Chromatography of photosynthetic pigments | lab report

The aim of this experiment was to separate and isolate the different photosynthetic pigments, found on spinach leaves and to extract them using the paper chromatography method. The aim was also to determine the relative amounts of chlorophyll a and chlorophyll b from their absorption spectra. Leaves of fresh spinach were used to separate its photosynthetic pigments and then measure their absorption and ratio between chlorophyll a and chlorophyll b. Apart from the paper chromatography method, another method called spectrophotometry was used in order to measure the absorption of light of each pigment. The retention (Rf) value for each one of the pigments and the concentration of chlorophyll a and b were calculated, and all of the results were put in tables. The ratio of chlorophyll a to chlorophyll b was then also calculated and the result was 2. 6: 1, which is quite close to the result expected.

Introduction:

It is necessary that some information on the experiment on chromatography of plant pigments is given, before the analysis of it. Spinach was used during this experiment and is a member of the family of ‘ Chenopodiaceae’. It comes from a leaf, winter annual plant, which evolved in the Fertile Crescent of the Middle East. Winter annuals are species of plants that grow in the coolness of autumn and grow stem, until the cold weather and short days of winter come to reduce their growth. In the spring, winter annual plants grow steadily by the time a combination of environmental factors urge them to the reproductive (frisk) phase of their life. The frisk of spinach begins primarily, depending on the day, and the ancestral forms of this plant crops frisk too soon, with less than 14 hours of light per day. This enables the seed of this

crop to grow up, before the advent of intense heat during summer in the Middle East. The seed then is left to latency and after its progress, it could grow only by the advent of cool, wet weather during the autumn. Modern forms of this plant crops have been selected to produce a vigorous and robust leaf vegetable, which is no longer adaptable to different environments and seasons, but some of the traits of its ancestors remain. Many varieties of spinach still produce well as a vegetable crop, sown in autumn and harvested in autumn, winter or spring. However, many varieties of spinach also grow, planted in spring and producing an abundant harvest, before the long summer days cause the frisk. Most modern European varieties do not start the frisk before the day duration reaches 16 hours (during the day in Northwest Washington near the summer solstice). Although it is still difficult to grow the seeds of spinach, when planted in warm soil, the seed grow a lot easier than these of the ancestral varieties.

As far as photosynthesis is concerned, it is the normal mode by which green plants obtain carbon and oxygen needed for nourishment. The plants that use photosynthesis have the ability to convert carbon dioxide into the atmosphere carbon compounds necessary for their development. The photosynthetic function requires the presence of oxygen and the energy of sunlight. The first observations of the phenomenon of photosynthesis were made by Priestley, in 1771. He first noticed that green plants cleaned the air, which was contaminated by the respiration of animals. The observations of Priestley were continued by Jan Ingenhousz (1730-1799), who was a Dutch doctor. Nicolas de Saussure demonstrated in 1804 that the weight of oxygen, which is expelled together with the weight of the plant during

photosynthesis, is greater than the weight of carbon dioxide absorbed. During the 20th century, the phenomenon of photosynthesis was studied on each side (biochemical, chemical, physiological, etc.). In 1941 experiments with radioactive isotopes and research on the complex series of the different reactions were made for the first time. Today, the mechanism of photosynthesis is in general when the water dissolves and carries carbon dioxide to the cells and chloroplasts in leaves. The light energy (hv) absorbed by the chlorophyll breaks down water (photolysis) in the data: H2O h. v [H] + 1 / 2 O2. The oxygen is released and the hydrogen atom bounds to various enzymes. Then the hydrogen is driven in reactions with carbon dioxide: CO2 + [H] (CL2O) x. In the second stage of the reaction solar energy is not needed, so these reactions are called “ dark”. Starch is one of the first compounds formed. This is transferred to other locations of the plant during the night when stopping the phenomenon of photosynthesis.

There are several pigments involved in biological reactions. The colors associated with photosynthesis and encountered in leaves and other parts of organisms are called photosynthetic pigments. The most important of these are the chlorophylls. Chlorophylls are common to all plants, primarily in cyanophytes and several bacteria. The organisms that contain no chlorophyll cannot do photosynthesis and are condemned to death. The main types of chlorophylls are chlorophyll a and chlorophyll b, which differ slightly in their structure. Chlorophyll a absorbs radiation with wavelengths near the two ends of the visible spectrum (i. e. red and blue). Besides chlorophyll a, plants use other colors, which absorb radiation with intermediate wavelengths. In this way there can be a better utilization of solar energy. These colors are

called complementary colors. Among the additional pigments are chlorophyll b and carotenoids that absorb photons of blue and blue-green and appear with a yellow to orange color. The xanthophylls are complementary colors derived from carotenoids, like beta-carotene, which is the predominant carotenoid in carrots. The fykovillines (phycocyanin and phycoerythrin) are complementary dominant colors in cyan bacteria and red algae.

The pigments get their colour by absorption, i. e. the removal of certain parts of the spectrum and the reflection or the passage of those remaining. Any colour, with a particular molecular structure, is tuned to a wavelength of colour, just like a radio can be tuned to a given radio frequency, to capture some brands. The frequency or frequencies, or set the colours that are absorbed by the stronger pigment molecule.

Chromatography is the separation of mixtures of compounds in pure components and their quantification is a central theme of chemical laboratory work. Only in this way scientists can properly analyze the purity of both chemicals and composition of mixtures with different content. The principles of chromatography as used today have as their starting point the botanist Michael Tswett (1872 – 1919). In 1906 he published a procedure involving the separation and isolation of yellow and green colours of leaves by chromatographic absorption.

Methods:

Firstly, the leaf extract was prepared by weighting out 3 g of fresh spinach. After that the spinach was cut into small pieces and placed in a mortar, while 15 ml of ice-cold acetone and a sprinkle of clean sand were added. Then, all

the ingredients were grinded together for a minute. The mixture then was transferred to a 50 ml stoppered test tube and was shaked for 10 seconds before being placed in the refrigerator or an ice bar for 10 minutes. After that, using a Pasteur pipette some of the dark upper layer (which contained the pigments was) was transferred to a small stoppered tube.

During the preparation of the chromatography paper, a line should be drawn across the width of the chromatography paper about 3 cm from the bottom. Then, 10 streak applications of pigment extract along the line were made using a capillary tube. The chromatography paper was then placed in the equilibrated chromatography jar and was left there for 30 to 45 minutes in dark or dim lightening conditions or until the separation of the 5 bands is visible. Afterwards, the chromatogram was removed from the jar and hold by the corner until dry. Then, the distance from the origin to the solvent front and to the centre of each band was measured.

During the elution and spectrophotometry, a spectrophotometer was used, which is a machine that measures the absorption of light of a solution at a specific wavelength. At first, the spectrophotometer was left for at least 10 minutes to warm up and after that the wavelength was set at 400 nm. After labelling five cuvettes (0, 1, 2, 3, 4), the five chromatogram bands were cut out and placed in the appropriate cuvette. The cuvette number one contained the first bang, which corresponded to chlorophyll b. The cuvette number two contained the second band, which corresponded to chlorophyll a. The cuvette number 3 contained the third and fourth band, which corresponded to violaxanthin and lutein respectively and finally the cuvette number four contained the fifth band and corresponded to beta-carotene.

But before the bands were put into the cuvettes, they should firstly be cut into small pieces in order to fit in them. Afterwards, 4 ml of acetone was put in each cuvette (to cuvette number zero also, which was blank) and then each tube was corked. The pigments were left to elute for five minutes and occasionally the tubes were swirled. Then the paper stripes were removed with forceps. After that the spectrophotometer was calibrated and the absorbance at each wavelength from 400 to 700 nm was read and all the results were in the end written in a table. Then, a graph including all of the results was plotted.

Finally, the separation of components was measured by the retention value (Rf).

Distance moved by the solute

Rf =

Distance moved by the solvent from the origin

Afterwards, the concentration of chlorophyll a and chlorophyll b was measured using the Beer – Lambert equation written as: A = acL and then the ratio between these two pigments was measured also.

Results:

Figure 1: The retention value is described, which can be defined as the ratio of the distance moved by the solute and the distance moved by the solvent along the paper. This is where both distances are measured from the

common origin or application baseline that is the point where the sample is initially spotted on the paper.

From the upper table it can be determined that Chlorophyll b has the lower retention value, which means that travelled a smaller distance than the other pigments on the chromatogram. On the other hand, B carotene travelled the biggest distance on the contrary with the other pigments on the chromatogram.

Graph of absorption spectra of different pigments:

Figure 2: The absorption of four different pigments is described (chlorophyll b, chlorophyll a, violaxanthin & lutein and B carotene) in different wavelengths from 400 to 700 nm.

In the upper graph, the differences in the absorbance between the 4 different pigments can be determined. It is obvious that chlorophyll a has the greatest absorption of all pigments. On the contrary, B carotene has the lowest absorption of all pigments. However, all the pigments show a decrease between the wavelengths from 500 to 620 nm.

The concentration of chlorophyll a and b were calculated using Beer-Lambert equation and consequently, the ratio of chlorophyll a to chlorophyll b is 2. 6: 1, according to the upper information.

Discussion:

This experiment has demonstrated the isolation of the photosynthetic pigments, following the method of chromatography on paper. First of all, the

leaf extract was prepared by grinding some leaves in acetone and some of the upper part of the leaf extract was added to the chromatography paper. Then, the paper was left in the equilibrated chromatography jar for some time until the pigments were separated into different bands. After that the chromatogram bands were cut out and put into different cuvettes with acetone. After the chromatogram bands were removed from the cuvettes, the absorbance of each pigment in different wavelengths (between 400 and 700) was measured using a spectrophotometer. After all the practical work was finished, the retention value (Rf) was calculated and the results were put in a table. Calculating the retention value of the pigments was made to read easier the results shown on the chromatography paper, as it is a scientific method of comparing the distance moved by the solute and the distance moved by the solvent front. According to the results, it is noticed that chlorophyll b had the smallest retention value and chlorophyll a had the second smallest retention value, which means that the lower the retention value is, the smallest distance from the baseline the pigment has on the chromatogram.

The absorption of each pigment that was isolated from the spinach leaves was plotted in a graph, which showed that chlorophyll a had the greatest absorption of all pigments. Chlorophyll b came with the second higher absorption in contrast with the other pigments and violaxanthin, lutein and B carotene came last with lower absorption. That means that a smaller amount of light managed to pass through the pigments of chlorophyll a and b. Afterwards, the concentrations of the two pigments chlorophyll a and chlorophyll b were calculated, so that the ratio of these could be also

calculated. Finally, the ratio seemed to be 2. 6: 1, which is quite close to the ratio expected to accrue. Each plant has a specific ratio of its pigments and does not change, but different plants do not have the same ratios.

Sometimes, small mistakes may be done during the processes used for an experiment and can affect the accuracy of the final results. There is a possibility in this experiment that wrong amounts of acetone were used, which would affect the results. There is also another possibility of making a mistake while reading the results on the spectrophotometer screen or making a wrong use of the chromatography paper by adding the leaf extract on it that would be able to completely change the results of the experiment. Furthermore, this can be the reason why people sometimes get different results while repeating the same experiment a few times.

Summing up, the inference of this process is that the results are enough accurate, because they do not seem to have a great variation of similar experiments done in the past on this subject. In general, the chlorophyll a to chlorophyll b ratio seems to be always around 3: 1, which is not much further from 2. 6: 1, which is the ratio of the two chlorophylls found by the end of this experiment.