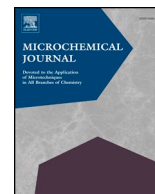




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Determination of iodine content in Fijian foods using spectrophotometric kinetic method

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ABSTRACT

The spectrophotometric kinetic method was validated for the determination of iodine in different food samples. The method is based on the iodide catalysed reduction of Ce^{4+} to Ce^{3+} by As^{3+} . The absorbance of the kinetic indicator reaction was measured at 370 nm for exactly 1 min at 37 °C. The change in absorbance per min, as a measure of initial rate, was plotted against the different iodine concentrations to achieve a linear calibration equation with the R^2 value of 0.9998 which showed excellent reproducibility. The limit of detection (LOD) was 1.54 ng/mL and the limit of quantification (LOQ) was 4.90 ng/mL. The incineration of the food organic matter was achieved by ashing the food samples at 600 °C using KOH and $ZnSO_4$ in steps for 3 h. Trace levels of iodine (ng) were determined successfully using the validated spectrophotometric kinetic method for the 9 food samples (36 sub-samples). The Fiji seaweeds, lumiwawa (brown seaweed) showed the highest iodine content being 6373.30 ± 0.39 ng/g followed by sea grapes (green seaweed) 1162.81 ± 0.61 ng/g, lettuce 114.81 ± 0.08 ng/g, English cabbage 108.40 ± 0.06 ng/g, Chinese cabbage 104.01 ± 0.06 ng/g, pumpkin 101.24 ± 0.08 ng/g, long bean 97.61 ± 0.10 ng/g, banana 76.18 ± 0.10 ng/g and tomato 40.32 ± 0.04 ng/g. The coefficient of variation for the sample analysis was $< 5.31\%$ with a mean and standard deviation of $2.55 \pm 0.17\%$ for the food samples analysed. The recovery analysis of iodine from standard samples ranged from $99.84 \pm 0.91\%$ to $100.24 \pm 5.92\%$ with an excellent average recovery of $100.06 \pm 3.16\%$. The analytical coefficient of variation was calculated to be 0.34% for the food samples analysed. This shows exceptional system analytical stability of the method used in this study.

1. Introduction

Iodine is an essential trace element and of much interest in nutritional research. In the human body, it is essential for the production of triiodothyronine (T_3) and thyroxine (T_4) hormones which are responsible to regulate body temperature and metabolic rate in adults and children and thus for the proper functioning and the development of the human body [1]. Iodine also helps in the maturation of the central nervous system, and the development of foetal and early postnatal life [2]. The most known adverse effect of iodine deficiency is goitre. Goitre is however just one effect of iodine deficiency, the others include endemic cretinism, infant mortality, infertility, miscarriage, mental retardation, neuromuscular defects, and dwarfism. All these are commonly known as Iodine Deficiency Disorders (IDDs) [3]. It is therefore particularly important that pregnant women, breast feeding mothers and young children have an adequate dietary iodine intake [4]. Therefore, the International Council for Control of Iodine Deficiency Disorders (ICCIDD), United Nations Children's Fund (UNICEF) and

World Health Organisation (WHO) recommended that the daily intake of iodine should be 90 µg for preschool children (0 to 59 months), 120 µg for school children (6 to 12 years), 150 µg for adolescents (above 12 years) and adults, 250 µg for pregnant and lactating women [5–7].

People get their iodine intake from iodised salt, however this does not always fulfil the requirements for recommended iodine intake levels. The natural dietary sources of iodine in foods include; milk, cereals, fruits, vegetables, eggs, meat, spinach and sea foods. These natural sources may not satisfy the requirements of iodine intake in humans as these iodine sources may not be bioavailable in a form as needed by the human body and also that the iodine concentrations are low [8]. As most iodine enters the human body through food intake so the knowledge of iodine contents in different foods and natural products is essential to estimate the daily iodine intake [4,9]. However, difficulties in the extraction and quantification explain why literature data on the iodine levels in foods are quite limited [10]. Therefore, various analytical methods have been used for the determination of trace amounts of iodine in different types of samples. These include inductively coupled

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plasma mass spectrometry (ICP-MS) [2,3,11–15], radiochemical neutron activation analysis (RNAA) [9,16], ion chromatography (IC) [17–21], high performance liquid chromatography (HPLC) [22], HPLC with UV detection [4], HPLC-diode array detection [23], inductively coupled plasma–atomic emission spectrophotometry (ICP-AES) [24] and atomic absorption spectrophotometry (AAS) [25]. Most of the techniques mentioned above, except RNAA, are not selective, suffer from interferences and need pre-concentration or separation procedures which lead to the loss of iodine [1]. In addition, all these methods require expensive instrumentation and complex sample preparation. Another reason is that iodine concentrations in food matrices are low and losses due to its high volatility make it challenging to analyse [11]. Thus, the majority of methods use the initial step for the determination of iodine in biological materials. This requires the conversion of iodine into iodide or iodate which may be reliably analysed. The conversions are mostly carried out either in dry alkaline medium in a muffle furnace at 600 °C or wet ashing involving digestion using a strong acid medium. Reproducible results have been obtained only when the losses of iodine are avoided at the incineration stage [26].

Being inexpensive, spectrophotometric kinetic methods to determine iodine content in food and dairy products has continuously been used [10,15,27–29]. Kinetic spectrophotometric procedures have also been utilized to determine the analytes such as drugs in different matrices which highlight the importance of kinetic spectrophotometry [30–32]. It is an attractive procedure because of its high sensitivity and accuracy without using expensive equipment [6]. Sometimes, the insufficient accuracy of the analytical methods used for iodine analysis contribute to the problem [26]. Since food is being the major contributor of the total iodine exposure for humans, ascertaining the iodine nutritional status of Fiji foods and dairy products is of great importance as part of the public health programs. Therefore, the present paper reports the validation of an inexpensive spectrophotometric kinetic method and the determination of iodine content in selected Fiji foods.

2. Method

2.1. Chemicals and reagents

All chemicals used were of high purity meeting the American Chemical Society (ACS) reagents requirements except arsenic trioxide (As₂O₃), potassium hydroxide (KOH) and zinc sulphate (ZnSO₄) which were analytical reagents (AR) purchased from Sigma-Aldrich, Australia. Thus, the ACS reagents potassium iodide (KI, ≥99.0%), diammonium ceric nitrate ((NH₄)₂Ce(NO₃)₆, ≥98.5%), nitric acid (HNO₃, 70%), hydrochloric acid (HCl, ≥37%), sulphuric acid (H₂SO₄, 95–98%), KOH (AR grade), As₂O₃ (≥99.0%) and ZnSO₄ (AR grade) were used in this study. The Standard Reference Material Iodised Table Salt (SRM No. 3530) was purchased from the National Institute of Standards and Technology (NIST), USA.

Iodine stock standard was prepared by dissolving 130.8 mg KI in 1 L Milli-Q-water (MQW). Iodine working standards (in the range of 2.5–25 ng/mL) were prepared by diluting its stock solution. H₂SO₄ and HCl combined reagent was prepared by adding 19.6 mL concentrated H₂SO₄ to 500 mL water, mixed well and cooled to room temperature. Then 5.4 mL concentrated HCl was added, mixed and diluted to 1 L with MQW. Ce⁴⁺ solution (0.05 M) was prepared from (NH₄)₂Ce(NO₃)₆ by dissolving 0.274 g in 10 mL MQW. To this, 50 mL concentrated HNO₃ was added followed by addition of 5 mL H₂SO₄. The solution was allowed to cool to room temperature and made up to 100 mL with MQW. 0.030 M As³⁺ solution was prepared by dissolving 0.593 g As₂O₃ and 0.6 g KOH in 100 mL MQW at 50 °C. 6 M KOH solution was prepared by dissolving 168.33 g KOH in 500 mL MQW. 0.52 M ZnSO₄ solution was also prepared by dissolving 74.76 g ZnSO₄ in 500 mL MQW.

2.2. Instrumentation

An oven with an automatic temperature control was used to maintain the temperature at 120 ± 0.1 °C. A high temperature Muffle furnace with a programmable temperature setup (Model: YC-1400S) was used for ashing of samples at 600 °C. Perkin Elmer Lambda 365 UV visible spectrophotometer equipped with 10 mm quartz cells with a thermostatic water bath (Thermoline, Australia) was used. The absorbance for the reduction of Ce⁴⁺ in the presence of As³⁺ at fixed time of 1 min was measured at 370 nm.

2.3. Food samples and sampling

The foods namely: leafy [(lettuce (*Lactuca sativa*), English cabbage (*Brassica oleracea*), Chinese cabbage (*Brassica chinensis*)], fruit [(tomato (*Solanum lycopersicum*), banana (*Musa*), long green bean (*Vigna unguiculata* ssp. *sesquipedalis*) and pumpkin (*Cucurbita moschata*)] and sea grapes/green seaweeds (*Caulerpalentillifera*) and lumiwawa/brown seaweeds (*Gracilaria maramae*) were analysed for their iodine contents. The samples were purchased randomly from the Suva Municipal market and supermarkets. For each food item, 4 samples of different brands or varieties from 4 different vendors were purchased. Thus, a total of 9 food samples consisting 36 sub-samples were analysed on a fresh weight basis.

Fresh food samples were collected into clean polythene bags and kept in an ice box. The samples were immediately transported to the laboratory. The edible part of each sample was individually taken out and pulverized to obtain particles of required size. The samples which could not be analysed on the same day were kept at –10 °C in clean acid washed screw capped plastic bottles until analysis.

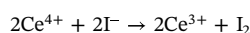
2.4. Ashing procedure and extraction

The alkaline ashing procedure reported by Mahesh et al. [10] as well as Nitschke and Stengel [4] was modified and used for the determination of iodine in food samples. The fresh food samples (1 g) were taken in clean dry test tubes in duplicate. For recovery studies, standard solutions containing 4, 12 and 18 ng/mL iodine were analyzed. To the food samples test tubes, 500 µL of 6 M KOH was added and mixed well. The test tubes containing samples were then placed in an oven at 120 ± 0.1 °C and the contents were allowed to dry completely for 24 h. The test tubes containing the samples were then transferred to the Muffle furnace, which was operating at 120 °C for 30 min. The temperature of the Muffle furnace was gradually increased to 600 °C over 30 min and incineration was continued for exactly 1 h.

Air was renewed in the Muffle furnace chamber every 15 min via a timer by opening the furnace door for 10–15 s. After 1 h the test tubes with incinerated samples were transferred to a desiccator and allowed to cool. Then 500 µL of 0.52 M ZnSO₄ was added to the samples in the test tubes and the contents dried at 120 °C for 3 h. The test tubes with samples were then transferred to the Muffle furnace and another ashing procedure was performed for 2 h at 600 °C renewing the air in the chamber every 15 min. The ashed samples which were white powder, free from any carbon, were dissolved in MQW in an ultrasonic bath for 10 min then the samples were centrifuged at 4500 rpm in centrifuge tubes for 10 min. The supernatant was filtered using a 0.45 µm filter and stored at room temperature for analysis of total iodine.

2.5. Analytical procedure sample analysis

The kinetic spectrophotometric method using the Sandell-Kolthoff reaction as shown below was used to determine the iodine concentration in food samples.



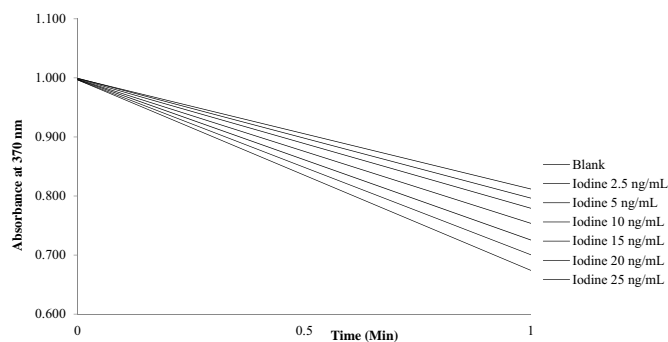
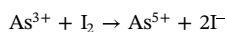


Fig. 1. Plot of average absorbance at 370 nm for the reduction of Ce^{4+} by As^{3+} against time in the presence of different iodine concentrations of 0, 2.5, 5, 10, 15, 20 and 25 ng/mL at analysis time of 1 min at 37°C.



The method was based on the catalytic effect of iodide on $\text{Ce}^{4+} - \text{As}^{3+}$ redox reaction. To initiate the indicator reaction, 0.25 mL MQW, 0.25 mL of $\text{H}_2\text{SO}_4\text{-HCl}$ mixture, 0.25 mL Ce^{4+} and 0.25 mL As^{3+} reagents were taken in sequence in a 10 mm path length cuvette. The contents of the cuvette were mixed for 10 s and pre-incubated at 37 °C for 2 min. The 0.25 mL catalyst iodide was added in blank/standard/sample to initiate the reaction. The decrease in the absorbance as a measure of iodine content i.e. the rate of disappearance of the yellow colour due to the reduction of Ce^{4+} to Ce^{3+} at different iodine concentrations (0 to 0.25 ng/mL) was monitored for 1 min at 370 nm [10] as shown in Fig. 1.

The initial rate of the indicator reaction i.e. $\Delta A/\text{min}$ was calculated from the time-absorbance (A) curves obtained for different iodine concentrations. Each standard was analysed 7 times and the average change in absorbance per minute was calculated and used for the calibration curve as shown in Fig. 2.

Then the iodine content of the samples was calculated using the following formula where ΔA_s is the absorbance change for the sample, ΔA_b is the absorbance change for the blank, m is the slope of calibration curve and d is the sample dilution (mL).

$$\frac{\Delta A_s - \Delta A_b}{m} \times 4 \times d = \text{iodine (ng/g)}$$

Table 1

Mean iodine contents ($n = 4$) in food samples analysed on a fresh weight basis.

Fresh samples (Scientific name)	Iodine content (ng/g)		
	Mean \pm SD ^a	Minimum (Mean \pm SD ^a)	Maximum (Mean \pm SD ^a)
Leafy vegetables			
Lettuce (<i>Lactuca sativa</i>)	114.81 \pm 0.08	40.15 \pm 0.03	178.17 \pm 0.14
English cabbage (<i>Brassica oleracea</i>)	108.40 \pm 0.06	56.92 \pm 0.03	145.81 \pm 0.07
Chinese cabbage (<i>Brassica chinensis</i>)	104.01 \pm 0.06	44.64 \pm 0.04	132.87 \pm 0.12
Fruits			
Tomato (<i>Solanum lycopersicum</i>)	40.32 \pm 0.04	24.17 \pm 0.02	53.62 \pm 0.04
Banana (<i>Musa</i>)	76.18 \pm 0.10	21.40 \pm 0.04	183.06 \pm 0.23
Long bean (<i>Vigna unguiculata ssp. sesquipedalis</i>)	97.61 \pm 0.10	37.64 \pm 0.04	183.98 \pm 0.19
Pumpkin (<i>Cucurbita moschata</i>)	101.24 \pm 0.08	48.87 \pm 0.03	183.58 \pm 0.20
Seaweeds			
Sea grapes (<i>Caulerpa lentillifera</i>)	1162.81 \pm 0.61	851.92 \pm 0.34	1525.83 \pm 0.83
Lumiwawa (<i>Gracilaria maramae</i>)	6373.30 \pm 0.39	2438.68 \pm 0.10	11,000.00 \pm 0.32

Confidence level at 95% with 3 degrees of freedom ($t = 3.182$).

SD - Standard deviation.

^a Mean of four samples of each food analysed four times ($n = 4$).

3. Results and discussion

3.1. Food samples ashing and analysis

Incineration of organic matter (OM) is generally based on acid or alkaline digestion. Acid digestion methods, as discussed in the literature, are generally effective in the destruction of OM but are not recommended for routine analytical purposes due to the fact that they require large amounts of concentrated acids for each sample [3,4,11–13,29,33–36]. Thus, the use of acid digestion becomes impractical when analysing a large number of samples manually. The use of alkaline dry ashing involving 30% K_2CO_3 and 10% ZnSO_4 resulted in incomplete ashing and poor recoveries of iodine from plants, biological materials and foodstuffs [10]. Incomplete ashing and poor recoveries were also observed when KOH was used as an ashing agent. Thus, to overcome all these problems, Mahesh et al. [10] used 0.1 mL 6 M KOH and 0.1 mL 0.5 M ZnSO_4 and ashing of the samples was carried out in two steps: 1 h with KOH and 2 h with ZnSO_4 .

In the current study, initially poor recoveries were obtained when the procedure specified by Mahesh et al. [10] was followed and this was attributed to incomplete ashing and the presence of OM. Thus, to overcome this problem, a modified alkaline ashing procedure reported by Nitschke and Stengel [4] was referred to which used < 200 mg of sample, 400 μL of 17 M KOH and dried at 150 °C for 48 h. This was followed by 4 h incineration at 600 °C. Finally, to overcome the poor recoveries, all the food samples in the present study were analysed by our developed ashing procedure discussed in Section 2.4. The iodine contents in the studied food samples (Table 1) were calculated using the calibration equation shown in Fig. 2.

The summary of the determined iodine contents in 9 food samples of three groups (vegetables, fruits and seaweeds) shown in Table 1 clearly shows that the brown seaweed lumiwawa had the highest iodine content being 6373.30 \pm 0.39 ng/g followed by sea grapes 1162.81 \pm 0.61 ng/g, lettuce 114.81 \pm 0.08 ng/g, English cabbage 108.40 \pm 0.06 ng/g, Chinese cabbage 104.01 \pm 0.06 ng/g, pumpkin 101.24 \pm 0.08 ng/g, long bean 97.61 \pm 0.10 ng/g, banana 76.18 \pm 0.10 ng/g and tomato 40.32 \pm 0.04 ng/g. A comparison of iodine contents for all the food samples is shown in Fig. 3. It has also been confirmed by other researchers that brown seaweeds generally have the highest iodine content [4,12,27,36,37]. The lowest iodine values were witnessed in fruits and vegetables. Almost similar results have also been reported by previous researchers while slight variation may be due to the food samples of different origins [13,38–41].

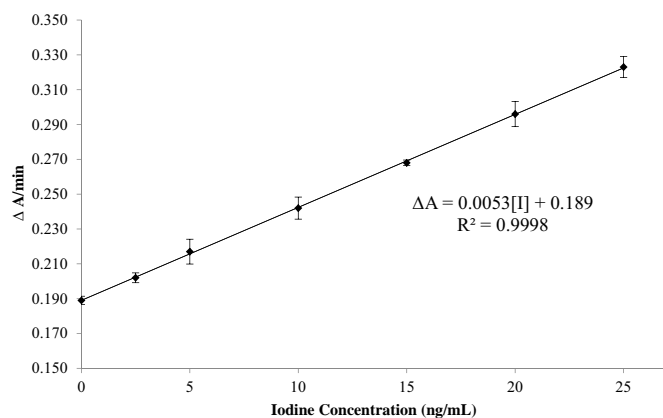


Fig. 2. Calibration curve i.e. plot of change in absorbance per minute ($\Delta A/\text{min}$) versus iodine concentration.

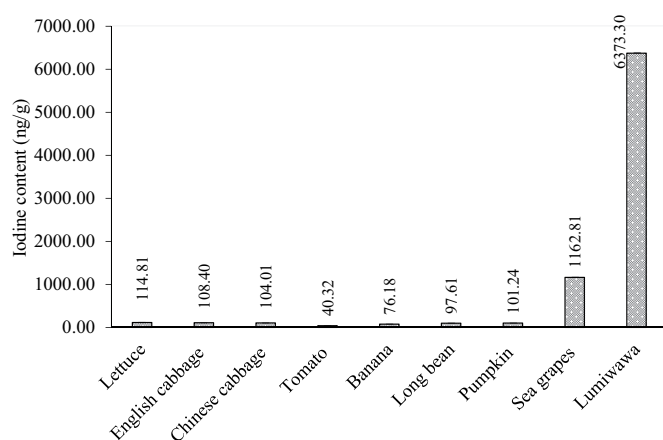


Fig. 3. Graphical representation of determined average iodine contents (ng/g) in the analysed food samples.

3.2. Precision, limit of detection (LOD) and limit of quantification (LOQ)

For all the food samples analysed, the coefficient of variation was calculated. It has been reported that a coefficient of variation $< 6.0\%$ with a mean and standard deviation of $3.4 \pm 1.77\%$ for > 20 foodstuff analysed on three or more occasions was sufficient to judge the precision. [10]. The coefficient of variation for the food samples analysis in the present study was also $< 6.0\%$ with a mean and standard deviation of $2.55 \pm 0.17\%$ for the 9 samples analysed each four times. Thus, this method was quite precise. The analytical coefficient of variation in the present study was worked out to be 0.34% for the 9 food samples analysed which showed exceptional system analytical stability.

The LOD was calculated by multiplying the sample standard deviation by the Student's t value. Thus, for seven replicates and six degrees of freedom, the student's t was taken as 3.143 at 98% confidence level [39]. The LOD was found to be 1.54 ng/mL . The LOQ was

Table 2

Recovery study from the standard iodine solutions at 4, 12, and 18 ng/mL.

Iodine concentration (ng/mL)	Nominal \pm SD ^a (ng/mL)	Recovery \pm RSD (%)	Standard analytical error (%)	Confidence interval ^b
4.0	4.01 ± 0.24	100.24 ± 5.92	0.60	0.38
12.0	11.98 ± 0.11	99.84 ± 0.91	0.23	0.17
18.0	18.02 ± 0.47	100.10 ± 2.64	0.88	0.76

^a Mean for four determinations ($n = 4$).

^b Confidence level at 95% with 3 degrees of freedom ($t = 3.182$).

determined following the method reported by Wisconsin Department of Natural Resources Laboratory Certification Program [42] expressed as 10 times the sample standard deviation and calculated to be 4.9 ng/mL .

3.3. Quality control

The analyses of known concentrations of 4, 12 and 18 ng/mL iodine were used to monitor the statistical control i.e. for the recovery study. In addition, each food sample was analysed 4 times ($n = 4$) from which the average iodine contents were calculated. The coefficient of variation and the confidence interval were also calculated for each sample to judge the degree of variation. The recovery ranged from 99.84 to 100.24%, as presented in Table 2, represents an excellent quality control.

3.3.1. Analysis of standard reference materials (SRM)

The Iodised Table Salt (SRM No. 3530) was analysed to judge the accuracy of the spectrophotometric kinetic method used for iodine determination. The adequacy of applied methodology was verified by usual measures of accuracy, reproducibility and recovery in which 300 mg of the SRM No. 3530 was dissolved in 1 L of MQW which gave 15.66 ng/mL iodine concentration. The sample was then placed in an ultrasonic bath for 10 min for complete dissolution of the salt prior to the spectrophotometric kinetic analysis. The recovery results obtained in terms of ng/mL which was further converted to mg/kg as reported in NIST SRM No. 3530 certificate are shown in Table 3.

Table 3 also compares the iodine content obtained from the SRM analysis by the present spectrophotometric kinetic method to the certified value of the Iodised Table Salt ($52.2 \pm 4.2 \text{ mg/kg}$) analysed using ICP-MS at NIST, USA. The recovery data shows great accuracy of the present method with excellent recovery of $99.68 \pm 2.38\%$. Thus, the method applied in the present study was very reliable for the determination of iodine contents in food samples.

4. Conclusions

It has been confirmed that trace levels of iodine (ng/g) can be analysed successfully using the modified ashing procedure and the spectrophotometric kinetic method validated. Thus, some commonly consumed Fiji foods were successfully analysed for their iodine content using the spectrophotometric kinetic method. The study confirmed that the seaweed lumiwawa (brown seaweed) had the highest iodine concentration of $6373.30 \pm 0.39 \text{ ng/g}$ among all the food samples studied while sea grapes (green seaweed) had iodine content of $1162.81 \pm 0.61 \text{ ng/g}$. Low iodine contents were shown by the fruit group where tomato showed the lowest iodine content as $40.32 \pm 0.04 \text{ ng/g}$. The data presented give clear indication of the iodine contents of some commonly consumed foods in Fiji and form a basic database on iodine levels from foods in the country. The spectrophotometric kinetic method used has definite advantages of being very sensitive, versatile and can be adapted as an inexpensive method for the determination of iodine in foods.

Table 3

Summary of the recovery results obtained from NIST SRM No. 3530 – (Iodised Table Salt) analysis.

Iodine concentration (ng/mL)	Iodine found \pm SD ^a (ng/mL)	SRM certified iodine \pm SD (mg/kg)	Iodine found in SRM \pm SD ^a (mg/kg)	Recovery \pm RSD (%)
15.66	15.61 \pm 0.37	52.2 \pm 4.2	52.03 \pm 0.37	99.68 \pm 2.38

^a Mean for seven determinations ($n = 7$).

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