
Vitamin C Detection with ItalSens Carbon SPE

Creating a calibration curve for ascorbic acid detection



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Application Note

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1 Introduction

1.1 Goals

This application note describes a simple experiment for the detection of Vitamin C (ascorbic acid). The experiment uses non-toxic solutions that can be safely disposed of in household waste or regular wastewater. For this reason, it is well suited as a hands-on electrochemistry experiment using safe and accessible substances.

1.2 Vitamin C

It is common knowledge that ascorbic acid, which is also known as vitamin C, cures scurvy. Actually scurvy is a consequence of vitamin C deficiency. The name ascorbic acid is referring to its anti-scurvy properties as well.
Already in 1753 it was observed that citrus fruits cure scurvy, but the responsible substance, vitamin C, was discovered much later, in 1912. It was isolated in 1928 and already in 1933 vitamin C was the first vitamin to be chemically produced.
Vitamin C is necessary for collagen production inside the human body, which is the reason why vitamin C deficiency causes scurvy. Collagen is an important component of strong tissue. Vitamin C is required for a properly running immune system and it acts as a reducing agent (antioxidant), for example as a free radical scavenger.
For this application, we explore the fact that vitamin C is easily oxidized while reducing other reactants. Vitamin C has also applications outside the human body or an electrochemical cell. Fruits, which turn brown when exposed to air like apples, won't turn brown when sprinkled with vitamin C powder or lemon juice.
Unfortunately, unlike many other animals, humans cannot make their own vitamin C and thus humans need to consume vitamin C containing products in their diet. Usually, fresh fruit and vegetables contain enough ascorbic acid or ascorbates to cover our vitamin C requirement. Different health organizations give different recommendations, but a daily amount of vitamin C of around 100 mg should be more than sufficient.
Vitamin C was promoted to be beneficial for preventing or reducing sicknesses especially the common cold when taken in higher dosage. There is very little to no scientific evidence to support this claim. However, the image of a health benefit due to a vitamin C rich diet makes ascorbic acid a great food additive. It makes the food sour, preserves it and promotes the idea that the product has health benefits.

1.3 Cyclic Voltammetry

Cyclic Voltammetry (CV) is the most popular technique in electrochemistry. It gives qualitative information, allows studying mechanism, kinetics and quantitative information. Before the boom of Electrochemical Impedance Spectroscopy (EIS) and Spectroelectrochemistry CV was said to be the electrochemist's spectroscopy. Just like an infrared (IR)- or nuclear magnetic resonance (NMR)-spectrum the shape of the CV, the position of the peaks and the amount of the current can tell an experienced electrochemist a lot about the inspected system.
A detailed explanation of the theoretical background and analysis of a CV is beyond the scope of this application note. Fortunately, all standard electrochemistry textbooks cover this topic, for example:

- Allen J. Bard, Larry R. Faulkner, *Electrochemical Methods: Fundamentals and Applications*, 2nd Edition, January 2001, ISBN: 978-0-471-04372-0
- Cynthia G. Zoski, *Handbook of Electrochemistry*, December 2006, ISBN: 978-0-444-51958-0
- Joseph Wang, *Analytical Electrochemistry*, 3rd Edition, June 2006, ISBN: 978-0-471-67879-3

The next paragraphs are a very brief introduction to CV.
While the name potentiostat suggests that it can hold a *constant* potential, a potentiostat can also create precisely controlled dynamic potentials. Popular dynamic potential profiles are a linear increase (ramp or sweep) and pulses, which are short jumps between different potentials.

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The measuring principle is the same as during constant potential measurements: The potential is applied between the working and reference electrodes, while the current is flowing and measured between the working and counter electrodes.

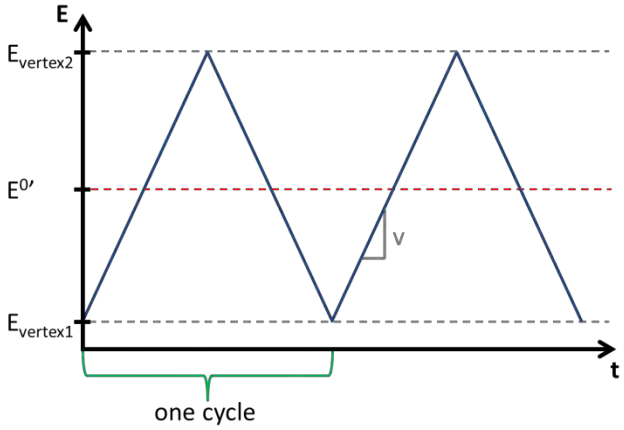

Figure 1 Potential profile of a cyclic voltammogram

CV is related to Linear Sweep Voltammetry (LSV). During a LSV a linear potential sweep is applied from an initial potential to a final potential while the current is measured. During a CV an additional sweep follows the first sweep. The second sweep has the same potential slope as the first one, but in the opposite direction. For example, a scan rate of 100 mV/s in the first sweep becomes –100 mV/s in the second sweep. Typically, the final potential of the second sweep returns to the starting potential of the first sweep, forming a cyclic process (see Figure 1).

The current is recorded during these potential sweeps. Currents during electrochemical experiments can be caused by two effects. It can be caused by a chemical reaction at the electrode- this is Faraday current. Or the current can be caused by the movement and accumulation of ions in front of the electrode, which makes the electrode behave like a capacitor. Every change of potential will create a current because of the capacitor storing more or less charge. This current is called capacitive charging current, which is often abbreviated to capacitive current.

Capacitive current is usually something that electrochemists try to avoid, because it creates a background current for the Faraday current, which is the signal of interest. During a CV the potential is changed with a constant rate- the scan rate, and so a constant capacitive charging current is present. The higher the scan rate the higher is the background current.

The potential range of the CV is usually chosen in a way that the reaction of interest happens around the center of that potential range. For example, if you are interested in the oxidation of an iron species and you expect that to occur at around 200 mV, you start the measurement at -200 mV and end at 500 mV.

As you know, potentials aren't absolute, but only potential differences can be measured. For a potentiostat the reference point or the potential of 0 V is the reference electrode. In this application note all the potential were measured versus a silver electrode covered with silver chloride and immersed in potassium chloride solution 3 mol/L. The abbreviation for that is Ag/AgCl/KCl3M or simply Ag/AgCl. Often you will find in literature the type of reference electrode together with the potential, for example -200 mV vs Ag/AgCl.

Usually, a starting potential is selected where no electrochemical reaction occurs, i.e. around 200 mV. As the potential approaches the redox potential of the active species, oxidation begins and a current starts to flow. In this region, the current depends on the applied potential and follows the behavior described by the Nernst equation.

As the potential continues to increase, the current rises until it reaches a peak. Initially, the electrode surface is surrounded by a high concentration of active species, so the reaction rate is mainly limited by the reaction kinetics. However, as the reaction proceeds, these species become depleted near the electrode surface.

After the peak, the current begins to decrease and eventually approaches a steady value. At this stage, the reaction is sustained by the transport of fresh species from the bulk solution to the electrode by

diffusion. Therefore, this region of the cyclic voltammogram is controlled by diffusion rather than by reaction kinetics (see Figure 2).

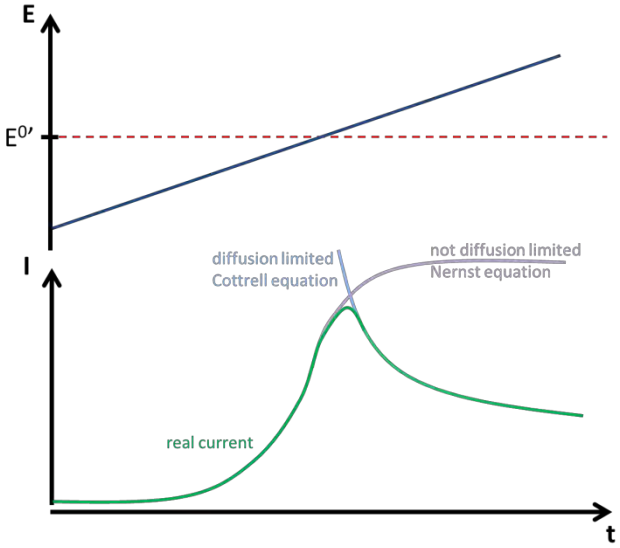

Figure 2 Relationship between a potential ramp and the measured current in an LSV

After the scan reverses and the potential gets closer to the redox potential the previously consumed species is now produced. This happens whenever the reaction triggered in the previous step is reversible. This will create a current as well, which is first potential limited and then limited due to the depletion of the consumed species. If you want to visualize this process, you could imagine the electrode is breathing out a cloud and in the next sweep inhaling the same cloud. The resulting current versus time is visible in Figure 3.

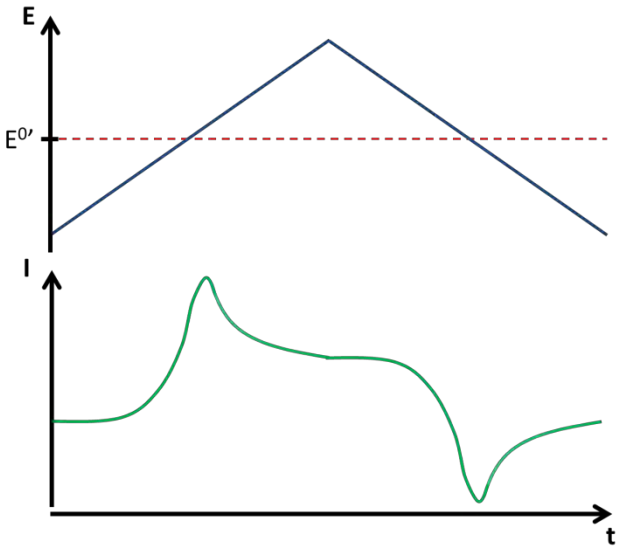

Figure 3 Current versus time during a cyclic voltammogram

The results of voltammetry techniques are plotted as a voltammogram, which is a plot of current versus potential. The shape of the current as described above and plotting I vs E leads to the iconic “duck” shape of the CV (see Figure 4).

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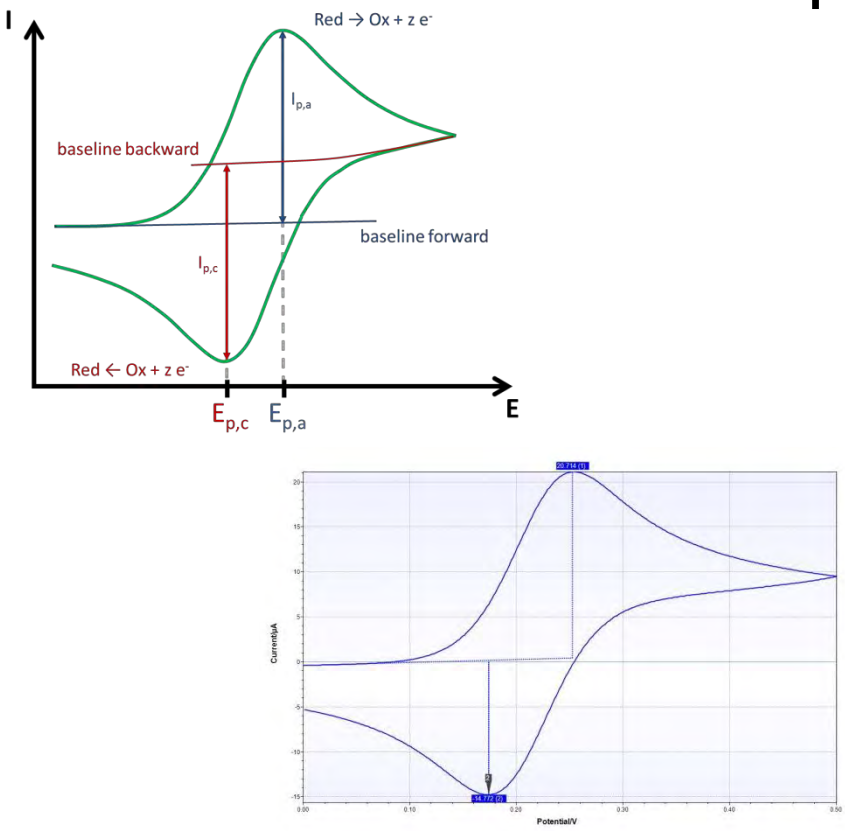

Figure 4 left scheme of a CV with some important parameters; right measured CV

Cyclic Voltammetry allows studying time dependency, because the scan rate can be adjusted. The behavior of the peaks with changes in the scan rate shows how fast certain reactions happens and if the species is on the surface or free diffusing. The potential of the peak is an indication for the species that is reacting. The presence of a peak during the anodic (towards positive potentials) and cathodic (towards negative potentials) sweep shows that a reaction is reversible. And the peak current is proportional to the concentration of the reacting species.
Usually oxidation reactions happen during the anodic sweep and reduction reactions during the cathodic sweep. The International Union of Pure and Applied Chemistry (IUPAC) has agreed that positive currents are oxidating currents and negative currents are reducing currents. That means when you measure a positive currents electrons are going into the working electrode while something in solution is oxidized and electrons go from the counter electrode into the solution while something in solution is reduced. If the measured current is negative, the working electrode reduces something in the solution and the counter electrode oxidizes.
However, there is another convention called the polarographic convention. In this case, positive current is reducing and negative oxidizing, plus the negative potentials are plotted on the right side and the positive potential on the left side of the abscissa.

1.4 Vitamin C Oxidation

The suggested mechanism for an electrochemical oxidation of ascorbic acid is shown in Figure 5. First the ascorbic acid is oxidized, losing 2 electrons and generating 2 hydronium ions (H^+). This is a reversible process, but the oxidized form of ascorbic acid reacts with water irreversibly. The product of this reaction is not electroactive and therefore cannot be reduced or oxidized at an electrode.

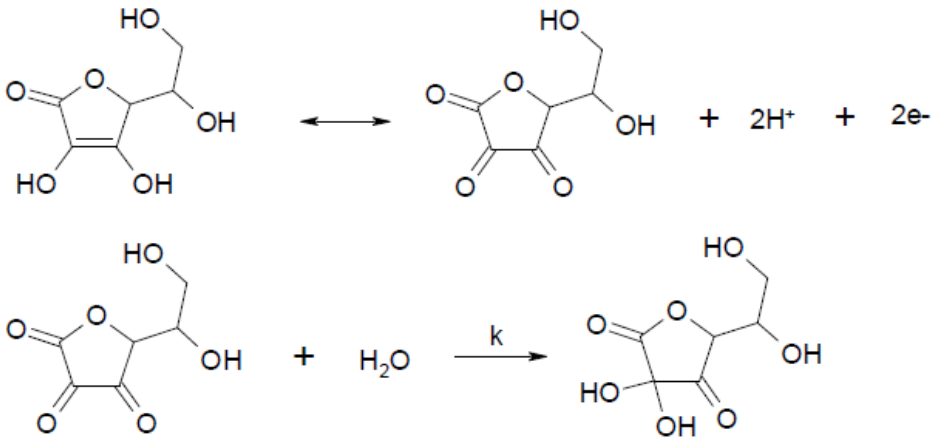

Figure 5 Electrochemical oxidation of ascorbic acid (source: Chem. Educator 2000, 5, 59–61)

Cyclic voltammetry supports this mechanism. In Figure 6 a cyclic voltammogram of a graphite paste electrode in a vitamin C solution (1 g/L in phosphate buffer) is shown. A clear oxidation peak is visible in the anodic sweep (from -0.5 to 0.9 V) at around 0.4 V. The reduction peak is entirely missing, because the reduced species went through an irreversible reaction and cannot be reduced again. As a result, no peak is observed during the second sweep from 0.9 to -0.5 V.

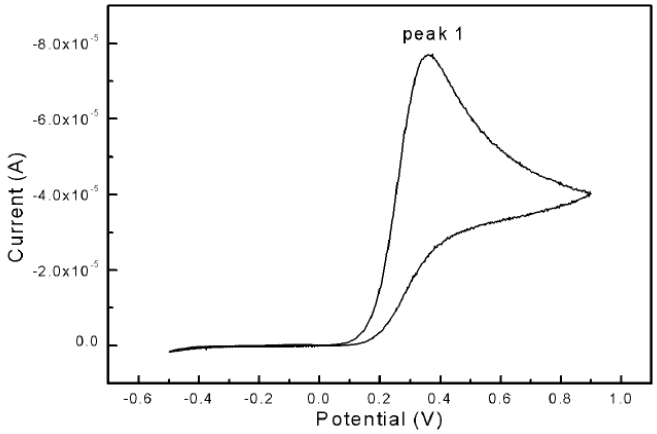

Figure 6 Cyclic Voltammogram of 1 g/L ascorbic acid solution at a graphite paste working electrode, potentials are versus Ag/AgCl (source: Chem. Educator 2000, 5, 59–61)

2 Experimental

2.1 Safety

As previously mentioned, this application note offers the opportunity to work with an electrochemical detection method without working with toxic substances or needing special disposal regulations. However, there are still some safety points to be considered:

- Don't drink or eat anything that has come into contact with laboratory equipment. You never know how well the beaker was cleaned, or if there was a toxic substance difficult to remove.
- Even harmless chemicals should be stored in an area where no eating or drinking is allowed.
- While the solutions in this experiment have low concentrations that are harmless, their respective solute powders or concentrated solutions can still cause irritations especially in your eyes.
- When making and handling your stock solutions, please wear eye protection and have an eye wash bottle or shower easily reachable. Keep in mind that if you get something in your eyes, you must use the eye wash bottle or shower with your eyes closed.
- To work in an informed way read the safety sheet that comes with the substances.

2.2 Professional or Amateur?

In this application note we present two ways of performing this measurement. If you have laboratory experience and equipment, the steps described from chapter 2.3 onwards are usually low cost and easier for you, because micro pipettes are a common item in chemistry and biology labs.
If you don't have the experience, the equipment or the budget to get the equipment, we recommend the version starting from chapter 2.4. It presents an easy and fast way to demonstrate the principle, but is less accurate and won't allow to make a real calibration curve, just the idea of the calibration curve.

2.3 Equipment for Professional Version

- EmStat Pico Development Kit
- ItalSens IS-C-3MM (screen printed electrodes based on graphite ink)
- vessels to store solution (closed)
- vessels to mix solutions (can be same as storage solution)
- volumetric cylinder (200 mL)
- micro pipette for around 100 µL, e.g. 20 µL to 200 µL
- micro pipette for around 800 µL, e.g. 100 µL to 1000 µL
- paper tissues

2.3.1 Solutions and Chemicals

2.3.1.1 Phosphate Buffer

You need a Phosphate buffer pH 7 with a concentration around 0.1 mol/L.
Phosphate buffer is very common and pH 7 is quite popular. There are many products that allow you to make phosphate buffer very easily. A powder or tablet will do the trick, because demineralized water is often available. You can also buy pre-made phosphate buffer directly.
For these experiments Phosphate Buffered Saline (PBS) by VWR (Netherlands) was used. It has the article number 0780-10L. 1.975 g of this powder was dissolved in 200 mL demineralized water to make a phosphate buffer with a concentration of 0.1 mol/L and pH 7.

2.3.1.2 Vitamin C Solution

Many vitamin C food supplements are available at drug stores, pharmacies and supermarkets. They are the easiest ways to get vitamin C solutions. Unfortunately, they often contain additional substances like aromas or colors that might show as well electrochemical activity. Choosing a product with high vitamin C content increases the ratio of vitamin C to other substances. Thus, it is a good idea to buy high concentration products. It is even better if you manage to get pure ascorbic acid powder.
How you will make your stock solution depends on your vitamin C containing product.
If you have powder or an effervescent tablet you can just dissolve it in around 30 mL of buffer solution. After dissolving the vitamin C product, fill up to 40 mL and calculate the concentration of your solution. In this application note, we use the mass concentration equation:

$$c = \frac{m_{(\text{vitamin C})}}{V_{(\text{solution})}}$$

where m(vitamin C) is the mass of vitamin C and V(solution) is the volume of the solution. The mass concentrations units are for example g/L or mg/L.
If you have a vitamin tablet, you need to grind it with a mortar and pestle or other methods or ways of turning it into a powder. The goal is to collect all the resulting powder and anything else rather than the tablet's powder, so make sure the used tools are clean. Put your powder in 30 mL of buffer solution and shake or stir for 10 minutes. Not all the binders will dissolve in the buffer, so your solution will have solid parts. The long stirring or shaking should make sure all the vitamin C is extracted from the powder. Afterwards fill the solution up to 40 mL and calculate its concentration using the equation above.
This is your stock solution.
Make 10 mL of a 200 mg/L solution by diluting a small amount of stock solution to 10 mL. If you have never done this, this sounds a bit abstract. An example will be given in the next paragraph. Generic

instructions cannot be given, because the vitamin C products have different amounts of vitamin C which results in different concentrations of your stock solution.
Practical example: Here is an example starting from an effervescent tablet with 1000 mg of vitamin C in it. The original 1000 mg effervescent tablet was dissolved in 30 mL buffer and then diluted to 40 mL. This means the 40 mL contain now 1000 mg vitamin C. 1000 mg / 40 mL = 25 mg/mL = 25 g/L. To make 10 mL of a 200 mg/L solution you need 2 mg of vitamin C. Which is calculated by multiplying the volume you want to have with the concentration you want to have: $V_{want} * c_{want} = m_{want}$
You need to know how much stock solution to add, so you calculate the volume like this:
$V_{stock} = m_{want} / c_{stock}$
This means you need 2 mg / 25 000 mg/L = 80 µL of stock solution. This will be diluted with buffer to 10 mL.

2.3.1.3 Calibration Solutions

Prepare multiple solutions that contain different known concentrations of vitamin C. We used the following concentrations in the range of 20 mg/L and 150 mg/L.
You can make different solutions from your 200 mg/L solution.
If you have vessels that are suitable for mixing 1 mL solution like Eppendorf tubes, this could be very convenient.
Mix the following concentration by adding the stock vitamin C solution to the buffer (Table 1):

Table 1 Guide for preparing calibration solutions

End Concentration vitamin C	200 mg/L stock solution	Buffer
20 mg/L	100 µL	900 µL
40 mg/L	200 µL	800 µL
60 mg/L	300 µL	700 µL
80 mg/L	400 µL	600 µL
100 mg/L	500 µL	500 µL
120 mg/L	600 µL	400 µL
140 mg/L	700 µL	300 µL

Should you find it difficult to handle such small volumes of solutions, just multiply the volumes by 10, for example 1 mL stock solution plus 9 mL of buffer will lead to 10 mL of 20 mg/L solution.
These solutions should be stored in a dark, cold place. We also recommend not using them for more than a week because vitamin C degrades over time.

2.3.2 Performing the measurement

2.3.2.1 Cut the Sensor

The SPE (Screen Printed Electrode) sensors (ItalSens IS-C-3MM) are delivered in stripes of 20 pieces each piece with working, reference and counter electrode. They are printed on polyester, so you can cut the electrodes with a pair of scissors. Don't touch the electrodes with bare hands to keep the electrodes clean. Wearing gloves during cutting is recommended. Even so, avoid touching the electrode surfaces around the disk shape.
Make the first cut to separate one set of 3 electrodes from the strip. Then make the part around the connection pads narrow enough to fit them into your SPE connector. Be careful and don't cut the leads or other conducting parts of the electrodes.
When you slide in the electrodes make sure the contact pads are aligned with the metal arcs in the connector.

2.3.2.2 Set the Parameters

Connect the potentiostat to your computer and open PSTrace.

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Connect the potentiostat with the software as described in your quick start.
Choose as technique Cyclic Voltammetry and set the following parameters (Table 2):

Table 2 Cyclic Voltammetry parameters

Parameter	Value
Technique	Cyclic Voltammetry
t equilibration	5 s
E begin	-0.2 V
E vertex1	0.7 V
E vertex2	-0.2 V
E step	0.005 V
Scan rate	0.05 V/s
Number of Scans	1

2.3.2.3 Background Measurements

First make a measurement of the phosphate buffer to know which signals are present without the presence of vitamin C.
Take 100 µL of the phosphate buffer and put it on the electrodes. The droplet needs to cover the reference, counter and working electrode.
Press the Run-Button.
This will run a CV for the electrode just with buffer. Typical features include peaks close to 0 V resulting from silver that is released from the reference electrodes. Increasing current above 400 mV is the result of carbon species on the electrodes surface being oxidized. A typical result is visible in Figure 7.

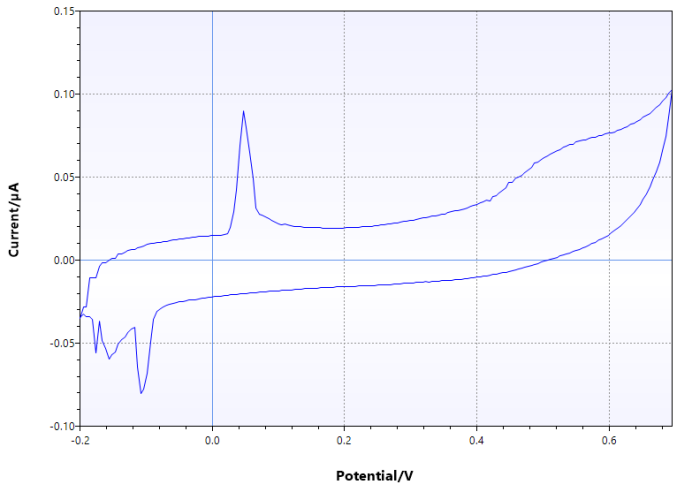

Figure 7 Typical Cyclic Voltammogram of Phosphate buffer pH 7 0.1 mol/L on an IS-C-3MM electrode

2.3.2.4 Measurements for a Calibration Curve

Remove the buffer drop by carefully using a paper tissue. Apply 100 µL of the 20 mg/L vitamin C solution.
Change the drop-down menu next to the Run-button to *Overlay*.
Run the measurement. You should observe a CV with a prominent peak on the anodic sweep at around 270 mV, that has no twin on the cathodic sweep. This is the vitamin C oxidation (see Figure 9).
It is handy to change the name of the measurement or curve to the vitamin C concentration used.

Remove the droplet with a paper tissue and repeat the measurement with a 100 µL droplet of the 40 mg/L solution.
Repeat this for all the solutions up to 140 mg/L.

2.4 Simple and Quick Version

In this chapter we will present a version of the experiment that is easy to perform even for someone without experience in a laboratory. It is also a very fast version of the experiment, because less solutions need to be prepared. A drawback is the missing information of the precise concentration used for the calibration.

2.4.1 Equipment

- EmStat Pico Development Kit
- ItalSens IS-C-3MM (screen printed electrodes based on graphite ink)
- vessels to store solution (closed)
- vessels to mix solutions (can be same as storage solution)
- volumetric cylinder (200 mL) / or other means to measure volume
- Pasteur pipette
- paper tissues

2.4.2 Solution and Chemicals

Prepare the phosphate buffer as described in chapter 2.3.1.1.
Read chapter 2.3.1.2 until “This is your stock solution.” to learn how to make your stock solution.
This vitamin C stock solution has a very high concentration. To use it, it needs to be diluted. A 10-fold dilution should work fine. Take 1 mL of this solution and add 9 mL buffer. For measuring low volumes, you can use:

- A kitchen or letter scale and work with the weight. You can assume that 1 mL weighs 1 g.
- A syringe, easily found in drugstores.
- Multiply the volumes by 10.

Vitamin C solutions should be stored in a dark, cold place. We also recommend not using them for more than a week because vitamin C degrades over time.

2.4.3 Running the Measurement

The steps described in the chapters 2.3.2.1, 2.3.2.2 and 2.3.2.3, i.e. cutting the electrodes, setting the parameters and performing the background scan, are exactly the same in this version and the one described in 2.3, but for one exception: during recording the background, instead of a precise 100 µL drop, just use the Pasteur pipette to place drop on the strip sufficient to cover all three electrodes.

2.4.3.1 Measurements for a Calibration Curve

Add to 10 mL buffer one droplet of your 10-fold diluted vitamin C solution. Mix well.
Remove the buffer drop from the strip by carefully using a paper tissue. Apply 1 droplet of the buffer spiked with one drop of the 10-fold diluted vitamin C solution.
Change the drop down menu next to the Run-button to *Overlay*.
Run the measurement. A CV with a prominent peak on the anodic sweep at around 270 mV, that has no twin on the cathodic sweep, should have appeared. This is the vitamin C oxidation (see Figure 9).
It is handy to change the name of the measurement or curve to the number of drops added.
Clean the pipette by taking up water and dispose of the water. Repeat this a few times. Or you can use one pipette for your stock solution and on to apply the sample to your electrode.
Add another droplet of the 10-fold diluted vitamin C solution to the 10 mL of buffer. Mix well.
Remove the droplet from the strip with a paper tissue and repeat the measurement with a new droplet of the solution containing 2 droplets of stock solution.
Repeat this 3 more times, so that the 10 mL buffer solution was spiked with a total of 5 drops.

This method is less reliable than solutions with known concentration. Due to this reason it can happen that the currents are too high and the instrument runs into the compliance voltage. This is usually visible due to a kink in the CV (see Figure 8). As long as that kink appears at a potential higher than the peak potential, the measurement should still be fine. To get rid of the kink dilute the vitamin C solution further than 10-fold, maybe 20-fold.

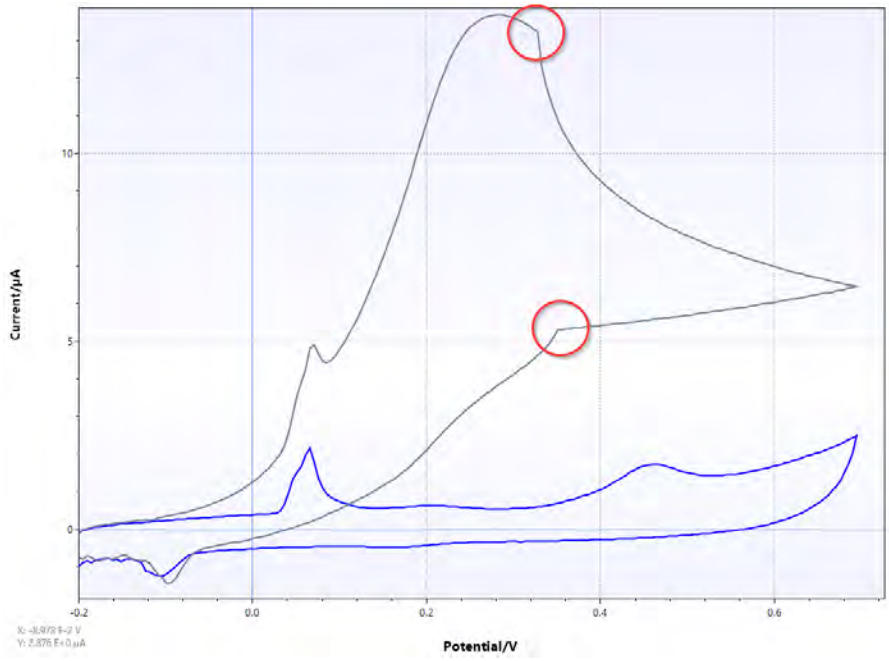

Figure 8 Cyclic Voltammogram from Figure 7 (blue) and a CV with a solution containing 10 droplets of vitamin C solution 10-fold diluted (grey), kinks by running into the compliance voltage are highlighted

3 Making a calibration curve

In a CV the peak, the current depends on the concentration in a linear way. Thus, for the calibration curve we can just plot the peak current versus the concentration of the vitamin C. The peak current is the current at the top of the peak. This can be easily obtained in Excel or in any other spread sheet software.
Note down the concentration of the droplet on the electrodes and the peak current in the CV of the corresponding measurement. If you have used the quick version described in 2.4, note down the number of drops used to spike the buffer instead of the exact concentration.
To find the peak current just choose the *Select point* tool and click on the peak. The peak current is displayed as a y axis value (see Figure 9).

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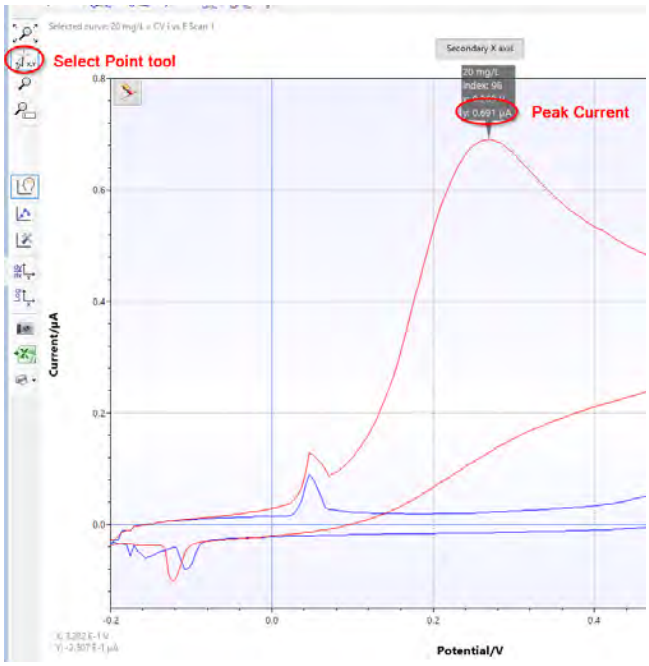

Figure 9 Cyclic Voltammogram from Figure 7 (blue) and a CV with a solution containing 20 mg/L vitamin C, peak current and point selecting tool are highlighted

Afterwards, plot in the spread sheet software the peak current versus the concentration. Then, perform a linear fit to obtain the function of the calibration curve (see Figure 10b).

3.1 Example

A series of measurements with different vitamin C concentrations is visible in Figure 10a. The peak currents of these measurements were taken to create a calibration curve visible in Figure 10b.

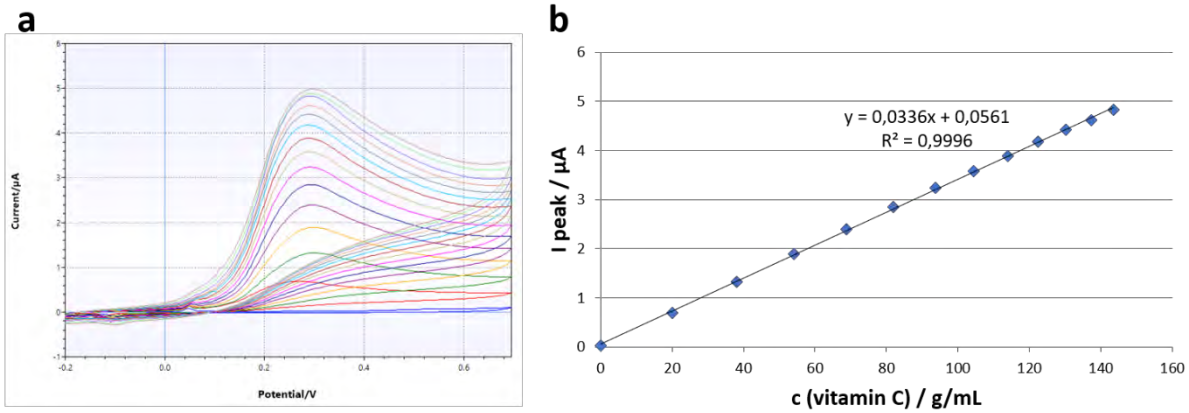

Figure 10 Series of CVs with increasing vitamin C concentration (a) and the matching calibration curve(b)

4 Extra Information

4.1.1 Linear Range

Most detection methods have a function to calculate the concentration from a signal or another parameter of interest. A linear function is easy to understand and to handle, so usually a linear dependency between signal and concentration is preferable.
Due to physical or chemical properties, the relationship of signal and concentration is not linear for all concentration ranges. If you develop a detection method, you need to check for which concentration range the calibration curve is linear.

4.1.2 Background Correction

Typically, results from this experiment don't require a background correction. The blank measurement (phosphate buffer, without vitamin C) shows negligible current compared to the measurement with vitamin C.
The oxidation peak is strong enough to render the baseline correction unnecessary. Baseline correction involves extrapolating the curve from the start of the measurement and measuring the peak current with that baseline as 0.
As an alternative the measurement of the phosphate buffer (concentration of 0 mg/L) could be subtracted from the other voltammograms. Experiments have shown that in this case only the intersection changes and not the slope of the calibration curve, so this step can be skipped.

4.1.3 Other Measurement Techniques

We chose CV here as a technique because it allows you to see that the peak associated with vitamin C has no reduction peak.
Linear Sweep Voltammetry (LSV) would have worked as well, because it corresponds to have just the cathodic sweep of the CV.
Differential Pulse Voltammetry (DPV) or Square Wave Voltammetry (SQV) could have worked as well, but they are usually more sensitive for reversible reactions.
A very different approach is Chronoamperometry (CA). In this technique, a constant potential is applied and the current is measured. If a potential higher than the peak potential of vitamin C is applied and the electrode is immersed in a buffer solution, the concentration of vitamin in this solution could be increased and the corresponding current measured. This requires stirring, a holder for the potentiostat, etc. Under these circumstances, we found it easier to work with drops of solution.

4.1.4 Selectivity Issues with Beverages

We used the ItalSens IS-C-3MM carbon electrodes. These electrodes are made from graphite paste and are not specific to vitamin C. This means other reactions can also take place at these electrodes.
There are several publications describing how to make electrodes that allow more specific quantification of vitamin C, which allowed measurements of vitamin C inside beverages like orange juice or vitamin water. Unfortunately, we have tried several drinks in different buffer concentrations, but we were not able to measure the vitamin C content with this simple method.