# **Exp. 1: QUANTITATIVE ASCORBIC ACID ANALYSIS**

## Solutions and Chemicals Used in the Experiment:

0.25% starch solution (0.625g of starch is weighed and dissolved in hot 250 mL water, boil until the solution is clear.)

0.7 M sodium thiosulfate (0.1 g  $Na_2CO_3$  and 11.08g  $Na_2S_2O_3$  / 1000 mL water)

0.2% KIO<sub>3</sub> solution (2g KIO<sub>3</sub> / 1000 mL water)

5% KI solution (2.5 g / 50 mL water)

0.1% Ascorbic acid standard solution (0.1g ascorbic acid / 100 mL water)

 $0.3~M~H_2SO_4$  solution (says 16.65 mL.  $H_2SO_4$  / 1000 mL)

## **Principle of the Experiment:**

The basic principle of the experiment is based on Ascorbic Acid being a strong reducing agent.

In the first reaction of the experiment;

 $KIO_3$  reacts with KI in acidic medium to form  $I_2$ .

 $KIO_3(aq) + 6 H^+(aq) + 5 I^-(aq) \rightarrow 3 I_2(aq) + 3 H_2O(I) + K^+(aq) (I_2 oluşumu)$ 

Then, some of the  $I_2$  formed as a result of this reaction is expected to react with Ascorbic Acid to reduce it to form  $I^{-}$ .

 $C_6H_8O_{6(aq)} + I_{2(aq)} \rightarrow C_6H_6O_{6(aq)} + 2 I^{-}_{(aq)} + 2 H^{+}_{(aq)}$  (C vitamininin oksidasyonu)

However, since the solubility of  $I_2$  in water is very low, it reacts with  $KIO_3$  and KI in acidic environment to form  $I^{3-}$  (Triiodide complex).

 $IO_3^-(aq)$  + 8  $I^-(aq)$  + 6  $H^+ \rightarrow I_3^-(aq)$  +  $3H_2O$ 

The Triiodide complex formed is reduced by reacting with Ascorbic acid just like I2;

Ascorbic acid +  $I_3^-$  +  $H_2O \rightarrow$  Dehydroascorbic acid +  $3I^-$  +  $2H^+$ 

The remaining amount of  $I^{3-}$  is also found by titrating with  $Na_2S_2O_3$ ;

 $|_{3}^{-} + 2S_{2}O_{3}^{2^{-}} \rightarrow 3|^{-} + S_{4}O_{6}^{2^{-}}$ 

#### **Experimental Procedure:**

Erlen No	0,3M H2SO4 (mL)	Ascorbic Acid (mL)	Orange Juice (mL)	Water (mL)	KIO3 (mL)	KI (mL)	Na2S2O3	Starch (mL)	Na2S2O3 	Waste (mL)
1	50	2,5	0	17,5	15	10	Titration (until light	itration 2 intil light ellow) 2		
2	50	5	0	15	15	10	yellow)			
3	50	10	0	10	15	10	2	colorless)		
Sample	50	0	20	0	15	10		2		

Reagents in the amounts given in the table were added to the four flasks. Standard Ascorbic Acid solutions were added to three of the flasks in the amounts given in the table. The fourth erlen was added with 20 mL of orange juice, which was squeezed as given in the table.

Then, these flasks were titrated with Standard  $Na_2S_2O_3$  (Sodium Thiosulfate) solution until it became a light yellow color. Then, 2 ml of starch was added to these flasks as indicators and titration was continued until it became colorless. Consumption values obtained as a result of titration are shown in the table.



Thiosulfate Consumption for Known Ascorbic Acid Quantities - Concentration Graph:

#### 1-Amount of Ascorbic Acid Contained in Fruit Juice Sample (mg / 100mL):

# 2- Comparison of Theoretical and Practical Ascorbic Acid Concentrations:

# Study Questions:

- 1) Ascorbic acid is synthesized by which organisms and by which metabolic way?
- 2) Briefly explain the collagen structure. What happens to the structure of collagen in ascorbic acid deficiency