Field Tests
For
Iodide, Iodate, And Iron

Phase 2 Final Report

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1. INTRODUCTION

Approximately one third of the world's population is suffering from deficiency diseases resulting from the shortage of key micronutrients in their diet. Nutrition and health experts working under the auspices of the United Nations identified iodine, iron and vitamin A as the key micronutrients lacking in the diet of many developing countries. This resulted in a series of research and development programs that should lead to a significant improvement of the health status of the population of developing countries through the fortification of common components of the local diet with these three micronutrients.

The developed countries have used food fortification for the prevention of several deficiency diseases. Effective fortification requires that a carrier be identified, which is consumed at a constant, predictable level by the target population. As typical examples, salt is iodized, and in Canada milk is fortified with vitamin D.

The dosage of micronutrients is important, since too low levels in the diet will have no noticeable benefit, while excessive doses are wasteful, and may actually result in detrimental health effects. In the low-technology setting of most developing countries, it is important that simple, effective tests be available for the determination of fortifying agents.

In 1993 PATH Canada has contracted our group to identify feasible approaches to rapid field testing of iodide, iodate and iron in salt, and for vitamin A. These methods were to be designed for use in the field, where they could be used to extend the capability of national Iodine Deficiency Disorders (IDD) programs to verify potency of iodized salt, both as means of quality control at the manufacturing level, and for field monitoring of delivered levels. Following successful validation of the above tests, the methods, which will be in the public domain, would be more widely disseminated to national IDD elimination programs.

The report presented in October 1994 reviewed the analytical chemistry of iodine, iron and vitamin A. We concluded that field testing for vitamin A can only be conceived in very specific circumstances, where both the food matrix and the form of added vitamin A are known. Tests for iodide and iodate in salt were found to be feasible, although the range and accuracy of the tests were difficult to control. The test for iron in salt was also possible, although the high concentrations (~1000 mg/kg) made the test more difficult.

As a result of this work, we embarked on a second phase of the project with objective to further develop, and test the field test concepts. The work was broadened to include tests that may be used in quality assurance during manufacture for iodide and iodate in salt. Rather than continue on testing salt for iron, in this phase we were asked to investigate the feasibility of developing a simple test for iron in fortified wheat flour. The following report summarizes our findings.

2. FIELD TEST REQUIREMENTS

The development of an effective field test depends on a clear understanding of the field conditions, and the requirements of the user in terms of the precision and accuracy of the test results. In the case of field tests for the measurement of the critical micronutrients, iodine and iron , we must accept that the 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 must be self contained in a kit, which does not require other utilities such as electric power. Although it would be desirable to require no further component, we realized that better tests could be devised if accept the need for water.

The field condition requires that the test kit be light, inexpensive and easy to use. The test must give acceptable results rapidly, and results must be readily interpreted.

The first step in developing a field test is the survey of the quantitative analytical systems that may be applied to the specific field conditions, sample matrix and expected concentration range. This is then followed by the laboratory testing of selected chemical systems. The laboratory identification of a feasible system must be followed up by the development of a test-kit that meets the needs of the client group. In the following sections the test development for iodate, iodide and iron will be described in separate sections.

3. DETERMINATION OF IODIDE IN SALT

Our mandate was to test the feasibility of developing a test system, that will allow the field identification of the iodide levels in salt in the range of 10 and 50 µg/kg iodine, with a sensitivity of ~10 µg/kg.

3.1 TECHNIQUES OF IODIDE ANALYSIS

We have reviewed the analytical chemistry of iodide in a variety of matrices. The principles of these techniques were examined with a view of identifying techniques that may be adapted to field tests.

3.1.1. Leuco crystal violet method

Iodide is selectively oxidized to iodine with potassium peroxymonosulfate. The elemental iodine reacts with the colourless leuco crystal violet indicator producing the highly coloured leuco crystal violet dye.

Procedure: STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER 17th edition, 1989 -

Measure 50 mL iodide solution into a 100 mL volumetric flask. Add 1.0 mL citric buffer and 0.5 mL of potassium peroxymonosulphate solution. Swirl to mix and let stand approximately 1 min. Add 1.0 mL leuco crystal violet indicator, mix, and dilute to 100 mL. For best results, read the absorbance (or compare the colour with standards) within 5 minutes after adding leuco crystal violet indicator solution.

Chloride interferes with colour development, and therefore this method is not suitable for field test of iodide in salt.

3.1.2. Catalytic reduction method

Iodide is determined based on its ability to catalyze the reduction of ceric ions by arsenous acid. The reaction is stopped after a specific time interval by the addition of ferrous ammonium sulphate. The resulting ferric ions, which are directly proportional to the remaining ceric ions, develop a colour complex with KSCN.

Procedure: STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER 17th edition, 1989 -

Prepare 10 mL iodide solution in a 25 mL test tube, keeping the iodide content of the diluted sample in the range 2 to 6 µg/mL. Add reagents to the sample in the following order: 1 mL NaCl solution, 0.50 mL arsenous acid solution, and 0.5 mL concentrated sulfuric acid. Place the reaction mixture and the ceric ammonium sulphate solution into a water bath at 30 °C, and allow to come to temperature equilibrium. Add 1 mL ceric ammonium sulphate solution, mix the contents of the test tube by inversion and start the stopwatch to time the reaction. After 15 ± 0.1 min. remove the sample from the water bath and add immediately 1.0 mL ferrous ammonium sulphate reagent with mixing, whereupon the yellow ceric ion colour should disappear. Then add, with mixing, 1.0 mL potassium thiocyanate solution. Replace sample in the water bath. Within 1 hr after the thiocyanate addition, read the red colour as percent transmittance

in a photometric instrument.

Unfortunately the method requires instrumentation. While relatively simple and inexpensive portable instruments are available, this approach unfeasible for a field test in the target markets.

3.1.3. Iodide oxidation with bromine water

Iodide is oxidized to iodine by the addition of bromine water. The excess of bromine is distilled (expelled) out of solution. The iodine is titrated with standardized sodium thiosulphate solution using starch solution as end-point indicator. The titration reaction is

$$2\ Na_2S_2O_3 + I_2 \rightarrow 2\ NaI + Na_2S_4O_6$$

Procedure: OFFICIAL METHODS OF ANALYSIS of the ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS 14th Edition Edited by Sidney Williams; Published by the AOAC, Inc. 1984;

Dissolve 50 gram sample in water and dilute to 250 mL in a volumetric flask. Take 25 mL aliquot for analysis. Place sample aliquot in 600 mL beaker and dilute to ca 300 mL. Neutralize to methyl orange with 85% phosphoric acid and add 1 mL excess. Add excess bromine water and boil solution gently until colourless, and than 5 min. longer. Add few crystals salicylic acid and cool solution to ~ 20°C. Add 1.0 mL 85% phosphoric acid and ~ 0.5 gram potassium iodide, and titrate iodine with 0.005 N sodium thiosulphate solution, adding starch indicator solution when liberated iodine colour is nearly gone.

1 mL 0.005 N $Na_2S_2O_3$ = 0.1058 mg iodine.

This method was extensively tested in the laboratory as an alternative to neutron activation analysis. The method was not very reproducible, giving high relative standard deviations (up to 30%) with well defined, clean purified salt samples. The need for distillation of free bromine, and the reactivity of bromine water makes the method impossible to adapt to a field test.

3.1.4. Iodide oxidation with iodate and hydrochloric acid

Iodide is oxidized to elemental iodine in the reaction with iodate in the presence of hydrochloric acid :

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

The free iodine is titrated with standardized sodium thiosulphate solution using starch solution as end-point indicator, as above.

Procedure: STANDARD METHODS OF CHEMICAL ANALYSIS N.Y. D.VAN NOSTRAND Comp. Inc. SCOTT, Wilfred, W. , 1939

A known amount of 0.1 N potassium iodate is added to the iodide solution, in sufficient amount to liberate all of the iodine combined as iodide, with several mL in excess. Hydrochloric acid and a piece of calcite are added. The mixture is

boiled until all of the liberated iodine has been expelled. To the cooled solution 2 or 3 grams of potassium iodide are added and the liberated iodine, corresponding to the excess of iodate in the solution, is titrated with standard thiosulphate. One mL of 0.1 N potassium iodate = 0.01058 gram iodine as potassium iodide.

Although the method may not be used directly in the field, the principle of this method seemed readily applicable to field testing.

3.1.5. Iodide decomposition by ferric (Fe^{+++}) salts

Iodide is oxidized to iodine in the reaction with ferric ions in the form of ferric ammonium sulphate:

$$2\ NH_4Fe(SO_4)_2 + 2\ KI \rightarrow I_2 + (NH_4)_2\ Fe\ (SO_4)_2 + FeSO_4 + K_2SO_4$$

The free iodine is titrated with standardized sodium thiosulphate solution using starch solution as end-point indicator, as above.

Procedure: STANDARD METHODS OF CHEMICAL ANALYSIS N.Y. D.VAN NOSTRAND Comp. Inc. SCOTT, Wilfred, W. (1939)

An excess of ferric ammonium sulphate is added to the sample in a distillation flask. The solution is acidified with sulfuric acid, then heated to boiling, and the iodine is distilled into a solution of potassium iodide. The free iodine in the distillate is titrated with standard thiosulphate, or by arsenous acid in presence of an excess of sodium bicarbonate.

The reagent is added from a burette until the titrated solution becomes a pale yellow colour. About 5 mL of starch solution are then added and the titration continued until the blue colour of the starch fades and the solution becomes colourless.

Further reagents may also be considered. For example iodine can be liberated from iodides by As^{5+}, Sb^{5+}, Bi^{5+}, Cu^{2+}, Cr^{6+}, $K_3Fe(CN)_6$ (potassium ferricyanide), HNO_3, Cl_2, H_2O_2, or ozone. In each of these approaches the resulting free iodine is titrated and the end point is detected using starch as indicator. The concentration of iodine may be also detected colourimetrically, (in a narrow range of concentrations) based on the colour density of the starch-iodine complex.

We did not do extensive testing of organic redox systems as these are very sensitive to both impurities and the sample matrix.

There are several non-colourimetric methods for the analysis of iodides using amperometric titration, ion-selective electrodes, ion-chromatographic or high performance liquid chromatographic techniques. As these techniques are not readily and inexpensively adaptable to field testing, they were not considered further.

Based on the theoretical background summarized above, and the results of our experiments during the first phase of this program we selected two approaches to the field measurement of iodide. In both iodide is oxidized and the resulting free iodine is detected colourimetrically. The two methods use potassium iodate and ferric chloride

respectively, as the oxidizing agent.

In our experience the iodate system is extremely sensitive to pH, including, of course the buffering action of the weak organic acid used. We optimized the pH of this system in the range from pH=1.8 to pH=3.4 in terms of colour development and colour differentiation. We then to devised an appropriate buffer system, keeping in mind the need to stabilize the starch both microbiologically and chemically.

We tested several systems using both pure Canadian and impure salt samples received from one or more of the target countries.

3.2 FIELD TEST FOR IODIDE IN SALT USING POTASSIUM IODATE

In the first phase of this program we have designed a preliminary kit, in which the buffering system was based on salicylic acid. This system gives a reddish colour with ferric iron, introduced either as an impurity, or as an ingredient in double fortification. The interference of the Fe^{+++} made this test kit vulnerable, and thus we opted for another organic acid buffer: sodium benzoate/benzoic acid, after testing several potential alternatives.

3.2.1. Principle:

Iodide (I^-) as potassium iodide in iodized salt is converted to elemental iodine by potassium iodate in an acidic medium :

$$5\ KI + KIO_3 + 6\ H^+ \rightarrow 6\ K^+ + 3\ H_2O + 3\ I_2$$

The free iodine will react with starch to form a deep blue/ purple complex. In this reaction system the colour intensity of the iodine-starch complex is directly proportional to the liberated iodine, and hence the iodide content of the salt, although the colour intensity and stability depends on several factors that must be controlled to achieve a reproducible result.

3.2.2. Interferences:

In the presence of $CaCO_3$, sodium silico aluminate or other alkaline substances the reaction mixture may require a small amount of dilute hydrochloric or sulphuric acid (~1%) solution in the reaction mixture to neutralize these alkaline substances. The buffer system in the kit should take care of this problem, unless the quantity of bases is very high. If this consistently happens on salts from a specific plant or region, the kits can be altered by adding extra salicylic, hydrochloric or sulphuric acid to the reagent. The presence of strong reducing agents other than iodide may also give a positive bias with iodate-based methods, as these free iodine from the excess iodate reagent.

3.2.3. Field test apparatus

a. metal pan (~8 cm o.d.)

b. 25 mL plastic flask with dropper in cap, containing the prepared reaction mixture

c. 10-50 ppm iodized salt colour standard chart (photograph)

3.2.4. Reagents

a. **Benzoic acid solution.** Add 400 mg ACS grade sodium-benzoate to a 100mL water, dissolve by gentle mixing, then adjust pH to 3.2 using ~ 0.1 N HCl. This forms a benzoic acid/ sodium benzoate buffer solution.

b. **Starch (iodide free) addition** - Heat the benzoic acid/ sodium benzoate buffer (a) solution to boiling. Triturate 3 gram of soluble potato starch (ACS grade) with 10 mL of cold water, and pour slowly, with constant stirring into the boiling buffer solution. Boil the mixture until a thin, translucent fluid is obtained. Excessive boiling may render the solution less sensitive. Allow to settle and filter the clean supernatant.

c. **Potassium iodate addition.** Dissolve approximately 3 g of KIO_3 in the buffered starch solution, completing the reaction mixture.

d. **Reaction mixture:** Mix thoroughly and dispense into 25 mL plastic dropper flasks.

e. **Iodized salt sample**

3.2.5. Procedure

a. Fill the metal pan with salt, creating a flat surface flush with its edge.

b. Apply a few drops of reagent solution (3.2.4 d) to the surface of the salt.

c. Compare the developed starch-iodide blue colour with the colour standard and determine the iodide concentration of the salt.

3.2.6. Results

With pure salt initial tests in our laboratory produced reproducible results that readily distinguished different levels of iodide in the salt in the range of 10 to 40 µg/g. In this test the colours developed are dark and the difference between 40 and 50 µg/g is almost indistinguishable. This may limit its usefulness in quality control, where target levels are typically 50 µg/g.

As a result, this approach is not recommended for universal application, eventhough it may be useful for testing of iodine retention in the field, due to its simplicity, and portability.

3.3. FIELD TEST FOR IODIDE IN SALT USING FERRIC CHLORIDE

3.3.1. Principle:

Iodide is oxidized to iodine by ferric (Fe^{+++}) salts

$$2\,FeCl_3 + 2\,KI \rightarrow I_2 + 2KCl + 2\,FeCl_2$$

The free iodine will react with starch to form a deep blue/ purple complex. In this reaction system the colour intensity of the iodine-starch complex is proportional to the liberated iodine, and hence the iodide content of the salt, although the colour intensity

and stability depends on several factors that must be controlled to achieve a reproducible result.

3.3.2 Interferences:

The method was remarkably free from interferences . Since the reagent solution does not introduce iodine, the test cannot obtain false iodine readings from strong reducing agents present in the salt as impurity or through deliberate attempts to replace KI with an inexpensive reducing agent.

3.3.3. Field test apparatus

a. pan (~8 cm o.d.) either metal or plastic

b. 100 mL plastic flask with dropper in cap, containing the prepared reaction mixture

c. 10-50 ppm iodized salt colour standard chart (photograph)

3.3.4. Reagents

a. **Ferric Chloride hexahydrate solution** Prepare a 0.1 M ferric chloride solution, by dissolving 2.70 g ferric chloride hexahydrate in 100 mL water at ~25°C. Let the solution stand and decant. Use only the clear supernatant.

b. **Starch solution (iodide free)** Triturate 3 gram of soluble potato starch (ACS grade) with 10 mL of cold water, and pour slowly, with constant stirring into 100 mL of boiling water. Add 400 mg sodium benzoate. Boil the mixture until a thin, translucent fluid is obtained. Excessive boiling may render the solution less sensitive. Allow to settle and filter the clean supernatant.

In the laboratory only, when solutions a and b are first made up, if they are cloudy, filter through Whatman 41 filter paper.

c **Reaction mixture:** Before use mix the above reagents thoroughly in the following proportion: 1 volume Solution a (ferric chloride hexahydrate), 1 volume Solution b (starch solution) in the mixing bottle.

d. **Iodized salt sample**

3.3.5. Procedure

a. Fill the petri dish with salt, creating a flat surface flush with its edge.

b. Apply a few drops of the reaction mixture (c) solution (3.3.4.c.) to the surface of the salt.

c. The final observed colour is the result of the effects of the starch-iodide blue and ferric chloride orange-yellow colours, which will vary from yellow through green to a dark greenish blue depending on concentration.

Compare the developed colour with the colour standards and interpolate the iodide concentration of the salt.

3.3.6. Results

Tests in our laboratory produced reproducible results that readily distinguished different levels of iodide in the salt in the range of 10 to 50 µg/g. Photographs of a series of standards are presented in Figure 1. We have found that the colour development requires about 10 minutes. The colour development is catalyzed by both acid and the iodide present, thus samples with higher iodine content respond faster - although the final results are consistent. Thus samples containing close to 50 ppm will develop a deep blue colour in 3-5 min., which will stabilize before 10 min., and stay at the same colour without fading or darkening for at least an hour.

The best colour clarity is obtained if the reagent solution is clear. Unfortunately, if any particulates are present, such as starch globules, then ferric oxychloride forms, which imparts cloudiness to the solution in the presence of starch. While this does not change the colour reaction, the perceived colour may change, making it harder to distinguish between higher iodine levels.

We tested both the ferric chloride and the starch solution separately, and they are stable and clear for a long period of time. The ferric chloride solution itself is stable for years without decomposing into ferric-oxychloride, the starch is much less stable due to microbial decomposition, and acid hydrolysis. While the presence of benzoic acid blocks the microbial activity, the acid hydrolysis continues, limiting its usefulness to ~ 6 months at high temperatures. However the reaction mixture can become cloudy with time, due to starch precipitation. If the solution is visibly cloudy, it can be filtered, or discarded.

3.3.7. Field Validation Results

A series of samples were prepared in our laboratory, and analyzed using both the kits and neutron activation analysis, using the SLOWPOKE reactor facility at the University of Toronto. The kit, and kits made up based on these instructions were used in tests by Dr. Hans Vanhassel's staff the laboratory of UNICEF in Quito, Equador. The samples prepared in Toronto were tested with locally made kits, and samples of local salt iodized to various levels were also tested in both Quito and Toronto, using both kits, and standard laboratory techniques. The results are presented in Tables 1 and 2.

All of the kits gave good results at low iodide levels. At higher iodine concentrations the limitations of our kit due to the poor colour charts became evident. The Equadorian laboratory consistently underestimated iodine values over ~ 30 µg/g, our laboratory obtained good, reproducible results, as we used actual standard salt samples rather than the photograph for estimating the colour, and hence, the iodide content of the samples.

We have also retested the kits after some 3 months storage of the samples. This gave some indication of the instability of both the samples and the kits. The kit results remained consistent with the analytical control results obtained at the same time, although there was some significant reduction in iodine content of the salts during this storage time, due to the impurities present in the uniodized salts. The results are presented in Table 3.

4. DETERMINATION OF IODATE IN SALT

4.1 TECHNIQUES OF IODATE ANALYSIS

There are several standard analytical techniques for the quantitative determination of iodine present in the form of iodate. The principles of these techniques were examined with a view of identifying techniques that may be adapted to field tests.

Iodate analysis is usually based on the reduction of iodate to elemental iodine, which is then determined by titrimetry or colourimetry. The acidulated iodate solution can also be reduced by KI, KSCN, a cold aqueous solution of SO_2, $K_4Fe(CN)_6$ Cu_2Cl_2, H_3AsO_3, or $FeSO_4$ to generate elemental iodine.

4.1.1. Reduction of iodate to iodine by potassium iodide

The procedure is the reciprocal of the one for the determination of iodide by means of an iodate, also based on the reaction :

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

Procedure:

For soluble samples STANDARD METHODS OF CHEMICAL ANALYSIS N.Y. D.VAN NOSTRAND Comp. Inc. SCOTT, Wilfred, W. (1939)

The solution containing the iodate is allowed to run into an excess of potassium iodide solution containing hydrochloric acid. The liberated iodine is titrated with sodium thiosulphate as usual. One mL 0.1 N sodium thiosulphate = 3.567 mg potassium iodate.

Qualitative test for flour OFFICIAL METHODS OF ANALYSIS of the ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS 14th Edition; S. Williams Ed.; AOAC, Inc. 1984

Reagent: 1 volume 1 % KI solution + 1 volume HCl (5%) solution.

Sift flour over surface of dry pan and spray mixed reagent onto flour from glass atomizer until particles are wetted. Black specks or purple spots indicate presence of iodate.

The quantitative method is not a suitable procedure for field application because of the titration. However, the reaction liberates iodine quantitatively, and the iodine-starch complex can form the basis of a quantitative or semi-quantitative colourimetric correlation suitable for a field test.

4.1.2. Iodate reduction to iodine by KSCN

Iodate is reduced to elemental iodine using diluted HCl and KSCN solution. The liberated iodine is observed by its colour (qualitative)

Procedure: OFFICIAL METHODS OF ANALYSIS of the ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS 14th Edition; Edited by Sidney Williams; Published by the AOAC, Inc. 1984

Qualitative test for flour

Reagent: 1 volume 1% KSCN + 4 volumes HCl (1%)

Distribute flour evenly over bottom of metal pan and cover with freshly prepared reagent solution. Break up any lumps with stirring rod and observe on white surface. Black specks or purple spots indicate presence of iodate.

The test for qualitative determination of iodate in flour can be readily adapted to the quantitative determination of iodate in salt, as chloride does not interfere with this test. A quantitative field test based on this reaction seems feasible, as long as deterioration of the KSCN can be prevented in the field.

Starch indicator forms an intense blue-black colour with iodine. In aqueous solution, iodine has the tendency to react with excess iodide anions to form the tri-iodide ion I_3^- according to the equation:

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

The triiodide ion can than react with another iodine molecule to form the linear penta-iodide anion:

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

This penta-iodide ion fits inside the helical beta amylose chain of starch. In the presence of excess iodide iodine in concentration as low as about 0.2 µg/mL will result in the formation of the dark-blue starch-iodine complex. The formation of this colour complex is reversible, and this is the basis for titrimetric methods: at the end of the titration the strong blue-complex fades to fully colourless.

4.2 EXISTING FIELD TESTS

To confirm that current iodate test kits are not reliable, we have obtained through PATH and ML several commercial or semi-official test kits for iodine in salt iodized by potassium iodate. All of these kits 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 a reducing agent, which reduces the iodine in the KIO_3 to I_2, and starch, which forms a deep blue-black complex with the released iodine and an acidified buffer. In the MBI kits both iodide and another reducing agent is present, which stabilizes the system. The kits received and tested are presented in Table 4.

These kits are quite sensitive, and readily produce a blue stain on the salt surface, indicating the presence of iodine. Unfortunately their quantitative measure-

ments are inaccurate, and irreproducible. Initially we believed that the problem may be due to the variability in the starch supply - thus if the amylose/amylopectin ratio changed, the iodine-starch complex colour would be shifted. The problem has turned out to be more substantial.

Actually at the low pH of these kits, (typically pH=2.6) salt with more than 15 or 20 ppm iodine will produce a dark blue colour, with no further visibly noticeable darkening due to higher concentrations. Thus concentrations of 15 ppm and higher all give the same dark colour. The deep colour is due to the fact that one molecule of iodate reacts with 5 molecules of potassium iodide, liberating 3 molecules of elemental iodine. Thus the elemental iodine is present in six times the concentration originally in the sample.

As indicated earlier, the way to overcome this problem is to dilute the salt 1:10 using a similarly coloured material. Iodine free salt or flour seem suitable for.

The kit we were sent from China uses a reaction with an organic dye - nevertheless, this kit also saturated at levels as low as 10µg iodine /g salt. As we only received one small, old kit, this result may not be representative.

An additional problem with the kits was the colour charts supplied with these kits. Rather than give photographs, showing the granular, irregular surface of actual samples, the chart shows clear, homogeneous coloured discs, which are not easily related to the actual appearance of the salt.

We followed three approaches to producing a viable kit: a liquid-phase colour development, a crude micro-titration method, and the solid state measurement of iodate using the method described above.

Working in a liquid medium reduces the problems due to uneven distribution of reagents. We tested the concept of dissolving a set volume of salt in a set volume of water in a flat-bottom plastic or glass vessel, added acid, KI and starch, and observed the colour thus developed. It is relatively simple to control volumes, and therefore this test is reproducible. The exact amount of iodine released could be estimated from the colour of the solution.

4.2.1 Proposed Improvements To Existing Field Tests

As indicated above the kits now manufactured for determining iodate in salt in the 50 µg/g concentration range are based on the development of the starch-iodine complex, and visual comparison of the colour obtained with printed standards. These tests work qualitatively reasonably well, but they produce inconsistent colours, and the depth of colour saturates, and values above 10 µg/g are not readily distinguished.

Based on our laboratory experience, and theoretical considerations, it is possible that the colour variations are also influenced by the composition of the starch indicator. The deep blue colour of starch solutions containing iodine is believed to arise from the absorption of iodine into the helical chain of β–amylose, a macromolecular component of most starches. The closely related α–amylose forms a

red adduct with iodine. Thus iodine complexes with α and β amylose, and amylopectin all have different absorption spectra, and different colour intensities. As a result, it is

critical that the type of starch used be well characterized, and the colour intensities be calibrated for it. In our work we used soluble potato starch.

Accordingly, to reduce this problem we suggest that the starch be well characterized, and only an β, such as soluble potato starch be used. This ensures that the starch-iodine complex formed has a single absorption maximum, and thus it has a well defined, reproducible colour. While this will improve the kits, the problem due to the kinetics of this reaction and its pH dependence remains a major limitation to these simple kits.

4.2.2. Principle of modified Iodate kit:

Iodate (I^{5+}) in salt is quantitatively converted to elemental iodine by potassium iodide by the same quantitative reaction as used in the iodide determination:

$$KIO_3 + 5\,KI + 6\,H^+ \rightarrow 6\,K^+ + 3\,H_2O + 3\,I_2$$

4.2.3. Interferences :

Strong oxidizing agents may interfere with the colour development by oxidizing the added iodide to elemental iodine.

4.3. FIELD TEST APPARATUS

a. metal pan

b. Two 100 mL plastic flasks containing the reaction solutions A and B respectively

c. Two disposable transfer pipettes, polyethylene, capable of delivering 1 mL solution

d. 10 mL plastic sampling vial

e 50 mL plastic sampling vial

f 0-50 ppm iodated salt colour standard chart (photograph)

g. sampling cap (1 mL)

h. sampling cap (9 mL)

4.3.1. Reagents

a. Solution A : Starch solution with KI - Triturate 3 gram of soluble potato starch (ACS grade) with 10 mL of cold water, and pour slowly, with constant stirring into 100 mL of boiling water. Add 400 mg Na-benzoate. Boil the mixture until a thin, (< 2 min.) translucent fluid is obtained. Longer boiling than necessary renders the solution less sensitive). Allow to settle and filter the clean supernatant through laboratory filter paper equivalent to Whatman No. 41. Add 5 g of KI, and adjust the pH to 7.5 with 0.1% Na_2CO_3 . Transfer the solution to a 100 mL plastic flask/vial.

b. Solution B. To 100 mL distilled water add 1 % HCl to pH = 1.2 Transfer the solution to a 100 mL plastic flask/vial.

4.3.2. Field Procedure

1. To the 50 mL plastic sampling vial add 1 small sampling cup (1 mL) of iodated salt and one large cup (9 mL) non-iodized salt. Add 2 drops of water and mix the salts with shaking. Transfer to the metal pan. (Salt must be granular, i.e. ground if necessary).

2 Add 1 mL of **solution A** and 1 mL **solution B** into the 10 mL sampling vial and mix.

3 Fill the metal pan with salt, creating a flat surface flush with its edge.

4. Apply several drops of the mixed reagent solution to the surface of the salt.

5. Compare the developed starch-iodide blue colour with the standard colour chart

4.4 PROPOSED NOVEL IODATE FIELD TEST FOR SALT

Based on the limitations of the solid-phase test, and several variants with other reducing agents, it became clear that for better reproducibility and accuracy we must develop a more quantitative test, that will be also suitable for in-plant quality control. While this introduces increased complexity, we felt that a valid field test may be developed.

4.4.1 Principle:

As indicated earlier iodate (I^{5+}) in salt is quantitatively converted to elemental iodine by potassium iodide by the same quantitative reaction as used in the iodide determination:

$$KIO_3 + 5\,KI + 6\,H^+ \rightarrow 6\,K^+ + 3\,H_2O + 3\,I_2$$

The free iodine will react with starch to form a deep blue/ purple complex. In this reaction system the colour intensity of the iodine-starch complex is proportional to the iodate content of the salt, although the colour intensity and stability depends on several

factors that must be controlled to achieve a reproducible result.

4.4.2. 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, polyethylene, capable of delivering 3 mL solution

d. 0-50 ppm iodated salt colour standard chart (photograph)

e. sampling cap (approximately 1 mL)

4.4.3. Reagents

a. Solution A : Starch solution with KI - Triturate 3 gram of soluble potato starch (ACS grade) with 10 mL of cold water, and pour slowly, with constant stirring into 100 mL of boiling water. Add 400 mg Na-benzoate. Boil the mixture until a thin, (< 2 min.) translucent fluid is obtained. Longer boiling than necessary renders the solution less sensitive). Allow to settle and filter the clean supernatant through laboratory filter paper equivalent to Whatman No. 41. Add 5 g of KI, and adjust the pH to 7.5 with 0.1% Na_2CO_3 . Transfer the solution to a 100 mL plastic flask/vial.

b. Solution B. To 100 mL distilled water add 1 % HCl to pH = 1.8 Transfer the solution to a 100 mL plastic flask/vial.

c Test solution B by checking the pH of a 1:1 mixture of Solution A and Solution B. The mixture should give pH 3.2- else adjust the pH of solution B with dilute HCl.

d. Boiled water - use as is (hot or cool) - containing little or no dissolved air.

4.4.4. Field Procedure

1. To the 250 mL tall form plastic (glass) vial add a 1 scoop (~1.8g) of salt. (Salt must be granular, i.e. ground if necessary).

2 Add 3 mL of **solution A** (with disposable transfer pipette)

3 Add 3mL of **solution B** containing HCl (with disposable transfer pipette).

4. Mix thoroughly with gentle swirling, stirring until dark colour develops (about 20 seconds) Add water to the 250 mL mark, close the cup and mix again. Wait until the colour becomes clear and transparent. as air bubbles dissipate.

5. Compare the developed starch-iodide blue colour with the similarly prepared colour standards on photo-chart and determine the iodide

concentration of the salt by matching the colours.

4.4.5. Observed Results

The sequence of steps is critical, since the proper pH at all stages of the process can influence the colour development. At very low pH the starch blue develops a pink tinge, while at high pH the colour development is very slow. We consider that the optimum pH range is 3.2 ± 0.1 after the dilution step. Therefore it is very important that the adjustment of solution A under laboratory condition will be done carefully. The pH meter will have to be calibrated with reliable pH buffers.

The background colour developed by adding only the reagents, without a sample must be determined, to ensure that the water used does not contribute to the final measurement. A positive reading can be due to dissolved oxygen in the water, and for this reason we suggest the use of boiled water, hot or cold. It may not be necessary to actually boil the water if the colour developed by the blank is not significant.

The starch-iodide solution is intrinsically unstable. To stabilize it we add sodium benzoate and buffer the pH. To minimize problems the reagent bottle must be kept closed. If the solution discolours, it can be reversed by adding a drop of sodium thiosulphate, however a slight discolouration does not interfere with the measurement. The alternative of adding KI separately either as a solid or as a concentrated solution is possible, but less convenient.

As the salt is measured volumetrically, large error in the weight of salt sampled can be introduced if the salt is not finely granular, or if it is very wet.

The standard colour chart is presented in Figure 2. Results of comparisons between titration and kit measurements are presented in Table 5.

4.4.6 Laboratory Validation To validate the rapid test methods for iodide and iodate developed at the University of Toronto we wanted to test the kits under realistic conditions typical of test, for use either in central laboratories or in the field appropriate in the context of developing country IDD elimination programs.

We wished to evaluate the

- ease of use of the test
- accuracy compared to the titrimetric method and/or neutron activation analysis

- precision

- reproducibility, in terms of intra- and inter-observer variation in readings)

The iodate tests were validated using samples of Canadian iodized salt, and iodized salt prepared by cooperating laboratories, which are likely to vary in level of iodate and iodide, consistency, colour, and level of impurities. Both the Canadian kits, and kits prepared locally according to our instructions were tested.

Field test kits for the liquid phase test were supplied to two participating laboratories: Dr. Nhari and T. Nyamandi, Government Analyst's Laboratory, Zimbabwe; and Dr. C.S. Pandav, and Dr. M. Karmarkar, Regional Coordinator, ICCIDD, New Delhi, India. In all cases six replicate analyses were performed, and the average and standard deviation were calculated. The summary of the results is presented in Table 6.

The results indicate that the tests work well within the target range of ± 10 mg/g in the range of 0 to 50 µg/g. Indeed, reproducible results within 5 µg/g were obtained when six measurements were averaged.

The titration results from India were not corrected for a blank reading for the un-iodated salt. Without this blank correction, due likely to an impurity in the Indian salt the kits actually gave a more accurate result than the titration method.

5. IRON

5.1 TECHNIQUES OF IRON ANALYSIS

5.1.1. Phenanthroline Method For Ferrous Iron

Ferrous ion (Fe^{2+}) is brought into solution and treated at pH 3.2-3.3 with 1,10 phenanthroline. Three molecules of phenanthroline chelate each atom of ferrous ion to form an orange-red complex. The coloured solution obeys Beer's law; its intensity is independent of pH from 3 to 9. Ferric ion does not react. A pH between 2.9 and 3.5 insures rapid colour development in the presence of an excess of phenanthroline. Procedure: STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER - 17th edition 1989

To determine ferrous iron, acidify aqueous sample with 2 mL concentrated hydrochloric acid to prevent oxidation. Withdraw a 50 mL portion of the acidified sample into a 100 mL volumetric flask and add 20 mL phenanthroline solution and 10 mL ammonium acetate solution (buffer) with vigorous stirring. Dilute to volume and measure the colour intensity within 5 min.

5.1.2. Phenanthroline method for total iron

Both ferrous and ferric ions are brought into solution, and the ferric forms is reduced to the ferrous state by boiling with acid and hydroxylamine. The solution is then treated with 1.10 phenanthroline at pH 3.2-3.3 as above.

Procedure: STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER - 17th edition 1989

Measure 50 mL of the aqueous sample containing ferrous and ferric ion into a 125 mL Erlenmeyer flask. Add 2 mL concentrated hydrochloric acid and 1 mL hydroxylamine solution. Add a few glass beads and heat to boiling. To insure dissolution of all the iron, continue boiling until the volume is reduced to 15 to 20 mL. Cool to room temperature and transfer to a 100 mL volumetric flask or Nessler tube. Add 10 mL ammonium acetate buffer solution and 2 mL phenanthroline solution and dilute to the mark with distilled water. Mix thoroughly and allow at least 10 min. for maximum colour development

4,7 diphenyl 1,10 phenanthroline (bathophenanthroline) may also be considered as a reagent since it has a higher colour absorbance, but unfortunately it is much more expensive.

5.1.3. Ferrozine Method For Total Iron

The sample is digested with nitric acid and hydrochloric acid, and all forms of iron are converted to the ferric form. The ferrozine reagent [3-(2-Pyridyl)-5,6-bis(4-phenylsulphonic acid)-1,2,4-triazine, monosodium salt, monohydrate] forms a purple coloured complex with ferric ion, and the colour is proportional to the iron content.

Procedure for aqueous samples: HACH WATER ANALYSIS HANDBOOK 1992 Hach Co. Loveland, Colorado U.S.A.

The procedure is based on a commercial portable kit including a

spectrophotometer: Collect samples in acid washed glass or plastic bottles. To preserve samples, adjust the sample pH to 2.0 or less with nitric acid (about 2 mL per liter). Before testing digest samples in nitric/hydrochloric acid, then adjust the sample pH to 3 to 5 with ammonium hydroxide, ACS. Adjust wavelength of spectrophotometer to 562 nm. Fill a sample cell to the 25 mL mark with sample. Add the contents of one Ferrozine iron reagent pillow to the cell (the prepared sample). Swirl to mix. Let stand for five minutes. Read the display in mg/L Fe ferrozine.

5.1.4. Thiocyanate Method For Total Iron

The iron content of the sample is oxidized to the ferric state. Thiocyanate is then reacted with the ferric ions forming to form an intense red complex. The colour fades rapidly especially in sunlight, due to the self-oxidation of thiocyanate and the subsequent reduction of the ferric ions to form a colourless complex. The fading is slower at low pH.

Procedure: E.B. Sandell. - COLOURIMETRIC DETERMINATION OF TRACES OF METALS. Interscience Publishers, Inc., N.Y. 1959

To a 30 mL of sample add 20 mL of 0.4 N hydrochloric acid and heat in water bath just below the boiling point for 20 minutes. Cool to room temperature and add a drop of 0.5 per cent potassium permanganate or enough to maintain a colour in the solution for one minute. Add 5.0 mL of potassium thiocyanate solution (30 gram in 100 mL) and compare the colour at once with a suitable series of standards. If less than 0.4 ppm of iron is present use 50 mL Nessler tubes for the comparison.

5.1.5. TPTZ Method For Total Iron

TPTZ (2,4,6-tripyridyl-s-triazine) reacts with ferrous ions to yield an intense violet colour over pH range 3.4-5.8. The indicator is combined with a reducing agent which converts precipitated or suspended iron to the ferrous state. The amount of ferric iron present can be determined as the difference between the results of a ferrous iron test and the concentration of total iron

Procedure: HACH WATER ANALYSIS HANDBOOK, Hach Co. Loveland, Colorado U.S.A. 1992

The procedure is based on a commercial portable kit including a spectrophotometer:

Collect samples in acid washed glass or plastic bottles. To preserve samples, adjust the sample pH to 2.0 or less with nitric acid (about 2 mL per liter). Adjust the sample pH to 2 or less with nitric acid (about 2 mL/L). Before testing, adjust the pH of the stored sample to between 3 to 4 with 5.0 N sodium hydroxide solution. Do not exceed pH 5 as iron may precipitate. Rotate the wavelength dial to 590 nm. Fill a sample cell to the 25 mL mark with sample. Add the contents of one TPTZ iron reagent powder pillow (which also contains the reducing agent) to the cell (the prepared sample). Cap and shake for 30 seconds. Compare reading with blank Read the display in mg/L total iron.

5.1.6. Further Colourimetric Reactions Which May Also Be Considered

For ferric ion: Deep blue colour with potassium ferrocyanide; violet colour with salicylic

acid; or green colour with potassium ferricyanide;

For ferrous ion: Blue colour with potassium ferricyanide

5.1.7 Other Approaches

We reviewed several other approaches to the analysis of iron based on gravimetric methods (AOAC), ion-selective electrodes for ferrous and ferric ions, fluorescence detection methods and HPLC techniques. However, we do not believe that these methods are adaptable to field testing, and therefore they are not describes in detail in this report.

5.2 FIELD TEST FOR IRON IN SALT

On theoretical and practical grounds we have selected, and tested the o-phenantroline method as the basis of a rapid field testing system for iron in salt.

5.2.1. Principle:

Ferrous ion (Fe^{2+}) in the salt is reacted with 1,10 phenanthroline in an appropriate reaction mixture. Three molecules of phenanthroline chelate each atom of ferrous ion to form an orange-red complex. Ferric ion and elemental iron do not react. A pH between 2.9 and 3.5 insures rapid colour development in the presence of an excess of phenanthroline. There are no known interferences with salt or its common impurities.

Initially we tried to base the test on the iodate field test kit, where a reagent is directly applied to the salt, and a colour is developed on the surface of the salt. Unfortunately the ortho phenantroline method is too sensitive, and coloured complexes formed with iron can be distinguished visually only between 0 and 20µg/g. Using this approach the test would be carried our as described here:

To reduce the iron concentration in the test samples, the iron-fortified salt must be diluted. While it is possible to mix a salt sample with some 50 parts of iron-free salt, in a field situation, the large amount of iron-free salt required would restrict the usefulness of the test. A liquid state dilution step would be more accurate, and water should be available locally, especially, as the test would not likely require water of high purity, although iron would, of course interfere.

Even so a large salt sample is not feasible, since the phenanthroline concentration must be approximately 30 times higher than that of the ferrous ion concentration for optimum colour development. This results in very high reagent use, if the size of the salt sample is not reduced. A quick calculation reveals that 1 millimole iron, 55.85 mg, requires 9 millimoles, or 1783.8 mg, of phenanthroline monohydrate. Thus one gram salt fortified with 1000 ppm iron 1 mg ferrous ion which would require 24 mL reagent solution.

Since it is impractical to take a representative field sample weighing less than 1 gram, the best approach would be to dissolve this amount in 250-500 mL water, and then take 5-10 mL from this solution for the colour development. This may be too arduous for a field test with minimally trained personnel.

The test, based on solid dilution is presented below.

5.2.2. Field Test Apparatus

a. Metal pan (8 cm o.d.)

b. 25 mL plastic flask containing the prepared reaction mixture

c. colour standard chart ; 500-1500 ppm iron in salt

d. 500 mL plastic sample container

e. sampling cap (to hold approximately 2 g salt))

5.2.3. Reagents

a. **1,10 phenanthroline solution:** weigh 200 mg 1,10 phenanthroline monohydrate (ACS grade) in a 100 mL volumetric flask. Add 40 mL distilled water and 2 drops of concentrated hydrochloric acid. Stir and dilute to volume with distilled water. (Note: one mL of this reagent is sufficient for no more than 60 microgram iron)

b. **ammonium acetate buffer solution** in a 150 mL Erlenmeyer flask dissolve 25 gram ammonium-acetate (ACS grade) in 15 mL distilled water. Add 70 mL concentrated (glacial) acetic acid (ACS grade)

c. **reaction mixture:** Mix the above reagents in the following proportion: 2 volume 1,10 Phenanthroline solution and 1 volume Ammonium acetate buffer solution. Mix thoroughly and dispense into 25 mL plastic dropper flasks.

d. **salt sample fortified with ferrous form of iron**

e. **iron free salt**

5.2.4. Procedure

a. Transfer a measuring cap (2 grams) of salt sample fortified with ferrous form of iron into a 500 mL plastic sample container containing 198 g iron free salt (1 to 100 "solid phase dilution") Wet the mixture with 3-4 drops of distilled water. Close the cap and shake and mix the content thoroughly approximately 15 minutes (the wetting helps to evenly distribute the fortified salt). Transfer a small sample of the salt mixture into a metal pan.

b. Apply several drops (10-20) of reagent solution to the surface of the salt.

c. Compare the developed orange colour of phenanthroline-ferrous complex colour with the similarly prepared colour standards and determine the iron concentration of the salt sample

5.2.5. Results

Initial tests in our laboratory produced reproducible results that readily distinguished different levels of iron in the salt in the range of 700 to 1300 µg/g.

5.3. TEST BASED ON LIQUID DILUTION

5.3. 1 Field test apparatus

a. sampling cap (to hold approximately 5 g salt)

b. 25 mL plastic flask containing the prepared reaction mixture

c. colour standard chart ; 500-1500 ppm iron in salt

d. 25 mL flat bottom tube (e.g. Nessler tube)

d. 500 mL volumetric flask

e. 1 mL and 5 mL pipettes (preferably Eppendorf or similar)

5.3.2. Reagents

a. **1,10 Phenanthroline solution:** weigh 200 mg 1,10 phenanthroline monohydrate (ACS grade) in a 100 mL volumetric flask. Add 40 mL distilled water and 2 drops of concentrated hydrochloric acid. Stir and dilute to volume with distilled water. (Note: one mL of this reagent is sufficient for no more than 60 microgram iron)

b. **Ammonium acetate buffer solution** in a 150 mL Erlenmeyer flask dissolve 25 gram ammonium-acetate (ACS grade) in 15 mL distilled water. Add 70 mL concentrated (glacial) acetic acid (ACS grade)

c. **Reaction mixture:** Mix the above reagents in the following proportion: 2 volume 1,10 Phenanthroline solution and 1 volume Ammonium acetate buffer solution. Mix thoroughly and dispense into 25 mL plastic dropper flasks.

5.3.3. Procedure

1. Transfer a measuring cap (5 grams) of salt sample fortified with ferrous form of iron into a 500 mL volumetric flask and dilute to volume with water. This should result in a solution containing 10 microgram ferrous ion per milliliter at a fortification level of 1000 ppm.

2. Transfer 1 mL of the prepared solution into a 25 mL flat bottom tube (e.g. Nessler tube) and add 5 mL of reagent solution and dilute to mark with distilled water. Transfer the liquid to a plastic cuvette, and compare the orange colour of o-phenanthroline-ferrous complex with the colour standard to determine the ferrous iron concentration of the salt sample.

5.3.4 Results

Although the differences in colour are not so clearly distinguishable as in the solid phase dilution, with some practice or training iron concentrations can determined to ±100 µg/g in the salt in the range of 500-1400 µg/g.

Photographs of a series of tests are presented in Figure 3.

5.3.5 . Recommendations for further work

We have performed some preliminary tests for the measurement of total iron (ferrous and ferric) in salt by reducing the ferric ion to ferrous form with hydroxylamine solution and hydrochloric acid without applying water-bath and heat. Our results have shown that the reduction of the ferric ion to ferrous ion in salt matrix is quite easily achieved. However, further recovery data will be necessary to establish the range and reproducibility of this method in a field environment. If double fortification of salt will come into wide-spread use, then work on this kit should be continued in an effort to develop a reliable kit, free from interference of oxidizing and reducing impurities present in the local salts.

6. TEST KIT FOR IRON IN FLOUR

We have completed the development of a technique for the colourimetric determination of iron in flour. The iron can be in the form of sulphate or fumarate, in the concentration range of 0-60 ppm iron. Although it would be desirable to extend this to electrolytically reduced, or elemental iron, the dissolution of elemental iron requires harsh reagents and excessive time, unsuitable for field use by minimally trained personnel.

6.1.1 Principle:

Ferrous iron (Fe^{++}) solubilized from flour reacts with α,α, bipyridyl (also called 2,2,dipyridine) to form a pink coloured complex.

$$3\ C_{10}H_8N_2 + Fe_2^{+} \rightarrow\ Fe(C_{10}H_8N_2)_3^{++}$$

In this reaction system the colour intensity of the iron-bipyridyl complex is proportional to the ferrous iron content of the flour, although the colour intensity and stability depends on several factors that must be controlled to achieve a reproducible result.

6.1.2 Field test apparatus

a. a,a bipyridyl solution

b. one disposable transfer pipette for delivering α,α, bipyridyl solution to the test tube,

c. 120 mL glass/plastic jar - used as a stand for test tube.

d. 50 mL test tube (glass or acrylic) marked at the 40 mL level

e. a small jar of glass beads

f. a small jar containing stannous chloride dihydrate crystals

g. 250 mL wash bottle for distilled water

h. plastic bags to contain the flour sample

i. small scoops for sampling flour (1 per sample bag)

j. plastic knife

k. a colour chart

l. sample of wheat starch as a reagent blank for iron content in water

6.1.3. Reagents

a. α,α, **bipyridyl solution** (10 mg/mL)

Weigh 1 gram of a,a, bipyridyl into a 150 mL beaker. Add 80 mL distilled water and adjust the pH to 2.4 with 9% HCl. Make up to 100 mL with distilled water - (water must be iron-free) . This is sufficient for approximately 300 measurements.

6.1.4 Procedure

1. To the test tube add 10 glass beads
2. Add 10 drops a,a, bipyridyl solution
3. add distilled water to the mark (40 mL total)
4. add one small scoop (850 mg) of flour. (Fill the scoop initially to nearly full by holding it by the built-in lid and then settle by gentle tapping. Repeat until there is a slight excess above the rim of the scoop. Use the knife to level the surface of the flour.)
5. Close cap on test tube, and shake vigorously until a bright pink colour develops. This should take 2-5 minutes.
6. Compare the developed pink colour with the similarly prepared colour standards on the photo-chart and determine the ferrous iron concentration of the flour by matching the colours.

The glass beads can be recovered, cleaned and reused. After completing the test empty the content of the test tube into the 120 mL jar, and flush out the flour with local water.

6.1.5 Use of local water:

Unfortunately most water contains iron. To eliminate the error due to the iron content of the local water, follow the above procedure, using the wheat starch as the "sample", and the local water instead of the distilled water.

Determine the equivalent iron content.

In subsequent tests using flour, subtract this blank value from the measured iron content.

6.1.6. Use of stannous chloride for determining total soluble iron.

After determining the ferrous iron content by the above procedure, retain the test tube containing the pink complex. Add a few crystals of stannous chloride to it, using the tip of the plastic knife.

Shake vigorously for ~2 min., then allow to stand for up to 10 minutes.

Read total soluble iron content from colour chart. Subtract the ferrous iron concentration from the total soluble iron, to obtain the soluble ferric iron concentration.

6.2. OBSERVATIONS AND RESULTS

Colour development in a series of standards is presented in Figure 4. The colourimetric method has been developed for ferrous sulphate as the flour fortificant.

The stability of ferrous sulphate affects the results. It can oxidize to the ferric (Fe3+) form which does not react with the α,α, bipyridyl , and has lower bioavailability.

Soluble ferric compounds, which are usually the result of the oxidation of the added ferrous iron, can be determined by the addition of a crystal of stannous chloride ($SnCl_2.2H_2O$), which reduces them to a ferrous form. By repeating the process with and without the stannous chloride the total soluble iron, and ferrous iron content can be measured. The soluble ferric iron can be calculated as the difference between these.

The inherent iron content of flour varies between 8 to 44 µg/g. Food proteins, carbohydrates and phytic acids bind the inherent iron as ligands, primarily as ferric iron, which is not detected by the test. These compounds are insoluble, and are reduced very slowly by even a strong reducing agent. The reaction does not go to completion for hours, therefore the test cannot be used to measure the inherent iron content of the flour matrix.

Elemental iron added as fortificant cannot be measured by this system, as the solubilization of the iron requires a very strong acid, which would be unsafe in a field kit.

6.3. IRON FORTIFIED FLOUR TEST KIT PROTOCOL VALIDATION

We provided five encoded samples with iron contents of 0-100 µg/g (ppm), a completed test kit and instructions for preparing a test kit to our collaborators at INCAP in Guatemala.

The collaborating laboratory was asked to use the test kit provided to analyze the flour samples provided. Each sample should be analyzed with the kit to obtain in six replicate results. The average and standard deviation was calculated. To evaluate the test kit at least two but preferably 5 or 6 persons were asked to judge the developing colour and concentration.

The collaborating laboratory was also asked to use their current analytical method to analyze these samples. Again each sample was analyzed in six replicates.

The collaborating laboratory also prepared its own test kits, and repeated the analyses with these locally made kits.

The field results were encouraging. The collaborating laboratory agreed that the test was simple to use, but was not in favour of a liquid phase test. The analytical results were acceptable in the critical range of 20-80 ppm, but were less definitive at

the higher concentrations. Some problems were due to the quality of the colour standards that were sent with the kit. The presence of the flour in the solution gives an opaque background, which alters the colour perception. This is mainly due to the reflected light from the sample. For better results, the chart must be better colour balanced, and the lighting for the measurement must be better specified. It is likely that if all observers made the measurements against a well-lit white background, the readings would be more consistent.

The results obtained are presented in tables 7 and 8.

The iron test is of questionable use in the field, as it does not readily see the iron originally present in the flour, and it can't see the added elemental iron. As elemental iron is often used as the fortifying agent, we are unlikely to develop a simple spot test that will be able to identify all forms of iron.

The ability to identify the ratio of ferrous to ferric iron may be useful in determining the degree of oxidation, hence the age of the flour. However, it is unlikely that this would be done in the field rather than in a laboratory.

Much further development may be needed. A more thorough validation would be useful before publishing the result.

7. CONCLUSIONS AND RECOMMENDATIONS

As indicated in the above report, the existing field test for iodate can be readily improved by standardization of the starch indicator used, and controlling the pH of the system, by inclusion of an appropriate buffer system. Since the reaction with iodide releases six times as much iodine in the field test for iodate, in our experience, the test can be used only for low levels of iodine, i.e. 5 to 20 µg/g. To overcome this problem we have suggested a liquid-phase system. We do not believe that this represents a problem in a field situation. In our laboratory we could readily differentiate iodine levels in increments of 10µg/g between 20 and 100 µg/g.

Our collaborators in India and Africa agreed that the test is relatively easy to use and provides reproducible readings in the specified range. Both laboratories emphasized that the use of liquid system in the field is much more cumbersome than the use of the simple dropper tests now in use. The added range and accuracy in the measurements must be useful to justify the extra effort required by the proposed tests.

Field tests for iodide were simple and reproducible. The ferric chloride-based test is preferable, as its colour range is broader, and visual differentiation is easier at the higher concentrations, than with the iodate-based kits. The kits must have better colour charts to be useful in the field.

Iron is readily detected using the colour reaction of ortho phenantroline. If anything, this test is too sensitive. The direct addition of drops of o-phenantroline to salt will give a quantitative measurement for iron in the 5 to 20 µg/g range. Unfortunately this would require a hundred-fold solid dilution in the field to enable us to do a reliable colour comparison. A viable alternative is to bring the salt and iron into solution, and thus achieving a large (up to 1000 fold) dilution, before colour development and quantitation by visual colour comparison. We have demonstrated the feasibility of both approaches, but the development of a working field testing kit will require further development and testing.

Iron determination in flour was based on a liquid-phase suspension of the flour, with colour development based on . This reaction is specific, and gives reproducible results for ferrous iron in the concentration range 0-100 µg/g. The main drawbacks of the technique are the requirements for the use of water, and the difficulty of interpreting the colour chart. This would be made easier with better colour charts, perhaps in the form of a transparency.

The discussion of the results with others in the field indicate that there is a well defined, limited need for reasonably accurate quantitative field kits. This need is separate from the requirement of very simple, inexpensive qualitative test kits, such as the dropper tests currently available. The reliability of these commercial qualitative kits could be improved as suggested earlier in this report, by the standardization of reagents, and improved colour charts.

With all kits further testing using a variety of field conditions would be desirable. The commercial, or wide distribution of the kits would require a professional packaging design - which should not be difficult.

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APPENDIX 1

Iodide test kits manuscript

Field Tests for Iodide in Salt

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Abstract

Rapid field tests for the determination of iodide in salt was developed, based on the oxidation of iodide to iodine and reacting the released iodine with starch to produce a coloured complex that could be visually related to the iodide content of the salt. Potassium iodate and ferric chloride were used as oxidizing agents in the two versions of the test that were examined in detail.

The reaction with ferric chloride gave a test with a wider useful concentration range. It had the added advantage of not producing false readings for iodine by reacting with reducing agents present in the salt as natural impurities, or due to fraud.

A simple test kit suitable for field use was developed, and tested with salts produced using local produced salts in Canada and Equador. The kit results were compared with results obtained by neutron activation analysis using the SLOWPOKE nuclear reactor facility of the University of Toronto, and by standard iodometric titration.

The test kits readily analyzed for iodine levels between 0 and 50 ppm, with an accuracy of ± 10 ppm and a reproducibility of ± 5 ppm. Inexpensive rapid tests using these kits may be useful in monitoring iodine levels in areas where salt iodization is based on potassium iodide, due to the presence of impurities in the salt supply that reduce the stability of the usual iodizing reagent, potassium iodate.

Introduction

At least one half of the world's population has insufficient iodine naturally present in their diet. The lack of iodine leads to iodine deficiency disorders (IDD) that range from mild thyroid enlargement to major developmental problems, such as cretinism. For many years the food supply of developed countries has been supplemented with iodine, by adding potassium iodide to salt at levels between 30 and 100 mg/kg. With the leadership of the United Nations salt iodization programs have been implemented in most of the countries of the world during the past decade, and it is hoped that all of the salt sold for food use will be fortified with iodine by the turn of the century, leading to the elimination of IDD. (Venkatesh Mannar, 1987)

The dosage of iodine is important, since too low levels in the diet will have no noticeable benefit, while excessive doses are wasteful, and may actually result in detrimental health effects. In the low-technology setting of most developing countries, it is often difficult to produce salt with well defined iodine content, due to the difficulty of evenly mixing in the low levels of iodine required, and the instability of iodine during distribution and retail (Diosady et al 1997 and 1998). The problem is compounded by the fact that not all of the salt supply is centrally controlled, and thus a significant fraction of the salt at the retail level may not be iodized. It is therefore important that simple, effective tests be available for the determination of iodine in salt.

As in developing countries potassium iodate is most often used for salt iodization, many simple test kits have been developed for the qualitative determination of iodate in salt. Iodide is used in several countries, where the impurities naturally present in the salt reduce the stability of potassium iodate (e.g. Equador and some plants in China). There are very few kits available for iodide. Although we understand, that MBI of India produces an iodide kit we have been unable to obtain a kit for testing.

PATH Canada has contracted our group to identify feasible approaches to rapid field testing of iodide in salt. These methods were to be designed for use in the field, where they could be used to extend the capability of national Iodine Deficiency Disorders (IDD) programs to verify potency of iodized salt, both as means of quality control at the manufacturing level, and for field monitoring of delivered levels.

Field Test Requirements

The development of an effective field test depends on a clear understanding of the field conditions, and the requirements of the user in terms of the precision and accuracy of the test results. In the case of field tests for the measurement of iodine, we must accept that the 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.

The field condition requires that the test kit be light, inexpensive and easy to use. It must be self contained, without a need for other utilities such as electric power. The test must give acceptable results rapidly, and results must be readily interpreted.

The first step in developing a field test is the survey of the quantitative analytical systems that may be applied to the specific field conditions, sample matrix and expected concentration range. This is then followed by the laboratory testing of selected chemical systems. The laboratory identification of a feasible system must be followed up by the development of a test-kit that meets the needs of the client group.

Our mandate was to test the feasibility of developing a test system, that will allow the field identification of the iodide levels in salt in the range of 10 to 50 µg/kg iodine, with a sensitivity of ~10 µg/kg.

Techniques of Iodide Analysis

Many analytical techniques are used for the determination of iodide in a variety of matrices. These include colourimetric methods, amperometric titration, measurements with ion-selective electrodes, ion-chromatography and high performance liquid chromatographic techniques. (Ali-Mohamed and Jamali, 1989, Han et al.,1987, Luther et al., 1988, Ruo et al. 1993, Sun and Field, 1984). Of these only colourimetric techniques can be inexpensively adapted to field testing.

Five colourimetric methods were reviewed, selected on the basis of their wide-spread acceptance as official analytical methods.

In the leuco crystal violet method iodide is selectively oxidized to iodine with potassium peroxymonosulfate. The elemental iodine reacts with the colourless leuco crystal violet indicator producing the highly coloured leuco crystal violet dye. (Anonymous, 1989, Hach, 1984) Unfortunately chloride interferes with colour development, and therefore this method is not suitable for use in a salt matrix.

Iodide can be determined based on its ability to catalyze the reduction of ceric ions by arsenous acid. The reaction is stopped after a specific time interval by the addition of ferrous ammonium sulphate. The resulting ferric ions, which are directly proportional to the remaining ceric ions, develop a colour complex with KSCN. (Dubravcic, 1955, Fischer et al.1986, Anonymous 1989) - Unfortunately the method is slow and time dependent, and thus it requires instrumentation, and it is therefore unsuitable for use in an inexpensive field test.

Iodide can be oxidized to iodine by the addition of bromine water. The excess of bromine is distilled (expelled) out of solution. The iodine is then titrated with sodium thiosulphate using starch as the end-point indicator. (Williams; 1984) The need for distillation of free bromine, and the reactivity of bromine water makes the method too dangerous to be used as a field test.

Iodide can also be oxidized to elemental iodine in the reaction with iodate in the presence of an acid such as hydrochloric acid. The free iodine can be then titrated with sodium thiosulphate solution using starch as end-point indicator (Scott, 1937).

This method seemed readily applicable to field testing.

Iodide can be oxidized to iodine in the reaction with ferric ions e.g. in the form of ferric ammonium sulphate:

$$2\ NH_4Fe(SO_4)_2 + 2\ KI \rightarrow I_2 + (NH_4)_2\ Fe\ (SO_4)_2 + FeSO_4 + K_2SO_4$$

The free iodine is titrated with sodium thiosulphate using starch as end-point indicator, as above. (Scott, 1939) This method also seemed applicable to field testing.

Iodine can be liberated from iodides by other oxidizing agents such as As^{5+}, Sb^{5+}, Bi^{5+}, Cu^{2+}, Cr^{6+}, $K_3Fe(CN)_6$ (potassium ferricyanide), HNO_3, Cl_2, H_2O_2, or ozone.

In each of these approaches the resulting free iodine is titrated and the end point is detected using starch as indicator. The concentration of iodine may be also detected colourimetrically, (in a narrow range of concentrations) based on the colour density of the starch-iodine complex. Unfortunately most of these redox systems are very sensitive to both impurities and the sample matrix.

After preliminary screening, we selected two approaches to the field measurement of iodide based on potassium iodate and ferric chloride respectively, as the oxidizing agent.

Field test for iodide based on potassium iodate

Iodide (I^-) as potassium iodide in iodized salt is converted to elemental iodine by potassium iodate in an acidic medium :

$$5\ KI + KIO_3 + 6\ H^+ \rightarrow 6\ K^+ + 3\ H_2O + 3\ I_2$$

The free iodine reacts with starch to form a deep blue complex. The depth and intensity of the blue colour of the system is directly related to iodine concentration, and in a narrow range of concentrations the colour differences are readily differentiated by eye.

The colour development is sensitive to pH. The optimum pH in terms of colour development and colour differentiation was in the range from pH=1.8 to pH=3.4. We then devised an appropriate buffer system, keeping in mind the need to stabilize the starch against microbial and chemical degradation.

Initially antimicrobial and pH adjustment system based on salicylic acid was tested. This gave a reddish colour with ferric iron, introduced either as an impurity, or as an ingredient in double fortification. The interference of the Fe+++ made this test kit vulnerable, and thus we opted for another organic acid buffer system: sodium benzoate/benzoic acid, after testing several potential alternatives.

In the presence of $CaCO_3$, sodium silico aluminate or other alkaline substances the reaction mixture may require a small amount of dilute hydrochloric or sulphuric acid (~1%) solution in the reaction mixture to neutralize these alkaline substances. The buffer system in the kit should take care of this problem, unless the quantity of base is very high. If this consistently happens on salts from a specific plant or region, the kits can be altered by adding extra salicylic, hydrochloric or sulphuric acid to the reagent. The presence of strong reducing agents other than iodide may also give a positive bias with iodate-based methods.

We opted for a simple dropper test, and tested it using salt samples iodized in our laboratory, using commercially obtained Canadian salt and impure salt samples received from target countries.

Field test apparatus

In its prototype format the kit consists of the following:

a. metal pan (~8 cm o.d.)

b. 25 mL plastic flask with dropper in cap, containing the prepared reaction mixture

c. colour standard chart (photograph), showing colours obtained with 0 to 50 µg/g iodine in 10µg/kg intervals

Reagents

The reagent solution was prepared as follows

a. **Benzoic acid buffer solution** was prepared by adding 400 mg ACS grade sodium-benzoate to 100 mL water. It was dissolved by gentle mixing, then the pH was adjusted to 3.2 using dilute (~ 0.1 N) HCl, to form the benzoic acid / sodium benzoate buffer solution.

b. **Starch addition** - The buffer solution (a) was heated to boiling. Three grams of soluble potato starch (ACS grade, iodide free) were triturated with 10 mL of cold water, and poured slowly, with constant stirring into the boiling buffer solution. The mixture was boiled until a thin, translucent fluid was obtained. This is a critical step. as excessive boiling may render the solution less sensitive. The solution was allowed to cool, settle and then filtered using a fast filter paper, e.g. Watman No. 41, to obtain a clear filtrate.

c. **Potassium iodate addition.** Approximately 3 g of KIO_3 was dissolved in the buffered starch solution (b), completing the reaction mixture. After thorough mixing it was dispensed into 25 mL plastic dropper flasks.

Procedure

a. The metal pan is filled with salt, creating a flat surface flush with its edge.

b. A few drops of the reagent solution are applied to the surface of the salt.

c. The developed starch-iodide blue colour is matched with the colour standards on the chart, and the iodide concentration of the salt is determined.

Field test for Iodide in salt using ferric chloride

Iodide is quantitatively oxidized to iodine by ferric (Fe^{+++}) salts according to the equation :

$$2\,FeCl_3 + 2\,KI \rightarrow I_2 + 2KCl + 2\,FeCl_2$$

The free iodine will react with starch to form a deep blue complex. Under controlled conditions the colour intensity is proportional to the amount of liberated iodine, and hence the iodide content of the salt. Since ferric chloride has a bright yellow colour, the colour of he sample will vary from yellow through green to a dark blue-green.

Field test apparatus

a. Three disposable transfer pipettes:
1 for delivering Solution "A" to the mixing bottle, 1 for delivering Solution "B" to the mixing bottle (both are marked with the required

volume), 1 for delivering drops of mixture to the iodized salt surface from the mixing bottle

b. Mixing bottle

c. Petri dish to contain the salt sample

d. Large scoop for mixing salt

e. 100 mL plastic flask with dropper in cap, containing the prepared reaction mixture

f. 10-50 ppm iodized salt colour standard chart ;

Reagents

a. **Ferric Chloride hexahydrate solution** A 0.1 M ferric chloride solution was prepared by dissolving 2.70 g ferric chloride hexahydrate in 100 mL water at room temperature. The solution was allowed to stand and the clear supernatant was decanted .

b. **Starch solution** was prepared by triturating 3 gram of soluble potato starch (ACS grade) with 10 mL of cold water, and pouring slowly, with constant stirring into 100 mL of boiling water. To prevent microbial degradation 400 mg sodium benzoate was added. The mixture was boiled until a thin, translucent fluid was obtained. The solution was allowed to cool, settle and then filtered to obtain a clear filtrate.

c **Reaction mixture:** Equal volumes of the two solutions were thoroughly mixed, and dispensed into the plastic dropper bottle. This mixture is not stable for long periods of time, and therefore it should be prepared, perhaps, daily.

Procedure

a. Add 1 volume Solution "a" to mixing bottle with the first transfer pipette, and 1 volume Solution "b" with the second transfer pipette. Mix well.

For salts with less than 50 ppm iodine as iodide:

b. Fill the petri dish with salt, creating a surface flush with its edge.

c. Apply several drops from the mixing bottle to the surface of the salt using the third disposable pipette.

d. After 5-8 minutes, compare the colour with the standard colour chart.

For salt with more than 50 ppm iodine as iodide:

b.Put one large scoop of sample salt and one large scoop of iodine-free salt in the petri dish. Mix in the petri dish after slightly wetting the salt with water.

c'. Apply several drops from the mixing bottle to the surface of the salt using the third disposable pipette.

d'. After 5-8 minutes, compare colour with standard colour chart.

e'. Multiply results by 2 to obtain the iodide levels.

Results

With pure salt iodized in our laboratory the iodate-based kit produced reproducible results that readily distinguished different levels of iodide in the range of 10 to 40 µg/g. The colours developed at high concentrations of iodine are dark and the difference between 40 and 50 µg/g is difficult to distinguish. This may limit its usefulness in quality control, where target levels are typically 50 µg/g.

Since potassium iodate is used as the oxidizing agent this system will release iodine from the reagent in the presence of many reducing agents, providing a false positive reading for iodine in the salt. As a result, this approach is not recommended for universal application, eventhough due to its simplicity, and portability, it may be useful for testing of iodine retention in the field.

The method ferric chloride method was remarkably free from interferences. Since the ferric chloride reagent solution does not introduce iodine, the test cannot obtain false iodine readings from strong reducing agents present in the salt as impurities or through deliberate attempts to replace KI with a less expensive, and physiologically useless reducing agent.

Tests in our laboratory produced reproducible results that readily distinguished different levels of iodide in the salt in the range of 10 to 50 µg/g. Table 1. Photographs of a series of standards that we produced were not of professional quality, despite colour correction during development, As a result the readings based on this crude colour chart were not nearly as accurate as samples that were compared with actual

salt standards on which the colour was developed by the kit's reagents.

We have found that the colour development requires about 10 minutes. As colour development is catalyzed by both acid and the iodide present, samples with higher iodine content reach the final colour intensity faster. Thus samples containing close to 50 ppm developed a deep blue colour in 3-5 min., which did not fade or darken for an hour.

The best colour clarity was obtained with a clear reagent solution. Unfortunately, particulates, such as starch globules, promoted the formation of ferric oxychloride, which, in the presence of starch. made the solution cloudy . While this did not change the colour reaction, the perceived colour changed, making it harder to distinguish between higher iodine levels.

We found that both the ferric chloride and the starch solution are stable and clear for a long period of time. The ferric chloride solution itself is known to be stable for years, thus starch stability limited the useful life of the kit. Despite the addition of benzoic acid as an antimicrobial agent, the starch breaks down after several months due to microbial degradation, and acid hydrolysis, limiting its usefulness to ~ 6 months at higher temperatures. We found that the reaction mixture occasionally became cloudy after extended storage, due to starch precipitation. Its storage life could be extended by removing the precipitate by filtration.

A series of samples were prepared in our laboratory, and analyzed using both the kits and neutron activation analysis, using the SLOWPOKE reactor facility at the University of Toronto. The kit, and kits made up based on these instructions were used in the UNICEF Laboratory by Dr. Hans Vanhassel in Quito, Equador. The samples prepared in Toronto were tested with locally made kits, and samples of local salt iodized to various levels were also tested in both Quito and Toronto, using both kits, and standard laboratory techniques. The results are presented in Table 2. All of the kits gave good results at low iodide levels. At higher iodine concentrations the limitations of our kit due to the poor colour charts became evident. The Equadorian laboratory consistently underestimated iodine values over ~ 30 µg/g, our laboratory obtained good, reproducible results, as we used actual standard salt samples rather than the photograph for estimating the colour, and hence, the iodide content of the samples.

The extended time between salt preparation and testing added to the inaccuracy of the kits. Iodide slowly loses iodine over time especially in impure salt stored at high temperature and humidity. Thus readings taken at different times may reflect actual differences due to loss of iodine between the time of initial analysis and final analysis by the kits. Despite these difficulties, the results were acceptable

Conclusions and recommendations

Two simple, inexpensive test kits for the analysis of iodide in salt have been developed, and successfully tested. The test results obtained were well within the specified accuracy of ±10 µg/g in the 0-50 µg/g range of iodine concentrations.

The ferric chloride based test gave a visually more readily distinguishable

range of measurements, as samples differed not only in colour intensity, but also in hue.

For full implementation of these test kits professionally made colour charts will be required, which would make it possible for untrained field operators to distinguish between salt samples with higher iodide content.

Acknowledgements

Samples of salt were made available for this study by UNICEF field offices in eight countries. The authors are grateful for the testing of the kits, and excellent comments and suggestions by Dr. Vanhassel of UNICEF Equador.

This work was initiated by Dr. Timothy R. Stone, Executive Director of Path Canada, who was tragically killed while on a humanitarian mission, in the crash of the hijacked Ethiopian plane in the Comoro Islands in November 1996.

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APPENDIX 2

Iodate test kits manuscript

Field Tests for Iodate in Salt

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Abstract

Field test kits for the determination of iodate in salt were tested. Dropper tests based on the blue colour of the starch-iodine complex formed with iodine released by excess potassium iodide gave rapid qualitative indication of the presence of iodate. Due to the dark colour of the complex, the tests were found to be quantitative only in a limited range, typically of 0 - 20 µg/g iodine in the salt.

Instructions for producing reliable kits of this type, with improved colour reproducibility are presented.

Improved qualitative tests in the range of 0-50 µg/g were obtained with a simple liquid-phase colourimetric system, based on the same chemistry. A field test kit was developed and tested in laboratories in India and Zimbabwe, as well as our laboratory in Toronto. The test gave reproducible results, comparable to titration, with a typical standard deviation of ± 5 µg/g. The range of measurements can be extended to 100 µg/g by dilution.

Instructions for preparing these kits is presented.

Introduction

As the diet of more than half of the world's population contains less iodine than required for developing and maintaining a healthy body, the iodine content of their diet must be supplemented with iodine to prevent iodine deficiency disorders (IDD). While the salt supplies of most industrialized nations have been iodized with potassium iodide for many years, 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). It is hoped that by the turn of the century severe forms of IDD will be eradicated through national salt iodization programs.

The dosage of iodine is important, since too low levels in the diet will have no noticeable benefit, while excessive doses are wasteful, and may actually result in detrimental health effects. In the low-technology setting of most developing countries, it is important that simple, effective tests be available for the determination of fortifying agents. In most of the developing countries iodine is added to salt in the form of potassium iodate, as it will not be oxidized to form volatile free iodine.

PATH Canada has contracted our group to test approaches to rapid field testing of iodate in salt. Despite of the superior oxidation resistance of iodate, iodated salt tends to lose its iodine content over time, and therefore it is important that the actual amount of iodine present be known (Diosady et al. 1998). Accordingly, these methods were to be designed for use in the field, where they could extend the capability of national IDD programs, to verify the potency of iodized salt, both as means of quality control at the manufacturing level, and for field monitoring of delivered levels.

Field Test Requirements

The development of an effective field test depends on a clear understanding of the field conditions, and the requirements of the user in terms of the precision and accuracy of the test results. In the case of field tests for iodate in salt, we must accept that the 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 must be self contained in a kit, which does not require other utilities such as electric power. Although it would be desirable to require no further component, we realized that better tests could be devised if we accept the need for water.

The field condition requires that the test kit be light, inexpensive and easy to use. The test must give acceptable results rapidly, and results must be readily interpreted.

The first step in developing a field test is the survey of the quantitative analytical systems that may be applied to the specific field conditions, sample matrix and expected concentration range. This is then followed by the laboratory testing of selected chemical systems. The laboratory identification of a feasible system must be followed up by the development of a test-kit that meets the needs of the client group.

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. The acidulated iodate solution can be reduced by KI, KSCN, a cold aqueous solution of SO_2, $K_4Fe(CN)_6$, Cu_2Cl_2, H_3AsO_3, or $FeSO_4$ to generate elemental iodine. The iodine may be determined colourimetrically directly, or by forming a coloured complex with starch or another reagent (Hatch 1984)

Reduction of iodate to iodine by potassium iodide

The procedure is the reciprocal of the one for the determination of iodide by means of an iodate, also based on the reaction :

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

The reaction liberates iodine quantitatively, and the iodine-starch complex can form the basis of a quantitative or semi-quantitative colourimetric correlation suitable for a field test. (Scott, 1937, Williams, 1984)

Reduction of iodate to iodine by KSCN

Iodate is reduced to elemental iodine using diluted HCl and KSCN solution. The liberated iodine is observed by its colour (Williams, 1984)

This test for the qualitative determination of iodate in flour likely can be adapted to the quantitative determination of iodate in salt, as chloride does not interfere with this test. A quantitative field test based on this reaction seems feasible, as long as deterioration of the KSCN can be prevented in the field.

Starch indicator forms an intense blue-black colour with iodine. In aqueous solution, iodine has the tendency to react with excess iodide anions to form the tri-iodide ion I_3^- according to the equation:

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

The tri-iodide ion can than react with another iodine molecule to form the linear penta-iodide anion:

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

This penta-iodide ion fits inside the helical beta amylose chain of starch. In the presence of excess iodide, iodine in concentration as low as about 0.2 µg/mL will result in the formation of the dark-blue starch-iodine complex. The colour intensity varies with the amount of free iodine, and can form the basis of a quantitative colourimetric measurement, in a narrow range of concentrations. The formation of this complex is reversible, and this is the basis for titrimetric methods: at the end of the titration which removes the iodine, the strong blue-complex fades completely to colourless.

Currently Available Field Tests

As the need for rapid field testing has been known, 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 ICC IDD 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 acidic buffer, a reducing agent, which reduces the iodine in the KIO_3 to I_2, and starch, which forms a deep blue-black complex with the released iodine.

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.

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.

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

Experimental Methods

Materials

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

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. After the iodate addition the mixture was further blended for at least 20 minutes to obtain a homogeneous, free-flowing solid.

These blends were analyzed by titration method AOAC 33.147 (Williams) 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 rapid field test kits, by dropping two to 10 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.

Inter-laboratory tests were conducted on the 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 fresh kit. The salt samples were analyzed in Toronto by both the kits, and the standard laboratory analytical technique.

The participating laboratories analyzed the salt samples with their standard

laboratory technique, the kit sent from Toronto, and a fresh 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 kits and the standard laboratory techniques.

Discussion of Results

Available test kits

The kits tested are listed in Table 1. All but one of these kits used the reaction with KI and starch for colour development. Initially we believed that the reported reproducibility problem may be due to the variability in the starch supply. If the amylose/amylopectin ratio changed, the colour of the iodine-starch complex would be shifted from the reddish colour of amylopectin to the blue of β amylase. The problem has turned out to be more substantial.

Actually at the low pH of these kits, (typically pH=2.6) salt with more than 15 or 20 ppm iodine will produce a dark blue colour, with no further visibly noticeable darkening due to higher concentrations. Thus concentrations of 15 ppm and higher all give the same dark colour. The deep colour is due to the fact that one molecule of iodate reacts with 5 molecules of potassium iodide, liberating 3 molecules of elemental iodine. Thus the elemental iodine is present in six times the concentration originally in the sample.

One way to overcome this problem is to dilute the salt 1:10 using a similarly coloured material. Iodine free salt, sand or flour would be suitable. Unfortunately, solid dilution is difficult to do evenly, and makes the kit bulky, as some 10-20 g of inert material must be carried for each test.

The kit we were sent from China used a reaction of iodate with an organic dye for colour development. Nevertheless, this kit also saturated at levels as low as 10µg iodine/g salt. As we only received one small, old kit, this result may not be representative.

An additional problem was the colour charts supplied with these kits. Rather than colour samples representative of the granular, irregular surface of actual samples, the charts show clear, homogeneous coloured discs, which are not easily related to the actual appearance of the salt.

We have followed three approaches to producing an improved field test system: we tried to improve the currently used iodide-based dropper test, and developed tests based on liquid-phase colour development, and a crude micro-titration method.

Improvement To Existing Field Tests

As indicated above the kits now manufactured for determining iodate in salt in the 50 µg/g concentration range are based on the development of the starch-iodine complex, and visual comparison of the colour obtained with printed standards. These tests worked well qualitatively, but they produced inconsistent colours, and the depth of colour saturated, and values above 20 µg/g were not readily distinguished.

Based on our laboratory experience, and theoretical considerations, it is possible that the colour variations are also influenced by the composition of the starch indicator. The deep blue colour of starch solutions containing iodine is believed to arise from the absorption of iodine into the helical chain of β–amylose, a macromolecular component of most starches. The closely related α amylose forms a red adduct with iodine, while amylopectin produces a blue colour with a different absorption maximum. Thus iodine complexes with α and β amylose, and amylopectin all have different absorption spectra, and different colour intensities. As a result, it is critical that the type of starch used be well characterized, and the colour intensities be calibrated for it. In our work we used soluble potato starch.

Accordingly, to reduce this problem we suggest that the starch be well characterized, and only a β–amylose, such as soluble potato starch be used. This ensures that the starch-iodine complex formed will have a single absorption maximum, and thus a well defined, reproducible colour. While this will improve the kits, the problem due to the kinetics of this reaction and its pH dependence remains a major limitation to these simple kits.

Strong oxidizing agents interfere with the colour development by oxidizing the added iodide to elemental iodine.

Field test apparatus

a. metal pan

b. Two 100 mL plastic flasks containing the reaction solutions A and B respectively

c. Two disposable transfer pipettes, polyethylene, capable of delivering 1 mL solution

d. 10 mL plastic sampling vial

e 50 mL plastic sampling vial

f 0-50 ppm iodated salt colour standard chart (photograph)

g. sampling cap (1 mL)

h. sampling cap (9 mL)

Reagents

a. Solution A : Starch solution with KI - Triturate 3 gram of soluble potato starch (ACS grade) with 10 mL of cold water, and pour slowly, with constant stirring into 100 mL of boiling water. Add 400 mg Na-benzoate. Boil the mixture until a thin, (< 2 min.) translucent fluid is obtained. Longer boiling than necessary renders the solution less sensitive). Allow to settle and filter the clean supernatant through a fast laboratory filter paper equivalent to Whatman No. 41. Add 5 g of KI, and adjust the pH to 7.5 with 0.1% Na_2CO_3 . Transfer the solution to a 100 mL plastic flask/vial.

b. Solution B. To 100 mL distilled water add 1 % HCl to pH = 1.2 Transfer the solution to a 100 mL plastic flask/vial.

Field Procedure

1. To the 50 mL plastic sampling vial add 1 small sampling cup (1 mL) of iodated salt and one large cup (9 mL) non-iodized salt. Add 2 drops of water and mix the salts with shaking. Transfer to the metal pan. (Salt must be granular, i.e. ground if necessary).

2 Add 1 mL of **solution A** and 1 mL **solution B** into the 10 mL sampling vial and mix.

3 Fill the metal pan with salt, creating a flat surface flush with its edge.

4. Apply several drops of the mixed reagent solution to the surface of the salt.

5. Compare the developed starch-iodide blue colour with the standard colour chart

Proposed Novel Iodate Field Test For Salt

Based on the observed limitations of the solid-phase test, including several variants with other reducing agents, it became clear that for better reproducibility and accuracy we must develop a more quantitative test, that will be also suitable for in-plant quality control. While this introduces increased complexity, we felt that a valid field test may be developed.

Working in a liquid medium reduces the problems due to uneven distribution of reagents. We tested the concept of dissolving a set volume of salt in a set volume of water in a flat-bottom plastic or glass vessel, added acid, KI and starch, and observed the colour thus developed. It is relatively simple to control volumes, and therefore this test is reproducible. The exact amount of iodine released could be estimated from the colour of the solution.

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, polyethylene, capable of delivering 3 mL solution

d. 0-50 ppm iodated salt colour standard chart (photograph)

e. sampling cap (approximately 1 mL)

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.

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 plastic (glass) 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, 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 standards.

Results

The sequence of steps was critical, since the pH at all stages of the process could influence the colour development. At very low pH the starch blue developed a pink tinge, while at high pH the colour development was very slow. Therefore the pH

adjustment of solution A under laboratory conditions must be done carefully.

Compensation for the effect of water quality available in the field could be made by measuring the background colour developed by performing the test, without adding a salt sample. A positive reading can be due to dissolved oxygen in the water. This effect may be reduced by using previously boiled water, or may be corrected mathematically, by subtracting the blank value from the measurement.

As the salt was measured volumetrically, large error 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.

The performance of the kit was checked against neutron activation analysis on salt samples iodated to various levels.

The results are presented in Table 2. The test kits and the laboratory analyses corresponded very well, and the reproducibility of the kit readings was good in the laboratory.

Test validation

Field test kits for the liquid phase test were supplied to two participating laboratories: Dr. Nhari and T. Nyamandi, Government Analyst's Laboratory, Zimbabwe; and Dr. C.S. Pandav, and Dr. M. Karmarkar, Regional Coordinator, ICCIDD, New Delhi, India. in all cases six replicate analyses were performed, and the average and standard deviation were calculated. The summary of the results are presented in Table 3.

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 titration results from India were not corrected for a blank reading for the un-iodated salt, due likely to an impurity in the Indian salt. The kits actually gave a more accurate result than the titration method without blank correction.

The results obtained in Toronto were closer to the laboratory measurements than those from the other two laboratories. There were two reasons for this discrepancy. 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 colour charts is critical for the accurate determination of iodine content.

There was an error introduced 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. Despite these problems, the reproducibilty of the results was acceptable.

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 testing compliance to regulations/recommendations, safety and iodine stability in the field.

Conclusions and recommendations

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 20 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. The reproducibility of the kit results may be improved by standardization of the starch indicator used, and controlling the pH of the system, by inclusion of an appropriate buffer system.

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. The test results are based on a volumetric measure of solid salt. Thus large errors can be introduced by salts of large particle size, that do not pack well into the sampling thimble, and therefore these samples should be ground.

The liquid phase system introduces significant complexity to the field test kit for iodate. The improved range, accuracy and precision of these tests justify its use in field situations, where a qualitative determination is inadequate, and a laboratory with trained personnel is unavailable.

A more extensive round of interlaboratory and field tests would be useful before the kit could be finalized in a commercially viable form.

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Samples of salt were made available for this study by UNICEF field offices in eight countries. The authors are grateful for the testing of the kits, and excellent comments and suggestions by Dr. Nhari and T. Nyamandi, Government Analyst's

Laboratory, Zimbabwe; and Dr. C.S. Pandav, and Dr. M. Karmarkar, Regional Coordinator, ICCIDD, New Delhi, India,

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APPENDIX 3

Iron test thesis, Meggie Yan

FEASIBILITY OF FIELD TESTS FOR IRON

by

MEGGIE YA N

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ABSTRACT

The purpose of this thesis was to develop the principles of field tests for iron in flour using colourimetric methods. Both the ferrous and ferric colourimetric tests have been reviewed and four different reagents associated with the ferrous iron have been tested and optimized. The four reagents were: 1,1O-phenanthroline, alpha,alpha'-bipyridyl, potassium ferricyanide and thioglycolic acid. The optimum pH value for 1,10-phenanthroline and a,a'-bipyridyl were tested and compared with previous references. The optimum pH for l,l0-phenanthroline was 3.2-3.3 which corresponded to the previous study. But the optimum pH value for a,a'-bipyridyl was found to be 1.8-1.9 which was deviated from the literature value of pH 2-9. The a,a'-bipyridyl was found to be the most suitable reagent for the development of the field test while thioglycolic acid was found to be nonapplicable due to the unstable results and toxicity.

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TABLE OF CONTENTS

1.0 INTRODUCTION

People in the developing countries are suffering from the shortage of the three micronutrients - iodine, iron and vitamin A. The consequences of the shortage of these micronutrients are some serious health problems. Iron deficiency anemia (IDA) is one of the most common types of detrimental effects resulting from the inadequate dietary intake of iron. It is also the most prevalent micronutritional problem that affects approximately one billion people world wide. Attention has been drawn toward these issues around the world and efforts have been made to eliminate these problems. Among the three micronutrients, correction of iron deficiency is the globally most achievable goal since it can be prevented effectively and cheaply. Simple and effective methods have been developed to deal with this problem and one of the examples of these methods is the provision of iron supplements in the diets of the populations suffering from this disease. Food products such as flour and salt have been fortified with iron and in some countries, laws have been passed to ensure that the flour and salt products have been fortified with a sufficient amount of micronutrients. These food products were chosen to be fortified because they are the most widely consumed food that even the people in third world countries would benefit from them. This is the start of the elimination of the micronutrients problem.

This next step of the micronutrients elimination program is the verifications of the actual amount of micronutrients that have been claimed by the manufacturers. The main focus of this report is the determination of the concentration of iron in flour. There are two basic techniques for the measurement of the amount of iron in flour. The first method is the analytical tests that are carried out in the laboratory by the professional personnel. The second method is the use of the rapid test kit (or the field test). The field test is an analytical method that is designed for the use of non- technical personnel to determine the approximate concentration of a substance in a sample. It is designed to be carried out in a field setting in which no water or other utility, such as electricity, is provided. All the commercially available field tests use the colourimetric methods and there are two main types of field tests: the qualitative and the semi-quantitative tests. The qualitative tests indicate only the presence of the compound in flour while the semi-quantitative tests allow the determination of the approximate concentration of the compound.

The objective of the project is to develop the principles for the field test methods for iron. Four reagents which form colour complexes with ferrous iron have been tested and they are: l,l0-phenanthroline, a,a'-bipyridyl, potassium ferricyanide and thioglycolic acid. Two forms of the ferrous salts have been used to test the above reagents and the two salts are ferrous fumerate and ferrous sulphate. The reason for testing two forms of ferrous salt is to confirm the feasibility of the reagent on different forms of iron that might be employed to fortify the flour in different countries. Moreover, for each reagent, the effects of the chelating agent, ethylenediaminetetraamine(EDTA) were also tested. The reason for employing EDTA into the development of the principles of field tests is due to the fact that EDTA forms a complex with metal ions in a 1:l ratio. If the EDTA complex is stronger than the colour complex, the uncomplexed iron can be detected using the colour reagents. This is especially useful if the sample contains high concentration of iron because at high concentration, the colour will be saturated and this makes the visual colour comparison no longer valid.

The development of the field tests is essential to the third world countries that are suffering from the micronutrients deficiency because the quality control of the fortified

flour should be carried out at a number of levels The generated results of the tests must reach the position which takes corrective action and therefore, field tests contribute to the elimination of the iron deficiency in the community.

2. LITERATURE SURVEY

Iron occurs as both the divalent and trivalent oxidation states and is combined to form ferrous and ferric compounds respectively. However, the human body is only capable of absorbing the ferrous and, therefore, food products are fortified with ferrous salt. In order to develop the principles for the field tests for iron in flour. analytical methods for ferrous iron have been investigated. More specifically, Section 2.l describes the various kinds of colourimetric methods associated with the ferrous iron.

Due to the fact that ferrous iron can be oxidized to the ferric form, analytical methods for ferric iron, therefore, have been reviewed as well. Section 2.2 summarizes the different types of the colourimetric methods for ferric iron Some methods are applicable for both ferrous and ferric iron and in Section 2.3, these methods are outlined. The determination of the effects of EDTA on iron are one of the objectives of this project. Therefore in Section 2.4 background information on EDTA is given.

2.1 ANALYTICAL METHODS FOR FERROUS IRON

2.1.1 PHENANTHROLINE METHOD FOR TOTAL IRON

The colour complex forms between the ferrous iron and 1,10-phenanthroline (C12H8N2) is an orange to red complex of(C12H8N2),Fe. Each colour complex consists of three mols of 1,10-phenanthroline and one mol of ferrous iron and it yields no colour with the ferric iron. The colour is proportional to the iron content of 0.02-0.25mg of iron and its intensity is constant at a pH 2.0-9.0. However, this method depends on various factors in terms of the colour development and these factors are: (l) the order of the addition of solutions. (2) time between additions; (3) temperature of the various solutions, (4) the presence of other compounds and (5) the amount of time required for the colour development. It is claimed that the colour becomes stable in fifteen minutes and does not change for at least forty eight hours. Moreover, it obeys Beer's law which has a maximum absorbance of 512 um. Procedure:COLORIMETRIC METHODS OF ANALYSIS - 3'd edition, 1949.

Transfer the sample or pipette an aliquot containing 0.02-0.25 mg of [total] iron into a 100 mL flask, and dilute to about 70 mL. Add 1 mL of a colourless 10 percent solution of hydrochloride to insure complete reduction to ferrous iron. Let the solution stand for 15 minutes. Then add 10 mL of a 0.25 percent colourless aqueous solution of 1,10-phenanthroline and mix well. Add sufficient 25 percent sodium citrate solution to adjust the pH to approximately 3.5. The adjustment need not to be accurate. The amount required will necessarily depend quite radically on the previous history of the sample. Dilute to volume, mix well, and let stand for 30 minutes at not less then 20"C.

2.1.2 PHENANTHROLINE METHOD FOR FERROUS IRON

Ferrous iron in a sample can be determined by using phenanthroline as the reagent. The procedure outlined in Section 2.1.1 can be applied but omit the addition of the hydroxylamine hydrochloride as the reducing agent.

2.1.3 α, or β-BIPYRIDYL METHOD FOR FERROUS IRON

Similar to the 1,10-phenanthroline test, three mols of a,a'-bipyridyl($C_{10}H_8N_2$) react with one mol of ferrous iron to form a red complex of $Fe(C_{10}H_8N_2)_3$ The colour is proportional to the iron concentration of 0.05-2mg of iron and the colour intensity is constant at a pH of 3-9. If the pH falls below 2.5 or above 9.5, the colour complex formed would fade. If the pH is within 3-9, the colour is stable for one year if the sample is not exposed to sunlight. The colour formed also obeys Beer's law and the maximum absorption for the colour complex is 552 um.

Procedure: Colorimetric methods of analysis - 3rd edition, 1949.

Select an aliquot of sample containing 0.05-0.1mg of iron. Add 1 mL of 1:10 sulfuric acid, 1 mL of 1.4 orthophosphoric acid, 0.4 mL of a 1 percent solution of a,a'-bipyridyl, and 10 mL of 20 percent ammonium acetate solution. Dilute to 100 mL and mix. Let this stand in the dark for 30 minuets for development of colour and read the transmittance. The addition of orthophosphoric acid stabilizes the ratio of ferrous and ferric ion. A similar solution will give the value for total iron if 2-3 mL of saturated sulfurous acid is added as reducing agent, and the whole is allowed to stand 24 hours for colour development.

2.1.4 α,α'-BIPYRIDYL FOR TOTAL IRON

The method describes in Section 2.1.3 is applicable in the determination of the total iron as well. Reducing agents such as titanous chloride, hydroquinone, hydroxylamine, ascorbic acid, p-hydroxyphenylglycine, sodium hyposulphite, sodium sulphite, and hydrazine sulphite can be used. Procedure: COLORIMETRIC METHODS OF ANALYSIS - 3'd edition, 1949.

Measure an aliquot of sample to contain 0.005-0. 12 mg of iron but preferably not over 20 mg of aluminum. Evaporate just to dryness on a steam bath, or with care on a hot plate. Cool and add 2 mL of 1:15 hydrochloric acid and 9 mL of water. Add 1 mL of 10 percent sodium sulphite solution, not over 3 days old, mix, and let stand for 2 minutes. Add 2 mL more of the sodium sulphite solution and transfer to a 50 mL volumetric flask. Dilute to volume and mix.

2.1.5 POTASSIUM FERRICYANIDE FOR FERROUS IRON

Potassium ferricyanide is another reagent which can be used to determine the presence of ferrous iron while ferric iron yields a green colour. A deep blue colour is formed of a colloidal dispersion of ferrous ferricyanide while the potassium ferricyanide solution is yellow in colour. The following reaction takes place:

$2Fe(CN)_6^{3-} + 3Fe^{2+} \rightarrow Fe_3(Fe(CN)_6)_2$

This method is independent of the pH of the solution. However, acid(carbon dioxide in the water) aids the decomposition of potassium ferricyanide and this, in fact, enhance the displacement reaction between the ferrous iron and the ferricyanide. However, the potassium ferricyanide solution is unstable due to the fact that it is easily oxidized by air. Procedure: COLORIMETRIC METHODS OF ANALYSIS -3'd edition, 1949. Prepare freshly boiled and cooled distilled water. Transfer 75 mL volumes to a series of Nessler tubes. To each add 10 mL of 1:5 sulfuric acid and suitable volumes of the ferrous standard. Mix 50 mL of the sample, after filtration to remove suspended matter if necessary, with 10 mL of sulfuric acid. To each standard and the sample add 15 mL of freshly prepared 0.5 percent solution of potassium

ferricyanide in recently bo iled and cooled distilled water. Dilute all to 100 mL with the oxygen-free water and compare.

2.1.6 DIMETHYLGLYOXIME METHOD FOR FERROUS IRON

A soluble red complex is formed between ferrous iron and dimethylglyoxime in ammoniacal solution or any organic bases solution. Two mols of the colour reagent react with one mol of ferrous iron to form the colour complex.

Procedure: SPOT TESTS ~ INORGANIC ANALYSIS- 5" edition, 1958.

A drop of the test solution (previously reduced if necessary) is mixed with a crystal of tartaric acid, and then a drop of 1 % alcoholic dimethylglyoxime is added, followed by a little ammonia. According to the iron content, a more or less intense red coloration appears. The colour fades on standing in the air because the ferrous complex oxidizes.

2.1.7 ISONITROSOBENZOYLMETHANE METHOD FOR FERROUS IRON

A blue complex is formed between the ferrous iron and isonitrosobenzoylmethane using benzene as the solvent.

Procedure: SPOT TESTS IN INORGANIC ANALYSIS - 5" edition, 1958.

One drop of the test solution is placed on filter paper and spotted with a 1.0 N alcoholic solution of the reagent. When held over ammonia, a green fleck appears. The test may be conducted likewise on a spot plate or in a microcrucible. In the latter case, benzene extraction is possible.

2.2 ANALYTICAL METHODS FOR FERRIC IRON

2.2.1 THIOCYANATE METHOD FOR FERRIC IRON

A red colour proportional to the amount of iron in the sample is formed between ferric iron and thiocyanate. For best visual result, samples containing 0.1-1.0 mg of ferric iron are considered to be more appropriate for this test. The pH value of the solution is between 1 and 2 and this can simply be achieved by adding acid solution to the sample. However, different types of acid use yield a different optimum pH value. When hydrochloric acid is used the optimum pH is 1.3-1.8. If nitric acid is used, the optimum pH is below 1.0. Ether or high alcohol can be used to extract the colour since the colour intensity increases by the reduction of the dielectric constant of the solvent. Moreover, ethylene glycol monobutyl ether can also be added to stabilize the ferric thiocyanate compound.

Procedure: COLORIMETRIC METHODS OF ANALYSIS - 3rd edition, 1949.

Prepare an amount of sample to contain O. 1-1.0 mg of iron to a 100 mL Nessler tube...and dilute to 80 mL. To sample add 5 mL of 20 percent ammoniumthiocyanate solution. Add 2 mL of ethylene glycol monobutyl ether to the sample before diluting to volume if it is desired that the colour be stable for 1 hour, and dilute to 100 mL. Protect from light and compare as usual. If the colour developed is not sufficiently intense repeat the preparation of sample but in dilution of the sample to volume use 50 mL of acetone. Colour may also be intensified by extraction. For this take 25 mL of the treated portions of sample in comparison tube. Add 5 mL of a mixture of equal volumes

of ether and amyl alcohol. Shake well and compare the colour of the upper layers by balancing. This procedure is particularly applicable for sample solutions containing less than 5 ppm of iron. The developed sample to which this technique is applied must be one to which no acetone has been added.

2.2.2 SALICYLIC ACID FOR FERRIC IRON

An amethyst colour is produced between the heme iron and salicylic acid. For visual comparison, the sample must have an iron content of 0. 15-3.0 mg. The colour developed obeys Beer's law and is affected by pH. Therefore, a buffer solution is used. If the colour is protected from sunlight it is stable for forty eight hours. Procedure: COLORIMETRIC METHODS OF ANALYSIS - 3'" edition, 1949.

Transfer a suitable aliquot of sample to contain 0.15-3.0 mg iron to a 100 volumetric flask. if a standard is to be run in parallel, prepare it with similar contents of reagents. If an equivalent acidity is not already present add 8 mL of I:1 hydrochloric acid. Add 1 mL of a 10 percent solution of sodium salicylate. The colour will be amethyst. Add 10 mL of 3 percent ammonium acetate solution. The colour becomes yellow. Now add 10 mL of 1:1 acetic acid. The colour returns to amethyst. The final pH is in the range of 2.5-2.8 and dilute to 100 mL and compare with the standard or read the transmittance around 530 um. The colour is stable for over an hour.

2.2.3 FERRON FOR FERRIC IRON

A green colour is formed when ferric iron is reacted with ferron, which is also known as 7-iodo-8 N acid. Saturated reagent solution containing about 0.2 percent ferron, is yellow in colour and Beer's law is followed. Three mols of the reagent are required to react with one mol of ferric iron. The system is highly pH selective: pH below 1.2 or above 8.3, the colour disappears. The optimum pH value is 2.0-1.0 since under these pH values the colour remains stable even if is exposed to sunlight. Therefore, a buffer is required. For visual comparison, the iron content is best if between 0.005-0.2 mg.

Procedure: COLORIMETRIC METHODS OF ANALYSIS - 3'd edition, 1949.

Transfer an aliquot of sample containing 0.005-0.2 mg of iron, and preferably 0.0075-0.05 mg to a 50 mL Nessler tube. Add I:1 ammonium hydroxide to render just acid to Congo red paper. Then add 10 mL of hydrochloric acid-potassium acid phthalate buffer for pH 2~6 and 1 mL of saturated aqueous solution of the reagent. Compare with a series of standards or read the transmittance. For more than 2 ppm of iron increase the reagent to 2 mL. Over 4 ppm of iron cannot be read. The effect of excess reagent on the colour can be eliminated with Coming HR filter 352.

2.2.4 SALICYLALDOXIME METHOD FOR FERRIC IRON

Under different pH conditions, different colour complexes form between ferric iron and salicylaldoxime. At pH of 3.0, a purple colour is formed; at pH of 10.0, a yellow colour is formed and at pH of 7.0, a red-orange complex is formed. Out of the three different colours, the red-orange one is the most commonly used since the pH can be easily achieved by using ammonium acetate. Beer's law is obeyed and the maximum absorbance is between 400-500 um. For visual comparison, the iron content in a sample should be 0.01-0.2 mg.

Procedure: COLORIMETRIC METHODS OF ANALYSIS - 3'd edition, 1949.

Transfer an aliquot of sample containing 0.01-0.2 mg of iron to a .100 mL flask. Add 10 mL of a fresh 0.1 percent solution of salicylaldoxime in 5 percent ethanol and mix well. Add 1 g of solid ammonium acetate and mix well to dissolve. If in doubt as to full intensity of colour having been developed add more reagent. Finally dilute to volume and compare. ...If measured by transmittance, suitable filters are Coming Signal Blue No. 556 or Coming Light Blue Green No. 428.

2.2.5 POTASSIUM FERROCYANIDE FOR FERRIC IRON

Prussian Blue, Fe4(Fe(CN)6)3, is a deep blue colour which forms between ferric iron and potassium ferrocyanide. The potassium ferrocyanide solution itself is yellow in colour and the following reaction takes place:

3Fe(CN)6j` + 4Fe3+ -) Fe4(Fe(CN),),

This method is independent of the pH of the solution. Maximum absorbance is 620 um and for visual comparison, the amount of iron in sample should be 0.001-0. 1 mg.

Procedure: COLORIMETRIC METHODS OF ANALYSIS - 3'd edition, 1949.

Transfer an aliquot of sample containing 0.001-0. 1 mg of iron to a Nessler tube and dilute to about 20 mL. Prepare a series of standards with 0.05, 0.1, 0.2, 0.4, 0.6 and O.5 mL of standard iron solution containing 0.1 mg of iron per mL. Each standard should contain the same volume of the same reagents as the sample. To each sample and standard add 1 mL of a 1 percent solution of potassium ferrocyanide and dilute to a uniform volume. Compare after 15 minutes. Alternatively read the transmittance at 620 um.

2.2.6 8-HYDROXYQUINOLINE FOR FERRIC IRON

In organic medium, an intense dark green colour is formed between ferric iron and 8-hydroxyquinoline. Colour comparison is performed after the extraction and the dissolution of the g-hydroxyquinoline complex in ethanol. The extraction is ideally carried out at a pH of 2.0-3.0. it obeys Beer's law and has maximum absorbance at 470 my and 570 mCl. 0.005-0.1 mg of iron is best suitable for visual comparison.

Procedure: COLORIMETRIC METHODS OF ANALYSIS - 3'd edition, 1949. Transfer an aliquot of sample solution containing 0.005-0.1 mg of iron to a 35 mL- 40 mL centrifuge tube.... Add 3 drops of glacial acetic acid, 3 drops of 0.2 percent solution of methyl red in ethanol, and 1 mL of 2 percent sodium oxalate solution. This will keep the iron in solution in the presence of phosphates and precipitate calcium. Add 5 percent sodium hydorxide solution until the indicator begin to change colour. Add 2 mL of a 2.5 percent solution of a-hydroxyquinoline in glacial acetic acid, then I percent sodium hydroxide solution, drop by drop, until the indicator changes colour. Iron hydroxyquinolate separates. Heat the tube in a boiling water bath for 10 minutes. centrifuge while warm to separate the iron hydroxyquinolate and calcium oxalate. Decant the upper layer and wash precipitate with water. Again centrifuge and decant. Dissolve the precipitate in 95 percent ethanol to which a drop or two of 1 percent sodium hydroxide solution has been added, and dilute to 50 mL or 100 mL. Compare or read transmittance at 470 um or 570 um.

2.3 ANALYTICAL METHODS FOR FERROUS AND FERRIC IRON

3.1 THIOGLYCOLIC ACID FOR IRON

This reagent forms a blue to purple colour with ferric iron and a red to purple colour with ferrous iron in ammoniacal solution. For visual colour comparison, the iron content in a sample is best between 0.02-0.2 mg. It follows Beer's law and it is not dependent on the pH value of the solution.

Procedure: COLORIMETRIC METHODS OF ANALYSIS -3'd edition, 1949.

Measure out an aliquot of sample to contain 0.02-0.2 mg of iron. Approximately neutralize, using an external indicator, and dilute to about 80 mL. If a standard is to be run in parallel, add the equivalent salts to it and dilute to the same volume. As reagent use 10 percent thioglycolic acid solution, neutralized with 1:1 ammonium hydroxide to about the phenolphthalein end point. Add 2 mL of the reagent and mix. Add 10 mL of 1.4 ammonium hydroxide, dilute to 100 mL and mix

2.4 EDTA

EDTA is a chelating agent which forms strong t1 complexes with metal ion.

Under low pH value, the EDTA complex becomes less stable. For ferrous iron, the minimum pH value is 5 and for ferric iron, the minimum pH is 2.

3.0 EXPERIMENTAL METHODS

3.1 PREPARATION OF FLOUR

3.1.1 MATERIALS

Flour samples with various concentrations of iron(II) were prepared from plain flour with no additives supplied by Robin Hood Multifood company. Ferrous fumerate and ferrous sulphate were used to prepare two identical sets of samples.

3.1.2 METHODS 3.1.2.1 SAMPLES FROM FERROUS FUMERATE

The stock flour of 1000 ppm of ferrous iron was prepared by mixing 1.82688 of ferrous fumerate with flour in a clean glass jar with lid. The total mass of pour and ferrous fumerate was 600g. The flour mixture was mixed by shaking for 30 minutes. The 20 ppm sample was prepared by mixing 4.0003g of the 1000 ppm stock flour with plain flour in a clean glass jar with lid. The total mass of 1000 ppm stock flour and plain flour was 205.908. The flour mixture was mixed well by shaking for 30 minutes. The 40 ppm sample was prepared by mixing 8.01 17 g of the 1000 ppm flour with plain flour to a total mass of 202.80 g in a clean jar. The flour mixture was then shacken for approximately 30 minutes. The 60 ppm sample was prepared by mixing 12.0207 g of the 1000 ppm flour with plain flour to a total mass of 200.02 g in a clean jar. The flour mixture was then shaken for approximately 30 minutes. The 80 ppm sample was prepared by mixing 16.0836 g of 1000 ppm stock flour with plain flour to a total mass of 200.96 g in a clean jar. The mixture was then mixed well by shaking for 30 minutes. Similarly, the 100 ppm sample was prepared by mixing 20.02 g of the 1000 ppm stock to a total mass of 200.03 g in a clean jar. The mixture was then shaken for 30 minutes.

Refer to Appendix I for sample calculations.

3.1.2.2 SAMPLES FROM FERROUS SULPHATE

The ferrous sulphate was ground by an electric grinder into powder form. 0.9974g of the ferrous sulphate powder was mixed with plain flour to a total mass of 200.96 g in a clean glass jar with lid to make a stock flour mixture of 1000 ppm. The mixture was well mixed by shaking for 30 minutes. The 20 ppm sample was prepared by mixing 3.9666 g of the 1000 ppm flour with plain flour in a clean jar with lid. The total mass of the plain flour and the stock flour was 199.86 g. The mixture was then shaken for 30 minutes. The 40 ppm sample was prepared by mixing 8.0007 g of the 1000 ppm flour with plain flour in a clean jar with lid. The total mass of the plain flour and the stock flour was 199.96 g. The mixture was then shaken for 30 minutes. The 60 ppm sample was prepared by mixing 12.0235 g of the 1000 ppm flour with plain flour in a clean jar with lid. The total mass of the plain flour and the stock flour was 200.78 g. The mixture was then shaken for 30 minutes. The 80 ppm sample was prepared by mixing 15.9584 g of the 1000 ppm flour with plain flour in a clean jar with lid. The total mass of the plain flour and the stock flour was 200. 11 g. The mixture was then shaken for 30 minutes.

3.2 FIELD TEST APPARATUS

The following set of apparatus was to be included with the test kids for all four types of reagents:

a. petri dish or 5 mL glass tube with screw lid b. a plastic stirring rod c. a few 25 mL plastic dropper flasks containing the prepared reaction mixture d. a plastic 5 mL graduate cylinder e. colour standard chart; 0-100 ppm iron in flour f. sampling cap (size

for approximately 1 g of flour)

3.3 1,10-PHENANTHROLINE TEST

3.3.1 MATERIALS

0.3% of 1,1O-phenanthroline was prepared by dissolving 0.3000 g of

1,10-phenanthroline in a 100 mL volumetric flask by freshly boiled distilled water. 6 drops of concentrated hydrochloric acid was added and the reagent solution was diluted to mark when it was cooled to room temperature.

Reducing agent was prepared by transferring 10.0 g of hydroxylamine hydrochlroride(HzNOH.HC1) to a 100 mL volumetric flask and was diluted to mark with distilled water. 27.2 g of sodium acetate (NaOAco3H20) was transferred to a 100 mL volumetric flask and was diluted to mark by distilled water to make a 2 M of sodium acetate buffer sol ution. 1000 ppm of EDTA was prepared by diluting 0.2000 g of EDTA to mark in a 200ml volumetric flask using distilled water.

3.3.2 METHODS

3.3.2.1 DEVELOPMENT OF THE FIELD TEST

This method was developed previously. However, modifications have been made for easier operation.

Reagents mixtures:

5 portions of 2 M sodium acetate solution were mixed with 3 portions of 3 %

1,10-phenanthroline. The solution was mixed thoroughly and transferred to a

25 mL plastic dropper flask.

2. flour sample

Procedure:

a. The given sampling cap was filled with the flour sample and the flour was transferred to the petri dish.

b. 2 mL of reagent mixture(2) were added to the sample and was let stand for 5 minutes for colour development. The above mixture was all measured by the graduate cylinder provided.

c. The colour developed was compared to the colour chart in which the samples were prepared in the same manner.

3.3.2.2 EDTA TEST

In two petri dishes, 2 sets of 1.4000 g of flour with the same concentration were weighed out and transferred to them. To each dish 4 mL of hydroxylamine

hydrochloride were added. After the addition of the hydroxylamine hydrochloride, the samples were stirred. They then waited 5 minutes so that the iron was totally reduced. Then 5 mL sodium acetate were added to each dish for buffering the system. 3 drops of HCl were added to each system to adjust the pH. 13 drops of 1000 ppm EDTA were added to one of the dishes while 13 drops of distilled water were added to the other dish. Then finally, 13 drops of 1,10-phenanthroline were added to both dishes. The samples were stirred and after fifteen minutes observations were made.

3.4 α,α'-BiPYRIDYL TEST

3.4.1 MATERIALS

1% a,a'-bipyridyl, was prepared by transferring 1.000 g of a,a'-bipyridyl to a 100ml volumetric flask. 1.0 mL of concentrated HCl was added to the volumetric flask and distilled water was used to dilute the solution to mark.

1:9 hydrochloric acid (HCl) was prepared by mixing 11.11 mL of stock HCl with 1 00.00 mL of distilled water.

10% of sodium sulphite was prepared by transferring 10.000 g of sodium sulphite to a 100 mL volumetric flask and it was diluted to mark by distilled water.

1000 ppm of EDTA was prepared by diluting 0.1000 g of EDTA in a 100 mL volumetric flask using distilled water.

3.4.2 METHODS

3.4.2.1 DEVELOPMENT OF THE FIELD TEST

Reagent mixtures

(1) 1 portion of 1% a,a'-bipyridyl solution was mixed with 1 portion of 1:9 HCl 5 portions of distilled water. After mixing, this solution was transferred to a 25 mL plastic dropper flask.

(2) the 10% sodium sulphite solution was transferred to a 25 mL plastic dropper flask

(3) flour sample

Procedu re:

a. The sample was transferred to the given tube by filling the sampling cap.

b. The sample was wetted with a few drops of distilled water.

c. 1.00 mL of reagent mixture (2) was measured by graduated cylinder and added to the flour sample. After putting on the screw cap, the mixture was shacken and let stand for 5 minutes.

d. 2.50 mL of reagent mixture(l) was measured by graduated cylinder and added to the tube. The mixture was shacken vigorously with lid and the colour was compared to the colour standard chart.

3.4.2.2 EDTA TEST

In two petri dishes, 2 sets of 1.4000 g of flour with the same concentration were weighed out and transferred to them. To each dish 2 mL of 1:9 HCl and 10.0 mL of distilled water were added. Then 5 mL of EDTA were added to one of the samples and 5 mL of distilled water were added to the other sample. The samples were then stirred. 2 mL of a,a'-bipyridyl were added to each set of samples and after the addition of the reagent, 1 mL of sodium sulphite was added to both samples. The samples were stirred and 5 minutes were waited for colour development.

POTASSIUM FERRICYANIDE TEST

3.5.1 MATERIALS

Sulfuric acid was prepared by mixing 10.0 mL of sulphuric acid with 50.0 mL of distilled water. 1000 ppm of EDTA was prepared by diluting 0.1000 g of EDTA in a 100 mL volumetric flask using distilled water.

Three different concentrations of potassium ferricyanide were made and they were 1.00/o, 0.5% and 0.05%. The 1.0% solution was made by dissolving 1.0g of potassium ferricyanide to 100 mL using distilled water. The 0.5% solution was made by diluting 0.5000g of potassium ferricyanide using distilled water to the mark of a 100 mL volumetric flask. The 0.05% was made by diluting 10.0 mL of the 0.5% solution to 100.0 mL by distilled water. All the flour samples were tested with all three concentrations with the addition of 1:5 sulphuric acid in each sample and the colours developed were compared. The concentration of the reagent which yielded the most obvious colour difference between the different concentrations of flour was used to develop the field

3.5.2 METHODS

3.5.2.1 DEVELOPMENT OF THE FIELD TEST

Reagent mixtures:

(1) 3 portions of the 0.5% potassium ferricyanide were mixed with 2 portions of sulphuric acid and 5 potions of distilled water.

(2) flour sample

Procedure:

a. Approximately 1 g of flour sample was transferred to the petri dish by the given sampling cap.

b. 2 mL of reagent mixture(2) were measured by the given graduated cylinder and transferred to the petri dish.

c. the mixture was stirred well with stirring rod.

d. the developed colour was compared to the standard colour chart

3.5.2.2 EDTA TEST

In two petri dishes, 2 sets of 1.40 g of flour of the same concentration were weighed out and transferred to them. 3 mL of 1000 ppm EDTA was added to one dish while 3 mL of distilled water was added to the other. To each dish 2 mL of the 1:5 sulphuric acid were added. Then 3 mL of the potassium ferricyanide solution were added.

The pastes were stirred by glass rod and the results were observed.

THIOGLYCOLIC ACID TEST

3.6.1 MATERIALS

10% of thioglycolic acid was prepared by diluting 10.00 mL of the stock thioglycolic acid to 100.00 mL in a 100 mL volumetric flask.

1:1 ammonium hydroxide was prepared by mixing 25.00 mL of stock ammonium hydroxide with 25.00 mL of distilled water.

1:4 ammonium hydroxide was prepared by mixing 10.00 mL of stock ammonium hydroxide with 40.00 mL of distilled water.

Phenolphthalein indicator was prepared by dissolving 8.5000 g of phenolphthalein a 100 mL volumetric flask with distilled water.

1000 ppm of EDTA was prepared by diluting 0. 1000 g of EDTA in a 100 mL volumetric flask using distilled water.

Thioglycolic acid was titrated with the 1:1 ammonium hydroxide to the phenolphthalein endpoint.

The following paragraphs describe the steps taken.

10.00 g of flour was transferred to a 250 mL Erlenmeyer flask which had previously been rinsed with diluted acid, then distilled water. (Kjeldahl flask was suggested by the AOAC Official Methods of Analysis. However, excessive foaming made the Kjeldahl flask an undesired piece of equipment to use) 20 mL of distilled water were added and mixed well. 5 mL of sulphuric acid was pipetted into the flask and the paste was mixed well. Then 25 mL of nitric acid was added and it was mixed well. After a few minutes, the flask was heated very gently at brief intervals until heavy evolution of nitrogen dioxide ($N0_2$) gas ceased. The material in the flask was charred after it had been heated continuously. Then a few mL of nitric acid were added cautiously at intervals and 5 min. until fumes evolved and very pale yellow liquid was obtained. The solution was cooled and 50 mL of distilled water were added as well as some anti-bumping chips.

The solution was then heated to until it fumed and it was then cooled. 25 mL of distilled water were added and the solution was filtered into a 100 mL volumetric flask. The solution was cooled to room temperature and diluted to volume by distilled water.

10 mL of this solution were pipetted to a 25 mL volumetric flask I mL hydroxylamine hydrochloride was added and the mixture was let stand for a few minutes. 9.5 mL of 2 M sodium acetate were added to the mixture and I mL of 1,l0-phenanthroline was added. All the above solutions were added by using pipettes. Distilled water was used to dilute the solution to mark and the mixture was let stand for more than 5 minutes.

It was then tested using a ultra violet spectrophotometer at h = 510 nm and h=396 nm.

The above digestion method was repeated for all the different concentrations of

flour. Refer to Appendix 3 for data.

CALIBRATION CURVE FOR IRON

3.8.1 MATERIALS

The visible lamp of the spectrophotometer was warmed up for at least half an hour. The wavelengths were set at 512 µm and 396 µm.

0.3% of 1,10-phenanthroline solution was prepared by dissolving 0.3000 g of the 1,10-phenanthroline solid with freshly boiled distilled water in a 100 mL volumetric flask. The solution was diluted to mark with distilled water when it was cooled to room temperature.

Standard iron solution was prepared by dissolving 0.7000 g of reagent grade ferrous ammonium sulphate hexahydrate in distilled water containing 0.3 mL of concentrated sulphuric acid and diluting to 100 mL with distilled water. Standard iron(III) solution was prepared by dissolving O. X600 g of reagent grade ferric ammonium sulphate dodecahydrate in distilled water containing 0.3 mL of concentrated sulphuric acid and dilution to 100 mL with distilled water. The iron concentration in each sample was 1000 ppm. 10.00 mL of the 1000 ppm solution were pipetted to a 100 mL volumetric flask and distilled water was used to dilute the solution to mark Standard buffer solution of pH 4 which consisted of potassium hydrogen phthalate was used.

3.8.2 METHODS

Solutions of ferrous and ferric iron complexes were prepared by adding to aliquots of the 100 ppm stock solutions, 10 ml of the 0.3% 1,10-phenanthroline solution, and 5 mL of buffer solution, and diluting to 25 mL with distilled water. The concentrations of iron in each of the two series of solutions were: 1 ppm, 3 ppm, 5 ppm, 8 ppm, 9.93 ppm and 12 ppm. The solutions were mixed thoroughly and let stand for at least 5 minutes. The absorbances for all the samples were than measured using the spectrophotometer. An absorbance versus concentration curve was them plotted. Please refer to the Appendix 2 for the actual plot.

4.0 RESULTS

4.1 1,10-PHENANTHROLINE TEST

The effect of EDTA was studied and it was found that for two samples of the same concentrations, one with the addition of EDTA and one with just the same amount of distilled water, the developed colours were visually identical for both samples.

Both ferrous fumerate and ferrous sulphate were tested with the field test method outlined in Section 3.3.2.1. The ferrous fumerate result is illustrated in Figure la where the concentrations were 0 ppm, 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm. In Figure lb, the ferrous sulphate is presented and the samples have concentrations of O ppm, 20 ppm, 40 ppm, 60 ppm and 80 ppm. It was observed that for the same concentration, the colour produced by the ferrous sulphate was more intense than the colour produced by the ferrous fumerate.

4.2 α,α–BIPYRIDYL TEST

The addition of EDTA had no effects on the system and the optimum pH was found to be 1.8-1.9 instead of 2-9 which was the optimum pH range claimed by the literature.

It was also found that the age of sodium sulphite contributed to the rate of colour development and the intensity of the colour. Freshly prepared sodium sulphite yielded the best result.

The field test method suggested in Section 3.4.2.1 were carried out on both ferrous fumerate and ferrous sulphate. Figure 2a illustrates the result from the ferrous fumarate and Figure 2b presents the result for ferrous sulphate. The concentrations of the samples were identical to the one for the 1,10-phenanthroline test. It was noticed that the colour complex formed from ferrous sulphate was stronger than the one formed from ferrous fumerate.

POTASSIUM FERRICYANIDE TEST

It was found that EDTA has no effects on the system and 0.5% potassium ferricyanide was found to yield the best colour when mixed with the flour.

Figure 3a and figure 3b represents the colour formed from ferrous fumerate and ferrous sulphate, respectively. The procedure given in Section 3.5.2.1 was used to prepare the samples. The concentrations of the samples were identical to the previous two methods that were described in this section.

4.4 THIOGLYCOLIC ACID TEST

The method given in Section 3.6.2.1 was used to test both the ferrous sulphate and ferrous fumerate. Figure 4a and figure 4b are the presentations of the usage of ferrous fumerate and ferrous sulphate, respectively.

The results obtained from the thioglycolic acid test were very inconsistent. Great variations of colour intensity were observed from one test to another. Similar to the other three reagents, the EDTA test did not work for this system as well.

4.5 CONCENTRATIONS OF IRON IN FLOUR SAMPLES

Table 1 listed the theoretical concentrations and the experimental concentrations of ferrous fumerate obtained by digestion.

Table 2 listed the theoretical concentrations and the experimental concentrations of ferrous sulphate obtained by digestion.

Refer to Appendix for raw data and sample calculation.

Table 1. Concentrations of ferrous fumerate in flour

THEORETICAL CONCENTRATION	EXPERIMENTAL CONCENTRATION
0	-0.l +/- 0
19.4	7.3 +/- 5. 1
39.5	37.6 +/- 2.8
60.1	57. 1 +/- 3.2
80.0	74.9 +/- 0.6
100.1	98.9 +/- 1.8

Table 2: Concentrations of ferrous sulphate

THEORETICAL CONCENTRATIONS (ppm)	EXPERIMENTAL CONCENTRATIONS
0	-0.1 +/- 0
19.8	17.9 +/- 1.5
40.0	37.7 +/- 0.5
59.9	57.7 +/- 3.0
79.7	78.6 +/- 0.7

5.0 DISCUSSION

5.1 FIELD TEST DEVELOPMENT

Four different reagents were tested in order to develop the principles of field tests, and for each reagent tests have been run in parallel to find out if they are reproducible. The 1,l0-phenanthroline test, a,a'-bipyridyl test and the potassium ferricyanide test yielded consistent visual results, while the results for the thioglycolic acid test were inconclusive. The reason for such results is due to the stability of the reagent itself.

Thioglycolic acid is unstable because it can be easily oxidized by air. However when it has been titrated with ammonium hydroxide to form a more stable salt, the temperature influences the stability of the solution. Thioglycolic acid is stable under 2"C, and therefore, under room temperature where the test is carried out, the reagent becomes unstable. As a result, thioglycolic acid is not suitable for the development of field test since the majority of field settings do not provide refrigeration. Some other properties of the other three reagents will be discussed in the following paragraphs.

1,10-phenanthroline has previously been studied and applied to the development of the field test. From the previous study, it was claimed that the optimum pH value is 3.2-3.3. This result has been verified and was found to be consistent: at a pH of 2.62, the colour development was slow and the intensity of the developed colour was not as strong. And at a pH value of 5.37, the same effects were observed. However, when the pH was 3.27 the colour developed instantaneously. Therefore the l,l0-phenanthroline test should be carried out at a pH of 3.2-3.3.

Other than the 1,1O-phenanthroline test, the pH value is also important to the α,α'-bipyridyl test. It was claimed by the literature that the optimum pH value is ranged between 3-9. However, from the experimental result, it was noticed at a pH of 1.8 the colour intensity was darker and the rate of colour development was faster. Therefore when performing the a,a'-bipyridyl test, the pH value should be at 1.8-1.9.

It was observed that the colour formed from the ferrous sulphate was much stronger than the colour forms from the ferrous fumerate. This condition held true for the potassium ferricyanide, α,α'-bipyridyl and 1,10-phenanthroline. This can be explained by the fact that the solubilities of ferrous sulphate and ferrous fumerate are very different. According to the MERCK's Index, the ferrous sulphate is soluble in water but the solubility of ferrous fumerate is only 0.14g/100ml of water at 25"C. For this reason, the colour intensity difference was observed. This effect can be reduced by increasing the solubility of the ferrous fumerate.

The solubility of ferrous fumerate can be increased by using test tube to carry out the test instead of using petri dish. More reagent was required because in the tube when shaking was required, a paste liked mixture was not desired. This method is exceptionally applicable to the a,a'-bypyridyl test. After shaking vigorously, the colour intensity of the solution has increased since the amount of ferrous dissolved has increased. Therefore, colour intensities formed by the two different types of ferrous salt were found to be visually identical. For potassium ferricyanide, this method was not applicable because the excess yellow background colour made the colour intensity visually impossible to distinguish.

Since the field tests results were based on colour development, other forms of colours such as the colour of the reagent itself, can affect the colour of the final product. This is the case of potassium ferricyanide. The potassium ferricyanide solution is yellow in colour. When it was reacted with the ferrous iron, a blue colour should be formed. However, when the yellow reagent was mixed with the blue colour, a green colour was formed instead. This explains why the field test result of the potassium ferricyanide test yielded a green colour was formed of the blue that was listed in the literature. Different concentrations of the reagent solution have been made so that the impact of this yellow colour was not as big. This is especially important when the concentration was low (e.g. 0 ppm and 20 ppm) because the excess yellow colour made the colours very hard to distinguish. Similarly, at high concentration, a reagent solution with pale yellow colour made it very difficult to distinguish the blue from the greenish-blue colour. Therefore, for the given the concentration range, the 0.5% potassium ferricyanide was most suitable because it satisfied both circumstances. However, due to the fact that the colour intensity difference between the ferrous fumerate and ferrous sulphate was big, modification is required before this test is applied to the field test.

The binding of EDTA to the ferrous iron has been tried and it was observed that EDTA had no effects on all the reagent systems. This can be explained by the pH value of the system. The minimum pH value for ferrous iron and EDTA was 5 which indicated that below pH 5, the EDTA complex becomes less stable than when the pH is greater than 5. It is because at low pH the conditional formation constant is small and the conditional formation constant describes the formation of metal ion and the EDTA at any particular pH. For the above reason, the field test environment which is suitable for the development of a strong 1,10-phenanthroline colour complex at a pH of 3.2-3.2 does not favour the formation of the EDTA complex. This also applies to the a,a'-bipyridyl test due to the fact that the pH value of that particular system is 1.8-1.9.

Other than the above factors, safety is another main concern for the development of field test. Thioglycolic acid could cause severe bum on skin and ammonium hydroxide has a very strong and unpleasant odor. Therefore, beside its unsuitability this is another factor which makes the thioglycolic acid test an undesirable one. However, all the other tests were used in an acidic environment, therefore gloves and safety goggles should be provided with the test kit.

One last factor to consider when designing a field test is the economic issue. Due to the fact that these tests are most likely to be purchased by third world countries, expensive reagents are not feasible. Based on the price list of SIGMA chemical company, for 1000 applications, the cost of 1,1 O-phenanthroline is B0.00. For a,a'-bipyridyl 1000 applications cost g.80 while for potassium ferricyanide the price is $0.07/1000 applications. From this aspect, potassium ferricyanide and a,a'-bipyridyl seen to be a lot more attractive.

5.2 OTHERS

According to the literature, three standard concentration curves should be obtained: one curve for the ferrous iron at h= 512 mR a single curve at k-512 mCl for ferric iron and a single curve for both ferrous and ferric iron at h=396 um. The theoretical slopes for these three curves are 0.196, 0.0004 and 0.054 ppm^{-1}, respectively.

The slopes obtained experimentally were: 0.1982, 0.0066 and 0.0531 ppm^{-1}, respectively. Therefore the calibration curve data for iron were accurate.

6.0 CONCLUSION

It is concluded that the thioglycolic acid is not suitable for the development of the principles for field test for iron. 1,10-phenanthroline and potassium ferricyanide are suitable but modifications are required. Out of the four different tests, it is concluded that the α,α'-bipyridyl test meets all the requirements to be used as an reagent for the field test for ferrous iron.

7.0 RECOMMENDATIONS

Chelating agents such as the sodium heptametaphosphate could be investigated. Oxidizing methods could also be applied so that the ferric tests could be employed into the development of the field test. The reproducibility of the above methods should also be tested.

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APPENDIX 1: : DATA FOR CALIBRATION CURVE

concentration (ppm)	ferrous iron (h=512)	ferrous iron (h=396)	ferric iron (h=512)	ferric iron (h=396)
0	0	0	O	O
1	0.2148	0.07	0.0202	0.0657
3	0.5917	0. 1872	0.0309	0. 1737
5	0.9986	0.3069	0.05	0.2886
8	1.5318	0.4677	---	---
9.93	1.9954	0.6082	0.0693	0.5306
12	2.3921	0.7185	---	---

APPENDIX 2: DATA FOR DIGESTION FOR FERROUS FUMERATE

CONCENTRATION	YELLOW	RED	absorbance =red-yellow	absolute absorbance	conc. (from graph)
0	0.021	0.01341	0. 122	0	-0.00555
20	0.01791	0.01704	0.1525	0.0305	0.148355
40	0.0161	0.4529	0.4368	0.3148	1.582745
60	0.0243	0.618	0 5937	0.4717	2.374369
80	0.0231	0.7434	0.7203	0. 5983	3.013118
100	0.244	0.9413	0.9169	0.7949	4.005045

SECOND RUN

CONC.	YELLOW	RED	absorbance =red-yellow	absolute absorbance	conc. (from graph)
0	0.0026	0.156	0.1534	0	-0.00555
20	0.0116	0.2524	0.2408	0.0874	0.435419
40	0.0221	0.4584	0.4363	0.2829	1.421796
60	0.0224	0.6117	0.5893	0.4359	2.193744
80	0.0354	0.7801	0.7447	0.5913	2.9778
100	0.0223	0.95	0.9277	0.7743	3.90111

AT 512

RUN#1CONC	RUN#2CONC	AVG	S.D	AVG*25
-0. 00555	-0. 00555	-0.00555	0	-0.1375
0. 148355	0.43541	0.291877	0.202999	7.296925
1.582745	1.421796	1.502271	0.113808	37.55676
2.374369	2.193744	2.284057	0.127721	57.10141
3.013118	2.9778	2.995459	0.024974	74.88648
4.005045	3.90111	3.953078	0.073493	98.82694

APPENDIX 3: DATA FOR DIGESTION FOR FERROUS FUMERATE
FIRST RUN(WAVELENGTH=396

CONCENTRATION	YELLOW	RED	absorbance	absolute absorbance =red-yellow	conc. (from graph)
0	0.739	0.77411	0.0351	0	-0.19962
20	0.544	0.40461	-0.1394	-0.1745	-3.48588

40	0.8886	1.00071	0.1121	0.077	1.250471
60	0.7174	0.87631	0. 1589	0.1238	2.131827
80	0.6974	0.91311	0.2157	0.1806	3.201507
100	0.9517	1.21491	0.2632	0.2281	4.096045

SECOND RUN

CONCENTRATION	YELLOW	RED	absorbance	absolute absorbance =red-yellow	conc. (from graph)
0	0.7286	1.0216	0.293	0	-0.19962
20	0.536	0.5033	-0.0327	-0.3257	-6.33333
40	0.8853	1.2647	0.3794	0.0864	1.427495
60	0.7113	1.0784	0.3671	0.0741	1.195857
80	0.6886	1.1493	0.4607	0.1677	2.958569
100	0.9447	1.4919	0.5472	0.2542	4.587571

AT 396

RUN#1CONC	RUN#2CONC	AVG	S.D	AVG*25
-0.19962	-0.19962	-0.19962	0	-4.9905
-3.48588	-6.33333	-4.90959	2.01343	-122.74
1.250471	1.427495	1.338983	0.125175	33.47458
2. 131827	1.195857	1.663842	0.661831	41.59605
3.201507	2.958569	3.080038	0.171783	77.00095
4.096045	4. 587571	4.341808	0. 34561	108.5452

APPENDIX 4:DATA FOR DIGESTION FOR FERROUS SULPHATE

FIRST RUN

CONCENTRATION	YELLOW	RED	absorbance	absolute absorbance =red-yellow	conc. (from graph)
0	0.02671	0.1277	0.101	0	-0.00555
20	0.0112	0.2472	0.236	0.135	0.67565
40	0.022	0.426	0.404	0.303	1.523165
60	0.0176	0.5942	0.5766	0.4756	2.394251
80	0.0199	0.7417	0.7218	0.6208	3.126494

SECOND RUN

CONCENTRATION	YELLOW	RED	absorbance	absolute absorbance =red-yellow	conc. (from graph)
0	0.0137	0. 1489	0. 1352	0	-0.00555
20	0.0266	0.3135	0.2869	0.1517	0.759611
40	0.0148	0.4468	0.4320	0.2968	1.492132
60	0.0353	0.612	0.5767	0.4415	2.222131
80	0.0198	0.7836	0.7637	0.6285	3.165451

STATISTICS

AT 512

RUN#1 CON	RUN#2CON	AVG	AVG*25	S.D
-0.00555	-0.00555	-0.00555	-0.13875	O
0.67565	0.759611	0.717631	17.9407625	0.059369392
1.523165	1.492132	1.507649	37.6912125	0.021943645
2.394251	2.222131	2.308191	57.704775	0.121707219
3.126494	3.165451	3.145973	78.6493125	0.027546759

3.6.2 METHODS

3.6.2.1 DEVELOPMENT OF THE FIELD TEST

Reagent mixture:

(I) 1 portion of the titrated thioglycolic acid was mixed with 5 portions of 1.4 ammonium hydroxide.

Procedure:

a. The given sampling cap was used to transfer approximately I g of flour sample to petri dish.

b. 2 mL of the reagent mixture (1) were measured by graduated cylinder and transferred to the sample.

c. The sample mixture was stirred well and the colour developed was compared to the standard colour chart.

3.6.2.2 EDTA TEST

In two petri dishes, two sets of 1.40 g of flour with the same concentration were weighed out and transferred to them. To one of the dishes, 3 mL of 1000 ppm EDTA was added and to the other dish, 3 mL of distilled water was added. Then 1 mL of the thioglycolic acid (after titration) was added to each dish followed by 5 mL of the 1:4 ammonium hydroxide. The pastes were stirred by glass rod and the results were observed.

3.7 CONFIRMATION OF CONCENTRATIONS OF FLOUR

3.7.1 MATERIALS

Flour of various concentrations, concentrated nitric acid and concentrated sulphuric acid.

0.3% of 1,10-phenanthroline was prepared by dissolving 0.3000 g of 1,10-phenanthroline in a 100 mL volumetric flask by freshly boiled distilled water. The reagent solution was diluted to mark when it was cooled to room temperature.

Reducing agent was prepared by transferring 10.0000g of hydroxylamine hydrochlroride(H_2NOH. HCl) to a 100 mL volumetric flask and was diluted to mark with distilled water.

27.2 g of sodium acetate (NaOAco3H20) was transferred to a 100 mL volumetric flask and was diluted to mark by distilled water to make a 2 M of sodium acetate buffer solution.

3.7.2 METHODS

The actual concentrations of iron in flour were determined by the wet digestion method illustrated by the AOAC Official Methods of Analysis with some modifications.