



# Estimation of Vit-c

Prof.Dr. May Jaleel



# CONTENTS



- INTRODUCTION
- PRINCIPLE OF THE EXPERIMENT
- EXPERIMENTAL PROCEDURE
- CALCULATION OF THE RESULTS





# INTRODUCTION

- Vitamins are important organic substances for normal growth and maintenance of life. If any of the vitamins are not taken into the body, there may be disruptions in growth and functions of organism due to the chemical reaction that the vitamin helps.
- Effects of vitamins on human health
  - Helping grow
  - Help create healthy generations
  - Normal functioning of the nervous and digestive systems, proper use of nutrients, and assistance to body resistance







• The effects of vitamins in functions of body are related to the regulation of biochemical reactions. The effects of vitamins on human health can be divided into three groups;

1. Helping grow
2. Help create healthy generations
3. Normal functioning of the nervous and digestive systems, proper use of nutrients, and assistance to body resistance.





# INTRODUCTION

- Vitamins are required in very low amounts in all metabolic activities. Today there are 15 compounds identified as vitamins. These compounds:
  - Active vitamin structure from the body can be taken from food
  - Provitamins (vitamin precursors) after being taken into the body undergoes a series of chemical changes into one or more compounds that show vitamin activity.
- Vitamins
  - Active vitamin
  - Provitamin







# INTRODUCTION

Vitamins are present in different amounts in foods and show different distributions. Although some foods are quite rich in one or several vitamins, they contain some vitamins in trace levels.

- These compounds, which are involved in many functions such as growth, development and repair in the body, should be taken regularly in certain amounts daily. For this reason, one should create a balanced diet from food.





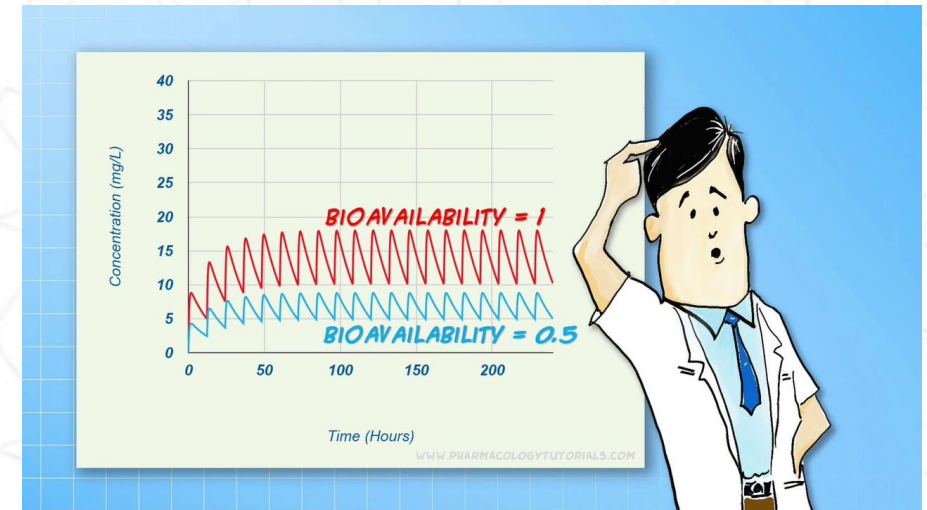
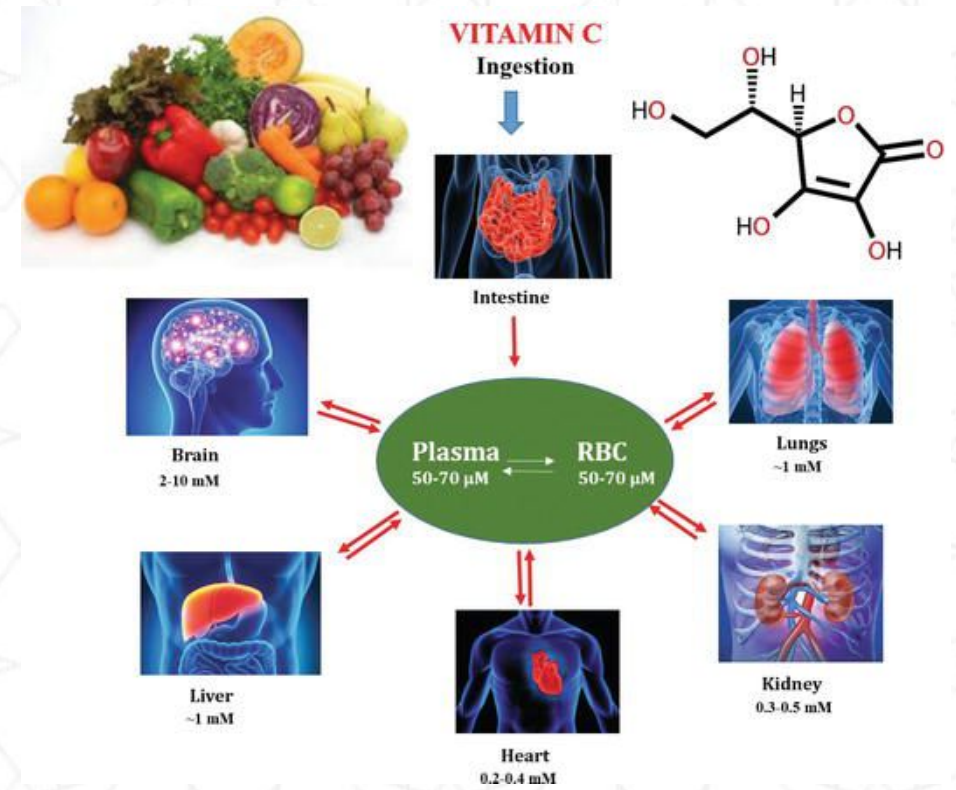
- Inadequate intake of vitamins with food causes absorption disorders or some metabolic disorders.
- Disease caused by vitamin deficiencies is defined as hypovitaminosis (taking vitamins below the normal minimum). For example; without fresh fruit and vegetables, sailors who had to manage dry foods for a long time had scurvy due to vitamin C deficiency. Since vitamin pills could not be made with the technology of the time, vitamin C had to be obtained from foods other than fresh fruits and vegetables. Vinegar, pickles began to be included in the list of supplies on the ships for this purpose





# INTRODUCTION

The amount of nutrients taken into the body differs from the amount used in the body. This is related to the bioavailability (bioavailability; absorption of any nutrient in the body and the level of its use in the organism). The need for vitamins in very low amounts increases the importance of bioavailability. Bioavailability of vitamins varies depending on;







- The composition of the individual's diet,
- Whether the vitamin is in active vitamin form or coenzyme form,
- How the vitamin interacts with other nutrients.

In addition, the bioavailability of vitamins may be affected by the way foods are processed and may also vary from individual to individual.







# INTRODUCTION

Vitamins are generally classified according to their physical properties as follows:

- Fat-soluble vitamins: A, D, E, K.
- Water soluble vitamins: B1 (Thiamine), B2 (Riboflavin), B6 (pyridoxine), B12 (cobalamin), Niacin, Folic acid, Biotin, Pantothenic acid, Vitamin C (Ascorbic acid).

Since vitamins are generally unstable substances, they can be damaged during processing of foods. In addition, care must be taken when preparing the extract in vitamin analysis. The amount of vitamins in foods can be expressed as gr /100gr, USP unit or I.U.

## FAT SOLUBLE VITAMINS

A	D	E	K
Fat Soluble	Fat Soluble	Fat Soluble	Fat Soluble
			
Vision, Reproduction, Bone Health, Immune System, Skin	Strengthens Bones, Calcium Absorption, Immune System	Immune System, Flushes Toxins	Blood Clotting, Bone Health

Water Soluble Vitamins	
Vitamin:	Name:
B1	Thiamine
B2	Riboflavin
B3	Niacin
B5	Pantothenic Acid
B6	Pyridoxine
B7	Biotin
B9	Folate
B12	Cobalamin
C	Ascorbic Acid



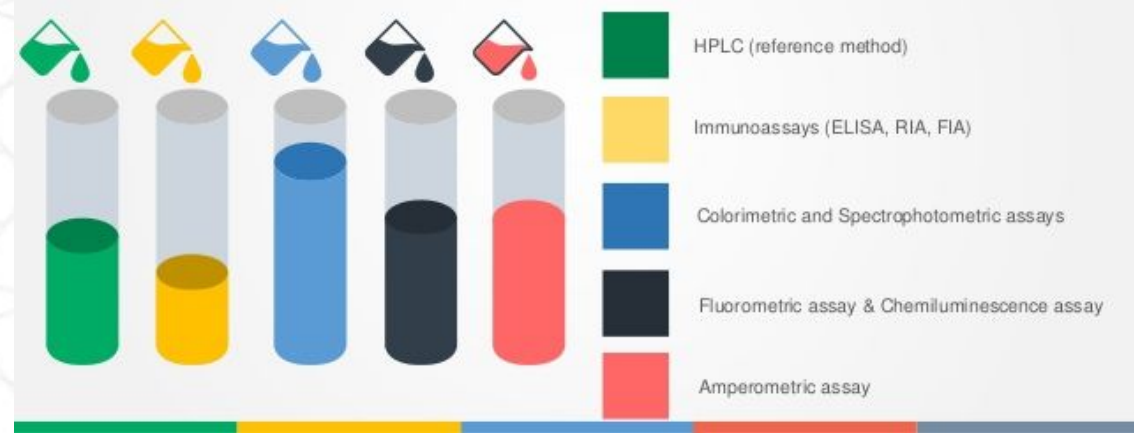


# INTRODUCTION

Vitamin analysis in foods can be determined by volumetric titration and instrumental analysis. Vitamin analysis in most foodstuffs can be performed by spectroscopic methods. For this purpose, vitamins in foodstuffs are extracted with suitable solvents (mostly in acidic medium) and taken into solution. The solution is passed through a special chromatographic column to separate the vitamins and quantify using fluometry or other optical methods.

## Vitamin Analysis Method

measure vitamins directly and give a quantitative measurement. These are recommended for use particularly for clinically useful vitamins. However these are expensive and not every laboratory can provide these assays. These methods include:



- HPLC (reference method)
- Immunoassays (ELISA, RIA, FIA)
- Colorimetric and Spectrophotometric assays
- Fluorometric assay & Chemiluminescence assay
- Amperometric assay





The most valid method for accuracy is HPLC. However, it is not preferred because it is very expensive.

The most common vitamin analyzes in foods;

- Determination of vitamin A (beta carotene) in soda and fruit and vegetable products
- Determination of vitamin A and D in vegetable margarines
- Vitamin C determination in fruit and vegetable products.







# INTRODUCTION

- For Vitamin C analysis there are usually three methods as follows:
  - Titration with Tillman Indicator (2,6-dichlorophenolindophenol dye)
  - Titration with iodine
  - Spectrophotometric method



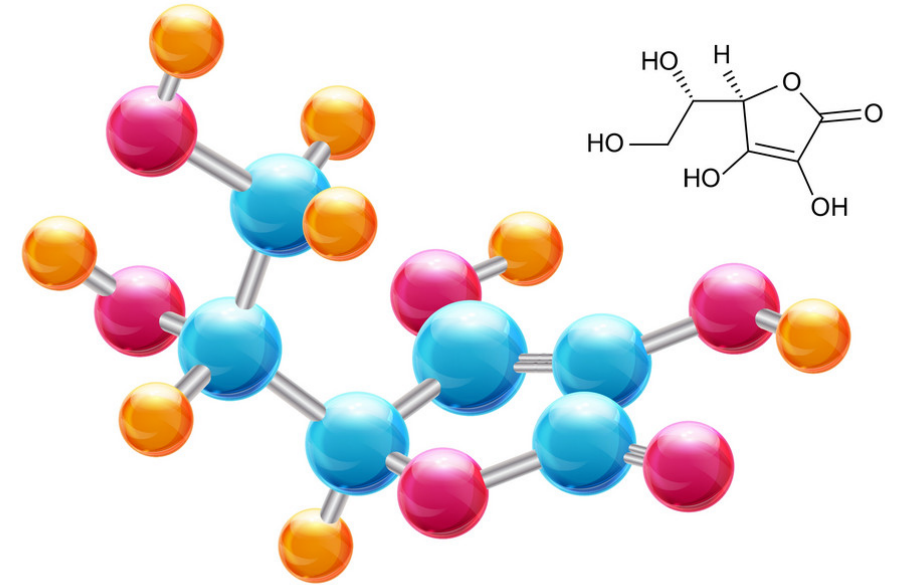


# INTRODUCTION

**Vitamin C:** Vitamin C, composed of colorless crystals with a melting point of  $192^{\circ}\text{C}$  and a molecular weight of 176, is an antiscorbut factor. It contains a dieneol group which has both reducing power and acidic properties.

- Easily soluble in water, methanol and ethanol.
- Insoluble in benzene, ether, petroleum ether, chloroform and oil.

Vitamin 







- Vitamin C (Ascorbic acid) is the most vulnerable of vitamins.
- Very sensitive to alkalis and oxidation, especially when catalysts such as Cu and Fe are present.
- More resistant as dry crystals.
- Very resistant to acid solutions (below pH 4).
- Ascorbic acid is also oxidized by the oxygen of the air. As a result of this oxidation, the molecule loses its vitamin activity.
- Available in nature as reduced ( $C_6H_8O_6$ ) and oxidized ( $C_6H_6O_6$ ).





# INTRODUCTION

## Deficiency:

- Capillary vessels can have a weak structure,
- Teeth gums can bleed easily, teeth can be lost
- It causes joint diseases.
- It is also necessary for the normal formation of collagen and protein, an important element of the skin and connective tissues.

## Vitamin C Deficiency

### SIGNS AND SYMPTOMS



Connective tissue defects



Poor immune function



Red patches on the skin



Dry and rough skin



Anemia



Depression and fatigue

vitaminsonly  
com.au





- Vitamin C increases the body's resistance to many diseases. Daily vitamin C requirement is 30–40 mg in adults.
- Vitamin C is the main vitamin contained in foods of plant origin.

*Richest resources:*

- Fresh fruits, especially citrus fruits such as oranges, tangerines, rose hips, strawberries
- Fresh vegetables are dark green leafy vegetables, tomatoes, green and red peppers, and potatoes

Animal products



Liver | Dairy products & Milk | Kidney

Plant products

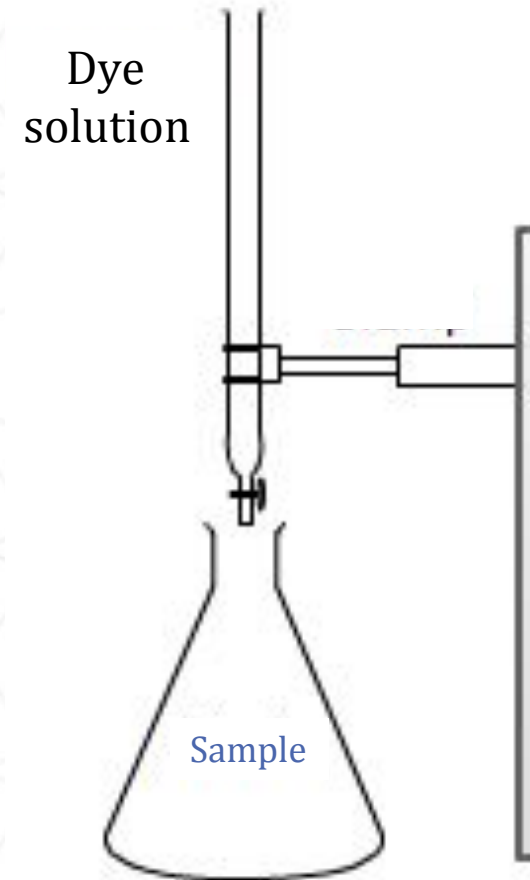
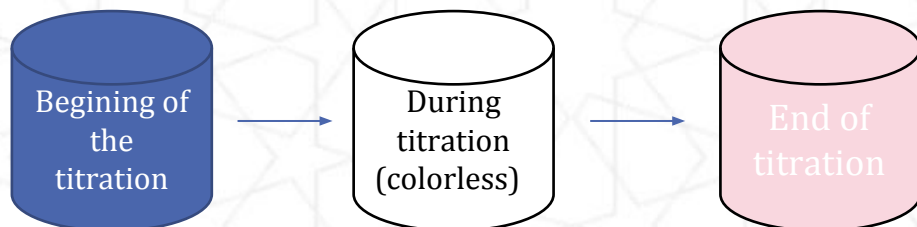
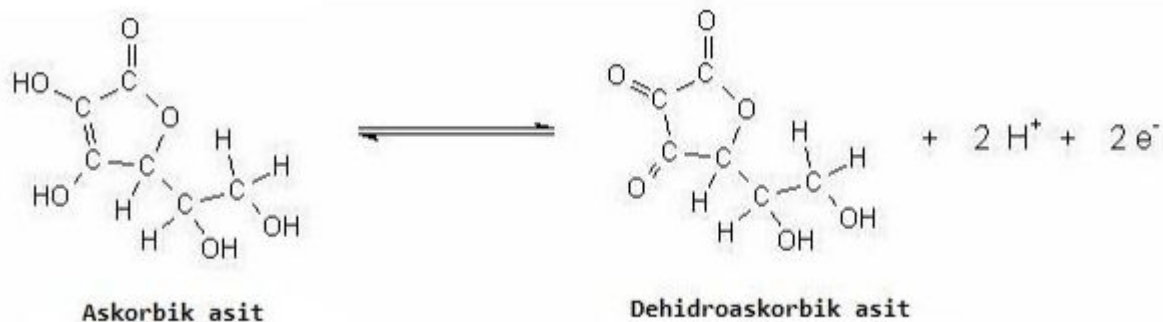


Citrus Fruits | Tomatoes | Green leafy vegetables

# PRINCIPLE OF THE EXPERIMENT

- It is a pink color produced by dehydro-ascorbic acid formed by titration with 2,6-dichlorophenolindophenol dye solution of ascorbic acid which is a strong reducing agent.

2,6-dichlorophenolindophenol dye is blue in alkali environment and pink in acidic environment



2,6-dichlorophenolindophenol dye is prepared with sodium bicarbonate (alkali environment), and at the end of the analysis it gives pink because of metaphosphoric acid in the environment





# EXPERIMENTAL PROCEDURE

## Acids Used For Extraction & Stabilization Of The Ascorbic Acid

### 1. Preparation of Sample (Extraction):

- Approximately 200 - 300 g of sample is weighed and put into a blender,
- Add 6% metaphosphoric acid solution up to the amount of sample weighed,
- Blender is turned into a homogenous crush by operating. (It is enough to run the blender for 2 minutes) **Metaphosphoric acid is used as stabilizer to prevent enzymatic oxidation of ascorbic acid during this process.**
- After weighing 10 - 40 grams of the mixture in the blender, add 3% metaphosphoric acid solution to a 100 ml measuring flask and fill the line with the same solution and filter through filter paper.
- Take 20 g or 20 ml directly and transfer to a 100 ml flask. Make up to volume with metaphosphoric acid solution.
- The sample is thoroughly mixed and filtered. In this way, the sample can be stored without oxidation until titration.

### • Metafosforic Acid (%6)

#### • AD(+):

- Better protection from metallic ions
- Better protection in general

#### • DISAD(-):

- Expensive
- Hard to dissolve
- Low Stability

### • Okzalic acid (%2)

#### • AD(+):

- Cheap
- Better stability

#### • DISAD(-):

- Blurry filtrate
- It is hard to determine end of titration

### • Acetic acid (%8)

#### • AD(+):

- Effective in the samples includes Fe ion

#### • DISAD(-):

- It can't stabilize the solution if it





# EXPERIMENTAL PROCEDURE

- **2. Standardization of dye solution:**
  - The aim of the standardization: In order to determine the factor of dye solution which is the amount of dye solution (ml) used to reduce 1 mg ascorbic acid the following steps should be taken:
  - Preparation of standard ascorbic acid solution: Take 10 mg ascorbic acid and solve it in 50 ml MFA solution. According to this 5 ml standard ascorbic acid solution contains 1 mg ascorbic acid.
  - 5 ml of MFA solution and 5 ml standard ascorbic acid solution is put into a flask.
  - Titrate to pink color with dye solution in the burette.
  - The amount spent is saved.  $(V_0) = \dots$  .m
- **3. Titration of the sample:**
  - Take 20 ml of the test sample and place 20 ml of MFA solution on top.
  - Mix well and filter.
  - 5 - 10 ml is taken from the filtrate and titrated to the light pink color with the dichlorophenolindophenol solution in the burette. (The resulting color should be permanent for at least 15 seconds and the titration should be completed as soon as possible.)
  - The amount spent is saved.  $(V_1) = \dots$  .ml







# CALCULATION OF THE RESULTS

M2: Amount of original sample in titrated filtrate (g)

M1: Amount of sample taken (g)

Vt: Amount of filtrate taken for titration

V: Completed flask volume (ml)

$$m_2 = \frac{m_1 * V_t}{V}$$

$$\text{Dye solution factor (F)} = \frac{1}{\text{Spent dye solution amount (ml)}}$$

$$\text{Ascorbic acid} \left( \frac{\text{mg}}{100\text{g}} \right) = \frac{V_1 * F}{m_2} * 100$$

V<sub>0</sub>: Spent dye solution amount for standard vitamin C solution (ml)

F: Factor

1: Amount of ascorbic acid in the flask (1 mg)





# CALCULATION OF THE RESULTS

ANOTHER WAY :

If  $V_0$  volume of dye solution oxidise 1 mg of ascorbic acid  
 $V_1$  volume of dye solution oxidise ??? mg of ascorbic acid

---

$$??? = (V_1 * 1) / V_0$$

This is for the amount of sample in the flask! If you made further dilution you should take those into consideration.

If M g of sample has ??? Amount of ascorbic acid  
100 g of sample has X amount of ascorbic acid

---

$$X = (100 * ???) / M$$

$V_0$ : Spent dye solution amount for standard vitamin C solution (ml)  
M: Amount of original sample in titrated filtrate (g)  
 $V_1$ : Amount of dye solution spent for the sample







# PPL

Spent dye solution amount for standard vitamin C solution (ml)=10

## PARALLEL 1

Amount of original sample in titrated filtrate (g)=5,1

Amount of dye solution spent for the sample=20,3 ml

## PARALLEL 2

Amount of original sample in titrated filtrate (g)=5,3

Amount of dye solution spent for the sample=20,6 ml

## PARALLEL 3

Amount of original sample in titrated filtrate (g)=5,0

Amount of dye solution spent for the sample=20,1 ml

Calculate the amount of ascorbic acid in the sample (mg/100g) ?

Discuss the results with the proof of academic journals, take sample as «lemon juice»

**USE AT LEAST 2 SOURCE FROM ACADEMIC JOURNALS!**



THANK YOU FOR YOUR  
ATTENTION

*Thank You*