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Analytical Methods Section

A Sensitive Kinetic Assay for the Determination of Iodine in Foodstuffs

D. L. Mahesh, Y. G. Deosthale & B. S. Narasinga Rao

Analytical Division, National Institute of Nutrition, Indian Council of Medical Research, Jamai Osmania PO, Hyderabad — 500007, India

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A spectrophotometric kinetic assay is presented for the determination of iodine present in foodstuffs. Incineration of organic matter was accomplished in successive steps with KOH and $ZnSO_4$. Quantitation of iodine was based on measurement of iodide-catalysed reduction of Ce(IV) to Ce(III) by As(III). The reaction was continuously monitored at 370 nm and initial velocity, i.e. rate of decrease in absorbance was plotted against concentration of iodine. The method showed good reproducibility. Recovery of iodine added to different foods ranged from 94% to 102% with a mean and standard deviation of 97 ± 3·3%. The method provides a detection limit of 0·4 ng and a sensitivity of 40 pg. The method is also applicable for the determination of iodine in serum, urine and other biological materials.

INTRODUCTION

Estimation of the iodine content of foods is beset with the difficulties of destruction of complex organic matter and quantitation of the iodine at ultra-trace levels. This perhaps explains why data on iodine content of foods in literature is limited. Several alkaline dry ashing methods are available for the incineration of organic matter present in serum and other biological material (Barker *et al.*, 1951; Foss *et al.*, 1960; Lauber, 1975). However, when these methods are adapted to foodstuffs ashing is often incomplete. This is observed even after 12–36 h of ashing at 600°C particularly in the case of cereals and oil seeds. To overcome this problem Moxon and Dixon (1980) suggested the use of K_2CO_3 and $ZnSO_4$ as an ashing aid. Moxon and coworkers have successfully used this combination for milk (1980) and other foodstuffs (Wenlock *et al.*, 1982), but poor recoveries and considerable losses of iodine varying from 10 to 30%, were observed in the authors' initial attempts of incineration plant food-stuffs with the above reagents.

Methods using ion-specific electrode (Crecelius, 1975; Miles, 1978), neutron-activation (Johansen & Steinnes, 1976), gas-liquid chromatography (Bakker, 1977), X-ray fluorescence (Crecelius, 1975), differential pulse polarography (Thompson *et al.*, 1983) and Auto Analyzer (Moxon & Dixon, 1980; Fisher *et al.*, 1986) have been reported for the determination of iodine content of foodstuffs. However, most of these methods demand sophisticated laboratory equipment. This indicates the need for a simple spectrophotometric method for the quantitative estimation of iodine in foodstuffs.

The iodide-catalysed reaction between Cerium (IV) and Arsenic (III) was first reported in the 1930s (Sandell & Kolthoff, 1934, 1937), and is extensively used for the quantification of iodine. The kinetics of this reaction in sulphuric acid have been studied in great detail by Rodriguez and Pardue (1969). Quantitation based on kinetic measurements has definite advantage over the usual two-point assay and the kinetic measurement of the catalytic activity of iodide was successfully employed for the determination of iodine in biological material by Lauber (1975).

This paper reports on a relatively simple kinetic method for the determination of iodine in foodstuffs. Various ashing procedures have been reinvestigated in an attempt to develop an efficient method of incineration. The authors have also reinvestigated the quantitative dependence of Sandell and Kolthoff's reaction upon the concentration of reactants to establish the optimal concentrations for the quantitative estimation of iodine.

MATERIALS AND METHODS

Apparatus

Corning glass tubes 14 × 125 mm. Muffle furnace. Hitachi Spectrophotometer with attached recorder and thermostatic water bath.

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Reagents

All chemicals used were of analytical grade. Scrupulous care was taken while washing the glassware. Deionised-distilled water was used throughout.

(1) Iodine stock standard, 0·1 mg ml^{-1}; KI, 130·8 mg was dissolved in 1 litre of water.
(2) Iodine working standard: stock standard was suitably diluted to prepare solutions containing 2–10 ng ml^{-1}.
(3) Potassium hydroxide, 6M.
(4) Zinc sulphate, 0·52M.
(5) Sulphuric-hydrochloric acid reagent: concentrated sulphuric acid (specific gravity 1.84, 19·6 ml) was added to water (500 ml), mixed and allowed to reach room temperature. Then concentrated HCl (specific gravity 1·18, 5·4 ml) was added and the solution finally made up to 1 litre with water.
(6) Ceric (IV) reagent, 5 mM: ammonium cerium sulphate $(NH_4)_4Ce(SO_4)_4 \cdot 2H_2O$ (0·316 g) was dissolved in water (10 ml). To this, concentrated HNO_3 (specific gravity 1·41, 50 ml) followed by concentrated H_2SO_4 (5 ml) were added and the solution was allowed to reach room temperature. Finally, the solution was made up to 100 ml with water.
(7) Arsenic (III) reagent, 30 mM: arsenic trioxide (As_2O_3) (0·593 g) and KOH (0·6 g) were dissolved in 100 ml water.

$Na^{125}I$ was purchased from Bhabha Atomic Research Centre (Bombay, India). Thyroglobulin was radiolabelled using Chloramine-T (Greenwood *et al.*, 1963). Unbound and non-covalently linked inorganic iodine was removed by precipitation of protein with uranyl acetate (Boratynski, 1987), and extensively dialysed against deionised glass distilled water. Small amounts of this ^{125}I-labelled thyroglobulin were used to check the recoveries.

Samples

Samples of foodstuffs were procured from the local market. The samples were dried in an oven at 65–70°C, and then ground in a cyclone mill and stored until analysis.

Procedure

Ashing

Samples (50–100 mg) were transferred to clean dry test tubes in duplicate. Standard solutions (1 ml containing 2–10 ng iodine) were taken into a series of test tubes; 6M KOH (0·1 ml) was added to all the tubes and mixed well with a fine stainless steel needle. The contents of the tubes were allowed to dry completely in an oven at 95 ± 5 °C. The tubes were then transferred to a muffle furnace maintained at 95°C. The temperature of the muffle furnace was increased gradually to 600°C over about 30 min and incineration was continued for 1 h. Air was renewed inside the chamber of the muffle furnace every 15 min, by opening the door for 10–15 s. After 1 h the tubes were transferred to a desiccator and allowed to cool. Then 0·52M $ZnSO_4$ (0·1 ml) was added and the contents dried (95°C) before another ashing step (1–2 h) was performed. The resultant ash, white and free from any carbon particle, was dissolved in water, centrifuged at 1500 *g* for 10 min, and the supernatant was collected for iodine determination.

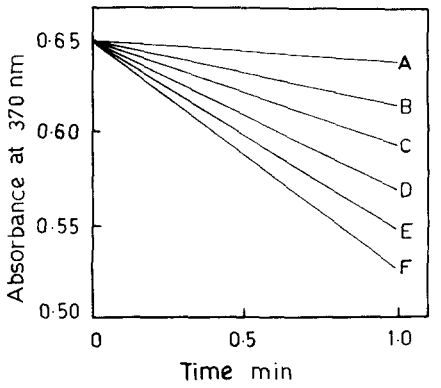

Fig. 1. Time-absorbance curves. Time course of reduction of Ce(IV) by As(III) in the presence of iodine. The final reaction mixture contained 1·0 mM Ce(IV); 6·0 mM As(III) and iodine: A, blank; B, 0.5 ng; C, 1·0 ng; D, 1·5 ng; E, 2·0 ng, F, 2·5 ng in total volume of 1·25 ml.

Kinetic assay

Deionised-distilled water (0·25 ml), H_2SO_4-HCl mixture (0·25 ml), Ceric (IV) reagent (0·25), and Arsenic (III) reagent (0·25 ml) were transferred into a 10 mm path-length curvette, mixed well and pre-incubated at 37°C for 2 min. The catalyst, i.e. iodine, solution (blank/standard/sample) (0·25 ml) was always added at the

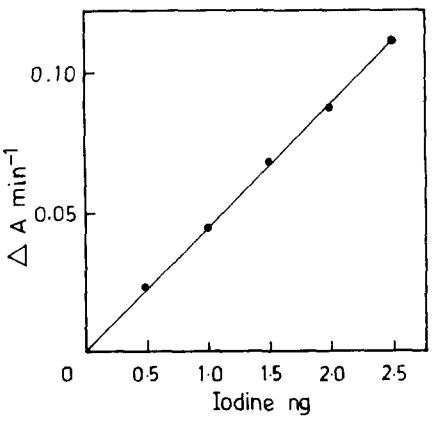

Fig. 2. Standard curve: a plot of initial velocities versus the concentration of iodine in ng.

end to initiate the reaction. The decrease in absorbance, i.e. reduction of Ce(IV) to Ce(III) was continuously monitored for 1 min at 370 nm. Initial velocities, i.e. Δ A.min^{-1} were calculated from time-absorbance curves (Fig. 1) and plotted against the concentration of iodine. Figure 2 gives a typical standard curve. (When the automatic recorder facility is not available, the reaction can be followed by manually recording the absorbance at 20 s intervals. These absorbance values can be used to draw time-absorbance curves.)

The iodine content of sample was calculated as follows:

$$\frac{\Delta A_s - \Delta A_b}{m} \times 4 \times d = \text{ng of iodine in the sample}$$

where s = sample; b = blank; m = slope of standard curve and d = dilution (in ml).

RESULTS AND DISCUSSION

Ashing

Incineration of organic matter is often based on acid digestion or alkaline dry ashing. Acid digestion methods (Zak *et al.*, 1952; Joerin, 1975; Fisher *et al.*, 1986) are in general effective in the destruction of organic matter, but not suitable for routine analytical purposes. They not only involve usage of large amounts (>12 ml) of concentrated acids for each sample (fumes of which pose a potential health hazard) but are also impractical when one wants to screen a large number of foodstuffs manually.

Alkaline dry ashing is generally used for the determination of iodine in biological material (Barker *et al.*, 1951; Foss *et al.*, 1960; Lauber, 1975). Among various studies that have reported alkaline dry ashing for the incineration of foodstuffs (Riesco *et al.*, 1976; Jones *et al.*, 1979; Moxon & Dixon, 1980; Belling, 1983; Aumont & Tressol, 1986), only Moxon and Dixon suggested the use of 30% K_2CO_3 and 10% $ZnSO_4$. The ashing was observed to be incomplete and recoveries poor when the same mixture was adapted for the incineration of organic matter present in plant foodstuffs. Carbonaceous particulate matter was observed even after 12 h of ashing, particularly in the case of cereals and oil seeds. The authors observed a loss of 10–30% of iodine when added in the form of KI. Incomplete ashing and the presence of fine carbon particles, which may interfere in the final estimation by absorbing the colour complex, may be responsible for the poor recoveries. In the authors' experience the use of Na_2CO_3 as an ashing agent suggested by Barker *et al.* (1951) also resulted in poor recoveries (30–70%). Incomplete ashing was also observed with KOH as an ashing reagent as suggested by Lauber (1975).

In the described method 6M KOH and 0·52M $ZnSO_4$ were used to overcome the above problems. Ashing in the presence of KOH and $ZnSO_4$ together results in the formation of very hard ash, hence ashing was carried out in two stages; first with KOH and then with $ZnSO_4$. A variety of samples differing in the nature of their organic matrix were successfully ashed with the given concentration of reagents. The total time required to complete the ashing ranged between 1 and 3 h. Samples such as fish, prawn, serum, urine and other biological materials were incinerated within 1·5 h, whereas foodstuffs such as cereals, pulses and oil seeds took 2–3 h.

Table 1. Recovery of Iodine in the Form of Na^{125}I and ^{125}I-Labelled Thyroglobulin

Source of ^{125}I	Percentage recovery[a]
Na^{125}I	95·2 ± 1·83
^{125}I-Thyroglobulin	98·0 ± 1·68

[a]Values are mean ± SD.

Recovery of iodine added

Iodine was added to the sample in the form of KI, Na^{125}I and ^{125}I-labelled thyroglobulin before ashing and carried through the complete procedure. The results are given in Tables 1 and 2. The recoveries were found to be in the range of 93·7–102·3% with a mean and standard deviation of 97·2 ± 3·32%. Lauber (1975) had also reported a 2·5% loss of iodine that is 97·5% recovery when ^{125}I thyroxine was incinerated as a test sample.

The performance of the method was also tested by analysing a reference standard material NBS-SRM-1549. The iodine content was found to be 3·18 ± 0·074 μg g^{-1} (n = 22), as against the certified value of 3·38 ± 0·2 μg g^{-1}. Casein (ICN Nutritional Biochemicals) was used as a laboratory material with every analysis. The coefficient of variation in iodine content (0·78 ± 0·030 μg g^{-1}) of the reference sample was only 3·5%. The analysis of IAEA-A-II, reference milk sample however gave a very low iodine content, 0·095 ± 0·0123 μg g^{-1} (n = 14) as against the non-certified value of 1·5 μg g^{-1}.

Table 2. Recovery of Iodine Added in the Form of KI to Different Foodstuffs and Water

Sample	Iodine present (ng)	Iodine added (ng)	Total iodine found[a] (ng)	Percentage recovery[a] (ng)
Rice	23·4	20	40·6 ± 0·47	93·7 ± 1·09
Tomato	1·5	3	4·6 ± 0·20	102·3 ± 4·5
Prawn	5 938	3 000	8 538 ± 201·88	95·5 ± 2·25
Casein	83·5	100	186 ± 2·18	101·4 ± 1·19
Fish	51·6	50	89·5 ± 1·20	97·7 ± 1·3
Water	5·7	5	10 ± 0·12	93·5 ± 1·12

[a]Values are mean ± SD.

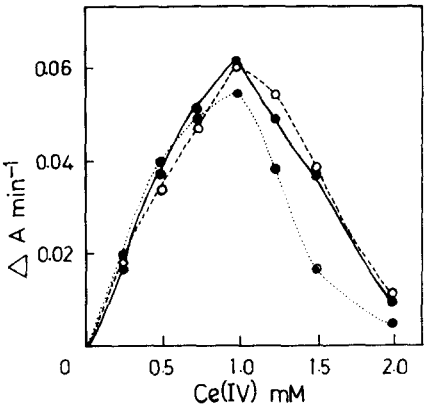

Fig. 3. Effect of varying concentrations of Ce(IV) on the initial velocity of the reaction. Initial velocities were measured in the presence of [As(III)]: (⊙), 9.99×10^{-4} M; (○), 6.00×10^{-3} M; (●), 1.00×10^{-3} M, keeping the concentration of iodine at 9.45×10^{-9} M.

Kinetics of the reaction

Rate dependence on [Ce(IV)]

Figure 3 represents the effect of varying concentrations of Ce(IV) 0–2 $\times 10^{-3}$M, on the rate of reaction. Initial velocities, i.e. a decrease in absorbance, were plotted as a function of Ce(IV) concentration. The effect of varying concentrations of Ce(IV) was studied at three different concentrations of As(III), viz. 9.99×10^{-4}M, 6.00×10^{-3}M and 1.00×10^{-2}M and a single iodide concentration of 9.45×10^{-9}M. It was observed that the rate of reaction increased as the Ce(IV) concentration increased to 9.99×10^{-4}M, but a further increase in the concentration to 1.99×10^{-3}M resulted in the loss of catalytic activity by more than 80% irrespective of the As(III) concentration. This may be due to the oxidation of iodine to a non-catalytic form by high concentrations of Ce(IV) (Rodriguez & Pardue, 1969).

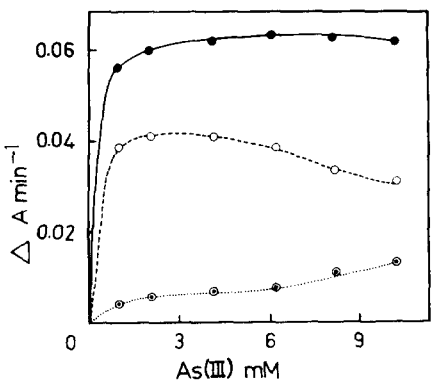

Fig. 4. Effect of varying concentration of As(III) on the iodide catalysed reduction of Ce(IV). Initial velocities were measured at three levels of [Ce(IV)]: (○), 4.99×10^{-4} M; (●), 9.99×10^{-4} M; (⊙), 1.99×10^{-3} M, keeping the iodide concentration at 9.45×10^{-9} M.

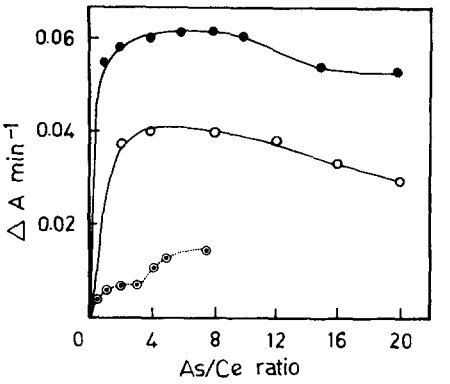

Fig. 5. A plot of [As(III)]/[Ce(IV)] ratios versus initial velocities. The concentrations are as for Fig. 4.

Rate dependence on [As(III)]

The effect of varying concentrations of As(III) on the initial velocity of iodide catalysed reaction is illustrated in Fig. 4. The reaction was carried out in the presence of three concentrations of Ce(IV), viz. 4.99×10^{-4}M, 9.99×10^{-4} and 1.99×10^{-3}M, and a single iodide concentration of 9.45×10^{-9}M. The reaction followed first order kinetics at low concentrations ($<1 \times 10^{-3}$M) of As(III). Catalytic activity of iodide was maximum at a Ce(IV) concentration of 4.99×10^{-4}M irrespective of the As(III) concentration. Thus, the initial velocity of the reaction was affected by the individual concentrations of the reactants and it was also found to be dependent on the ratio of the concentrations of the reactants (Fig. 5). Hence, for the rate of reaction to be proportional only to the concentration of iodine, the ceric concentration was fixed at 9.99×10^{-4}M and that of arsenic at 6.00×10^{-3}M, keeping the ratio of their concentration at 6.

Other factors such as reaction time, temperature and concentrations of acids such as H_2SO_4, HCl, HNO_3 are known to affect the reaction rate (Lauber, 1975). Therefore in the present procedure the reaction time (1 min), temperature (37°C) and concentrations of HNO_3 (1·55M), H_2SO_4 (0·25M) and HCl (0·013M) were kept constant.

Precision and detection limit

The coefficient of variation was calculated to be less than 6% with a mean and standard deviation of 3·4 ± 1·77%, when more than 20 foodstuffs were analysed on three or more occasions. The sensitivity of the present method was calculated according to Aumont and Tressol (1987). The sensitivity of the kinetic assay is 0·04 ng per ml at an iodine level of 0·4 ng per ml of ash solution. Samples having ≥ 20 ng of iodine per gram of dried foodstuff can be successfully analysed using the above described method.

Applicability

More than 20 samples varying in complex organic mat-

Table 3. Iodine Content of Food Samples[a]

Sample	Scientific name	No. of analyses	μg I per 100 g Mean ± SD	Coefficient of variation
Food grains				
Rice	*Oryza sativa*	6	43·8 ± 1·95	4·45
Ragi	*Eleusine coracara*	3	18·3 ± 0·94	5·12
Pea	*Pisum sativam*	3	34·7 ± 1·24	3·57
Vegetables				
Amaranth leaves	*Amaranthus gangeticus*	3	16·3 ± 0·94	5·77
Mint leaves	*Mentha spicata*	3	8·7 ± 0·09	1·08
Tomato	*Lycopersicum esculentum*	3	1·5 ± 0·04	2·61
Ladies finger	*Abelmoschus esculentus*	3	4·5 ± 0·14	3·11
Roots and tubers				
Beetroot	*Beta vulgaris*	3	16·7 ± 0·94	5·65
Potato	*Solanum tuberosum*	3	1·9 ± 0·09	4·89
Spices				
Red chillies (dry)	*Capsicum annuum*	5	40·0 ± 1·41	3·53
Clove	*Syzygium aromaticum*	4	6·1 ± 0·21	3·53
Pepper	*Piper nigrum*	3	35·6 ± 0·28	0·79
Oil seeds				
White mustard	*Sinapsis alba L.*	5	50·6 ± 1·09	2·16
Ground nut	*Arachis hypogia*	3	55·3 ± 0·33	0·61
Fruits				
Grapes	*Vitis vinifera*	3	9·6 ± 0·04	0·48
Apple	*Malus sylvestris*	3	5·3 ± 0·16	3·23
Foodstuffs, animal origin				
Milk		10	12·3 ± 0·70	5·68
Egg (hen)		3	50·7 ± 0·94	1·85
Fish (fresh water)		3	79·0 ± 3·26	4·12
Prawn (marine)		5	11·3 ± 0·36[b]	3·21
Tissues and body fluids				
Serum PBI		5	5·8 ± 0·16[c]	2·81
Urine		10	66·8 ± 0·32[d]	1·98
Thyroid tissue (bovine)		3	14·3 ± 0·95[b]	6·57

[a] Iodine content of food grains, spices, oil seeds, egg, fish, prawn and bovine thyroid tissue was expressed on a dry weight basis; vegetables on a 90% moisture basis; roots, tubers and fruits on an 80% moisture basis.
[b] mg per 100 g dried powder.
[c] μg per 100 ml of serum.
[d] μg per gram creatinine.

ter were analysed for their iodine content. The results are shown in Table 3. In addition, the present method was also found to be applicable to the estimation of iodine content in serum, urine and other biological material. Twenty serum samples (from normal healthy volunteers) were analysed for their protein bound iodine (PBI). The PBI content of serum ranged from 4·6 to 9·3 μg per 100 ml with an average of 6·88 μg.

The above-reported spectrophotometric kinetic assay has definite advantages in being very sensitive and quite versatile. In addition to this it can be easily adapted with the minimum of equipment.

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REFERENCES

Aumont, G. & Tressol, J. C. (1986). Improved routine method for the determination of total iodine in urine and milk. *Analyst*, **111**, 841–3.

Aumont, G. & Tressol, J. C. (1987). Rapid method for the direct determination of inorganic iodine in plasma using ion-exchange chromatography and the Sandell and Kolthoff reaction. *Analyst*, **112**, 875–8.

Bakker, H. J. (1977). Gas–liquid chromatographic determination of total inorganic iodine in milk. *J. Assoc. Off. Anal. Chem.*, **60**, 1307–9.

Barker, S. B., Himphrey, M. J. & Soley, M. H. (1951). The clinical determination of protein-bound iodine. *J. Clin. Invest.*, **30**, 55–62.

Belling, G. B. (1983). Further studies on the recovery of iodine-125 after alkaline ashing prior to assay. *Analyst*, **108**, 763–5.

Boratynski, J. (1987). A micromethod for the determination

of an unbound radioactive iodine in radiolabelled glyproteins by means of uranyl acetate. *Anal. Biochem.*, **160**, 35–8.

Crecelius, E. A. (1975). Determination of total iodine in milk by X-ray fluorescence spectrometry and iodide electrode. *Anal. Chem.* **47**, 2034–5.

Fisher, P. W. F., L'able, M. R. L. & Giroux, A. (1986). Colorimetric determination of total iodine in foods by iodide-catalysed reduction of Ce^{4+}. *J. Assoc. Off Anal. Chem.*, **69**, 687–9.

Foss, O. P., Hankes, L. V. & Van Slyke, D. D. (1960). A study of the alkaline-ashing method for determination of protein-bound iodine in serum. *Clin. Chem. Acta*, **5**, 301–26.

Greenwood, F. C., Hunter, W. M. & Glover, J. S. (1963). The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. *Biochem. J.*, **89**, 114–23.

Joerin, M. M. (1975). A rapid method of determining total iodine in bovine milk. *Analyst*, **100**, 7–11.

Johansen, O. & Steinnes, E. (1976). Determination of iodine in plant material by a neutron-activation method. *Analyst*, **101**, 455–7.

Jones, G. B., Belling, G. B. & Buckley, R. A. (1979). Recovery of iodine as iodine-125 from biological materials prior to assay. *Analyst*, **104**, 469–71.

Lauber, K. (1975). Iodine determination in biological material. Kinetic measurement of the catalytic activity of iodide. *Anal. Chem.*, **47**, 769–71.

Miles, P. (1978). Determination of iodide in nutritional beverage products using an ion selective electrode. *J. Assoc. Off. Anal. Chem.*, **61**, 1366–9.

Moxon, R. E. D. & Dixon, E. J. (1980). Semi-automatic method for the determination of total iodine in food. *Analyst*, **105**, 344–52.

Riesco, G., Taurog, A. & Larsen, P. R. (1976). Variations in the response of the thyroid gland of the rat to different low-iodine diets. Correlation with iodine content of diet. *Endocrinology*, **99**, 270–80.

Rodriguez, P. A. & Pardue, H. L. (1969). Kinetics of the iodide-catalyzed reaction between Cerium(IV) and Arsenic(III) in sulphuric acid medium. *Anal. chem.*, **41**, 1369–76.

Sandell, E. B. & Kolthoff, I. M. (1934). Chronometric catalytic method for the determination of microquantities of iodine. *J. Am. Chem. Soc.*, **56**, 1426.

Sandell, E. B. & Kolthoff, I. M. (1937). Microdetermination of iodine by a catalytic method. *Microchim Acta*, **1**, 9–25.

Thompson, D., Lee, S. & Allen, R. (1983). Differential pulse polarographic determination of iodine in foods and nutritional products. *J. Assoc. Off. Anal. Chem.*, **66**, 1380–4.

Wenlock, R. W., Buss, D. H., Moxon, R. E. & Bunton, N. G. (1982). Trace nutrients 4. Iodine in British food. *Br. J. Nutr.*, **47**, 381–90.

Zak, B., Willard, H. H., Myers, G. B. & Boyle, A. J. (1952). Chloric acid method for determination of protein-bound iodine. *Anal. Chem.*, **24**, 1345–8.