Phytochemistry-II

Carbohydrates !

Quantitative estimation of sugars … (Conc. ?!)

Sugars can be estimated by

- Titrimetric methods
  - Iodimetry
  - Cupper reduction
- Gravimetric methods
- Colorimetric methods
  - Enzymatic Method

Titermetric methods

I. Iodimetric method

Iodimetry

a sample is directly titrated with an iodine solution (I_2 is a titrant)

- Direct method
- To quantify reducing agents

Iodometry

a sample is treated with an iodide solution (KI) → then the released iodine is titrated with $Na_2S_2O_3$

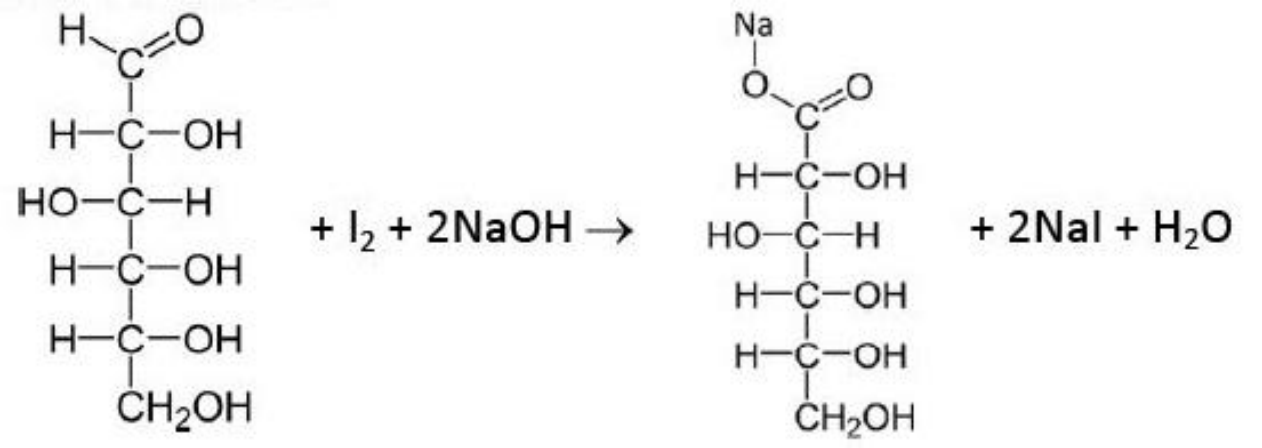
- Indirect method
- To quantify oxidizing agents

Principle of assay

- The method is based on the fact that iodine can oxidize aldoses in alkaline medium, whereas it has no effect on ketoses.

- I_2 oxidizes glucose in alkaline medium into gluconic acid

$$C_6H_{12}O_6 + 3NaOH + I_2 \longrightarrow C_6H_{11}O_6OK + 2NaI + 2H_2O$$



Benefit of using Iodimetry ?

To determine Aldoses in presence of ketoses

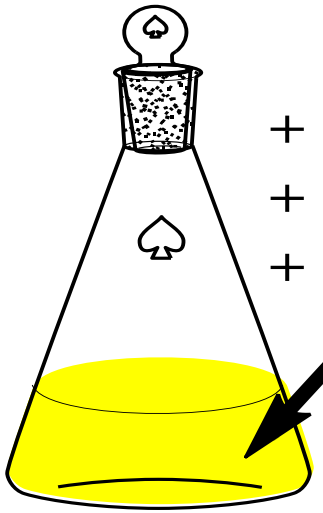
e.g. Mix of Glucose & Fructose

1- Its (back titration).

2- Titrant is $Na_2S_2O_3$ & Starch is used as indicator.

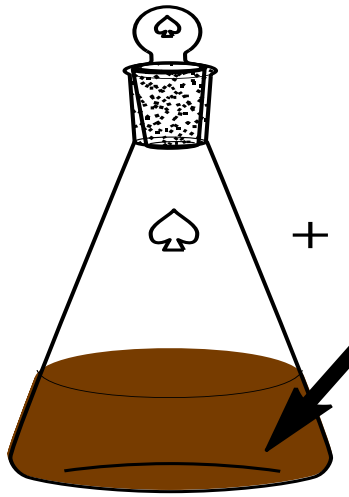
3- Two reactions take place (I_2/sugar & I_2/$Na_2S_2O_3$)

procedure



+ 10 ml sugar sol.
+ 10 ml 0.1N I_2 sol. (Known xss)
+ 10 ml Na_2CO_3

Incubate at **dark** for 10 min.



+ 10 ml 2N H_2SO_4

Excess unused I_2 is back titrated against $Na_2S_2O_3$ using **starch as indicator (Near E.P)**

Procedure

1. Load a burette with standard sodium thiosulfate solution.

2. To conical flask pour in 10 ml aliquot of sample (Bulb pipette), add 10 ml of 0,1 N iodine standard solution (Bulb pipette), and 10 ml of 2N Na_2CO_3solution (Grad. Pipette).

3. Cover the flask with a stopper and put into dark place at 10 min.

4. Add 10 ml of 2N H_2SO_4 solution and titrate the sample with thiosulfate until the solution becomes pale yellow (Straw yellow = Near E.P).

5. Introduce 5 drops of starch indicator, and titrate with constant stirring to the disappearance of the blue color = E.P.

E.P

Titration colors:

Brown → Faint yellow / Colorless →

Brown → Straw yellow → Blue /

Greenish blue → Colorless (E.P)

Comments:

Why do we use alkaline medium?

In alkali solution, iodine disproportionate into hypoiodite ion (oxidized) and iodide ion (reduced). (disproportionate means Redox reaction where one substance partly oxidized and partly reduced)

$$I_2 + 2NaOH = \underline{NaOI} + \underline{NaI} + H2O$$

I^+ I^-

Aldehyde group (aldoses) is oxidized with formed hypoiodite ion

$$HCHO + NaOI = HCOOH + NaI$$

Then excess hypoiodite in alkali solution disproportionate into periodate (Colorless)

$$3NaOI = NaIO_3 + 2NaI$$

Why do we do acidification before titration?

To reconvert excess unused periodate to Iodine (Brown), (by enhancing its reaction with iodide)

$$NaIO_3 + 5NaI + 3H_2SO_4 = I_2 + Na_2SO_4 + 3H_2O$$

Also, Sodium thiosulphate needs a neutral or weak acid environment.

Blank

Do blank experiment, How and Why?

How: Replace the sugar by water

Why: KI (Used to dissolve I_2 as KI_3) may contain appreciable amounts of iodate ion (IO_3^-) which in acid solution will react with iodide and yield iodine. The liberated iodine would react with thiosulfate and thereby causes error in E.P.

Sources of error in the iodimetric method:

1. Loss of iodine by evaporation from the solution.

- This can be minimized by having a large excess of iodide (KI) ➔ to keep the iodine tied up as tri-iodide ion (soluble form).
- The titrations involving iodine must be made in cold solutions ➔ to minimize loss through evaporation, also stoppered flask should be used.

2. Atmospheric oxidation of iodide ion in acidic solution.

- Quick titration of the liberated iodine is necessary ➔ to prevent oxidation.

3.The indicator not behave properly at the endpoint and a quantitative determination is not possible.

- Starch solutions are no longer fresh or improperly prepared

Calculations

$$C_6H_{12}O_6 + 3KOH + I_2 \longrightarrow C_6H_{11}O_6OK + 2KI + 2H_2O$$

Factor:

1 molecule I_2 = 180 gm glucose

I = 90 gm glucose

1 ml 0.1N Iodine = 0.009 gm glucose (monosacch.)

$$\% \text{ of glucose} = \frac{(10 - E.P) \times 0.009 \times 100}{V}$$

10 : ml of I_2 sol. added

E.P:ml of 0.1N $Na_2S_2O_3$ used in the titration

V: volume taken "of sugar solution"

Required equipments

- Burette
- ***Stoppered*** Conical Flasks (250 ml).
- 2 Bulb Pipette (10 ml).
- 2 Grad. Pipette (10ml).
- Dropper

Thanks