The Investigation of the Vitamin C Concentrations of Distinctive Layers (Flavedo, Albedo& Locule) of Different Citruses (*Citrus limon*, *Citrus sinensis*, *Citrus paradisi* and *Citrus tangerine)*

Extended Essay (Biology)

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ABSTRACT

In our daily lives, most of the dietitians claim that the most abundant part of a fruit, in terms of vitamin, is the outermost peel. The focus of this study is to test whether the vitamin concentrations changes as different parts of a fruit is investigated. So the aim of this study is to investigate the vitamin C concentrations of distinctive layers (flavedo, albedo& locule) of different citruses (*Citrus limon*, *Citrus sinensis*, *Citrus paradisi* and *Citrus tangerine)* by a redox titration using a solution of iodine of accurately known concentration and starch solution as an indicator. In the experiment, different layers were extracted with the help of a scalpel and liquefied in presence of 100 mL %3 metafosforic acid solution. Then, titration method is used. Values obtained from titration were then processed and the amount of ascorbic acid concentrations in each layer were calculated.

The mean value of ascorbic acid concentration of *Citrus limon* from the outermost layer to the locule found to be: 166.390mg/100g ,102.260mg/100g, 55.140mg/100g; for *Citrus sinensis*: 232.980 mg/100g ,169.72mg/100g , 49.840mg/100g ; for *Citrus paradise*: 118.960mg/100g, 108.880mg/100g, 40.926mg/100g ;for *Citrus tangerine*: 232.980mg/100g, 169.720mg/100g and 49.840mg/100g. According to the obtained data and calculated mean values, in this investigation the accuracy of the hypothesis is confirmed; the ascorbic acid amount of different citrus fruits (Citrus limon, Citrus sinensis, Citrus paradisi and Citrus tangerina) decreases as inner parts of fruit is investigated. The least concentrated layer is found to be locule (the innermost layer) while the most concentrated one is found to be flavedo (the outermost layer). Overall, the results of this study advocated the hypothesis that the ascorbic acid amount of different citrus fruits (Citrus limon, Citrus sinensis, Citrus paradisi and Citrus tangerina) decreases as inner parts of fruit is investigated.

Word Count: 294

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INTRODUCTION

I was always interested in vitamins. My mother have always followed the upcoming news about nutrients and their vitamin viability since I was a little child. A few months ago, while my mother and I was reading one of the latest magazines about this issue, she showed me a news which claimed that mostly the outer layer of a fruit contains the highest vitamin levels. Therefore she began to boil the outermost layer of citruses (like orange) and drank it in order to benefit from the most nutritious part of the fruit. After doing some research I saw that without doing any research, people were accepting this fact and they were doing the same as my mother did. From that day on, I wonder how different layers of the same fruit can contain distinctive amount of vitamin.

A vitamin is an organic compound required by an organism as a vital nutrient in limited amounts.[1] An organic chemical compound (or related set of compounds) is called a vitamin when it cannot be synthesized in sufficient quantities by an organism, and must be obtained from the diet. Thus, the term is conditional both on the circumstances and on the particular organism. For instance, ascorbic acid (vitamin C) is a beneficial vitamin for humans, but not for most of the other animals. [2]

Ascorbic acid and dehydroascorbic acid are both trivial names for Vitamin C. Compound can both donate or gain hydrogen atoms, therefore it can be found in both states as shown in Figure 1.1. Ascorbic acid is actually a simple compound to monosaccharide which is chemically related to the empiric formula of $C_6H_8O_6$. They both are soluble in water, glycerol, ethanol whereas insoluble in fat solvents such as ether. Both of them exists in L and D form, however the biologically active ascorbic acid is the L- form which distinguishes this vitamin from glucose. The vitamin is stable under pH level of 4; as pH level increases the stability of the ascorbic acid decreases .Moreover, it can be easily oxidized by metals such as copper or iron. Fortunately, those food rich in ascorbic acid are relatively acidic and lack of iron and copper.[3]

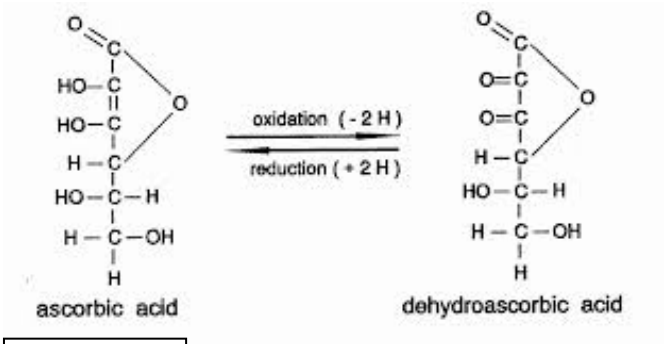


Figure1.1

[1] S, Liebermann, and Bruning N. *The Real Vitamin & Mineral Book*. NY: Avery Group, 1990, pg 3-4.

[2] "Vitamin and Mineral Supplements in the Primary Prevention of Cardiovascular Disease and Cancer,".*Annals of internal medicine***159,** pg12.

Figure1.1: http://chemistry.oregonstate.edu/courses/ch130/old/VITCTEXT.htm

[3] Carolyn D. Berdanier, Advanced Nutrition Micronutrients, CRC Press, pg 76

Ascorbic acid readily converts between the free and dehydro form, it functions in hydrogen ion transfer system and aids in the regulation of states in the cell. [4]Therefore, it is essential as needed for the repair of the tissues and growth. As it is a water-soluble antioxidant it is also responsible for the protection of naturally occurring antioxidants.

Vitamin C functions as an amino acid in the production of a collagen which is used to make up the skin and tendons as well as blood vessels. Moreover, this vitamin is needed for the incorporation of iron into ferritin (a protein responsible for iron storage).[5] It is also responsible for repairing and maintaining cartilage, bones and teeth. Thus, in vitamin C deficiency "scurvy disease" occurs which causes spongy gums and bleeding from the mucous membranes. However, human body cannot synthesize this vitamin by itself as human body is lack of one of the amino acids needed for the synthesis of ascorbic acid; also and it cannot be stored in body. Therefore, it has to be consumed regularly on daily basis and one of the highest source of vitamin C is citrus fruits.[6]

The role of citrus fruits in providing nutrients has been recognized since ancient times. Citrus fruit belonging to the genus *Citrus* of the family Rutacae, are well known for their refreshing fragrance , thirst quenching ability and providing adequate vitamin C as per recommended dietary allowance.[7] Citrus flower buds begin to form in early winter and develop through late winter and spring as the optimum temperature for citrus root growth is about 79 Fahrenheit and the minimum temperature at which citrus roots will elongate is about 54 Fahrenheit. Citrus will grow in most soils from sandy to adobe clay, provided it drains well. Citrus fruits are produced all over the world; according to UNCTAD, in 2004 there were 140 citrus producing countries while Brazil can be considered as the largest citrus producer. [8]

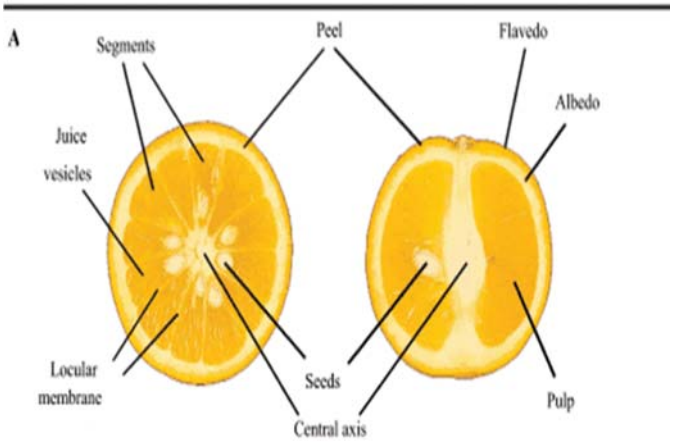


Figure 1.2

Citrus fruit arises through the growth and development of the ovary and consists of 8-16 carpel clustered around and joined to the floral axis, which forms the core of the fruit. The carpel forms locules in which seeds and juice sacs grow. The peel is divided into exocarp, or flavedo, and mesocarp, or albedo. [9]The flavedo

[4] Advanced Nutrition Micronutrients , Carolyn D. Berdainer (2000), pg 75-77

[5] Advanced Nutrition Micronutrients, Carolyn D. Berdanier, CRC Press, pg 76

[6] Advanced Nutrition Micronutrients, Carolyn D. Berdanier, CRC Press, pg 76

[7] Milind L. , Citrus Fruit , pg 1.

[8] http://en.wikipedia.org/wiki/Citrus_production

[9] Citrus Fruit- Biology, Technology and Evalutaion, Milind Ladaniya, (2008) , pg 106

consists of the outermost coloured part while albedo is the inner, white layer and the locule part is the innermost juicy part (Figure 1.2).[10]

Each of these layers' development consists of different pathways so I desired to determine whether these layers vitamin C availability differ as well. **Do different layers (flavedo, albedo& locule) of *Citrus limon*, *Citrus sinensis*, *Citrus paradisi* and *Citrus tangerina* contain dissimilar concentration of ascorbic acid measured by a redox titration using a solution of iodine of accurately known concentration and starch solution as an indicator?** This research question will be discussed throughout this paper.

[10] figure1.2: http://www.scielo.br/img/revistas/bjpp/v19n4/a06fig07.gif

HYPOTHESIS

The outer seed coat is composed primarily of the outer epidermis of the ovular wall. The epidermal cells form a secondary wall which makes up a woody, cream- or yellow-colored tough covering.[11] This layer is mostly composed of cellulosic materials like glucose, but also consists of oil glands, pigments and protein. According to the 'Function of ascorbic acid in collagen metabolism' article by M. J. Barnes, ascorbic acid acts as an amino acid for collagen production, so it has an inexorable role in protein synthesis and structurally similar to cellulose. In plants, ascorbic acid has similar functioning as well. As protein is mostly condensed at the outermost layer of the fruit (flavedo), it can be stated that ascorbic acid most abundantly found in the flavedo.

In a citrus, the sun exposure is highest in flavedo. For the protection and development of the fruit, the damage caused by the UV light should be induced. As stated in the introduction, vitamin C help prevention and threatening of ultraviolet-induced photo damage. Therefore, the vitamin C dense in flavedo expected to be the highest. Eventually, it is logical to state that ascorbic acid concentration in a citrus decreases as inner parts of the fruit is evaluated.

Ascorbic acid's activities, such as prevention from excessive UV radiation or collagen synthesis, are mostly needed in the outermost layer of the fruit. Hence, it can be hypothesized that **the ascorbic acid amount of different citrus fruits (Citrus limon, Citrus sinensis, Citrus paradisi and Citrus tangerina) decreases as inner parts of fruit is investigated.** Flavedo is expected to be the most abundant layer in terms of vitamin C while locule is expected to be the least.

In human beings ascorbic acid plays an important role in the maintenance of collagen which represents about one third of the total body protein. It is essential for the constitution of the principal protein of skin, bones, teeth and cartilage. Moreover, because of global warming, the amount of dangerous UV radiation that reaches to the surface is increasing. Excessive UV exposure results in skin cancer and unpreventable cornea problems. To minimize the effect of this phenomena, the consumption of ascorbic acid should be increased. Vitamin C cannot be stored in the body or body cannot produce it by itself so it has to be taken on a daily basis. Consumption of 100 mg/day of ascorbic acid is found to be sufficient to saturate the body in healthy individuals. [12] According to this hypothesis, in order to achieve the necessary amount of ascorbic acid consumption on daily basis, expenditure of the outermost layer of the citrus fruits will be more logical.

[11] . http://irrec.ifas.ufl.edu/flcitrus/pdfs/short_course_and_workshop/citrus_flowering_97/Jackson-Seed_Development.pdf
[12] Vitamin C in Human Health and Disease Is Still a Mystery ? An Overview."*Nutrition Journal*. N.p., n.d. Web. 14 Dec. 2014

METHOD DEVELOPMENT AND PLANNING

To test the research question "Do different layers (flavedo, albedo& locule) of *Citrus limon*, *Citrus sinensis*, *Citrus paradisi* and *Citrus tangerina* contain dissimilar amount of ascorbic acid by carrying out a redox titration using a solution of iodine of accurately known concentration and starch solution as an indicator?", same titration method should be applied to same volume of different layers of distinctive citruses. Titration is a method which determines the amount of acid in a certain mixture by the help of neutralization. Here, it will be used to find out the amount of ascorbic acid in different layers of each citrus fruit. I chose this method as this is the most accurate way to determine the amount of acid in the albedo and flavedo as well as locule.

I investigated this research at Düzen Norwest Laboratory and acquired laboratory acces from Selin Miran,a scientist working at Düzen Norwest laboratory. The equipment used in this experiment , that were designed by me, were supplied by the Düzen Norwest Laboratory and Merck Company.

The measurements in flavedo & albedo layers will be more complicated than just measuring the vitamin C concentration in the juice. Therefore, it would be better to take measurements of the peel in the first hand.

The flavedo and albedo layers of the fruit should be separated carefully by the help of a scalpel. After all of the solid parts are weighed on the electronic balance, the mass values should be recorded. However, as mentioned in the introduction part, ascorbic acid can be oxidized in presence of oxygen. Therefore, after doing some research I figured out that certain acids (metafosforic acid and ethandioic acid) prevent this loss of vitamin C for a short period of time. And the ideal amounts of acids found to be mL %3 metafosforic acid and %1 ethandioic acid in a 100 mL solution.[13] So without waiting, 100 mL %3 metafosforic acid and %1 ethandioic acid containing solution should be added to these layers.

Then sample should be prepared for titration. Firstly, 100 mL %3 metafosforic acid should be added to the peel (to make it ready for homogenization) and homogenized in a blender. Approximately 25 g from this mixture will be enough for the titration. Then this solution should be completed to 100 mL by the help of mL %3 metafosforic acid and %1 ethandioic acid. Then, this mixture have to be soared by the help of a filter paper. 25 mL should be obtained from this supernatant and titrated with the 0.05 M (0.1N) iodine solution in presence of %1.1 mL starch indicator. When ascorbic acid completely reacts with the iodine solution(at equilevance point) the color of the sample changes to dark violet from transparent. When end point is reached, neutralization becomes complete and the number of moles of used iodine becomes equal to the number of moles of acid in the flask. When

[13] Article: The affect of the metaphosporic acids and some other inorganic acids on the catalytic oxidation of ascorbic acid , Carl M. Lyman, M. O. Schultze and C. G.King, J. Biol. Chem. 1937,pg 760

equilevance point is reached, addition of titrant should be stopped and the volume of the used titrant should be saved. During these steps, some air bubbles may be observed in the flask which can cause random errors. To prevent this from happening, flask should be examined in a delicate manner.

Furthermore, the base of titration is constructed upon the observational skills of human. The end point is revealed via color differentiation, therefore can only be detected by the human eye. Not only color change is delicate and tedious but also different people have distinctive sensitivity toward colors. Therefore, in this experiment at least two helpers should observe the titration process to obtain more accurate results.

After these steps, mass of titrated sample can be calculated and the amount of ascorbic acid in 100 g can be found by simple ratio operations.

For locule part, the juice is squeezed immediately and directly titrated with the 0.05 Molar iodine solution in presence of %1 .1 mL starch indicator.

METHOD

Materials Used in the Experiment

- 0.05 M Iodine solution
- 1% starch solution in 100 mL distilled water
- 3% Metaphosphoric acid solution in 1 L distilled water
- 3% Metaphosphoric acid solution + %1 Ethandioic acid in 1 L distilled water
- Electronic balance (± 0.001)
- Test mixer
- Erlenmeyer flask (250 mL ± 0.01)
- Volumetric flask (100 mL ± 0.01)
- Funnel
- Filtration paper
- Blender
- Pipe (100-1000 microlitre)
- Citrus limon, x5 (1 for each trial)
- Citrus sinensis, x5 (1 for each trial)
- Citrus paradisi, x5 (1 for each trial)
- Citrus tangerina, x5 (1 for each trial)
- Graduated cylinder (50 mL ± 0.01)
- Scalpel x1

PROCEDURE

-For flavedo and albedo parts;

1) Seperate the flavedo and albedo layers of all fruits carefully by the help of a scalpel.
2) Weigh mass of the solid part (20-50 g) by an electronic balance and record the value.
3) Without waiting add 100 mL %3 metafosforic acid and %1 ethandioic acid containing solution to the peel.
4) To make the sample is ready for titration, homogenize it with a blender.
5) Take approximately 25 g from the homogenized mixture.
6) Add 100 mL solution containing %3 metafosforic acid to the 25 g homogenized mixture.
7) Soar the mixture by using a filter paper.
8) Obtain 25 mL from the supernatant.
9) Your sample is ready for titration.

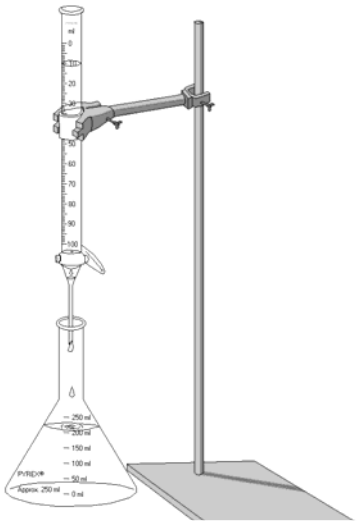


Figure2.1: Titration system set-up

For titration:

1) A standard titration method will be used (App 1).
2) 25 mL of supernatant is pipette into 250 cm^3 conical flask which contains %1 .1 mL starch as an indicator.
3) Prepare your burette, it should be conditioned and filled with 1.00 mL 0.05 M iodine solution. You should check for air bubbles and leaks, before proceeding with the titration to avoid error in volume.
4) Note the initial iodine solution in the burette and open the tap.
5) Start dropping the solution from the burette to the flask until dark violet colour is reached in the flask.
   When end point is reached the colour of supernatant changes from orange/yellow to dark violet. When end point is reached, the violet colour should be observable for approximately 10 to 20 seconds and disappear again. Be careful not to add excessive iodine solution which will turn the solution into violet permanently. If it happens, restart to the titration process.
6) Repeat steps 1-5 for five times for albedo and flavedo .

-For locule:

1) Squeeze the juice and obtain 10.00 mL.
2) 10.00 mL of juice is pipette into 250 cm^3 conical flask which contains %1 .1 mL starch as an indicator.
3) Prepare your burette, it should be conditioned and filled with 1.00 mL 0.05 M iodine solution. You should check for air bubbles and leaks, before proceeding with the titration to avoid error in volume.
4) Note the initial iodine solution in the burette and open the tap.
5) Start dropping the solution from the burette to the flask until dark violet colour is reached in the flask. When end point is reached the colour of juice changes from orange/yellow to dark violet. When end point is reached, the violet colour should be observable for approximately 10 to 20 seconds and disappear again. Be careful not to add excessive iodine solution which will turn the solution into violet permanently. If it happens, restart to the titration process.
6) Record the final volume of titrant. Then by extracting the final volume from the initial volume value find the volume of the used titrant.
7) Repeat steps 1-6 for five times for the locule.

RESULTS

Type of *citrus* fruits	Name of the layer	Trials	Mass of weighed sample (±0.001g)	Mass of weighed homogenized metaphosporic acid - peel solution (±0.001g)	Volume of the titrated solution (±0.01 mL)	Initial volume of the titrant (±0.01 mL)	Final Volume of the titrant (±0.01mL)	Used Volume of Titrant (±0.01mL)	Volume of added %3 metaphosporic acid and %1 ethandioic acid solution(±0.01mL)	Volume of added %3 metaphosporic acid solution (±0.01mL)
Limon	Flavedo	1	25.110	27.411	25.00	1.00	0.74	0.26	72.58	100.00
		2	25.126	27.461	25.00	1.00	0.75	0.25	72.54	
		3	25.128	27.482	25.00	1.00	0.73	0.27	72.52	
		4	24.998	27.592	25.00	1.00	0.74	0.26	72.42	
		5	25.132	27.273	25.00	1.00	0.74	0.26	72.73	
	Albedo	1	30.441	27.037	25.00	1.00	0.82	0.18	72.96	100.00
		2	30.431	27.102	25.00	1.00	0.82	0.18	72.89	
		3	30.450	27.998	25.00	1.00	0.81	0.19	73.00	
		4	30.330	27.112	25.00	1.00	0.81	0.19	72.89	
		5	30.270	27.047	25.00	1.00	0.82	0.18	72.95	
	Locule	1	**No need**		10.00	1.00	0.38	0.62	0.00	00.00
		2			10.00	1.00	0.38	0.62		
		3			10.00	1.00	0.37	0.63		
		4			10.00	1.00	0.38	0.62		
		5			10.00	1.00	0.36	0.64		

Table1: indicating the mass of weighed samples of flavedo & albedo, mass of the obtained homogenized metaphosporic acid- peel solution, the difference in the volume of the titrant, the volume of added %3 metaphosporic acid and %1 ethandioic acid solution and the volume of the added %3 metaphosporic acid solution with their uncertainity values for *citrus limon*.

Type of *citrus* fruits	Name of the layer	Trials	Mass of weighed sample (±0.001g)	Mass of weighed homogenized metaphosporic acid-peel solution (±0.001g)	Volume of the titrated solution (±0.01mL)	Initial volume of the titrant (±0.01 mL)	Final volume of the titrant (±0.01mL)	Used volume of titrant (±0.0mL)	Volume of added mL %3 metaphosporic acid and %1 ethandioic acid solution(±0.01mL)	Volume of added %3 metaphosporic acid solution (±0.01 mL)
Sinensis	Flavedo	1	48.368	26.484	25.00	1.00	0.42	0.58	73.51	100.00
		2	48.356	26.480	25.00	1.00	0.44	0.56	73.52	
		3	48.359	26.482	25.00	1.00	0.43	0.57	73.51	
		4	48.379	26.489	25.00	1.00	0.42	0.58	73.51	
		5	48.367	26.488	25.00	1.00	0.43	0.57	73.51	
	Albedo	1	38,617	25.164	25.00	1.00	0.66	0.34	74.83	100.00
		2	38.613	25.160	25.00	1.00	0.65	0.35	74.84	
		3	38.614	26.161	25.00	1.00	0.66	0.34	73.83	
		4	38.616	25.162	25.00	1.00	0.66	0.34	74.83	
		5	38.619	25.169	25.00	1.00	0.67	0.33	74.83	
	Locule	1	**No need**		10.00	1.00	0.43	0.57	0.00	0.00
		2			10.00	1.00	0.44	0.56		
		3			10.00	1.00	0.44	0.56		
		4			10.00	1.00	0.43	0.57		
		5			10.00	1.00	0.43	0.57		

Table2: indicating the mass of weighed sample flavedo & albedo, mass of the obtained homogenized metaphosporic acid- peel solution, the initial and final volume of the titrant, the volume of added %3 metaphosporic acid and %1 ethandioic acid solution and the volume of the added %3 metaphosporic acid solution with their uncertainity values for *citrus sinensis*.

Types of *citrus* fruits	Name of the layer	Trials	Mass of weighed sample (±0.001g)	Mass of weighed metaphosporic acid &homogenized peel solution (±0.001g)	Volume of the titrated solution (±0.01mL)	Initial volume of the titrant (±0.01 mL)	Final Volume of the titrant (±0.01mL)	Used Volume of Titrant (±0.0mL)	Volume of added mL %3 metaphosporic acid and %1 ethandioic acid solution(±0.01mL)	Volume of added %3 metaphosporic acid solution
Paradisi	Flavedo	1	35.821	26.648	25.00	1.00	0.76	0.24	73.35	100.00
		2	35.835	26.630	25.00	1.00	0.76	0.24	73.37	
		3	35.794	26.671	25.00	1.00	0.77	0.23	73.33	
		4	35.825	26.629	25.00	1.00	0.77	0.23	73.37	
		5	35.892	26.661	25.00	1.00	0.76	0.24	73.34	
	Albedo	1	23.793	24.671	25.00	1.00	0.86	0.14	75.33	100.00
		2	23.791	24.682	25.00	1.00	0.85	0.15	75.32	
		3	23.692	24.785	25.00	1.00	0.86	0.14	75.22	
		4	23.164	24.671	25.00	1.00	0.85	0.15	75.33	
		5	23.338	24.714	25.00	1.00	0.85	0.15	75.29	
	Locule	1	**No need**		10.00	1.00	0.55	0.45	0.00	00.00
		2			10.00	1.00	0.50	0.45		
		3			10.00	1.00	0.53	0.47		
		4			10.00	1.00	0.55	0.45		
		5			10.00	1.00	0.52	0.48		

Table3: indicating the mass of weighed sample flavedo & albedo, mass of the obtained homogenized metaphosporic acid- peel solution, the initial and final volume of the titrant, the volume of added %3 metaphosporic acid and %1 ethandioic acid solution and the volume of the added %3 metaphosporic acid solution with their uncertainity values for *citrus paradisi*.

Types of *citrus* fruits	Name of the layer	Trials	Mass of weighed sample (±0.001g)	Mass of weighed homogenized metaphosporic acid-peel solution (±0.001g)	Volume of the titrated solution	Initial volume of the titrant (±0.01 mL)	Final Volume of the titrant (±0.01mL)	Used Volume of Titrant (±0.0mL)	Volume of added %3 metaphosporic and %1 ethandioic acid solution(±0.01mL)	Volume of added %3 metaphosporic acid solution
Tangerina	Flavedo	1	32.439	29.795	25.00	1.00	0.76	0.24	70.21	100.00
		2	32.273	29.938	25.00	1.00	0.72	0.28	70.06	
		3	32.461	30.123	25.00	1.00	0.73	0.27	69.88	
		4	32.237	29.723	25.00	1.00	0.75	0.25	70.28	
		5	32.182	29.681	25.00	1.00	0.75	0.25	70.32	
	Albedo	1	35.554	25.531	25.00	1.00	0.86	0.14	74.47	100.00
		2	35.728	25.585	25.00	1.00	0.86	0.14	74.42	
		3	35.463	25.453	25.00	1.00	0.85	0.15	74.55	
		4	36.528	25.828	25.00	1.00	0.84	0.16	74.17	
		5	35.533	25.152	25.00	1.00	0.86	0.14	74.85	
	Locule	1	**No need**		10.00	1.00	0.67	0.33	0.00	0.00
		2			10.00	1.00	0.67	0.33		
		3			10.00	1.00	0.67	0.33		
		4			10.00	1.00	0.67	0.33		
		5			10.00	1.00	0.66	0.34		

Table4: indicating the mass of weighed sample flavedo & albedo, mass of the obtained homogenized metaphosporic acid- peel solution, the initial and final volume of the titrant, the volume of added %3 metaphosporic acid and %1 ethandioic acid solution and the volume of the added %3 metaphosporic acid solution with their uncertainity values for *citrus tangerina*.

During the experiment, one can realize some changes with her/his senses. This qualitative data can be:

- -During titration of the sample, 1.0 mL 0.1 N iodine is used as an indicator. While iodine was dropping into the flask, each drop caused a very volatile violet colour appearance until the end point is reached. When end point is reached, neutralization becomes complete. Therefore, the violet colour was less volatile (observable for 10 to 20 seconds) when end point was reached but still not permanent!

Calculation Sample for trial 1 *Citrus sinensis*:

-For Flavedo:

1) After separating the flavedo with the help of a scalpel, 48.368 g peel is measured and 100 mL metaphosporic acid is added to the peel. Before the titration part, 26.484 g is obtained from the homogenized peel-metaphosporic acid solution and by the addition of 73.51 mL %3 metaphosporic and %1 ethandioic acid solution it is completed to 100 mL. Then the solution is filtrated and 25 mL of the supernatant is used for titration. Now, the amount of sample in the titrated solution can be determined:

$$\frac{48.368\ x\ 26.484}{100+48.368}\ x\ \frac{25}{100} = 2.16\ g\ \text{sample}$$

2) For the solid parts, 1 mL 0.1 N iodine solution is equal to 8.806 mg ascorbic acid. Therefore the amount of ascorbic acid in 0.58 mL iodine solution can be calculated like:

$$\frac{0.580mL\ x\ 8.806mg}{1mL} = 5.06\ mg\ ascorbic\ acid$$

3) In order to find the amount of ascorbic acid 100 g sample:

$$\frac{100g\ x\ 5.06\ mg}{2.16g} = 234\ mg/100g$$

-For Locule:

1) Amount of ascorbic acid = $\frac{V1\ x\ 8.806\ mg\ x\ 100}{V0}$ where V1is the volume of the used 0.1 N iodine solution and V0 is the volume of the titrated solution. So, the ascorbic acid amount in locule of sinensis can be calculated by:

$$\frac{0.57\ mL\ X\ 8.806\ mg\ X\ 100}{10} = 50.2\ \text{mg / 100 mL}$$

After doing necessary calculations for each layer of each citrus, table5 is obtained:

Trials	Mass of Ascorbic Acid in 100 g sample of citrus (mg/100g)											
	Limon			Sinensis			Paradisi			Tangerina		
	Flavedo	Albedo	Locule	Flavedo	Albedo	Locule	Flavedo	Albedo	Locule	Flavedo	Albedo	Locule
1	167,1	103,0	54,6	234,0	171,0	50,2	123,0	108,0	39,6	115,9	73,8	29,0
2	159,5	100,2	54,6	228,4	176,1	49,3	120,7	104,0	39,6	135,0	73,4	29,0
3	172,0	100,6	55,5	232,5	164,5	49,3	115,7	104,7	41,4	129,2	79,3	29,0
4	166,0	106,6	54,6	236,5	171,2	50,2	115,4	114,6	39,6	121,6	81,6	29,0
5	167,2	100,9	56,4	233,5	165,8	50,2	120,0	113,1	42,26	121,9	75,2	29,9

Table5: calculated ascorbic acid values in a 100 g sample for distinctive layers (flavedo,albedo,locule) of citrus limon, citrus sinensis, citrus paradise and citrus tangerine.

Using the Microsoft Excel 2007 program, one can determine the statistical relationship between the ascorbic acid concentration and the different layers of citruses. The following formulas are used to obtain the values in Table6.

Mean:

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

where;

n is the largest number of trials (5 for this experiment)

x_i is the ascorbic acid amount in the corresponding layer

Standard Error:

$$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{n}}$$

where;

n is the largest number of trials (5 for this experiment)

x_i is the ascorbic acid amount in the corresponding layer

σ is the Standard deviation of the corresponding group/data

Standard Deviation:

$$\sigma = \sqrt{\frac{\Sigma\ (x - \bar{x})^2}{n}}$$

where;

n is the largest number of trials (5 for this experiment)

x_i is the ascorbic acid amount in the corresponding layer

$\bar{x}$ is the mean value of the corresponding group/data

	Statistical Analysis											
	Limon			Sinensis			Paradisi			Tangerina		
	Flavedo	Albedo	Locule	Flavedo	Albedo	Locule	Flavedo	Albedo	Locule	Flavedo	Albedo	Locule
Mean	166.390	102.260	55.140	232.980	169.720	49.840	118.960	108.880	40.926	232.980	169.720	49.840
Median	167.100	100.900	54.600	233.500	171.000	50.200	120.000	108.000	39.600	121.900	75.200	29.000
Range	12.500	6.400	1.800	8.100	11.600	0.900	7.600	10.600	2.660	19.100	8.200	0.900
Variance	20.063	7.058	0.648	8.272	21.787	0.243	10.933	23.147	1.584	55.307	13.078	0.162
Standard Deviation	4.479	2.656	0.804	2.954	4.667	0.492	3.306	4.811	1.258	7.430	3.616	0.402
Standard Error	2.003	1.188	0.360	1.321	2.087	0.220	1.478	2.151	0.562	3.325	1.617	0.180

Table6: The mean, median, range, variance, SD, SE of the data table5.

For Citrus *limon ;*

ANOVA Summary					
Source	SS	df	MS	F	P
Between Groups	31164.99	2	15582.49	1683.441	2.01E-15
Within Groups	111.076	12	9.256333		
Total	31276.06	14			

Table7: The Anova: One-Way Analysis of Variance for Independent Samples results obtained from the data in Table 6 for *Citrus limon*. The P-value being numerically less than 0.05 indicates the truth of hypothesis.

For *Citrus sinensis*;

ANOVA Summary					
Source	SS	df	MS	F	P
Between Groups	86522.1693	2	43261.0847	4219.63	8.2E-18
Within Groups	123.028	12	10.2523		
Total	86645.1973	14			

Table8: The Anova: One-Way Analysis of Variance for Independent Samples results obtained from the data in Table 6 for *Citrus sinensis*. The p-value being numerically less than 0.05 indicates the truth of hypothesis.

For *Citrus paradisi*;

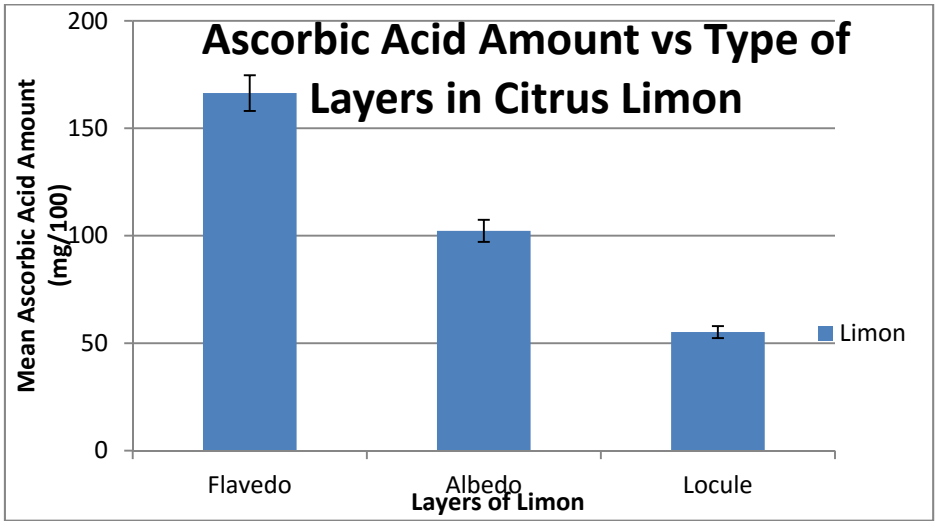
ANOVA Summary					
Source	SS	df	MS	F	P
Between Groups	18226.2533	2	9113.1266	766.58	2.19E-13
Within Groups	142.6573	12	11.8881		
Total	18368.9106	14			

Table9: The Anova: One-Way Analysis of Variance for Independent Samples results obtained from the data in Table 6 for *Citrus paradisi*. The p-value being numerically less than 0.05 indicates the truth of hypothesis.

For *Citrus tangerine*;

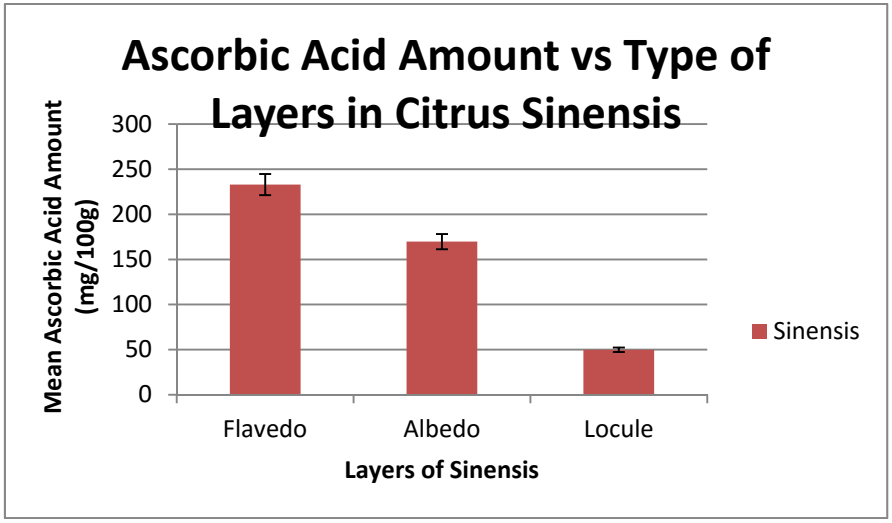
ANOVA Summary					
Source	SS	df	MS	F	P
Between Groups	22820.0093	2	11410.0047	499.37	2.8E-12
Within Groups	274.188	12	22.849		
Total	23094.1973	14			

Table10: The Anova: One-Way Analysis of Variance for Independent Samples [14] results obtained from the data in Table 6 for *Citrus tangerine*. The P-value being numerically less than 0.05 indicates the truth of hypothesis.

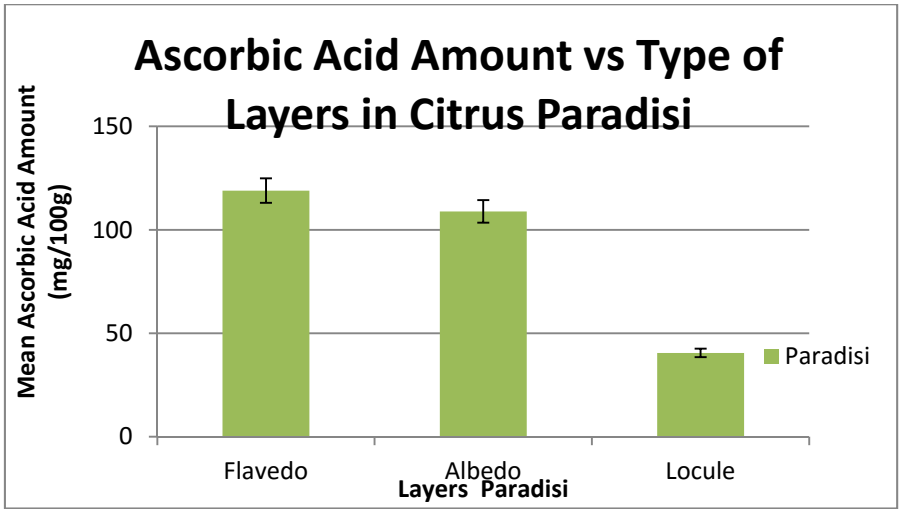


Graph1: Indicating the distinctive amount of ascorbic acid concentration in different layers (flavedo, albedo and locule) of Citrus limon.

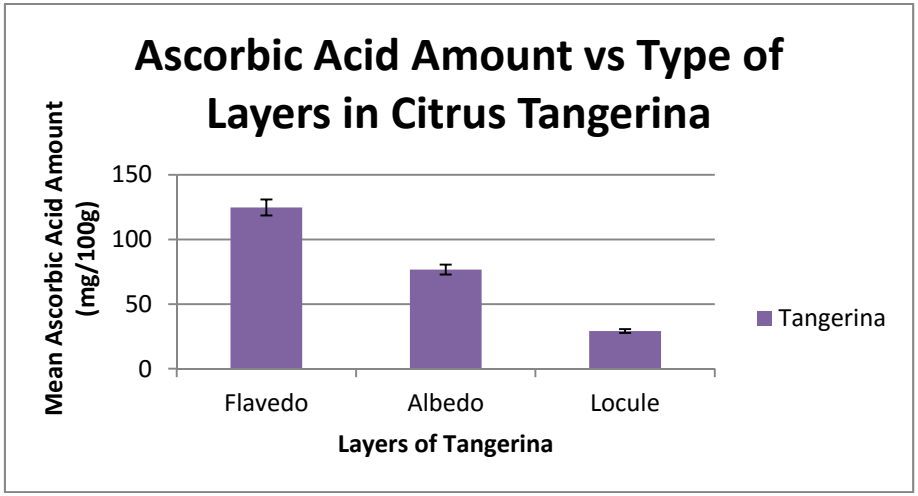
[14] http://vassarstats.net/anova1u.html



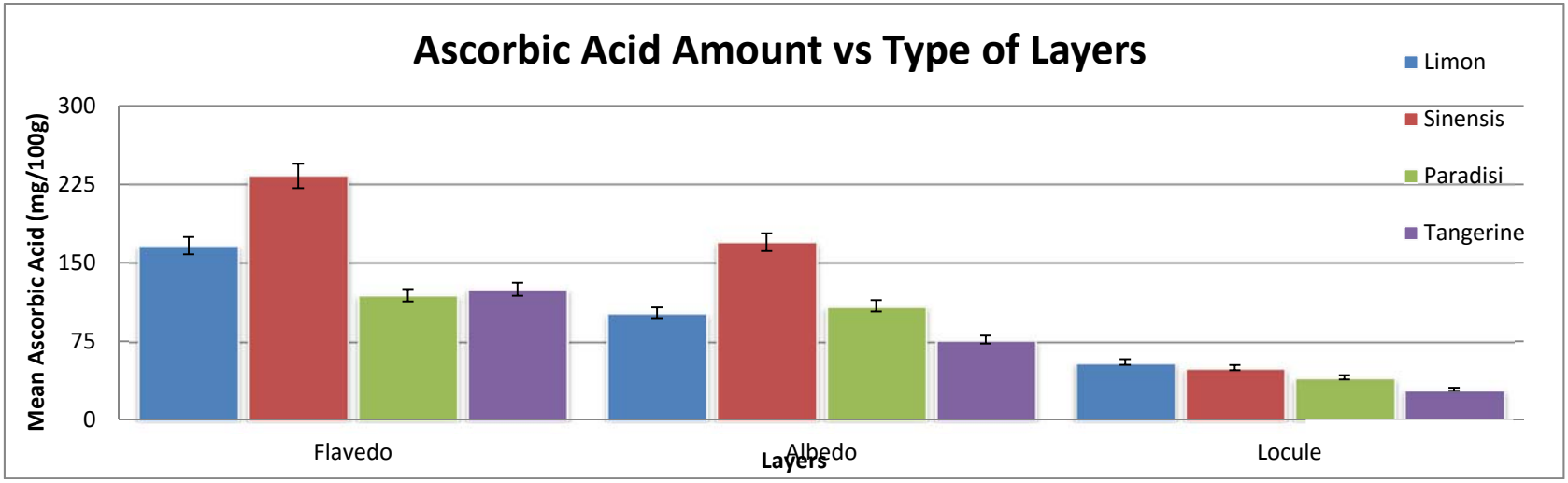
Graph2: Indicating the distinctive amount of ascorbic acid concentration in different layers (flavedo, albedo and locule) of Citrus sinensis.



Graph3: Indicating the distinctive amount of ascorbic acid concentration in different layers (flavedo, albedo and locule) of Citrus paradisi.



Graph4: Indicating the distinctive amount of ascorbic acid concentration in different layers (flavedo, albedo and locule) of Citrus tangerina.



Graph5: Indicating the distinctive amount of ascorbic acid concentration in different layers (flavedo, albedo and locule) of different citruses (limon, sinensis, paradise, tangerine)

CONCLUSION & EVALUATION

In this experiment, the relation between the amount of ascorbic acid (Vitamin C) and the different layers (flavedo, albedo& locule) of *Citrus limon*, *Citrus sinensis*, *Citrus paradisi* and *Citrus tangerina* was investigated. Different layers of distinctive citruses were homogenized and titrated to determine the amount of ascorbic acid by using the amount of titrant needed for the neutralization of acid.

Four different types of citruses (*Citrus limon*, *Citrus sinensis*, *Citrus paradisi* and *Citrus tangerine)* were chosen. Before the titration of the solid layers (flavedo, albedo), they were cut by a scalpel and homogenized by a blender. By completing the homogenized parts to 100 mL with addition of %3 metafosforic acid and %1 ethandioic acid and by obtaining 25 mL supernatant of the soared solution, they were ready for titration with 0,05 M (0.1N) iodine solution in presence of %1, 1 mL starch indicator.

The result of the experiment depict that the Vitamin C concentration increases as the distance from the center of the fruit increases, which can be summoned from the data in Tables 5 & 6. The ascorbic acid concentration of *Citrus limon* from the outermost layer to the locule is: 166.390mg/100g ,102.260mg/100g, 55.140mg/100g; for *Citrus sinensis*: 232.980 mg/100g ,169.72mg/100g , 49.840mg/100g ; for *Citrus paradise*: 118.960mg/100g, 108.880mg/100g, 40.926mg/100g ;for *Citrus tangerine*: 232.980mg/100g, 169.720mg/100g and 49.840mg/100g. As observed, even though the vitamin C concentrations are distinctive in every citrus fruit, there is an observable increase in ascorbic acid concentration from the innermost layer to the outermost in every investigated citrus, which can be also seen from Graph1-5.

Moreover, the hypothesis advocated by the source, which explicitly stated "*Only %25 of ascorbic acid in the fruit is in the juice, remainder is found in the peel, especially in the flavedo."*[15] The literature value in this source is numerically close to the value found in this investigation.

According to the P- value less than 0.05 for every citrus (which is obtained from the anova statistical analysis), the hypothesis proven to be true since the value is smaller than the alpha value of 0.05 . In short, one can state that different layers (flavedo, albedo& locule) of *Citrus limon*, *Citrus sinensis*, *Citrus paradisi* and *Citrus tangerina* contain dissimilar amount of ascorbic acid.

The Error and Uncertainity

In the investigation, there were several errors and uncertainties due to the environment the experiment was performed in, the equipment used or the random errors caused by the experimenter. Even though digital devices such as electronic scales minimized the

[15] Y. H. Hui, Handbook of Fruit & Fruit Processing, (301)

uncertainty of the results, still there are some sources of error which possibly "deflected" the results. After doing statistical analysis, it can be seen that the standard errors are not high which reflects the accuracy of the results in the study. As can be seen in Table6, the flavedo of *Citrus tangerine* has the highest standard error of 3.325 and the locule of *Citrus tangerine* has the lowest standard error of 0.18 .

The statistical analysis also gives information about precision. Standard deviation values can be found in the range of 7.430 and 0.402 . As smaller values indicate higher precision of the data group, it can said that the investigation is slightly precise.

Although the standard error and standard deviation values indicate the accuracy and the precision of the data and the reliability of the experiment can be shown by the p-value, there are both systematic and random errors exist in the experiment that can be inferred from the error bars in Graph1-5.

The errors and uncertainties in the investigation were minimized by stabilizing the controlled variable such as:

- The type of the indicator (1% starch solution) is constant in each trial, as every indicator has distinctive range which can affect the results.
- Type and the molarity of the titrant base (0.05M Iodine solution) is constant in each trial so that the amount needed for the neutralization of acid can be compared.
- Type of genus is kept constant (*Citrus*), as citruses are one of the genus with highest vitamin C concentration.

Even though these factors were controlled, it is known that an experiment consists of error-posing components as long as it consists of measurements and one cannot acquire infallible results.

- The colour differentiation during the titration is decided by the experimenter which reveals its dependence on human senses. So, even though experiment was repeated for several times, end point may not be detected precisely in every trial.

In order to improve this investigation, errors should be decreased for increased accuracy of experiment.

- Titration method is highly dependent on human observational skills. For instance, the end point is revealed via color differentiation, therefore can only be detected by the human eye. Not only color change is delicate and tedious but also different people have distinctive sensitivity toward colors. Therefore, "Conductometry Titration" method can be used next time.

FURTHER INVESTIGATIONS

Today, one of the most efficient sources of Vitamin C is citrus fruits. As human body is unable to produce ascorbic acid by itself, it had to be taken from the outside; mostly from citruses. Results of the experiment ' Do different layers (flavedo, albedo& locule) of *Citrus limon*, *Citrus sinensis*, *Citrus paradisi* and *Citrus tangerina* contain dissimilar amount of ascorbic acid measured by a redox titration using a solution of iodine of accurately known concentration and starch solution as an indicator?' indicates that the ascorbic acid most abundantly found in the outermost layer of citrus fruits. So according to the conclusion of this experiment, it would be logical to state that people should consume flavedo of citruses. However, it is widely known that absorption of each ingredient in human body is different. Therefore, their functioning mechanisms and usage eras in the body differentiates. In this case, even though ascorbic acid is most abundantly found in the flavedo, the rate of consumption of this layer may not be as efficient as the locule part. So a new question arises: Do different layers (flavedo, albedo& locule) of *Citrus limon*, *Citrus sinensis*, *Citrus paradisi* and *Citrus tangerine* have different absorption rates in human body?

APPENDICES

Appendix 1: Titration Method

Titration is a common laboratory method of quantitative chemical analysis that is used to determine the unknown concentration of a known reactant. Because volume measurements play a key role in titration, it is also known as *volumetric analysis*. A reagent, called the *titrant* or *titrator*, of a known concentration (astandard solution) and volume is used to react with a solution of the analyte or*titrand*, whose concentration is not known. Using a calibrated burette orchemistry pipetting syringe to add the titrant, it is possible to determine the exact amount that has been consumed when the *endpoint* is reached. The endpoint is the point at which the titration is complete, as determined by an indicator (see below). This is ideally the same volume as the equivalence point—the volume of added titrant at which the number of moles of titrant is equal to the number of moles of analyte, or some multiple thereof (as in polyprotic acids). In the classic strong acid-strong base titration, the endpoint of a titration is the point at which the pH of the reactant is just about equal to 7, and often when the solution takes on a persisting solid color as in the pink of phenolphthalein indicator. There are however many different types of titrations. Many methods can be used to indicate the endpoint of a reaction; titrations often use visual indicators (the reactant mixture changes color). In simple acid-base titrations a pH indicator may be used, such as phenolphthalein, which becomes pink when a certain pH (about 8.2) is reached or exceeded.

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