

MI – ICCIDD

**Training Manual for
Laboratory Personnel in
Iodine Estimation of Salt**

This Manual is prepared by
Prof. Madhu G. Karmarkar
Dr. Denish Moorthy
Ms. Suraksha Shukla

Contents

1. Background Material	4
2. Principle and Laboratory Procedures for Iodine Estimation in Salt	6
I) Principle	6
II) Equipment and Chemicals	6
III) Preparation of Reagents	8
IV) Procedure	9
3. Iodine content in Parts Per Million	10
4. Calculations	12
5. Precautions	13
6. Quality Assurance for Iodine Estimation in Salt	14
1) Internal Quality Assurance	
a) Preparation of known value samples for Salt Iodine Estimation	14
b) Procedure for Internal Quality Assurance	15
c) Internal Quality Assurance at Production Level	16
2) External Quality Assurance	
a) External Quality Assurance at Production Level	17
b) External Quality Assurance Programme at Salt Testing Laboratories	18
7. Graphical Representation of the Procedure to Measure the Iodine Content of Salt	19

Background material:

Iodine is one of the first minerals recognized as essential for human health. Iodine belongs to the family of halogens (Chlorine, bromine, iodine and fluorine) placed in the seventh group of period table. Most of the iodine exists in the ocean. It was present during the primordial development of the earth but large amounts were leached from the surface soil by glaciations, snow, or rain and were carried by rivers, floods and winds into the sea.

Iodine is an essential micronutrient for humans and is required in a very small amount i.e. 150 µg per day. The only role of iodine in the body, known at present is for the synthesis of thyroid hormones. The human body contains 15 to 20 mg of iodine of which almost 80% is in the thyroid gland. The iodine taken in the diet is absorbed throughout gastrointestinal tract and circulates as plasma Inorganic Iodide (PII) in the body. The PII is mainly cleared by two organs in the body - Thyroid and Kidney. The iodine taken up by the thyroid gland is used for making thyroid hormones, thyroxine and triiodothyronine. Thyroxine is called T₄ because it has 4 iodine atoms in its structure while triiodothyronine is called T₃ because it has 3 iodine atoms in its structure. Iodine taken up by the kidney is excreted in the urine. The level of excretion in urine correlates well with level of intake. Thus urinary iodine can be used to assess the level of iodine intake.

Thyroxine has two major roles in the body one is for development of brain, central nervous system and skeletal system during the early developmental stages and the second is calorogenic effect i.e. increase in oxygen uptake by tissues thereby controlling all metabolic processes in the body.

Iodine deficiency occurs mainly as an environmental deficiency. Iodine present in upper crust of earth is washed/leached out by repeated flooding and glaciation. Thus making the soil deficient in iodine. Crops grown on such soil will have less iodine and when consumed by humans or animals lead to iodine deficiency. Thus it is the deficiency of soil which is responsible for iodine deficiency in humans and animals.

Iodine deficiency during pregnancy and first two to 3 years of life leads to derangement in the development of brain and central nervous system & skeletal. These changes are irreversible. No amount of iodine or thyroxine can revert back the damage done during the developmental period leading to several disabilities like deaf mutism, squint, gait defects etc. but the most important of these is the loss of learning ability with loss of 13 IQ points. These can be easily prevented by supplying proper amount of iodine during this period.

Since there is no natural food item which will help in supplying adequate amount of iodine, it is essential to supplement or fortify the food item for meeting the iodine requirements of the individual.

There are several modes of iodine supplementation used. These are: Iodised oil, Iodised capsules, Iodised bread, Iodised water, Iodised salt

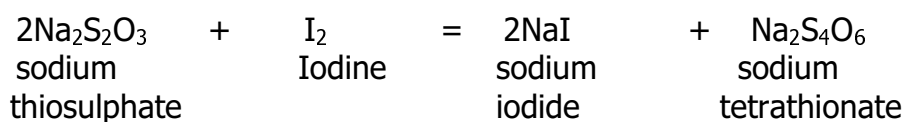
Out of these the most effective method is iodised salt. This is because salt is one food item which is taken in a fixed amount everyday by everybody whether rich or poor, old or young. Thus it is an ideal vehicle for supply of constant amount of iodine to everybody daily. It is also most economical way of supplying iodine. Government of India had taken a decision of supply of iodine via iodised salt from 1962 under the National Goitre Control Programme.

The iodised salt may loose iodine due to moisture and heat during transportation. To avoid this the PFA (Prevention of Food Adulteration) Act states that iodine content of salt should not be less than 30 ppm at production and 15 ppm at consumer level. It is essential to monitor the amount of iodine in salt by quantitative method (Iodometry). The details of procedure are given.

Principle and Laboratory Procedures for Iodine Estimation in Salt

I) Principle

The iodine content in iodated salt is estimated by a process called iodometric titration. Free iodine reacts with sodium thiosulphate solution as follows:



II) Equipment and Chemicals

a) Equipment

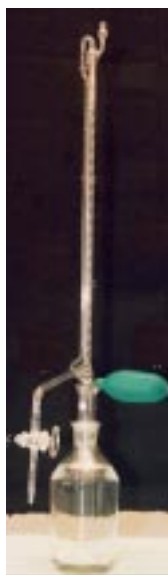
1. Laboratory balance for preparing reagents
2. Beakers – 100, 200, 500 ml
3. Glass bottles with stoppers for reagents:
1,000 ml
250 ml
4. Open pan balance for weighing salt samples



5. Measuring cylinders with stopper 50 ml
6. Wash bottle 500 ml
7. Conical flasks with stopper 200 ml
8. Glass or plastic funnel
9. Auto dispensers



10. Burette 10 ml auto zeros



b) Chemicals

1. Sodium thiosulphate - $\text{Na}_2\text{S}_2\text{O}_3$, Analytical Reagent Grade (AR)
2. Concentrated sulphuric acid – H_2SO_4 , (AR)
3. Potassium iodide – KI, (AR)
4. Soluble chemical starch
5. Boiled double-distilled water, pharmaceutical grade

The approximate cost of reagents would be Rs. 1200, which would analyze 100 salt samples.

III) Preparation of Reagents

a) Sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$): Dissolve 1.24 grams in 1 litre boiled double-distilled water. This volume is sufficient for testing 200 salt samples. Store the solution in a cool and dark place. Normality may change as time progresses. Properly stored, the solution can last for two weeks. Reagents may be prepared fresh in case there is a change in normality.

b) 2.N Sulphuric acid ($2\text{H}_2\text{SO}_4$): To 90 ml double-distilled water, add 5.56 ml concentrated H_2SO_4 slowly. Add double-distilled water to make 100 ml. This volume is sufficient for testing 100 salt samples. Store in a cool dark place. The solution may be kept indefinitely.

Caution: To avoid violent and dangerous reaction always add acid to water, never water to acid.

c) Potassium iodide (KI, AR): Dissolve 10 grams KI in 100 ml double-distilled water. This volume is sufficient for testing 20 salt samples. Store in a cool, dark place. Properly stored, the solution may be kept for 6 months.

d) Saturated Sodium Chloride (NaCl): To boiling double distilled water go on adding sodium chloride (NaCl AR Grade) while stirring until no more of it dissolves. Cool the solution

e) Soluble Chemical Starch: Weigh 1 gram of soluble starch and dissolve in 10 ml double distilled water. Add 90 ml of saturated sodium chloride solution to make it up to 100 ml. Add a pinch of sodium benzoate as a preservative.

IV) Procedure

Weigh 10 grams of salt and put it in a stoppered conical flask. Add 50 ml of distilled water and dissolve the salt. Add 1 ml of 2N H₂SO₄ followed by 5 ml of 10% KI solution with the help of an automatic dispenser and close the flask with a stopper. If the iodine is present, the solution will turn yellow. Keep the flask in the dark (for e.g. in a closed cupboard) for 10 minutes to avoid exposure to light. Remove the flask and titrate against 0.005N Na₂S₂O₃. During the titration, when the yellow colour becomes pale, add 2 drops of the starch solution as an external indicator. The solution will become purple. Continue titration till the solution becomes colourless. Note the burette reading. To calculate the iodine content in parts per million (PPM), refer to the **Table – 1**.

Table 1: Iodine Content in Parts Per Million (PPM)

Burette reading	Parts Per Million (PPM)	Burette reading	Parts Per Million (PPM)
0.0	0.0	2.6	27.5
0.1	1.1	2.7	28.6
0.2	2.1	2.8	29.6
0.3	3.2	2.9	30.7
0.4	4.2	3.0	31.7
0.5	5.3	3.1	32.8
0.6	6.3	3.2	33.9
0.7	7.4	3.3	34.9
0.8	8.5	3.4	36.0
0.9	9.5	3.5	37.0
1.0	10.6	3.6	38.1
1.1	11.6	3.7	39.1
1.2	12.7	3.8	40.2
1.3	13.8	3.9	41.3
1.4	14.8	4.0	42.3
1.5	15.9	4.1	43.4
1.6	16.9	4.2	44.4
1.7	18.0	4.3	45.5
1.8	19.0	4.4	46.6
1.9	20.1	4.5	47.6
2.0	21.2	4.6	48.7
2.1	22.2	4.7	49.7
2.2	23.3	4.8	50.8
2.3	24.3	4.9	51.9
2.4	25.4	5.0	52.9
2.5	26.5	5.1	54.0

Burette reading	Parts Per Million (PPM)	Burette reading	Parts Per Million (PPM)
5.2	55.0	7.7	81.5
5.3	56.1	7.8	82.5
5.4	57.1	7.9	83.6
5.5	58.2	8.0	84.6
5.6	59.2	8.1	85.7
5.7	60.3	8.2	86.8
5.8	61.4	8.3	87.8
5.9	62.4	8.4	88.9
6.0	63.5	8.5	89.9
6.1	64.5	8.6	91.0
6.2	65.6	8.7	92.0
6.3	66.7	8.8	93.1
6.4	67.7	8.9	94.2
6.5	68.8	9.0	95.2
6.6	69.8	9.1	96.3
6.7	70.9	9.2	97.3
6.8	71.9	9.3	98.4
6.9	73.0	9.4	99.5
7.0	74.1	9.5	100.5
7.1	75.1	9.6	101.6
7.2	76.2	9.7	102.6
7.3	77.2	9.8	103.7
7.4	78.3	9.9	104.7
7.5	79.4	10.0	105.8
7.6	80.4		

Calculations

The table of iodine content as determined by the burette reading has been prepared based on the following:

- 1) 1 ml of 0.005N $\text{Na}_2\text{S}_2\text{O}_3$ = 0.1058 mg of iodine
- 2) Thus, the burette reading X 0.1058 will give the amount of iodine in 10 gm of salt
- 3) To get the iodine value in million, one has to multiply by 1,00,000 to either sides

Gms of salt	I_2 in mg
10 X 1,00,000	0.1058 X 1,00,000

- 4) To convert mg in to gm, divide by 1000

Equation becomes:

$$\begin{aligned}\text{Gms of salt (10,00,000)} &= \text{Burette Reading} \times 0.1058 \times 100 \\ &= \text{Burette Reading} \times 10.58\end{aligned}$$

Thus Burette reading X 10.58 will give the iodine content in parts per million.

Precautions

Adding sulphuric acid to a solution of iodated salt liberates iodine, which is titrated with sodium thiosulphate. Starch is used as an external indicator. Potassium iodide solution is added to keep the iodine in the dissolved state.

1. The starch solution must be added near the end of the titration, when very little iodine is left and the solution has a faint-yellow colour. If starch is added earlier, the iodine starch complex becomes very strong and reacts too slowly with sodium thiosulphate, resulting in false high readings.
2. The titration should be done in a comfortably cool room because iodine is volatile and the sensitivity of the starch indicator diminishes as the temperature rises.
3. Potassium iodide (KI) is used because of the low solubility of iodine. The liberated iodine forms an unstable complex KI_3 with KI:
$$KI + I_2 = KI_3 \text{ and } I^- + I_2 = I_3^-$$
As free iodine is used up in the reaction with thiosulphate, the equilibrium between I_2 and I_3^- ions is disturbed and more iodine is dissolved in order to maintain the equilibrium.
4. A few minutes should be allowed before titration, since the rate of reaction between I^- ions and the oxidant is slow.
5. The reaction mixture should be kept in the dark before titration because light accelerates a side reaction in which iodide ions are oxidized to iodine by atmospheric oxygen.

Quality Assurance for Iodine Estimation in Salt

Quality assurance is a proactive and continuous process of monitoring a system for reproducibility and reliability by:

- a) Setting standards of performance & designating possibility
- b) Ensuring definitive corrective actions are taken when the criteria not met
- c) Performing measurements within a stated level of confidence

A quality control system is an indicator system for documented performance and actions that:

- a) Provides a record of consistency of performance
- b) Records action taken when performance fails to meet standard

This system uses a non-blinded system with a potential for bias.

There are essentially two types of Quality Assurance protocols:

- 1) Internal Quality Assurance
- 2) External Quality Assurance

Internal Quality Assurance:

Preparation of known value samples for Salt Iodine Estimation

1. A sample of salt (1/2 – 1 kg) collected from the market
2. Analyze iodine in the salt samples for 20 times
3. Calculate mean \pm S.D.
4. Store the samples in aliquot of 1 gms each in ziplock plastic bags

5. Preserve at room temperature away from heat and moisture
6. Take out one ziplock packet of salt each time for internal quality assessment, to be run with the analysis of unknown samples
7. The ziplock bags are stable if stored properly, for 6-12 months

Procedure for Internal Quality Assurance

1. By running the known value sample along with every batch of test sample analysis (25-30 unknown samples)
2. Quality control results can be reported by the system in several different ways. Graphically, data can be presented in Levy-Jennings format

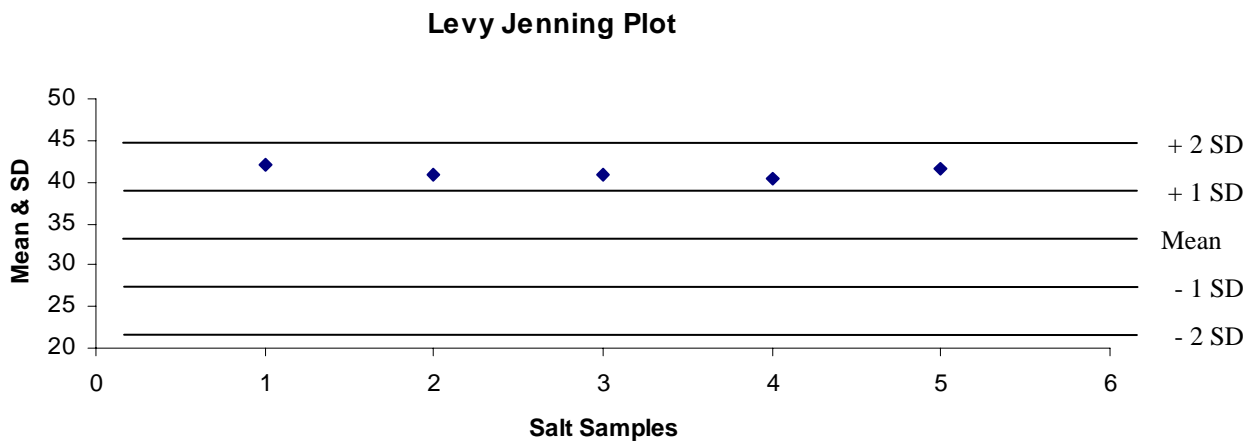


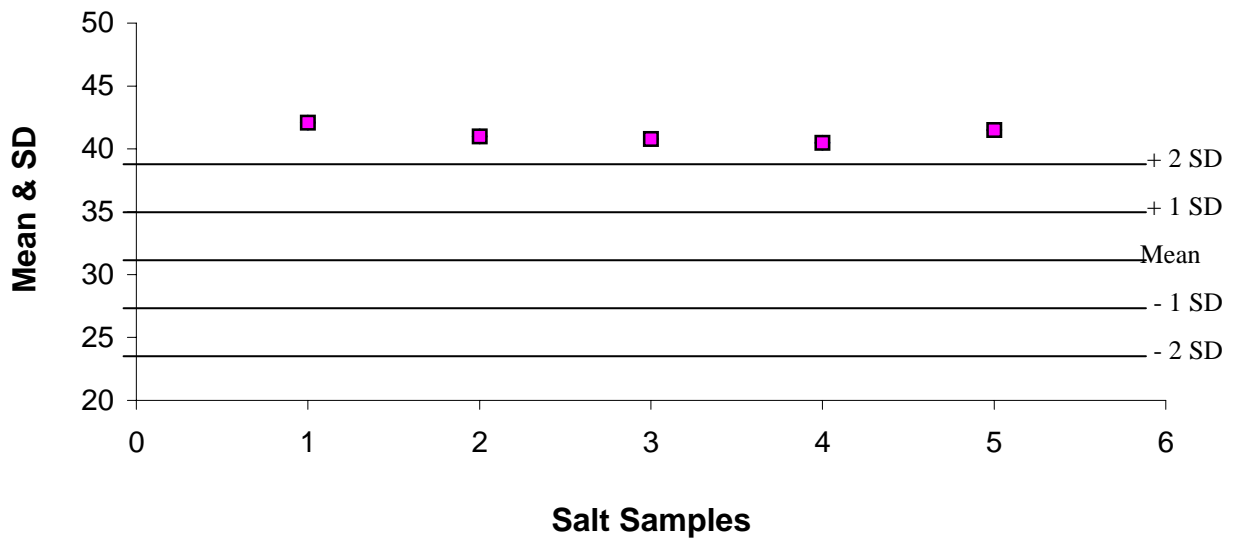
Fig. 1

3. If the value falls between ± 2 S.D. as shown in Fig. 1
 - ▶ Indicates consistency of method
 - ▶ Reagents quality
 - ▶ Performers ability

4. If the value falls outside ± 2 S.D.

- ▶ Check aliquots for internal quality
- ▶ Check reagents for contamination
- ▶ Pipetting error
- ▶ Any other

Levy Jenning Plot



Internal Quality Assurance at Production Level:

Production site laboratories should analyze known value samples each day and maintain Levy Jenning Plot.

External Quality Assurance

External quality assurance is a process by which the quality of results from each laboratory can be ensured. The known value samples are exchanged between the laboratories participating in the quality assurance program with a central laboratories functioning as a reference laboratory.

The functions of reference laboratory:

- i) Prepare samples on same lines as internal quality assurance (Mean \pm S.D.)
- ii) Send one sample to each participating laboratory at regular interval
- iii) Send the computed chart to each participating lab once a month
- iv) Training of laboratory staff at periodic intervals
- v) Providing quality reagents
- vi) Providing external quality assurance samples prepared fresh every time
- vii) Analyzing certain number of samples from each participating laboratory on a regular basis
- viii) Coordination of all laboratories

External Quality Assurance at Production Level:

Six salt samples from production site collected randomly on six different days and analyzed in duplicate at production site laboratory, should be sent to the reference laboratory each month (at least 50gms). The reference laboratory will analyze these salt samples in duplicate and then compare the results with the values obtained by production site laboratory.

The reference laboratory will analyze six salt samples (different brands) purchased from the market six times each. Find the Mean and the Standard Deviation for each salt sample. Reference laboratory will send 50 gms of each salt sample in ziplock plastic bags to the production site laboratory each month. Production site laboratory will analyze these salt samples in duplicate and send the results to reference laboratory. Reference laboratory then will compile the results, compare the values, and advise any remedial measures.

External Quality Assurance Programme at Salt Testing Laboratories:

Similar procedure should be followed for exchange of salt samples between salt testing laboratories and reference laboratories using similar protocol.

Estimation of Iodine Levels in Salt by Iodometric Titration



Step 1: Carefully weigh 10 grams of the salt sample to be tested using an electronic open pan micro-balance



Step 2: Carefully pour the 10 grams of salt into a 250 ml conical flask, which has a stopper



Step 3: Carefully measure 50 ml of double distilled water with a measuring cylinder



Step 4: Pour the 50 ml of double distilled water into the conical flask



Step 5: Shake the flask till ...



Step 6: ...all the salt dissolves



Step 7: Using the automatic dispensers...
(for 2N Sulphuric Acid on the left
and for 10% Potassium Iodide on the right)...*



*Step 8: Add 1 ml of
2N Sulphuric Acid ...*

** (One can also use a single dispenser if it is properly rinsed with double distilled water between adding the two reagents)*



Step 9: ...and 5 ml of 10% Potassium Iodide & close mouth of the flask with a stopper...



Step 10: ...the solution will turn yellow (if iodine is present)



Step 11: Keep the flask in the dark (e.g. in a closed cupboard) for 10 minutes to avoid exposure to light



Step 12: Using the automatic zero burette...



Step 13: ...fill the burette tube with 0.005N sodium thiosulphate, with a pumping action...



Step 14: ...till the level of sodium thiosulphate is at zero



Step 15: After 10 minutes, take the flask out of the dark...



Step 16: Titrate the solution in the flask with the sodium thiosulphate



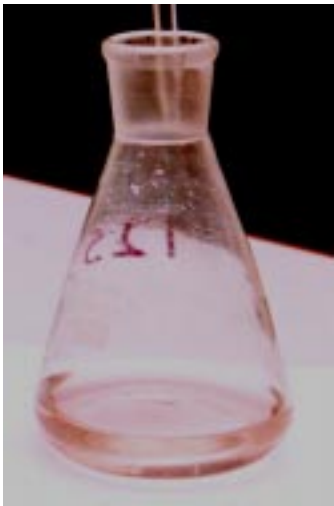
Step 17: ... till the solution is pale (faint) yellow in colour



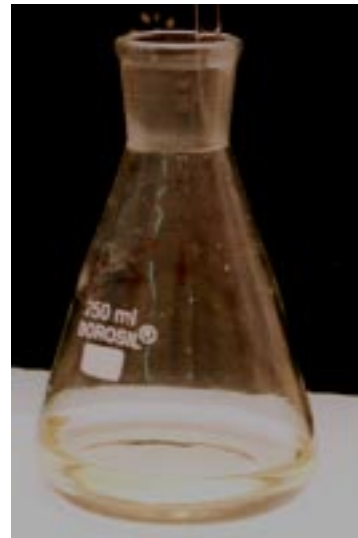
Step 18: Add 2 drops of the 1% starch solution...



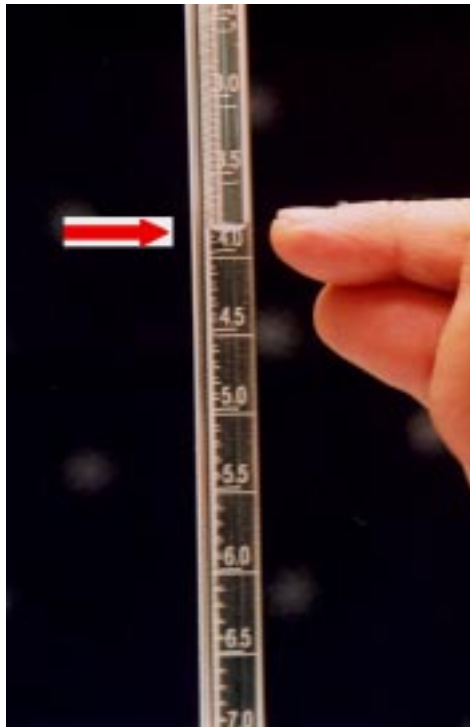
Step 19: The solution will turn purple in colour.



Step 19: Continue titration till the solution colour starts fading...



Step 20: ...and eventually becomes colourless



Step 21: Note down the burette reading (in this case 3.9) and record the corresponding iodine level as given in Table – 1 (in this case 41.3)