

Determination of Trace Copper in Biological Samples by On-line Chemical Vapor Generation-Atomic Fluorescence Spectrometry

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INTRODUCTION

Chemical vapor generation (CVG) is a prevalent sample introduction technique in analytical atomic spectrometry owing to its efficient matrix separation, enhanced analyte transport efficiency (approaching 100%), more efficient analyte atomization, and potential speciation analysis (1,2). Hydride generation-atomic fluorescence spectrometry (HG-AFS) is a representative application of CVG in analytical atomic spectrometry. It has additional advantages of simple instrumentation, flexible sampling amount, wide linear dynamic range, good stability, and low instrumental and running costs. Although CVG, especially the hydride generation techniques, has been used with atomic spectrometry for routine analysis of various samples, the elemental coverage of this technique is still limited, usually the eight traditional hydride generation elements, plus mercury, cadmium, and zinc. Recently, the range of elements amenable to CVG has been expanded to more transition elements and some noble elements (3,4).

Copper is an essential element for the functions of the human body such as hematopoiesis, cyto-genesis, and endocrine, whereas toxicity would attack in case of excess intake. Therefore, it is important to develop sensitive, rapid, simple, and reliable analytical methods to monitor copper in food, biological, geological, or envi-

ABSTRACT

On-line chemical vapor generation atomic fluorescence spectrometry (CVG-AFS) was, for the first time, used to determine trace copper in biological samples by merging acidified sample solution with potassium tetrahydroborate aqueous solution in the presence of micro-amounts of 1,10-phenanthroline. Nitric acid, for both sample digestion and chemical vapor generation, was used as the acid medium. CVG conditions and instrumental parameters were optimized for the best CVG efficiency, good gas/liquid separation, and efficient atomization/excitation. Under the optimized conditions, a limit of detection of 4 ng mL^{-1} was obtained for copper, with a linear dynamic range of over three orders of magnitude. The proposed method was successfully applied to the determination of copper in biological certified reference materials.

ronmental samples. In 1996, Sturgeon et al. (5) were first to detect volatile copper species by merging an acidified sample solution into an aqueous sodium tetrahydroborate solution at room temperature, and successfully applied this method to determine copper in environmental matrices by CVG inductively coupled plasma optical emission spectrometry (CVG-ICP-OES). Subsequently, the significant enhancement (10-fold) of the copper-containing vapor generation efficiency was observed by adding trace amounts of 1,10-phenanthroline (6,7). It has further been found

that the sensitivity can be increased by an order of magnitude when low concentrations of acetic acid, instead of hydrochloric or nitric acid, is used for CVG for copper (8).

In this work, copper CVG is for the first time coupled to atomic fluorescence spectrometry (AFS) by using a commercial intermittent-flow CVG-AFS instrument. The intermittent-flow method is a compromise sample introduction method, which has characters of both flow injection and continuous-flow methods, demonstrating less cross contamination, less sample consumption, low running cost, and the possibility of adjusting the pump rate at different stages to optimize the vapor generation conditions (9). The influence of experimental conditions on the analytical performance was studied in detail, and it was successfully applied to the determination of trace copper in biological samples.

EXPERIMENTAL

Instrumentation

A Model AFS-2202 double-channel non-dispersive atomic fluorescence spectrometer with a programmable intermittent-flow vapor generation apparatus (Beijing Kechuang Haiguang Instrument Co., Beijing, P.R. China) was used throughout this work. Details about the construction of the instrument can be found elsewhere (10). A copper hollow cathode lamp, specially designed for AFS, was employed as the excitation source. The argon carrier and shield gas were controlled individually by two flow meters which could operate at a low flow rate.

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Figure 1 shows the schematic diagram of the intermittent-flow vapor generation apparatus. Two gas-liquid separators were used in order to eliminate the interference of vapor and the fluctuation of Ar/H₂ flame atomizer. A PTFE tubing (i.d. 1.0 mm) was used as the storage coil (90 cm in length) and transport tubing (40 cm in length), whereas the silicone rubber tubing (i.d. 4.5 mm, 15 cm in length) was used as the reaction coil. The operation of the pump can be programmed in several steps in which the duration and rotation rate of the pump can be manipulated, and the working program is described in Table I.

Reagents

A 100-mg L⁻¹ working solution of copper was prepared by serial dilution of a copper stock standard solution of 5000 mg L⁻¹. A 1% 1,10-phenanthroline solution was prepared by dissolving 0.5 g of 1,10-phenanthroline (AR, Tianjin Kermel Chemical Reagent Co., Tianjin, P.R. China) in 50 mL of 20% ethanol solution. Potassium tetrahydroborate solution was prepared daily by dissolving appropriate amounts of KBH₄ (AR, Chengdu Kelong Chemical Reagents Co., Chengdu, P.R. China) in 0.05% potassium hydroxide solution. Nitric, hydrochloric, sulfuric, formic, propionic, and malonic acids were also purchased from Chengdu Kelong Chemical

Reagents Co., and acetic acid was ordered from Chengdu Changlian Chemical Reagents Ltd. (Chengdu, P.R. China). The certified reference materials used in this work were GBW 07604 Poplar Leaf (Institute of Geophysical and Geochemical Exploration, CAGS, Langfang, P.R. China) and GBW(E) 080193 Bovine Liver (Institute of Rock and Mineral Analysis, CAGS, Beijing, P.R. China).

Procedure

For one measurement cycle, the acidified sample solution of 1.4 mL (determined by the pump rate and sampling time) was sucked in Step 1 to fill the storage coil but not mix with the reductant, and Step 2 was of 6-second duration to change the sampling tube over to the carrier. At Step 3, the sample solution of the fixed volume was pushed by the carrier into the reaction coil to mix with the reductant for the CVG process. The volatile species and hydrogen gas separated from the gas-liquid separator was swept by the carrier gas into the atomizer and ignited at the outlet of the quartz tube (the atomizer). It is not necessary to add any auxiliary hydrogen to maintain the argon-hydrogen (Ar/H₂) flame, since there is enough of hydrogen produced in the CVG process. When Step 3 finished, the pump was stopped again and made ready for the next measurement cycle.

Sample Pretreatment

Certified reference materials GBW 07604 Poplar Leaf (0.2500 g) and GBW(E) 080193 Bovine Liver (0.1000 g) were accurately weighed and dissolved in 10 mL of a 4:1 nitric and perchloric acid mixture, and subsequently the sample solutions were heated at 170 °C to near dryness. After cooling, the residue was diluted to 25 mL with 1% nitric acid and 0.0005% 1,10-phenanthroline for the measurement by CVG-AFS.

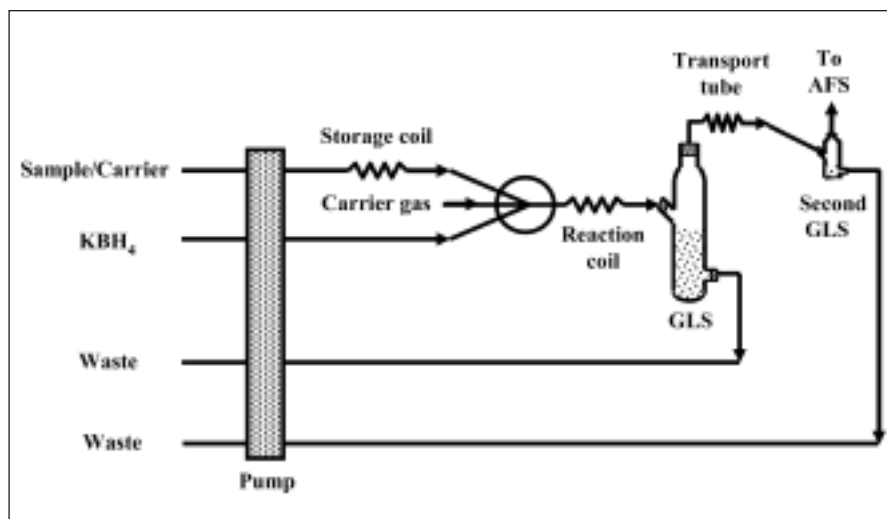


Fig. 1. Schematic diagram of the intermittent-flow vapor generation for atomic fluorescence spectrometry. GLS: gas/liquid separator.

TABLE I
Working Program for the Intermittent-Flow Reactor

Step	Flow Rate (mL·min ⁻¹)		Time (s)	Function
	Sample/Carrier	KBH ₄		
1	10.3	8.6	8	Put the sampling tube into sample solution
2	0	0	6	Change-over of sampling tube into carrier solution
3	10.3	8.6	16	Readout and cleanup
4	0	0	6	Get ready to return to Step 1

RESULTS AND DISCUSSION

Optimization of Chemical Reaction Conditions for CVG

Previous reports (5,6) showed that either hydrochloric acid or nitric acid could be used in vapor generation of copper with a tetrahydroborate system, though nitric acid is generally avoided as its decomposition products interfere with hydride generation. Several organic acids were also tested by Xu et al. (8), and it was found that the acetic acid of relatively low concentration could enhance the signal of copper significantly over a broader concentration range compared to nitric acid. In this work, the influence of several inorganic and organic acids, including hydrochloric, nitric, sulfuric, formic, acetic, propionic and malonic acids, were investigated on the atomic fluorescence intensity of 500 $\mu\text{g L}^{-1}$ copper, and the results are shown in Figure 2. It can be seen from Figure 2 that each inorganic acid or malonic acid provides good performance while the effect of acetic acid or propionic acid is unsatisfactory. The exact identity of the volatile copper species is unknown, but the copper alkyl might be generated when organic acids (such as acetic or propionic acid) are used for the acidic medium. Presumably, the good performance of acetic acid in Xu's work was probably due to the efficient atomization of the resulting volatile species in ICP, compared with the relative lower temperature of the Ar/H₂ flame in this work. The significant impact of the atomizer temperature on the signal response of copper has also been validated by Luna et al. (11). As shown in Figure 3, the generation efficiency of copper volatile species depended strongly on the organic acidity. Taking into consideration that it is essential to use nitric acid in many digestion procedures and thus no need to remove it from ana-

lyte solution prior to analysis, nitric acid was chosen for further study in this work. High acidity leads to the generation of a large amount of hydrogen gas which dilutes the analyte and causes the instability of the Ar/H₂ flame, while low acidity results in insufficient generation of hydrogen to maintain the flame. It was found that 1% nitric acid was the optimal reaction medium, for both high vapor generation efficiency for copper and the stability of the Ar/H₂ flame.

In this work, potassium tetrahydroborate is used not only as a reductant but also as the hydrogen supply to maintain the Ar/H₂ flame. Figure 4 shows the effect of its concentration on the atomic fluorescence intensity of 100 $\mu\text{g L}^{-1}$ copper. Abundant hydrogen is produced when its concentration is over 1.5%, resulting in a dramatic dilution and shorter residence time of the volatile copper species in the atomizer. Therefore, 1.25% potassium tetrahydroborate was chosen for further experiments for the highest signal intensity of copper.

Several complexing reagents, such as ethylene diamine tetraacetic acid (EDTA), thiourea, L-ascorbic acid, 2-picolinic acid, 1,10-phenanthroline and potassium iodide, were investigated in this work as sensitivity-enhancing reagents, but only 1,10-phenanthroline showed a significant enhancement effect as reported by Zhou and colleagues (6). Figure 5 shows that the optimal concentration of 1,10-phenanthroline is 0.0005%, with an enhancement factor of about 12.

Optimization of Instrumental Parameters

As expected, the signal intensity of copper increases with the lamp current. The signal-to-noise ratio reached a maximal plateau when the lamp current was around 80 mA. Observation height is the distance

from the quartz furnace outlet to the point where the atomic fluorescence signal is measured. The closer observation point (< 9 mm) results in a higher blank because of the scatter of the radiation source on the atomizer outlet, but the signal intensity of copper is remarkably reduced as the observation height increases. As a consequence, an observation height of 10 mm was chosen for this study.

The carrier gas flow rate greatly affects the efficiency of CVG, as well as transport and atomization of volatile species. At a low flow rate, the resultant volatile species might decompose quickly in the reaction solution due to a lack of effective separation from the reaction media. However, at a high flow rate, the dilution of the volatile species and the short residence time of the analyte in the Ar/H₂ flame would be dominant, resulting in the decrease of the signal intensity. Considering both CVG and atomization efficiency, 200 mL min⁻¹ was the best carrier gas flow rate. In addition, 400 mL min⁻¹ argon was used as the shield gas in order to prevent extraneous air from entering the flame, alleviating the interference from the alien environment.

Rapid mixing of sample and reductant solution is of paramount importance to the overall efficiency of the generation process as well as fast gas/liquid separation of the volatile species from spent solution because the nascent volatile species is relatively unstable and easily decomposed in the aqueous phase (4,11-13). Increasing sample / reductant flow rate by adjusting the pump rotater speed of the intermittent-flow system at Step 3 is the easiest way to enhance the efficiency of this process, while an auxiliary carrier gas also assists the transport of the resultant volatile species to the detection unit. Therefore, the maximal rotater speed of the programmable pump of 10.3 mL min⁻¹

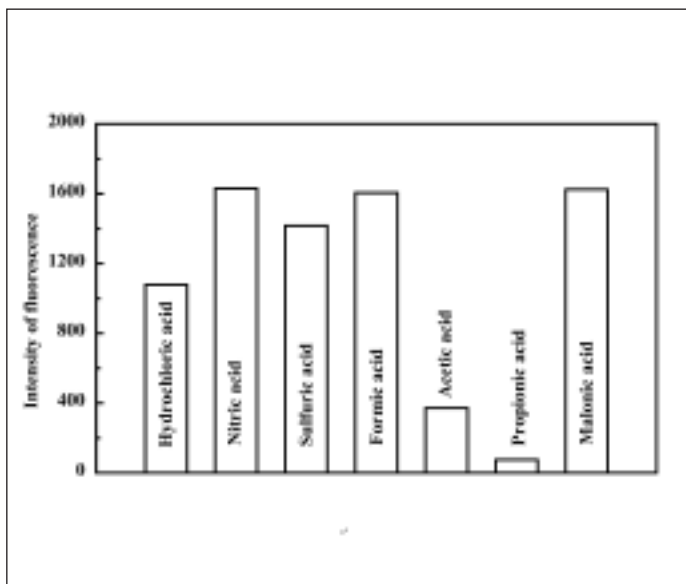


Fig. 2. Effect of acid medium on the fluorescence intensity of $500 \mu\text{g L}^{-1}$ copper. Concentration of acids: 1% hydrochloric acid; 1% nitric acid; 0.5% sulfuric acid; 0.5% formic acid; 0.75% acetic acid; 0.5% propionic acid; and 1% malonic acid.

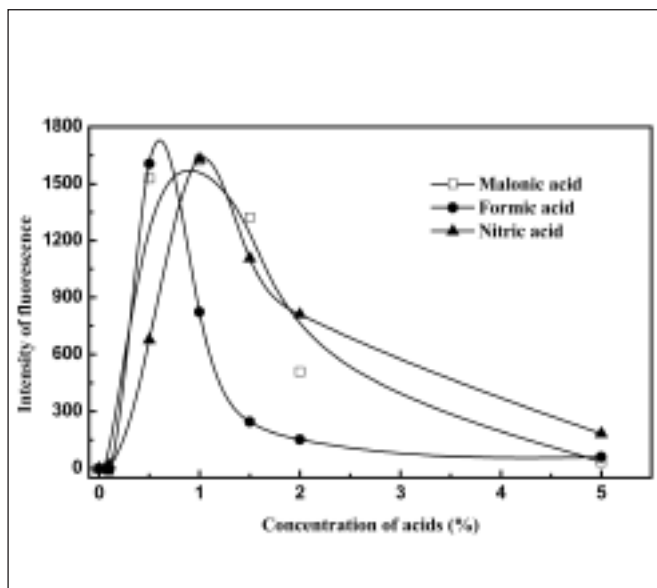


Fig. 3. Effect of concentration of nitric, formic or malonic acid on the fluorescence intensity of $500 \mu\text{g L}^{-1}$ copper.

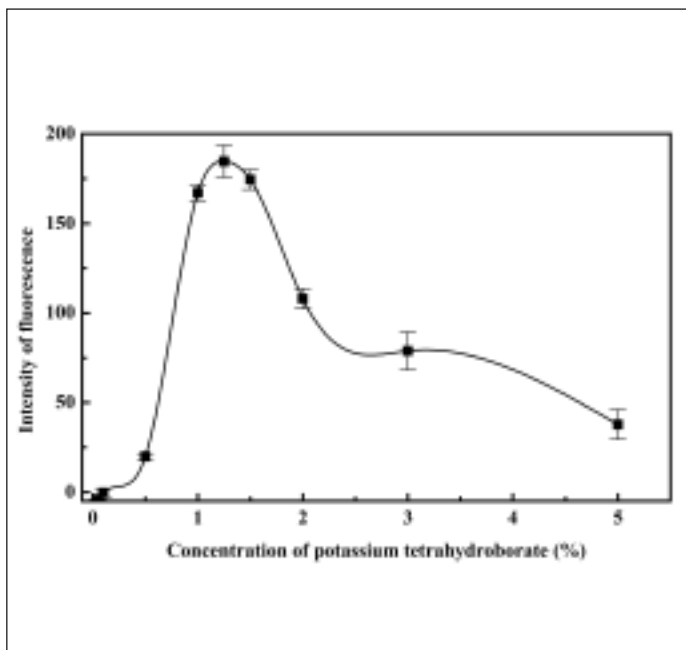


Fig. 4. Effect of potassium tetraborate concentration (in 0.05% potassium hydroxide) on the atomic fluorescence intensity of $100 \mu\text{g L}^{-1}$ copper. Error bars denote one standard deviation (same for Figure 5).

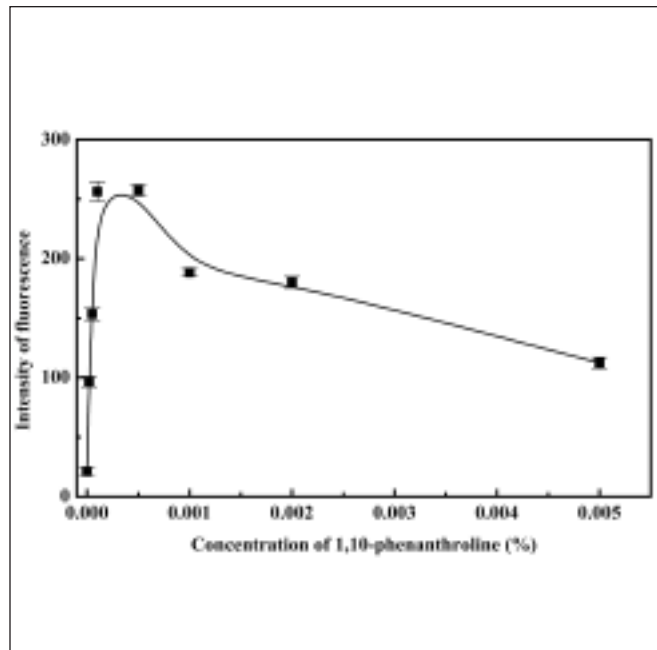


Fig. 5. Effect of 1,10-phenanthroline concentration on the fluorescence intensity of $100 \mu\text{g L}^{-1}$ copper.

(corresponding to the sample / reductant ratio of 1.2) was adapted in this work.

Little difference was found between the silicone rubber and Teflon® tubing for the efficiency of copper volatile generation when optimizing the reaction coil. The length of the reaction coil from 5 to 30 cm on the atomic fluorescence intensity of copper was studied, and 15 cm was found optimal. Two gas-liquid separators were used in order to eliminate the interference of vapor and the fluctuation of Ar/H₂ flame atomizer. The effect of the length of the transport tube between the first and second gas/liquid separators was also investigated. Good signal size can be obtained even if the transport tube is as long as 40 cm. A short transport tube led to instability of the flame, thus the measurements. Considering both the sensitivity and precision of the measurements, a 40-cm transport tube was selected for use. The optimized instrumental parameters are summarized in Table II.

Evaluation of Interference

In the present work, the potential interferences caused by some metals in environmental, biological, and geological samples were studied carefully, and the results are listed in Table III. There is no significant interference of most common co-existing metals except Ni(II) at high concentrations. Although masking reagents, such as thiourea, ammonium fluoride, and organic acids (formic, acetic, propionic, and malonic acid), were used in an attempt to minimize the interferences from Ag(I) and Ni(II), none was found to be effective.

Analytical Applicability

Under the optimized conditions, the analytical figures of merit of the proposed method for the determination of copper were obtained

TABLE II
Optimized Instrumental Parameters of the Proposed Method
(100 µg L⁻¹ Cu)

Lamp Current	80 mA
Observation Height	10 mm
Carrier Gas Flow Rate	200 mL min ⁻¹
Shield Gas Flow Rate	400 mL min ⁻¹
Sample / Reductant Flow Rate	10.3 mL min ⁻¹
Length of Reaction Coil	15 cm
Length of Transport Tube	40 cm

TABLE III
Interference Study of Various Co-existing Metal Ions
on the Proposed Method (500 µg L⁻¹ Cu)

Co-existing Metal Ions	Concentration (mg L ⁻¹)	C _{metal ion} / C _{Cu(II)}	Recovery (%)
Ca(II)	500	1000	92
K(I)	500	1000	93
Na(I)	500	1000	87
Mg(II)	100	200	94
Zn(II)	100	200	96
Mn(II)	100	200	90
Al(III)	50	100	91
Cr(III)	25	50	95
Fe(III)	25	50	91
Co(II)	25	50	94
Ni(II)	25	50	82
Ag(I)	1	2	86
Au(III)	1	2	95

and compared with similar atomic spectrometric techniques, as summarized in Table IV. At least 0.99 of R² (linear correlation coefficient) showed good linearity of the calibration curves. The limit of detection (LOD) of 4 µg L⁻¹ is better than or equivalent to those by atomic absorption spectrometric detection, and the linear dynamic range from LOD to 1000 µg L⁻¹ are much better.

In order to validate the potential application of the proposed method, two certified reference materials, GBW 07604 Poplar Leaf and GBW (E) 080193 Bovine Liver, were analyzed for trace copper. As seen from Table V, good agreement

was obtained between the determined and certified values.

CONCLUSION

The commercial HG-AFS instrument was successfully employed for the determination of trace copper by using the CVG technique, thus expanding its measurable elements to non-traditional hydride generation element. The proposed method has the advantage of high sensitivity, fairly broad linear dynamic range, low sample consumption, cost-effectiveness, and ease of use. It has potential application in the biological analysis for trace copper. Further work should be directed to the identifica-

TABLE IV
Analytical Figures of Merit of the Proposed Method
in Comparison With Similar Techniques

Method	Upper Linear Range ($\mu\text{g L}^{-1}$)	LOD (3σ) ($\mu\text{g L}^{-1}$)	Linear Correlation Coefficient (R)	RSD (%)	Reference
^a FI-CVG-AAS	150	1.8	0.9997	2.6 (100 $\mu\text{g L}^{-1}$)	(6)
^b CVG-QT-AAS	15,000	800	-	9 (10,000 $\mu\text{g L}^{-1}$)	(11)
^c CVG-GF-AAS	200,000	2800	0.9958	11 (5000 $\mu\text{g L}^{-1}$)	(14)
^d CVG-AFS	1000	4	0.9978	2.5 (500 $\mu\text{g L}^{-1}$)	This work

^a Flow-injection Chemical Vapor Generation Atomic Absorption Spectrometry;

^b Chemical Vapor Generation Quartz Tube Atomic Absorption Spectrometry;

^c Chemical Vapor Generation Graphite Furnace Atomic Absorption Spectrometry;

^d Chemical Vapor Generation Atomic Fluorescence Spectrometry.

TABLE V
Analytical Results for Reference Materials

Sample	Matrix	Found by this Method (mg kg^{-1})	Certified Value (mg kg^{-1})
GBW 07604	Poplar Leaves	9.1 \pm 1.0	9.3 \pm 3.0
GBW (E) 080193	Bovine Liver	92.2 \pm 5.7	91.6 \pm 5.7

tion of the generated volatile species, thus elucidating the CVG mechanism.

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