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Ammonium persulfate: a safe alternative oxidizing reagent for measuring urinary iodine

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The chloric acid method is most commonly used to obtain accurate and reproducible measurements of iodine and remove interfering substances. Unfortunately, chloric acid is a potential hazard, requiring an explosion-proof hood, among other precautions. We have developed a simple, convenient, and economic method for measuring urinary iodine by using 1 mol/L ammonium persulfate, a nonexplosive, nonhazardous chemical, as the oxidizing reagent. The oxidation procedure can be completed in 30 min at a temperature of 91–95 °C. The iodine in the urine is then measured by a modification of the traditional colorimetric method of Sandell and Kolthoff. Urine samples (110) collected from a mixed population of healthy males and females, ranging in age from 6 to 79 years and living in the US, were analyzed for urine iodine content by two methods: the proposed ammonium persulfate method and the chloric acid method. The ammonium persulfate method has an intraassay CV of 9.1% at 0.42 ± 0.04 μmol/L (mean ± SD), 7.8% at 1.46 ± 0.11 μmol/L, and 4.0% at 3.54 ± 0.14 μmol/L. The interassay CV is 10.2% at 0.46 ± 0.05 μmol/L and 7.9% at 3.27 ± 0.26 μmol/L. Recovery of iodine added to urine in vitro was 107%, 94%, and 97% for 0.42 μmol/L, 0.77 μmol/L and 3.64 μmol/L, respectively. The lower limit of detectability was 0.0034 μg of iodine. Values for iodine in 110 urines measured by the reference chloric acid method ranged from 0.06 to 8.03 μmol/L and by the ammonium persulfate method from 0.05 to 7.4 μmol/L. The persulfate method (y) correlated extremely closely with the reference chloric acid method (x) by the Pearson correlation (y = 0.923x + 0.810 μmol/L, and r = 0.994, $S_{y|x}$ = 1.841).

INDEXING TERMS: chloric acid • colorimetric assay • methods comparison

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Measurement of urinary iodine is the most common method to monitor dietary iodine intake *[1–4]*. However, the methods used are usually affected by interfering substances present in urine. These substances may contribute positively to the iodine catalytic effect when using the traditional method of Sandell and Kolthoff *[5]*, which depends on the reduction of ceric sulfate by arsenite in the presence of iodine. Therefore, to obtain accurate measurements it is necessary to eliminate the interfering substances before the colorimetric analysis. We have routinely used the standard method of Benotti et al. *[6]*, which involves a manual chloric acid digestion at 105–115 °C to remove the interfering substances before the automated colorimetric analysis with a Technicon autoanlayzer.

Although chloric acid digestion eliminates the interfering substances effectively to provide accurate measurements of urinary iodine *[6, 7]*, it has drawbacks. Chloric acid is difficult to locate from chemical vendors and hence has to be prepared in the laboratory by using perchloric acid and potassium chlorate. Chloric acid is a health hazard, requiring a special fume hood for the liberation of fumes, including chlorine gas, during the sample digestion. Both chloric acid and potassium chlorate are also potentially explosive. Our aim was to seek an alternative oxidizing agent that provides an accurate measurement of urinary iodine and is nonhazardous, nonexplosive, and economical.

The present method includes ammonium persulfate as the oxidizing agent to eliminate the interfering substances in urine before the colorimetric measurement by the Sandell–Kolthoff reaction.

Materials and Methods

APPARATUS

Oxidation of iodine calibrators and urine samples was performed manually in 16 × 100 mm glass test tubes, using either a thermolyne aluminum top hot plate (Fisher Scientific, Pittsburgh, PA) with an attached improvised sand bath, or a Dri Block heater (Tecan Dri Block DB-3, Triangle Park, NC).

The automated colorimetric measurements were done by a Model I Technicon AutoAnalyzer, which consists of a sampler proportioning pump, a 60-ft glass coil [~3 mm (o.d.) × 2 mm (i.d.); Technicon, Tarrytown, NY] immersed in a water bath (Model 1295 PC; VWR Scientific, Boston, MA), a colorimeter

with a 420-nm filer, and a Model I Technicon recording system. A spectrophotometer (Model 1295 PC, VWR Scientific) set at 420 nm was used for the manual procedure.

CHEMICALS

Analytical-grade ammonium persulfate [$(NH_4)_2S_2O_8$], arsenic trioxide (As_2O_3), concentrated sulfuric acid (98%), and sodium chloride were obtained from Fisher Scientific (Itasca, IL). Potassium iodate (KIO_3) was obtained from Sigma Chemical Co. (St. Louis, MO), and ceric ammonium sulfate [$(NH_4)_4Ce(SO_4)_4 \cdot 2H_2O$] from GFS Chemicals (Columbus, OH). Glass-distilled deionized water was used for preparation of reagents and dilution procedures.

REAGENTS

Sulfuric acid (2.5 mol/L) was prepared in an ice bath by carefully adding 280 mL of concentrated sulfuric acid to 1000 mL of water, using a 3000-mL Florence flask as the reaction vessel. The cooled mixture was then diluted to 2000 mL with water.

Ceric ammonium sulfate (0.0158 mol/L) was prepared by dissolving 10 g of ceric ammonium sulfate in 1000 mL of 1.25 mol/L sulfuric acid.

Ammonium persulfate (1 mol/L) was prepared by dissolving 228.2 g of ammonium persulfate in water to a volume of 1000 mL.

Arsenious acid (0.0253 mol/L) was prepared in a 3000-mL Florence flask by heating on a hot plate a mixture of 5 g of arsenic trioxide, 25 g of sodium chloride, and 200 mL of 2.5 mol/L sulfuric acid until dissolved. After cooling, the mixture was diluted to 1000 mL with water.

The screening reagent was prepared by dissolving 16 g (0.025 mol/L) of ceric ammonium sulfate in 1000 mL of 1.25 mol/L sulfuric acid.

All reagents, including calibrators, were stored in amber bottles at ambient temperature.

PREPARATION OF IODINE CALIBRATORS

The stock iodine calibrator (A) was prepared by dissolving 168.6 mg of potassium iodate in a 100-mL volumetric flask with water, resulting in an iodine concentration of 7.87 mmol/L (1000 μg/mL iodine). For stock B, 1.0 mL of stock A was diluted in 100 mL of water; the iodine concentration was 78.74 μmol/L (10.0 μg/mL iodine).

The working calibrators, ranging from 0.02, 0.04, 0.06, 0.08, and 0.10 μg/0.2 mL iodine or 0.78, 1.57, 2.36, 3.18, and 3.94 μmol/L, were prepared by diluting 1.0 mL, 2.0 mL, 3.0 mL, 4.0 mL, and 5.0 mL of stock B calibrator, respectively, with water to 100 mL. Water is used for the zero calibrator.

AUTOMATED PROCEDURE

Calibrators, urine samples, and urine controls (200 μL each) are added to 16 × 100 mm glass tubes, followed by the addition of 1.0 mL of 1 mol/L ammonium persulfate to all tubes. All samples are oxidized for 30 min in a 91–95 °C heating block so that the temperature of the sample mixture inside the tubes is stable (±0.5 °C). A blank tube containing water equivalent to the volume of the sample mixture is used to check the temperature by inserting a thermometer into it.

The samples are then cooled to room temperature and 2.0 mL of arsenious acid is added. The test samples, including calibrators and controls, are transferred to 2.0-mL conical autoanalyzer cups. The leftover mixture in the test tubes is used to screen for urines that have grossly increased iodine content. The screening is performed by adding 1 drop of screening reagent. If any of the samples turn from a yellow color to colorless in 1.0 min, these samples are removed and further diluted.

The sampler module is set at a sampling rate of 40 samples/h. The water bath temperature is set at 37 °C to increase the sensitivity of the calibration curve. The percent transmission is measured by using a continuous flow cell with a 10-mm light path at 420 nm (fixed filter). Water is used to adjust the recorder to 100% transmission.

The calibration curve is prepared daily by plotting the percent transmission vs iodine content ranging from 0.00, 0.02, 0.04, 0.06, 0.08, and 0.10 μg of iodine on the x-axis. Sample concentration is interpolated from the calibration curve and multiplied by a factor of 500 and reported as μg/dL iodine. To convert μmol/L to μg/dL, multiply by a factor of 12.7, and to convert μg/dL to μmol/L, multiply by a factor of 0.07874. Sample readings exceeding 0.10 μg are diluted with water to produce a concentration within the range of the calibration curve. A typical calibration curve is shown in Fig. 1.

Values for samples reading <0.005 μg of iodine off the calibration curve are usually repeated with a 400-μL sample size. The calibrators are then made up to the same volume (400 μL) by adding 200 μL of water to each calibrator.

MANUAL PROCEDURE

Samples are treated as described in the automated procedure. After oxidation is completed, the samples are cooled and 2.0 mL of arsenious acid, 1 mL of 1.25 mol/L H_2SO_4, and 1 mL of water are sequentially added. The tubes are then placed in a 32 °C water bath and incubated for 10 min. The manual method is kept at 32 °C for ease in handling by slowing down the reaction rate of iodine.

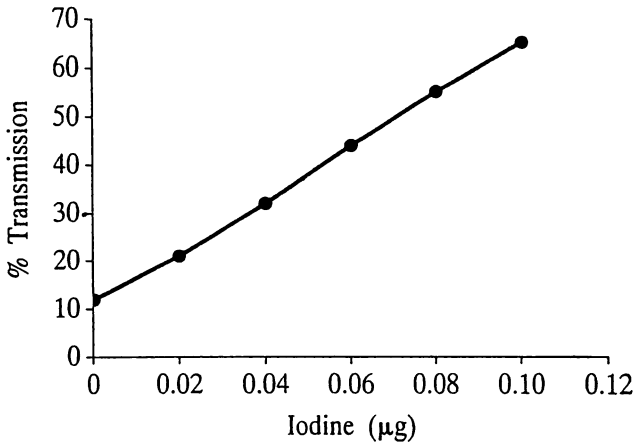

Fig. 1. Calibration curve for various concentrations of iodine.

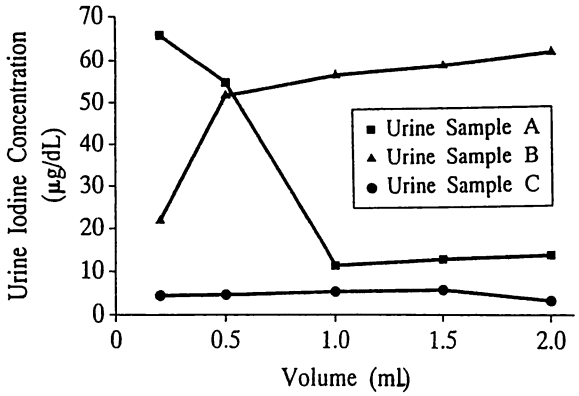

Fig. 2. Effect of volume of ammonium persulfate (1 mol/L) oxidation reagent on urine iodine content.

Iodine values for urine samples are as follows: A = 16.2 μg/dL (1.28 μmol/L); B = 61.0 μg/dL (4.80 μmol/L); and C = 5.0 μg/dL (0.39 μmol/L).

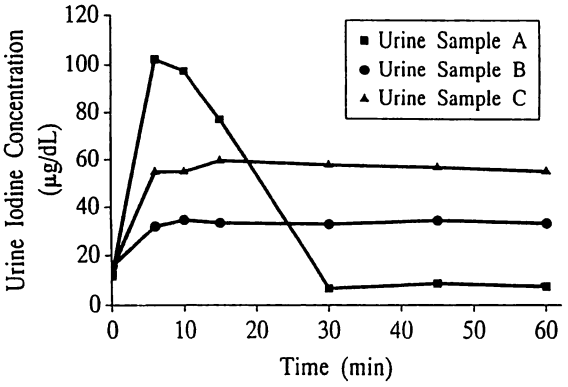

Fig. 4. Effect of oxidation time of ammonium persulfate on urine iodine content.

The 30-min incubation time is required to eliminate the urine straw color, which increases the apparent urine iodine concentration. Iodine values for urine samples are as follows: A = 16.2 μg/dL (1.28 μmol/L); B = 32.6 μg/dL (2.56 μmol/L); and C = 61.0 μg/dL (4.80 μmol/L).

The reaction is started by adding 0.5 mL of ceric ammonium sulfate to all tubes, which are incubated precisely for 10 min. To process a batch of samples accurately and comfortably, the addition of the ceric ammonium sulfate should be done at 1- or 2-min intervals.

At the end of the incubation, the percent transmission is read at 420 nm in a 10-mm light path cuvette. Water is used to adjust the spectrophotometer to 100% transmission. The results are calculated as described in the automated procedure.

Results

OPTIMIZATION STUDIES

Optimal oxidation is defined as having (*a*) eliminated the straw color of the urine sample mixture for the color reaction measurement, (*b*) eliminated the interfering substances, and (*c*) achieved acceptable iodine content recoveries. For this purpose, the reagent volume, digestion time, and temperature were studied. Figs. 2, 3, and 4 illustrate the effect of the reagent-to-sample volume ratio for the ammonium persulfate oxidation, the length of time to achieve optimal oxidation to avoid complete evaporation of the solution mixture, and the optimum temperature for the oxidation, respectively. The optimum conditions for the oxidation reaction are 1 mL of 1 mol/L ammonium persulfate to eliminate interfering substances as observed in sample A, temperature of 95 °C, and oxidation time of 30 min.

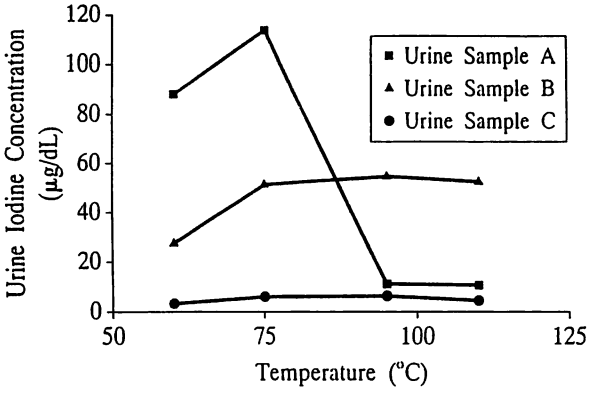

Fig. 3. Effect of temperature on urine iodine content.

The higher temperature of 95 °C is required to eliminate the urine straw color, which increases the apparent urine iodine concentrations. Samples are as in Fig. 2.

ANALYTIC PERFORMANCE

Intra- and interassay variability at different urine concentrations are shown in Table 1. These samples were assayed at the low, middle, and upper end of the calibration curve. Each of the samples was assayed 9–10 times on the same day and 6 times on different days.

The detection limit, 0.0034 μg of iodine, was taken as 2 SD of the mean derived from 10 measurements of water samples for iodine content.

LINEARITY

To evaluate linearity, serial dilutions of a urine sample (3.976 μmol/L) with water were measured for urine iodine content. The results were multiplied by the appropriate dilution factors and compared with the expected value (Table 2). The result was highly linear.

Table 1. Assay precision.

	n	Urine I, μmol/L	CV, %
Pool Intraassay			
1	9	0.42 ± 0.04*	9.1
2	10	1.46 ± 0.12	7.8
3	9	3.54 ± 0.14	4.0
Pool interassay			
1	6	0.46 ± 0.05	10.2
3	6	3.26 ± 0.26	7.9

* Mean ± SD

Table 2. Linearity of diluted urinary iodine by ammonium persulfate oxidation method.

Dilution ratio	Iodine, μmol/L: Measured	Theoretical	Recovery, %
1	3.976	3.976	100
1:1.5	2.598	2.650	98
1:3	1.299	1.325	98
1:6	0.646	0.663	97
1:12	0.378	0.331	114

INTERFERING SUBSTANCES

We assessed the effects of potassium thiocyanate *[8]* and L-ascorbic acid *[9]* on the measurement of urine iodine by adding known amounts of these compounds to water or urine to a final concentration ranging from 0.0128 to 0.206 mmol/L for potassium thiocyanate and 1 and 20 mmol/L for L-ascorbic acid. As shown in Table 3, these potentially interfering substances did not affect the assay.

RECOVERIES

Recoveries of iodine added to urine samples in vitro at different concentrations are shown in Table 4. The mean recovery was 99% (range 94–107%).

COMPARISON OF STANDARD CHLORIC ACID AND AMMONIUM PERSULFATE METHODS

Validity of the ammonium persulfate method was assessed by comparing the persulfate method with the standard chloric acid method (Fig. 5) *[6]*. Pearson's correlation was then applied to the results. There was an excellent correlation in 110 urine samples between the two methods, the coefficient of correlation (r) being 0.994. The regression equation with our standard chloric acid oxidation method (y) was $y = 0.923x + 0.810$, $r = 0.994$, $S_{y|x} = 1.841$, $P < 0.0001$, where x is the value measured by the ammonium persulfate method. Furthermore, when the paired t-test was used for the two methods for each sample, $P < 0.004$.

Table 3. Effects of potassium thiocyanate and ascorbic acid on urinary iodine measurement.

	Urine controls: Low iodine	High iodine	Water sample
L-ascorbic acid, mmol/L			
0	0.34 ± 0.039*	3.86 ± 0.12	ND#
1	0.34 ± 0.016	3.88 ± 0.16	ND
20	0.31 ± 0.032	3.71 ± 0.11	ND
Thiocyanate, mmol/L			
0	0.34 ± 0.039	3.87 ± 0.12	ND
0.01	0.32 ± 0.024	3.89 ± 0.13	ND
0.21	0.33 ± 0.024	3.71 ± 0.12	ND

* Mean ± SD, μmol/L
\# ND = None detected = <0.008 μmol/L

Table 4. Recovery of iodine added to urine in vitro.

Urine sample	Conc, μmol/L	KIO_3 enriched, μg of iodine	Detected, μmol/L	Recovery, %
1	0.34	0.010	0.45	107
2	0.54	0.030	0.72	94
3	2.85	0.100	3.54	97

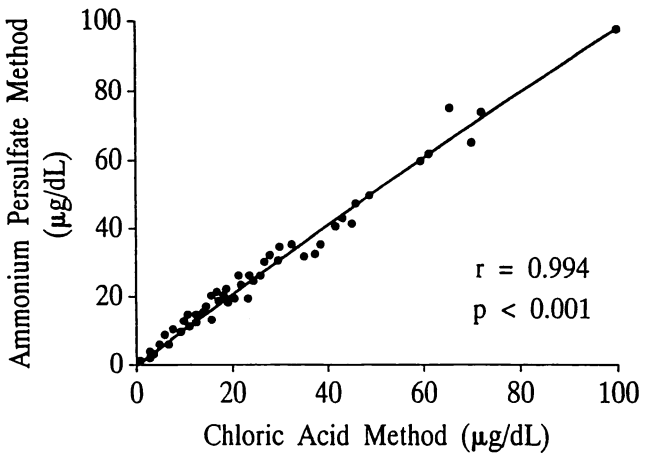

Fig. 5. Correlation between urine iodine concentration measured by chloric acid digestion (x) and ammonium persulfate (y) method. n = 110 urine samples.

Values for urine iodine were <3.9 μg/dL or 0.31 μmol/L in 11 urines. Values for these 11 samples ranged from 0.6 μg/dL to 3.6 μg/dL (0.05 to 0.31 μmol/L) by the persulfate method, which correlated extremely well with the chloric acid method ($r = 0.923$; $P < 0.001$).

In comparing the automated vs the manual method in 12 urine samples, the correlation coefficient was 0.994 ($P < 0.0001$).

Discussion

Various techniques have been proposed for the measurement of urinary iodine. Each method has its own advantages and limitations. The automated dialysis system does not completely eliminate the interfering substances, since they cross the membrane barrier *[8]*. The alkaline ashing method *[10]* with potassium hydroxide depresses the catalytic effect of iodine in the Sandell–Kolthoff reaction, thus requiring a correction factor for the calculations.

The Technicon automated digestion system converted the manual procedure into an automated method by using corrosive concentrated mineral acids at 300 °C *[11]*. The measurement of iodine was as accurate and as sensitive as the semiautomated method. However, this equipment is no longer commercially available. More recently, a modified automated digestion system has been reported *[12]* that utilizes potassium persulfate with ultraviolet irradiation to digest the samples and measures the iodine content by the Sandell–Kolthoff method. One disadvantage of this method is the potential cross-contamination of other samples by a grossly increased urine iodine concentration of a sample digested in this closed system. Furthermore, the initial cost to establish this method is expensive and may be prohibitive for some laboratories.

Persulfate compounds have long been used in analytical chemistry *[13]*. They are used extensively in organic oxidation

reactions, in bleaching processes, and as an oxidizer in the measurement of organic nitrogen *[14, 15]* and manganese *[16]*. Most authors used potassium persulfate as the oxidizer *[12]*. The potassium persulfate reagent is also safe but is far less soluble in water than ammonium persulfate and requires ultraviolet irradiation for complete oxidation *[12]*. At the recommended concentration of 0.1 mol/L potassium persulfate, incomplete oxidation of the urine resulted and interfering substances remained. Thus, we selected ammonium persulfate because it is nonhazardous, not potentially explosive, economical, very soluble in water (which makes it easy to prepare more concentrated solutions), a more potent oxidizer, avoids ultraviolet irradiation, and eliminates the requirement of an exhaust fume hood.

The temperature of the heating block apparatus must be uniform throughout the oxidation step (±0.5 °C) to assure reproducible and accurate results. If a fluctuation in heating occurs, the final volume of the digested sample mixture should be adjusted with water when the volumes of the mixtures are significantly less than others so as to minimize the variation caused by volume differences. Allowing the oxidized mixture to evaporate to dryness will result in loss of iodine. The present method is analytically sensitive (>0.0034 μg of iodine), on the basis of the Sandell–Kolthoff reaction, and is accurate (r = 0.994 compared with chloric acid digestion) *[6]*, reproducible (intraassay CV = 9.1% to 4.0%; interassay CV = 10.2% to 7.9%), affords excellent recovery (mean 99%), is linear up to 0.10 μg of iodine, and is safe and economical. There is no interference from either thiocyanate or L-ascorbic acid. Adaptation of this semiautomated method to measure the color reaction to other autoanalyzer systems is feasible by using the new sample-to-reagent ratio and the same set of conditions described in our semiautomated procedure.

Many laboratories, especially in developing countries, do not have the appropriate equipment or the resources to purchase the special perchloric acid fume hood required by standard laboratory safety regulations. The new ammonium persulfate oxidation method will give comparable results with the chloric acid method. However, it can only be used for urine samples.

In spite of the hazardous chemical properties of chloric acid, it has been our experience that it is an extremely versatile and effective oxidizer for providing accurate iodine determinations, and is useful for the determination of iodine in other biological materials such as blood, tissues, food and food products, and plants *[6, 17]*.

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