

## Sine “quinone” test by Roxane Andersen

Life sciences

Experiment

Senior 1 Age 17

Collège François-Xavier-Garneau

Quebec City and Chaudière-Appalaches

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### **Project summary**

Experiment on the effects of ubiquinone on photosynthetic systems (isolated chloroplasts) using data gathered with a pH meter. Semi-quantitative analysis of results (graphic and mathematical) and extrapolation for the purpose of their concrete application.

## **Project Report**

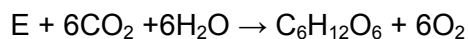
### **Introduction**

There are many different types of metabolisms on this planet, but the two most common are photosynthesis and cellular respiration. These very similar and complex reaction chains are the very basis of life: they are the key to energy production in almost all organisms. These two metabolic pathways use a series of chemiosmotic, biochemical and other mechanisms and principles to draw energy from the environment. Of all the molecules that participate in the process, one is particularly interesting: plastoquinone (or ubiquinone in cellular respiration). This molecule, in combination with a number of other parameters, is responsible for the creation of an environment favourable to the synthesis of energy.

The goal of this experiment is to determine semi-quantitatively the effect of adding ubiquinone to a photosynthetic system in order to verify the following hypothesis: more ubiquinone (or plastoquinone) in this type of system should increase its speed and productivity. The different data gathered from chloroplast solutions using a pH meter must therefore be processed. Since the analysis of such results is highly technical, the first part of this report is devoted to the basics necessary to understand the results and their interpretation.

### **Theoretical and methodological contexts**

In general, metabolisms develop according to certain basic principles. Photosynthesis, the principal metabolism in question, is a reaction series that transforms energy, CO<sub>2</sub> and water into sugar and O<sub>2</sub>.



Photosynthesis takes place in specialized organelles, or chloroplasts, made up of an outer membrane and an inner membrane, the latter forming compartments called thylakoids. Photosynthesis can be broken down into two major reaction series: reactions that require light and those that do not. Light-dependent reaction series are the ones that transform solar energy into ATP (an energy-rich molecule). They take place inside the

chloroplast, more specifically in two photosystems located between the thylakoids (photosystems are pigment systems that release electrons to form an electron transport chain between them. This chain has a specific function: to produce ATP through photophosphorylation. ATP is synthesized in part by the movement of electrons and protons between carriers.

One of these carriers is plastoquinone, whose equivalent in cellular respiration is ubiquinone. More specifically, these molecules are carrier coenzymes, which means that they move between reactions, allowing electrons and protons to move around. In chloroplasts, they act as hydrogen carriers and are partly responsible for a mechanism that allows the cell to produce energy, the electrochemical gradient. This gradient is a difference in electrochemical potential—electrical potential (mV) and chemical potential (pH)—which allows specialized complexes to synthesize ATP. Without this potential, it is impossible to produce energy in this form:



The aim of my experiment is to determine the effects of ubiquinone on photosynthesis (and, by extrapolation, on cellular respiration). My hypothesis is as follows: considering the theory of potential electrochemical gradients and the essential presence of benzoquinones (plastoquinone and ubiquinone) in metabolisms, one might assume that the addition of such a molecule to a solution of isolated chloroplasts would increase energy production. In fact, since plastoquinone creates the potential that governs energy production, adding plastoquinone to a solution of chloroplasts should increase the electrochemical gradient, thereby making it possible to synthesize more ATP or to synthesize it more quickly. To verify this hypothesis, it is necessary to gather certain data. The experiment is divided into two parts. While the first part—isolating the chloroplasts—is essential, it does not contribute to achieving the goal. Once a sufficient quantity of chloroplasts has been collected, the experiment can begin. At this point, it is necessary to develop an effective experimental method to refute or confirm the hypothesis. The method used is based on the acid-base nature of chloroplast solutions from which it is possible to gather the necessary data using a pH meter. The analysis of the experiment will be based on a graphic analysis establishing a comparison between chloroplasts with and without quinone, using a boiled chloroplast solution as a chloroplast control and a solvent solution as a second control. Analysis of the curve (speed in u. pH/sec.) will make it possible to establish a relationship between the two solutions. It will also be possible to establish the relationship between the concentration of H<sup>+</sup> ions over time, using the following formula:

$$-\text{pH} \text{ antilog} = [ ] \text{ H}^+$$

It will also be necessary to establish the causes of errors and uncertainties that might alter certain results. It will be interesting to consider possible parallels with cellular respiration and to evaluate possible concrete applications based on the results obtained.

The material used in the experiment includes fresh spinach leaves, sucrose (a sugar), water, a 10% propylene glycol solution and ubiquinone. A mixer, a centrifuge, centrifuge tubes, beakers, graduated cylinders, a stirring plate, an electronic balance accurate to 0.1 g, an electrode pH meter (with a pH 7 buffer solution) and patience are also required. A set-up diagram and details about the steps in the experiment will be presented later.

## **Analysis and discussion**

The analysis will be divided into two parts: first, a comparison between the pH values of the same solution over time, then a comparison of values of the two types of solutions. These are semi-quantitative analyses. A first comparison of the graphs of the solutions without quinones reveals a trend. The pH values are of the same order of magnitude and all the curves have a reproducible general shape. In fact, it appears that the pH first decreases—the acidity of the medium increases—then, after a certain amount of time (between 400 s and 700 s), the pH oscillates before stabilizing. The difference between the pH values and between the more specific shapes can be explained by the causes of errors (listed below in the report). Then, a comparison of the graphs of solutions with added ubiquinone reveals that the pH values remain of the same order of magnitude. The general trend here is even more evident than with the solutions without quinones: the pH value decreases, then increases before stabilizing. This trend can be explained by the following principle: photosynthesis in chloroplasts occurs on a permanent basis. When the chloroplasts are isolated, the reactions remain the same but the media change: interactions between the membranes and the cytoplasm become interactions between the membranes and the solvent (10% propylene glycol). First, H<sup>+</sup> ions are released into the medium (potential electrochemical gradient), then ATP synthetase produces ATP by pumping the ions through the chloroplast membranes. Finally, the chloroplasts appear to favour acyclic photophosphorylation, which results in a slight decrease in the pH due to the production of new H<sup>+</sup> ions and a new search for equilibrium (oscillation). The reproducible nature of these results will be determined more specifically in the presentation. It will be necessary to derive the functions and obtain acceleration values or use existing curves to determine the average values. With these average values, the uncertainties and differences will be more quantitative (because they will have been processed mathematically). Second, a comparison between the graphs for the two types of solutions reveals that the solutions with added ubiquinone always have lower pH values (so they are always more acidic) than the solutions without quinone. There is also a greater difference in pH between the quinone solutions than between purely chloroplast solutions over the same period of time (so the reaction is quicker). A graphic analysis of the concentration of H<sup>+</sup> ions over time leads to the same type of conclusion. The reproducibility of this trend will also be determined more specifically during the presentation. Despite the reproducible general shape, the results show a certain variance: the causes of error in this case have a greater effect than the causes of uncertainty on measurements and results.

Some parameters can affect results by increasing the acidity of a medium or by accelerating reactions. The most important causes of error are lighting, the freshness of the solutions, temperature, the partial insolubility of ubiquinone and the rinsing of the pH meter electrode. If there is a lot of light, photosynthesis occurs more rapidly, as does the decrease in pH. It is therefore necessary to ensure that, if the lighting is not identical in all experiments, it is as close as possible for solutions used in a single step of the experiment. Lettuce or spinach leaves that are not fresh can alter or even completely falsify the results. Since there are more dead cells in older leaves, photosynthesis is no longer possible, which decreases the speed of the reactions as well as the concentration of chloroplasts in the solutions. This results in higher pH values. In addition, the speed of photosynthesis varies in proportion to temperature. The pH will therefore vary in indirect proportion to temperature: the higher the temperature, the lower the pH, up to a certain point. Therefore, as with lighting, it is necessary to restrict temperature variations in a given step and to take such variations into account in the overall interpretation of results. Also, ubiquinone is only partially hydrosoluble (because of its nonpolar hydrocarbon

chain), which results in a suspension in the chloroplast solution and which might reduce the quantity of light reaching the chloroplasts as well as the proportion of ubiquinone that reacts to it, thereby decreasing the speed of the reaction. Finally, by rinsing the electrode with distilled water, the solution is diluted and the pH is slightly higher.

Causes of uncertainty are not as important as causes of error, but they can still influence results. The uncertainty on the pH meter is  $\pm 0.02$  units, and the uncertainty related to time is  $\pm 2$  s. The longer the time span, the less important the relative value of uncertainty. Consequently, the uncertainty on the pH meter has more influence overall. Its relative importance varies according to the evolution of the reaction.

Despite the differences observed, the results are consistent with the theory. A number of improvements could be made to the method used. For example, it would be advisable to ensure constant lighting and temperature, perhaps by conducting the experiment somewhere where outdoor light does not penetrate and where temperature can be controlled. Using fresh chloroplasts would be an improvement, but since the date on which spinach leaves are picked is not indicated on the packaging, this parameter is difficult to control. To reduce the suspension of ubiquinone, a magnetic stirring bar makes it possible to maintain at least a certain uniformity in the solution (this improvement is currently being tested). In order to restrict the relative value of uncertainty with the pH, the number of data could be increased and a pH meter accurate to 1 mg could be used. In terms of the experiment as such, working simultaneously with several pH meters, one for each solution, would improve the quality of readings and prevent errors that could be introduced by rinsing the electrode. It would also decrease the relative significance of errors over time. Ideally, data collection could be computerized so that errors caused by repeated readings are minimized. This would also make it possible to establish a larger database, since the time required to collect data could be devoted to preparing the solution.

## **Conclusion**

Although the experiment is not quite finished and there are still a few elements to be verified, it is now possible to draw a general conclusion and to consider that the goal of the experiment has been achieved. In fact, we observed that adding ubiquinone had an effect on photosynthesis via effective readings. In addition, in light of our observations, despite certain differences between the curves, the hypothesis is almost certainly confirmed: adding ubiquinone to a solution of isolated chloroplasts increases the production of  $H^+$  ions and therefore the speed of photosynthesis reactions. We can therefore extrapolate and state positively that adding ubiquinone increases the energy efficiency of a photosynthesis system. Additional information will be available at the presentation.

It is also possible to further extrapolate and state that cellular respiration, which is based on the same principles as photosynthesis, could also be more effective with added ubiquinone.

Although the details of concrete applications cannot be presented here, there are a few interesting possibilities. Certain reservations, however, apply. Despite the precautions taken in the experiment, the results may be obtainable only under certain very precise conditions. Nevertheless, it is possible to imagine a variety of applications, for example, producing plants that are better adapted to certain conditions and that could maximize photosynthesis and produce more energy over a shorter period of time. Similarly, in the

very long term, this type of plant could reduce the concentration of CO<sub>2</sub> in the atmosphere.

Only by experimenting will we be able to acquire more in-depth scientific knowledge. I would like to thank all scientists, since science would not be such a fantastic world of discovery without them.