

## DETERMINATION OF IODINE IN HUMAN SWEAT BY SPECTROPHOTOMETRY

I-FANG MAO<sup>1</sup>, MEI-LIEN CHEN<sup>1</sup>, YI-CHANG LIN<sup>2</sup>,  
CHUNG-FU LAN<sup>3</sup>

*A spectrophotometric method for the determination of trace quantities of iodine in human sweat has been developed. The samples were completely homogenized by ultrasonication. Various forms of iodine in sweat were converted into iodate by acid digestion. The converted iodate then acted as a catalyst for the reduction reaction of Ce (IV) in the presence of As (III) to develop color change for colorimetric analysis.*

*The accuracy and precision of this method were tested with the spike method. From the experimental results, this method has been proved to be accurate enough for the determination of total iodine concentration in human sweat samples ranging from 2 to 10 ng/mL. A total of 40 sweat samples of 10 volunteers were examined, and the average concentration of iodine was found to be  $36.6 \pm 6.7$   $\mu\text{g/L}$ . These results suggest that this method is a simple, reliable, feasible and sensitive method for the determination of iodine concentration in human body fluids. (Chin J Public Health (Taipei): 1996; 15(1): 40-46)*

**Key words:** total iodine determination, sweat

### INTRODUCTION

Iodine is one of the fifteen essential elements in the diet and is a constituent of the thyroid hormones [1]. These thyroid hormones are crucial for the normal development of the skeletal, muscular, reproductive, circulatory and nervous systems [2]. The level of thyroxine or triiodo-thyronine in blood is a diagnostic index for some thyroid diseases [3]. The 24-hour urinary iodine content or the iodine/creatinine ratio in urine is also a measurement for the diagnosis of the severity of iodine deficiency and the classification of endemic goiter [4].

The spectrophotometry of catalytic reduction reported by De Visscher [5] for urine and by Benotti [6] for serum has been the frequently used method for body fluid iodine determination. The iodide ion selective electrode method was reported for the determination of iodine in some organic substances [7] including thyroxine [8]. The method of cathodic stripping voltammetry was developed for the determination of both iodide and iodate in water [9]. A colorimetric procedure was developed by Hashmi for the determination of iodate with the mixture of isonicotinic acid and 2,3,5-triphenyltetrazolium chloride [10] and a method for determining radioactive iodine solution has also

<sup>1</sup> Department of Social Medicine, School of Medicine, National Yang-Ming University, Taipei, Taiwan

<sup>2</sup> Department of Public Health, College of Public Health, National Taiwan University, Taipei, Taiwan

<sup>3</sup> Institute of Public Health, National Yang-Ming University, Taipei, Taiwan

Address correspondence and reprint requests to: I-Fang Mao, Associate Prof., Department of Social Medicine, School of Medicine, National Yang-Ming University, Shi-Pai, Taipei, Taiwan

Tel: 886-(02)-8212301 ext. 7057

Fax: 886-(02)-8221942

been described by Arino [11-14].

Literature review revealed that assays of iodine concentration in sweat are not available. Although some trials were conducted in this respect, many problems were found to be solved. In this study, a better method for iodine assay in human sweat has been developed. With this new technique, it would enable us to investigate the relationship between iodine loss from profuse sweating and some physiological conditions of iodine deficiency or endemic goiter [15].

## METHODS AND MATERIALS

### Sweat Samples

The human sweat samples were obtained from 10 male volunteers, who were 16-18 years old high school students. Each sweat sample was collected after one hour exercise training daily. To facilitate rapid collection of fresh sweat, a plastic collector (5×9×2 cm) was used holding next to the skin of back and chest. Five to ten milliliter sweat was collected for each player within three minutes. Each sweat sample was transferred to a 20-mL plastic tube, and one drop of xylene was added as a preservative.

### Apparatus

A Tecator system with 12 digesters (model 1009) was used for digesting the sweat samples. A Perkin-Elmer model 35 UV-VIS spectrophotometer was used to determine the absorbance change due to discoloration of ceric ammonium sulfate by sweat iodine.

A heat system sonicator with 1.6 mm tapered microtip probe was used to homogenize the sweat sample. A Millipore RQ system was used to obtain pure water.

### Reagents

All the reagents used in this study were prepared from chemicals of E. Merck, GR grade. Chloric acid solution (28%) was prepared by adding 500 g of potassium chlorate to 910 mL pure water. The solution was poured into a 3L Florence flask and dissolved by heating and stirring, 375 mL of 70% perchloric acid was then added from a separatory funnel

at a rate of approximately 15 mL per minute. The acid was cooled and placed in a freezer at a temperature of  $-23^{\circ}\text{C}$  for 12 hours. The chloric acid was filtered and suctioned through a Buchner funnel with a piece of Whatman No.1 filter paper. This preparation yielded approximately 1 L of 28% chloric acid.

A 0.5% sodium chromate solution was prepared by dissolving 5 g sodium chromate in water and making up to 1 L. A 0.025 M arsenous acid solution was prepared by dissolving 4.946 g  $\text{As}_2\text{O}_3$  in pure water, followed by adding 200 mL 2.5 M sulfuric acid and diluted to 500 mL, with heating and stirring for complete dissolution. It was then cooled down to room temperature and diluted to a volume of 1 L. A 0.02 M ceric ammonium sulfate solution was prepared by dissolving 13.38 g  $\text{Ce}(\text{NH}_4)_4(\text{SO}_4)_4 \cdot 4\text{H}_2\text{O}$  in pure water, adding 44 mL conc.  $\text{H}_2\text{SO}_4$  and making up to 1 L.

An iodine free sodium chloride solution (20% w/v) was prepared by dissolving 100 g of NaCl, which had been recrystallized in water/ethanol mixture, in pure water and diluted to 500 mL. Three stock solutions were prepared. Iodide standard stock solution I: 0.2616 g potassium iodide, pre-dried in dessicator, was dissolved in pure water and diluted to 1000 mL (1.00 mL = 200  $\mu\text{g}$  of iodine). Stock solution II containing 4000 ng of iodine in 1.00 mL solution, and stock solution III containing 100 ng of iodine in 1.00 mL solution. Stock solution III should be freshly prepared every week, while working standards are prepared freshly daily from stock solution III by an appropriate dilution. All the working standards, the arsenous solution, and ceric ammonium sulfate solution were kept in a water bath at  $30\pm 1^{\circ}\text{C}$  until the test was finished.

### Analytical Procedures

The human sweat samples (5~10 mL), each collected in a 15 mL test tube, were stirred vigorously with a vortex for 30 seconds. An aliquot of 3 mL sweat was then transferred to a 12×75 mm test tube and homogenized with a sonicator for five minutes. An aliquot of 0.5 mL of the homogenized sweat solution was immediately transferred to a 2×15 cm diges-

tion tube, to which 3 mL chloric acid and 0.5 mL sodium chromate solution were then added.

The digestion was proceeded in a Tecator digester at a temperature between 115°C and 118°C. At the end of one hour, digestion mixture was carefully watched for color changes. The sample should be discarded if the orange-yellowish color changed to light-greenish or colorless. One to two drops of chloric acid would, however, be added to avoid iodine loss if the orange-yellowish color was restored. The heating was continued for one additional hour. Red crystals of chromium trioxide should appear on the glass wall whenever the tubes are transferred to a cool water bath. The digestion must be continued until red crystals appear.

Approximately 0.5 mL of the solution remained in a tube at the end of digestion. The following reagents were then added to these tubes: 1 mL sodium chloride solution, 1.5 mL arsenous solution and 0.5 mL conc. sulfuric acid. After cooling, the mixture was made up to 10 mL, mixed with 3 mL ceric ammonium sulfate solution, and incubated at  $30 \pm 1^\circ\text{C}$ . The absorbance was measured with a spectrophotometer at 420 nm after 15 min of incubation.

A calibration curve was established from the standard iodide solutions of 0, 2, 4, 6, 8, and 10 ng/mL. The precision was expressed as percent coefficient of variation (% CV), respectively. The accuracy was ascertained through analysis of aliquot coming from each sweat sample spiked with potassium iodate ranging from 3 to 5 ng/mL.

## RESULTS AND DISCUSSION

The dynamic detection range of urinary iodine has been reported to be 20-60 ng/mL by De Visscher et al. [5]. And 40-120 ng/mL by Benotti et al. [6]. These methods have been re-examined in this study and found unstable for the determination of iodine content of the sweat samples because of the rapid discoloration and poor recovery. It has been demonstrated, under some volume ratios of 0.025M arsenous to 0.02 M ceric ammonium sulfate solution, the oxidation-reduction reaction (The

reaction equation:  $2 \text{Ce (IV)} + \text{As (III)} \rightarrow 2 \text{Ce (III)} + \text{As (V)}$ ) will proceed with iodine as a catalyst [16]. The most adequate combination of As/Ce ratio and reaction time were examined for the iodine concentration of 0~10 ng/mL by means of observing the degree of discoloration of yellow color of ceric ion at some set points of time. A highly significant correlation between absorbance and iodine concentration could be obtained whenever As/Ce volume ratio and observation time had been set constant. Four different volume ratios of As/Ce (0.6/3.0, 0.9/3.0, 1.5/3.0, 2.0/3.0) and some different observation period of time (from 4 to 20 minutes) were evaluated. The data are shown in Figure 1.

The volume ratio of As/Ce = 1.5 mL/3.0 mL (Fig. 1-d) and an observation time of 15 minutes have been judged in this study to be the most suitable condition for the determination of absorbance based on the data of sensitivity and correlation coefficient of the regression line. The correlation coefficient calculated from the data shown in Figure 2 is 0.999.

An aliquot of 100  $\mu\text{L}$  of standard iodide solution (300 ng/mL or 500 ng/mL) was added to the 10 mL diluted sweat solutions, i.e., 30 ng and 50 ng, and the recovery efficiency was measured. Sweat specimens were obtained from 3 individuals and multiple determinations were made. The best recoveries are shown in Table 1, obtained with an As/Ce ratio of 1.5 mL/3.0 mL. With the spike of 30 ng, the recovery was 106.9-110.2% and the standard deviation was 3.7-6.0%. In comparison, when a spike of 50 ng was used, the recovery was 105.6%-107.6% and standard deviation was 4.0-4.6%.

The microtip of the ultrasonics was used for homogenizing the sweat sample in this study. This is an important factor to obtain good accuracy and recovery, since sweat is excreted as a suspension. Poor accuracy and recovery would occur if this step was omitted.

The total iodine concentration of sweat samples from ten subjects have been determined by means of the proposed procedures. The data are shown in Table 2, and the average of iodine levels of the sweat samples is

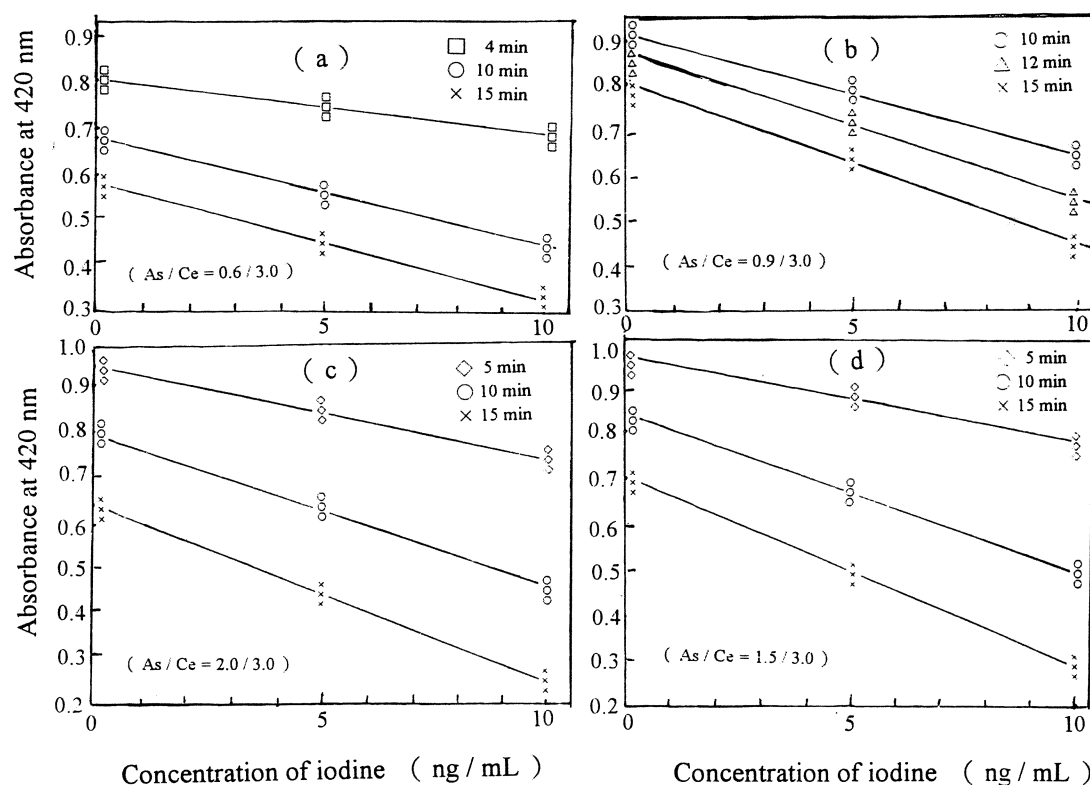


Fig. 1. Effect of As/Ce ratio and observation time on the relationship between absorbance and iodine concentration.

(a) (b) (c) (d) diagram represented different volume ratios of As/Ce.

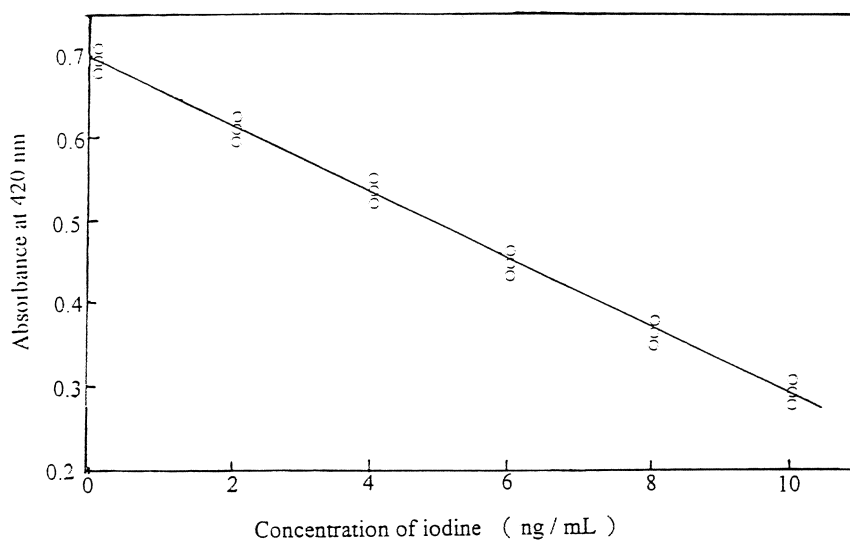


Fig. 2. Standard curve of sweat iodine determination

The determination was done by using a volume ratio of As/Ce = 1.5 ml/3.0 mL observation time was 15 minutes. Each experiment was carried out in triplicates.

Table 1. Recovery of iodide added to the sweat samples<sup>a</sup>

Sample No.	Recovery of the "spike" (%) <sup>b</sup>			
	30 ng "spike"		50 ng "spike"	
	mean $\pm$ SD	CV (%) <sup>c</sup>	mean $\pm$ SD	CV (%) <sup>c</sup>
1	106.0 $\pm$ 6.0 (n=12)	5.7	105.6 $\pm$ 4.6 (n=12)	4.4
2	107.8 $\pm$ 5.6 (n=12)	5.2	106.9 $\pm$ 4.0 (n=12)	3.7
3	110.2 $\pm$ 3.7 (n=12)	3.4	107.6 $\pm$ 4.1 (n=12)	3.8
Average	108.0 $\pm$ 5.2	4.8	106.7 $\pm$ 4.3	4.0

<sup>a</sup> Values given represent the average of triplicate analyses of 4 test runs.

<sup>b</sup> For each test, 100  $\mu$ L of iodide solution (300 ng/mL or 500 ng/mL) was added to a 10mL sample solution containing 0.5 mL sweat to give the final iodide concentration as indicated.

<sup>c</sup> CV is coefficient of variation.

Table 2. Sweat iodine concentration by the newly modified catalytic reduction method developed in this study

Subjects	No. of samples	Iodine conc ( $\mu$ g/L)
1	4	41.2 ( 8.6)*
2	4	44.1 (11.1)
3	4	24.9 (22.0)
4	4	37.2 (28.5)
5	4	41.4 ( 3.7)
6	4	38.1 ( 2.7)
7	4	28.6 (15.6)
8	4	28.7 (26.4)
9	4	42.1 ( 8.5)
10	4	39.8 (11.5)
Average		36.61 ( 6.72)

\* ( ) Values shown as mean coefficient of variation (%).

36.6 $\pm$ 6.7  $\mu$ g/L. If each sportsman excreted 3~5 liters sweat per day, the sweat iodine loss would be in the range of 110~180  $\mu$ g per day.

Daily fecal iodine excretion has been reported to be about 20  $\mu$ g [17], and normal male adults excrete more than 50  $\mu$ g I/g creatinine in daily urine. An average adult excretes about 400 to 600 mL of sweat daily through insensible perspiration, and excretes about 22

$\mu$ g iodine in the sweat. Therefore, 195  $\mu$ g to 270  $\mu$ g iodine would be excreted from each of the athletes by calculating iodine loss from daily excretion in the fecal, urine and sweat. And if the daily intake of iodine in the food were lower than 200  $\mu$ g, iodine deficiency would ensue, leading to goiter. However, the US FDA recommended daily allowance of iodine is only 150  $\mu$ g.

Digestion with chloric acid and sodium chromate is necessary to estimate the non-susceptible bound form of iodine in addition to the usual iodide ion. Procedures for this application are available. The formation of non-catalytic forms of iodine is reduced by adding an excess of sodium chloride that sensitizes the reaction.

In conclusion, this method has met the requirement of high accuracy in determining the amount of sweat iodine. It is a reliable and feasible method not only suitable for the study of iodine in human sweat; but the method could also be used for the determination of iodine in other body fluids.

## REFERENCES

1. Guyton AC. Textbook of Medical Physiology. 7th ed., Philadelphia: W.B. Saunders, 1986; 1017-1018.
2. Martini F. Fundamentals of Anatomy and Physiology. London: Prentice Hall, 1989; 479-481.
3. Henry JB. Clinical Diagnosis and Management by



- Laboratory Method. 17th ed., Philadelphia: W.B. Saunders, 1984; 307-312.
4. Stanbury JB, Hetzel BS. Epidemiological Surveys in Endemic Goiter and Cretinism. New York: John Wiley & Sons, 1980; 165-169.
  5. De Visscher M, Beckers C, Van Der Shrink HG. Determination of urine iodine, *Endocrinology* 1961; **21**: 175-179.
  6. Benotti J, Benotti N. Protein-bound iodine, total iodine, and butanol extractable iodine by partial automation. *Clin Chem* 1963; **9**: 408-416.
  7. Sharefkin JG, Schwerz HE. Qualitative detection of iodine in organic compounds. *Anal Chem* 1960; **32**: 996-998.
  8. Paletta B, Pazenbeck K. Electrometric determination of iodide in organic material. *Clin Chim Acta* 1969; **26**: 11-14.
  9. Hasty RA. Reduction of iodate by hydrazine. Application of the iodide ion selective electrode to the uncatalyzed reaction. *Mikrochimica Acta* 1973; **6**: 925-933.
  10. Hashmi MH, Ahmad H, Rashid A, Azam F. Simultaneous colorimetric determination of iodate and bromate. *Anal Chem* 1964; **36**: 2471-2473.
  11. Arino H, Kramer HH. Determination of specific activity of iodine solutions via an iodide electrode. *Nucl Applicat* 1968; **4**: 356-359.
  12. Inui T, Ochiy, Morishima M. Determination and clinical significance of anti- idiotype antibody in model experiments of thyroglobulin and TSH. *Rinsho Byori* 1990; **38**: 11411-8.
  13. Czech W. Determining serum TBG level using the charcoal-cellulose I-125 thyroxine uptake test. *Endokrynol Pol* 1989; **40**: 107-14.
  14. Passath A. The role of binding protein in radio-immunologic determination of the free thyroxine concentration using thyroxine analog method: thyroxine immunoextraction versus thyroxine analog immunoextraction. *Wien Med Wochenschr* 1989; **139**: 98-102.
  15. Mao IF, Ko YC, Chen ML. The stability of iodine in human sweat. *Jap J Physiol* 1990; **40**: 693-700.
  16. Mao IF, Chen ML. Ultramicro-determination of iodide in water by a catalytic reduction method. *Toxicol Environ Chem* 1994; **49**: 241-45.
  17. Vought RL, London WT, Lutwal L. Reliability of estimates of serum inorganic iodine and daily fecal and urinary iodine excretion from single casual specimens. *J Clin Endocrinol* 1963; **23**: 1221-1228.

# 人體汗中碘分光光度儀分析法研究

毛義方<sup>1</sup> 陳美蓮<sup>1</sup> 林宜長<sup>2</sup> 藍忠孚<sup>3</sup>

本研究是建立使用分光光度儀測量人體汗液中碘含量之方法。將汗液樣本使用超音波振碎機使汗液均質化，然後使用強酸加入汗液中，使各類碘化合物轉化成碘酸物(iodate)，然後以此碘酸物催化As及Ce之氧化還原反應發生之呈色作為汗液中碘含量之測定依據。

結果顯示，本研究發展之方法對汗液中

含碘量可作適當之定量，其準確度介於105.6～110.2%，其精確度CV值為3.4～5.7%，測定範圍為2～10 ng/mL，利用本法取10名自願者之汗液樣本共40個，測得其汗液中含碘量，平均值為 $36.6 \pm 6.7 \mu\text{g/L}$ 。此研究提供了測量體液中含碘量的一個簡單、精確及可行之測定方法，可應用於流行病學及生理學上之研究。(中華衛誌 1996；15(1)：40-46)

關鍵詞：碘濃度測定、人體汗液

---

<sup>1</sup>國立陽明大學社會醫學科

<sup>2</sup>國立台灣大學公共衛生研究所

<sup>3</sup>國立陽明大學公共衛生研究所

聯絡人：毛義方

聯絡地址：國立陽明大學社會醫學科，台北市石牌立農街二段155號

聯絡電話：(02) 821-2301 ext. 7057

傳真：(02) 822-1942

收稿日期：84年9月

接受日期：84年12月

