Development of Rapid Test Kits for Monitoring Salt Iodization

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The iodine content in iodized salt has to be monitored from production to consumption level to ensure retention of adequate iodine for prevention of Iodine Deficiency Disorders (IDD). As in most countries there is insufficient technical Infrastructure or financial resources to establish laboratory based measurements, there is a need for inexpensive rapid, portable analytical techniques.

A number of commercial kits have been developed based on the reaction of iodine with starch. These kits are simple, easy to use, but are subject to interferences and are useful only in a limited concentration range. We tested the performance of a number of typical commercially available kits. The best kits are suitable for measurements only in the range 0-20 ppm Iodine. There is a need for a more accurate kit with a wider range suitable for in-plant quality control. We have developed a simple, more quantitative kit suitable for in-plant use. The description of the kit and its alidation in four countries is described. The kit still requires the development of an acceptable packaging for commercial use.

1. INTRODUCTION

More than half of the world's population receive less iodine than required for developing and maintaining a healthy body through the normal diet. To prevent iodine deficiency disorders the diet must be supplemented. During the past twenty years there has been a strong effort, lead by the United Nations, to iodize all salt for human consumption (Venkatesh Mannar, 1987). The iodine addition level is important, since too low levels will have no noticeable benefit, while excessive doses are wasteful, and may be detrimental. In most developing countries, there are few means to accurately determine the iodine level in salt. Despite the superior oxidation resistance of iodate, iodated salt tends to lose iodine over time, (Diosady et al. 1998) and it is therefore important that simple, effective tests for iodine during manufacturing, distribution, retail and at the household level be developed.

We have worked with the Program for Appropriate Technologies in Health, PATH Canada, and the Micronutrient Initiative to review the availability of rapid field technologies for iodate analysis, and on the development of improved analytical techniques suitable for in-plant quality control of iodate addition

1.2. Field Test Requirements

The success of a field test depends on a clear understanding of the field conditions, and the requirements of the users in terms of the precision and accuracy of the test results. We must accept the fact that field tests will be carried out by personnel with limited training, under conditions where laboratory services, utilities or even simple supplies will not be readily available. As a result all of the equipment and supplies for the test should be contained in a light, inexpensive, portable kit, which does not require utilities such as electric power or other reagents or vessels. The test must give accurate results rapidly, and results must be readily interpreted

1.3. Iodate Analysis

There are several standard analytical techniques for the quantitative determination of iodine present in the form of iodate. Iodate analysis is usually based on the reduction of iodate to elemental iodine, which is then determined by titrimetry or colourimetry. Acidified iodate solution can be reduced to generate elemental iodine. The iodine may be determined colourimetrically directly, or by forming a coloured complex with starch or other reagents (Hatch 1984).

The most widely used procedure is based on the reaction with potassium iodate which quantitatively liberates the iodine in the iodate:

$$KIO_3 + 5\,KI + 6\,HCl \rightarrow 6\,KCl + 3\,H_2O + 3\,I_2$$

The liberated iodine has a faint yellow colour, which may be measured directly. More often, starch is used as an indicator. Starch forms an intense blue-black colour with iodine, through a series of rapid reactions. First, the iodine reacts with theexcess iodide ions

$$I_2 + I^- \rightarrow I_3^-$$

The tri-iodide ion thus formed reacts with another iodine molecule to produce the linear penta-iodide anion:

$$I_3^- + I_2 \rightarrow I_5^-$$

which fits inside the helical ß- amylose chain of starch. The complex will be visible in the presence of 0.2 µg/mL iodine. The colour intensity varies with the amount of free iodine, and can form the basis of a quantitative colourimetric measurement. The formation of this complex is reversible, and this is the basis for titrimetric methods.

1.4. Currently Available Field Tests

A number of test kits have been developed and distributed either commercially or as a part of the national IDD strategies of several developing countries. All of the kits that we could obtain through cooperating UNICEF and ICCIDD offices, consist of a means of adding a few drops of a reagent onto the surface of the salt sample, and visually comparing the colour developed with a calibration chart. The reagent typically consists of an acid, a reducing agent, and starch.

These kits are quite sensitive, and readily produce a blue stain on the salt surface, indicating the presence of iodine. It was our understanding, through discussion with experts in the field, that the quantitative measurements made by currently available kits are inaccurate, and irreproducible.

More sophisticated and accurate test kits are available commercially , however these tests require either a high initial investment in a portable instrument, or require expensive supplies, that make these tests less suitable for use in developing countries.

2. OBJECTIVES

In this work we have tested a large number of kits from several countries in an effort to evaluate their performance, and to devise improvements to their range and accuracy.

3. EXPERIMENTAL METHODS

3.1 Materials

Food grade, un-iodized salt was obtained from Toronto Salt Chemical Co., Toronto. All reagents were analytical grade, obtained from BDH Chemicals, Toronto ON.

3.2 Testing of commercial kits

Iodated salt samples with various levels of iodine were prepared by weighing 1-3 kg salt into a laboratory ribbon blender (LeRoy-Somer, Montreal PQ) and slowly adding the calculated amount of potassium iodate as a 30 g/L aqueous solution, while blending. The mixture was blended for an additional 20 minutes to obtain a homogeneous, free-flowing solid.

These blends were analyzed by titration (AOAC 33.147) and neutron activation analysis, using the SLOWPOKE nuclear reactor facility of the University of Toronto (Hancock 1978)

Samples of the analyzed salt were spread out in a 5-8 mm thick layer in a petri dish, and their iodine content was measured using the commercially available rapid field test kits, by dropping two to ten drops onto the salt surface, producing wet spots of ~ 10 mm in diameter. The colour of the spots was compared to the colour chart provided with the kit to give the iodine content.

We have also carried ten calibrated samples to manufacturers' plants in India, Thailand and Indonesia (2 locations). The accuracy of kits manufactured in these locations were tested in informal "blind" tests of local salt field test kits.

3.3 Testing of improved kits

Inter-laboratory tests were conducted on a test kit developed in our laboratory. Test kits were made up in Toronto, and sent to the participating laboratories, together with a series of pre-analyzed salt samples and instructions for making up a kit. The salt samples were analyzed in Toronto by both the kits, and standard analytical techniques.

The participating laboratories analyzed the salt samples with their standard laboratory technique, the kit sent from Toronto, and a kit made

in situ. They also made up a series of iodated salt samples using local, unpurified salt, and analyzed them, as above. Some of the salt was also sent to Toronto, where it was also analyzed by both the improved kits and the standard laboratory techniques.

4. RESULTS AND DISCUSSION

4.1 Commercial test kits

Some 80 test kits from India, Pakistan, Nepal, Thailand, Bangladesh, China and Indonesia were tested in Toronto. A further four were tested at the manufacturers' site. All but one of these kits used the reaction with KI and starch for colour development. The kit we received from China used a reaction of iodate with an organic dye for colour development. This kit was unable to distinguish between levels above 10µg iodine/g salt. As we only received one small, old kit, this result may not be representative.

At low pH (typically pH=2.6) salt with 15 ppm of iodine or more will produce a dark blue colour when the kit reagent or reagents are dropped on the salt surface. Unfortunately in all kits tested all concentrations above 15 ppm gave the same dark colour. The colour saturation is due to the fact that one molecule of iodate reacts with 5 molecules of potassium iodide, liberating 3 molecules of elemental iodine. Thus, during the colour measurement or estimation, elemental iodine is present in six times the concentration originally in the sample.

An additional problem with these kits was the quality and size of their colour charts,which were not easily related to the actual appearance of the salt.

The simplest kits, with widest distribution are manufactured by MBI Kits in India. Their kits contain a "retest" solution, which lowers the pH in salts with high alkalinity. These kits provide good qualitative indication of the presence of iodine and can discriminate between salt levels in the range of 0-15 ppm. Unfortunately the kit cannot discriminate between levels above 15 ppm.

The kit develped by Mahidol University in Thailand is more sophisticated, as it carefully controls both pH and starch colour. The presentation background is controlled, as is the actual quantity tested. This kit works best with finely divided, refined salt - where it gives reproducible results in the 0-15, perhaps 0-25 ppm range. Unfortunately this kit is significantly more expensive than the MBI kit.

Clearly, none of the commercially available kits are suitable for in-plant quality control of production. Small operators that cannot afford the skilled manpower and equipment requirements of the standard titration method, need a semi-quantitative test kit with a reproducibility of say ± 15% in the 10-50 or 10-100ppm range. Accordingly, we tried to improve the currently used iodide-based dropper test, and developed tests based on liquid-phase colour development.

4.2. Improvements To Existing Kits

As indicated above the curremtly available kits are based on the formation of the starch-iodine complex, and visual comparison of the colour obtained with printed standards. The kinetics of this reaction is pH dependent, which limitats the reprocibility of the results.

One way increase the range of measurement is to dilute the salt 1:10 using a similarly coloured material, such as odine free salt, sand or flour. Unfortunately, solid dilution is difficult to do evenly, and would make the kit bulky, as some 10-20 g of inert material is needed for each test.

The colour development is influenced by the composition of the starch indicator. The colour arises from the absorption of iodine to the b-amylose, a macromolecular component of most starches. The closely related a-amylose forms a red adduct with iodine, while amylopectin produces a blue colour with a different absorption maximum. As a result, reproducible results may be obtained only if the starch is well characterized, and the colour intensities shown on the colour charts are calibrated with the actual kit reagents. In our work we used soluble potato starch.

While this will improve the kits, the problem due to the kinetics of this reaction and its pH dependence remains a major limitation To improve reproducibility, the kits should contain a

buffer system that allows the colour development to take place at a controlled pH value.

Strong oxidizing agents interfere with the colour development by oxidizing the added iodide to elemental iodine, providing false positive readings. Further improvements to basic dropper kits may be made by substituting another reducing agent for KI. Unfortunately there are few simple reagents with the required redox potenial, that react fast enough.

Finally, the kit colour charts can be improved, to match the size and texture of the colured salt surface. The salt schould be tested on a uniform background, and its surface should be completely wetted by either a few drops of water, or the test solution. The sample presentation could be standardized by the use of a small white plastic cap.

4.3. Proposed Novel Iodate Field Test

The solid-phase dropper tests depend to a large extent on diffusion, to give a reproducible surface colour. An improved kit must be based on a quantitative measurement to give better reproducibility and accuracy. I This may be best achieved by using volumetric solutions, which eliminates the uneven distribution of reagents.

We developed a simple test based on the concept of dissolving a measured volume of salt in a set volume of water, and estimating the colour developed in a flat-bottom plastic or glass vessel, after acid, KI and starch addition. IThe system and its performance are briefly described in the following:

4.3.1. Field test apparatus

a. 250 mL tall form plastic vial (glass optional)
b. Two 100 mL plastic flasks containing the reaction solutions A and B respectively
c. Two disposable transfer pipettes, capable of delivering 3 mL solution
d. 0-50 ppm iodated salt colour standard chart - prepared by photographing a set of six standard solutions, and making colour corrected prints.
e. sampling cap (approximately 1 mL)

4.3.2. Preparation of reagents

a. Solution A: Starch solution with KI - Soluble potato starch (ACS grade) (3g) was triturated with 10 mL of cold water, and poured slowly, with constant stirring into 100 mL of boiling water. To prevent spoilage, 400 mg Na-benzoate was added to the solution. The mixture was boiled for ~2 min., to obtain a non-viscous translucent fluid. Extensive boiling reduces the sensitivity of the test. The solution was allowed to cool, and the settled solids were removed by decanting, and filtering the supernatant using Whatman No. 41 filter paper, or equivalent. Five g KI, were added to the filtrate, and its pH was adjusted to 7.5 with 0.1% Na_2CO_3 .
b. Solution B. The pH of 100 mL distilled water was adjusted to 1.8 with 1 % HCl.
c Solution B was then mixed 1:1 with solution A.

4.3.3. Field Procedure

1. One scoop (~1.8g) of salt. (Salt must be granular, i.e. ground if necessary) was added to the 250 mL tall form vial.
2 With the disposable transfer pipettes 3 mL of **solution A** and 3mL of **solution B** were added,
3 The solution was thoroughly mixed with gentle swirling, until a dark blue colour developed (about 20 seconds).
4. If no colour developed then more solution B was added, to a maximum of 12 mL.
5. Local water, preferably de-aerated by boiling, was then added to the 250 mL mark, and the solution was mixed.
6. After the air bubbles entrained by the mixing have cleared, the iodide concentration of the salt was determined by matching the colour of the solution with the colour charts.

4.3.4 Test validation

As the salt was measured volumetrically, large errors in the weight of salt sampled could be introduced if the salt was not finely granular, or if it was very wet.

We found that some highly alkaline salt samples did not develop the blue colour, underestimating the iodine content. This was readily remedied by adding extra acid. In the test kit instruction we have allowed for this by suggesting that extra solution B be added prior to dilution. It could also be remedied by the use of a "recheck solution" - but this may lead to a dilution error.

Field test kits for the liquid phase test were supplied to participating laboratories. In all cases

Laboratory	Measurement used	Relative standard deviation
University of Toronto	U of T kit	2.81
University of Toronto	Titration	1.39
University of Toronto	Spectrophotometer	0.58
Zimbabwe	U of T kit	1.30
Zimbabwe	Titration	5.84
Zimbabwe	Zimbabwe kit	12.33
India	U of T kit	10.31
India	Titration	4.26
India	Indian kit	22.33

Table 1. - Comparison of measurement techniques.

six replicate analyses were performed, and the average and standard deviation were calculated. The performance of the kit was checked against the standard laboratory analysis on salt samples iodated to various levels. The results are presented in Table 1.

The results indicate that the tests worked well within the target range 0 to 50 µg/g iodine. The developed colours were compared to a set of six colour standards set at 10µg/g intervals, and the observers interpolated between these 10µg/g levels. Indeed, reproducible results within 5 µg/g were obtained when six measurements were averaged.

The kit results obtained in Toronto were closer to the laboratory measurements than those from the other collaborating laboratories. There were two reasons for this discrepancy: the chart colours, and sample degradation. Our laboratory could compare the observed colours with the colours of actual standards, not only photos of these standard solutions. We found that the quality of the colur charts is critical for the accurate determination of iodine content.

There was an error inherent in the iodine determination of salts prepared by another laboratory, due to the degradation of the sample during shipping. We found that iodated salts, especially unrefined local salts, lose iodine with time, and therefore the measured iodine content may have been actually different at the time of reading in the three participating laboratories. (Diosady et al. 1999 adn 1999a) Despite these problems, the reproducibilty of the results was acceptable.

4.4. Photometric quantitation

The colour developed in the liquid-phase kits is compared with colour standards to obtain a reading of iodine content.

This measurement by eye could be readily made more accurate by using an instrument for the colour measurement. Ideally a spectrophoto-meter measuring the absorption maximum of the starch-iodine complex would give the best results. While spectrophotometers are too expensive, with recent advances in electronics several inexpensive filter photo-meters have become available. We have tested a photometer specifically developed for the determination of iodate in salt, manufactured in Tienjin China, and compared its results with that of our kits, the standard laboratory tests and results from another photometer. As expected, the photometric measurements were reproducible, and depending on accurate calibration, very reliable. The Chinese unit underestimated the iodine content by about 2.5% compared to the NAA method. Their use is simple, and the cost in large quantities should be accessible to most salt producers (~$100) . It seems that the liquid-phase kit developed in our laboratory would be a simple useful tool for in-plant quality control, which may be later upgraded, by replacing the visual colour measurement, with an instrumental technique, using a simple colorimeter or photometer, such as the Chinese instrument.

5. CONCLUSIONS

The existing field tests for iodate are small, and simple to use, and readily detect iodate in salt at levels above 5µg/g. These tests were found to be quantitative only at low levels of iodine, i.e. 5 to 15 µg/g. The range of the tests may be extended by diluting the salt sample with another iodine-free solid of similar colour and texture, such as salt, sand or flour, but this makes the kits more bulky, and the

test less reproducible. The current kits may be improved by standardization of the starch indicator used, controlling the pH of the system, and controlling the quantity and presentation of the salt samples.

The liquid phase colour development gave reproducible results in the range of 0-50µg/g iodine. This range can be readily extended to 100µg/g by dilution. There is no doubt that the liquid phase test is more cumbersome, and in some applications the

extra quantitative information may not be worth the increased cost and complexity. However, the proposed kits provide an inexpensive and simple method of obtaining valid quantitative measure of iodine content useful for in plant quality control.

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