Sensing Technique of Silver Nanoparticles as Labels for Immunoassay Using Liquid Electrode Plasma Atomic Emission Spectrometry

ARTICLE *in* ANALYTICAL CHEMISTRY · FEBRUARY 2012

Impact Factor: 5.64 · DOI: 10.1021/ac202782b · Source: PubMed

5 AUTHORS, INCLUDING:

Tung Nguyen Hoang
Vietnam Academy of Science and Technology
4 PUBLICATIONS **127** CITATIONS
SEE PROFILE

Miyuki Chikae
Japan Advanced Institute of Science and Tech…
39 PUBLICATIONS **963** CITATIONS
SEE PROFILE

Yoshiaki Ukita
University of Yamanashi
77 PUBLICATIONS **278** CITATIONS
SEE PROFILE

Pham Hung Viet
Vietnam National University, Hanoi
159 PUBLICATIONS **4,367** CITATIONS
SEE PROFILE

analytical chemistry

Letter

pubs.acs.org/ac

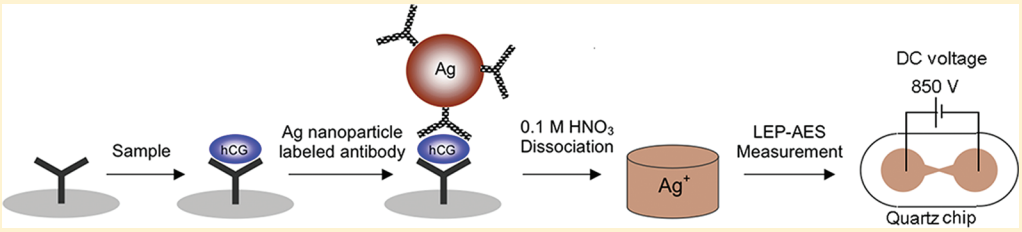
Sensing Technique of Silver Nanoparticles as Labels for Immunoassay Using Liquid Electrode Plasma Atomic Emission Spectrometry

Nguyen Hoang Tung,[†,‡] Miyuki Chikae,[†] Yoshiaki Ukita,[†] Pham Hung Viet,[‡] and Yuzuru Takamura*[,†]

[†]School of Materials Science, Japan Advanced Institute of Science and Technology, 1-1 Asahidai, Nomi, Ishikawa 923-1292, Japan

[‡]Research Center for Environmental Technology and Sustainable Development (CETASD), Hanoi University of Science, 334 Nguyen Trai Str, Thanh Xuan Dist, Hanoi, Vietnam

Supporting Information



ABSTRACT: We report the use of liquid electrode plasma-atomic emission spectrometry (LEP-AES) in protein sensing studies employing Ag nanoparticle labeling. LEP-AES requires no plasma gas and no high-power source and is suitable for onsite portable analysis. Human chorionic gonadotropin (hCG) was used as a model target protein, and the immunoreaction in which hCG is sandwiched between two antibodies, one of which is immobilized on the microwell and the second is labeled with Ag nanoparticles, was performed. Sensing occurs at the narrow pass in the center of a quartz chip following oxidative dissolution of the Ag nanoparticles by nitric acid. hCG was analyzed in the range from 10 pg/mL to 1 ng/mL, and the detection limit for hCG was estimated at 1.3 pg/mL (22.8 fM). The proposed detection method has a wide variety of promising applications in metal-nanoparticle-labeled biomolecule detection.

Inductively coupled plasma-mass spectrometry (ICPMS) has been applied as a powerful tool for trace analysis of various elements. ICPMS offers a large dynamic range (several orders of magnitude) and high sensitivity and is widely used in industry, for clinical diagnosis, for environmental measurements, etc. Recently, ICPMS has been proposed for sensitive and quantitative element-tagged bioassays, such as determination of gold-nanoparticle-tagged antibodies and lanthanide (Eu, Tb, Dy, and Sm)−chelate antibody conjugates, and for protein assays in biological samples.[1−11] However, ICPMS requires the use of bulky equipment for plasma gas such as argon and a high-potential and high-frequency power supply.

We have developed a new technology based on atomic emission spectrometry that can be used instead of the conventional ICP-AES and have termed it liquid electrode plasma-atomic emission spectrometry (LEP-AES).[12,13] One of the major advantages of the LEP-AES system is its portability, which is derived from the fact that it requires no plasma gas and no high-power source. The sample is introduced into a quartz chip with a narrow center area and bilateral wells, and high voltage is applied between the sample solution in the wells, which act as liquid electrodes, using a platinum electrode. The plasma is inducted by the concentrated voltage and current at the narrow center pass. The atoms in the sample solution are excited by the plasma, and subsequent emission of light of the appropriate frequency returns the atoms to a lower energy level. The applicability of LEP-AES has been illustrated in heavy metal measurements in the microfluidic channel[13] and detection of trace amounts of sodium and lithium in zirconium dioxide.[14]

Ag nanoparticles have been proposed as a label material for biosensing using the LEP-AES assay. Ag nanoparticles have generally been considered as useful labels in analytical biochemistry because of their unique performance in the sensitive electrochemical detection using differential pulse voltammetry,[15] surface-enhanced Raman scattering,[16] and optical scanning of the grayscale.[17] Additionally, our group reported the sensitive electrochemical detection of Ag nanoparticle labels in a metalloimmunoassay and confirmed the stability, specific binding with antigen, and analytical performance of the silver-nanoparticle-labeled antibody.[18]

Here, we report, for the first time, the use of LEP-AES in the quantitative detection of proteins, following a sandwich-type immunoassay using Ag-nanoparticle-labeled antibodies. A scheme of the process is shown in Figure 1A. The efficacy of the new methodology was evaluated using human chorionic

Received: October 20, 2011
Accepted: January 13, 2012
Published: January 13, 2012

   dx.doi.org/10.1021/ac202782b | *Anal. Chem.* 2012, 84, 1210−1213

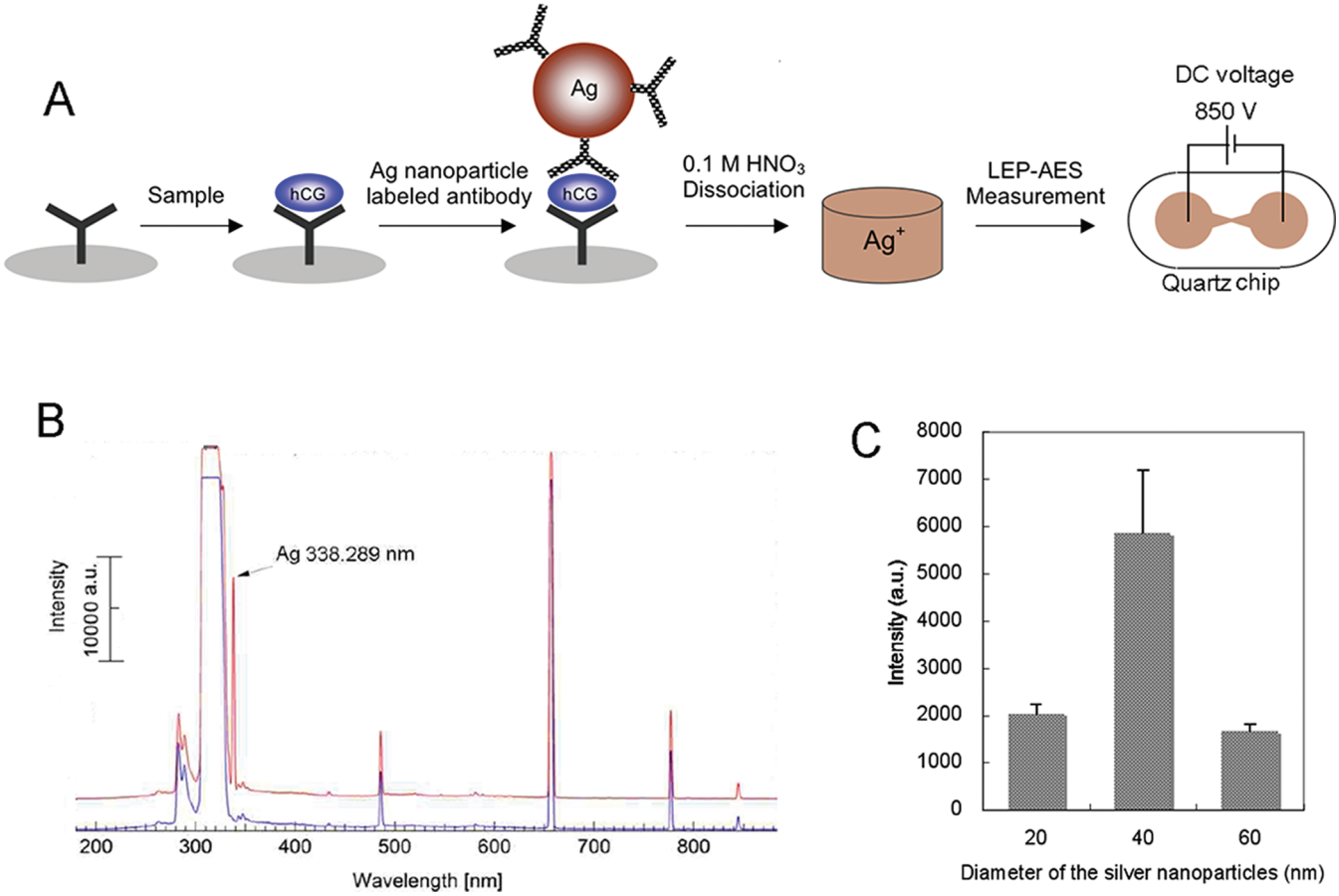


Figure 1. (A) Schematic representation of the immunoassay system. hCG was sandwiched between two antibodies, of which one was immobilized onto the microwell and the other was conjugated to Ag nanoparticles. The Ag nanoparticles were dissociated oxidatively, and the silver ion concentration was measured. (B) Emission spectrum of the Ag ion (I). The upper spectrum corresponds to 5 mg/mL and the lower spectrum corresponds to 0 mg/mL, in 0.1 M HNO_3. The arrow indicates the signal of the silver ion at 338 nm. (C) Variation in the analytical signal with different sizes of the silver nanoparticles at a concentration of 1 ng/mL hCG.

gonadotropin hormone (hCG), which was selected as the target analyte of interest because the details of its behavior, such as selectivity, epitopes for the binding of two antibodies, and the quantitative relation to the silver nanoparticle labeled antobody are well documented in our laboratory.[18,19]

EXPERIMENTAL SECTION

Instruments. Experimental measurements were performed using the MH-5000 ultracompact elemental analyzer (Micro Emission Ltd., Japan), which was of a portable size with a length of 20.4 cm, width of 10.5 cm, height of 11.4 cm, and weight of 1.4 kg including batteries (Figure S-1A, Supporting Information). Analysis was carried out using the software of LepiSuite LEP_Analyzer. A quartz chip (LepiCuve-C cuvette) for measurement was purchased from Micro Emission Ltd. (Japan) (Figure S-1B, Supporting Information).

Immunoassay Procedure. Ag-nanoparticle-labeled hCG antibody (Ag-Mab-hCGs) was prepared in a manner similar to that described in a previous report by our group.[18] The full method of the preparation and materials used are available in Supporting Information. Another antibody of hCG, monoclonal antihuman α-subunits of a follicle-stimulating hormone (Mab-FSH), was immobilized on the microwell. In brief, 50 μL of a 100 μg/mL Mab-FSH solution in 50 mM phosphate buffered saline (PBS, pH 7.4) was incubated in polystyrene microwells (Nunc) at 4 °C for 12 h. After rinsing three times with PBST (PBS with 0.05% Tween 20), the wells were blocked for the suppression of nonspecific adsorption with 100 μL of blocking solution (1% BSA in PBS) overnight. Mab-FSH-immobilized microwells were stored at 4 °C until use.

The sandwich-type immunoreaction was performed using the general procedure. The Mab-FSH-immobilized microwells were rinsed three times with PBST, after which 50 μL of the samples was added and incubated for 30 min at room temperature with moderate shaking. After rinsing three times with PBST, 50 μL of the Ag-Mab-hCG was added at a 1:5 dilution of the stock solution and incubated for 30 min at room temperature with moderate shaking. After rinsing three times, the sandwich-type immunocomplex-labeled Ag nanoparticles were formed according to the hCG concentration in the microwell.

LEP-AES Analysis. After the immunoreaction, the amount of labeled Ag nanoparticles was determined by LEP-AES. A 50 μL aliquot of 0.1 M HNO_3 was pipetted into the microwell for oxidative dissociation of Ag nanoparticles. After 5 min, 60 μL of the solution containing the released silver ion (I) was applied to the quartz chip for measurement.

LEP-AES measurement was performed under the following conditions: applied voltage of 850 V, pulse duration of 30 ms, pulse interval of 270 ms, 20 pulses per exposure, and 5 exposures per measurement. The total measurement time was within 3 min after sample introduction.

dx.doi.org/10.1021/ac202782b | *Anal. Chem.* 2012, 84, 1210–1213

RESULTS AND DISCUSSION

The emission spectra of the silver nitrate in 0.1 M HNO_3, which contained 0 or 5 μg/mL of silver ion, are shown in Figure 1B. A sharp peak corresponding to the silver ion was observed at 338 nm (indicated by an arrow).

Ag-Mab-hCGs were prepared using Ag nanoparticles of various sizes (20, 40, and 60 nm diameter), and the signal intensities were compared after the immunoreaction with 1 ng/mL of hCG. As shown in Figure 1C, the highest signal intensity was obtained with the 40 nm diameter Ag nanoparticle label. Generally, increasing the diameter of the Ag nanoparticle is expected to increase the signal intensity of the silver ion correspondingly. Indeed, the signal intensity from the 40 nm diameter Ag nanoparticle label was higher than that from the 20 nm label. In contrast, the signal intensity from the 60 nm diameter Ag nanoparticle label was lower than that from the others. It appears that the immunoreaction was sterically inhibited by the large size or mass of the particle. The 40 nm diameter Ag nanoparticle was adopted as the optimal label material in this study.

The analytical performance of the LEP-AES system was investigated by measuring various concentrations of hCG between 0 and 10 ng/mL with 0.1% BSA in PBS. A linear relationship between the logarithmic signal intensity and the logarithmic concentration of hCG was obtained in the range from 10 pg/mL to 1 ng/mL (Figure 2). The regression

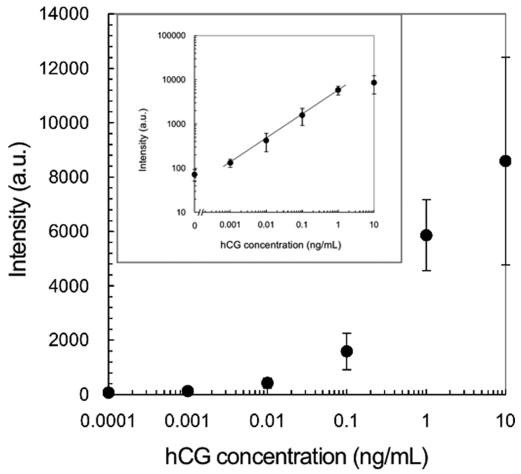


Figure 2. Calibration plots of hCG determined from the peak intensity of the silver ion. Error bars indicate the relative standard deviation of three measurements (n = 3) performed with three different samples. The low concentration region is also displayed as the inset.

equation was $y = 5696.3x^{0.5611}$, and the correlation coefficient was 0.9995. Under optimal conditions, a detection limit of 1.3 pg/mL (22.8 fM) for hCG was estimated from 3SD (SD was the standard deviation of 7 measurements of a blank solution).

Generally, target human proteins can be detected at levels as low as 0.1−0.5 ng/mL using the conventional ICPMS-based immunoassay, which uses gold-cluster antibodies and lanthanide (Eu, Tb, Dy, and Sm)−chelate antibody conjugates, and a linear response to protein concentration over 3 orders of magnitude can be obtained.[20] For example, 3 ng/mL of peanut allergens can be detected using an Eu-labeled immunoassay,[4] and 0.1 ng/mL of rabbit-antihuman IgG can be detected using Au nanoparticle labels.[7] The detection limit of the proposed method was lower than the reported values for the detection using the ICPMS-based immunoassay. The obtained high sensitivity may possibly be attributed to the fact that there is no need to dilute the sample with argon gas. Additionally, LEP-AES sensing of silver can become widely applicable for the detection of silver ions along with silver enhancement of gold nanoparticle labels[21−23] and the detection of biocatalytically deposited silver metal using an HRP or ALP label.[24,25]

In summary, we have demonstrated, for the first time, the use of LEP-AES for high-sensitivity protein sensing using the Ag-nanoparticle-labeled immunoassay of hCG. LEP-AES is a compact elemental-analysis method that may serve as an alternative to ICP-AES. A linear correlation between the logarithmic signal and the logarithmic concentration can be obtained over 3 orders of magnitude, and a low detection limit for hCG of 1.3 pg/mL (22.8 fM) can be achieved. Ag nanoparticle labeling is readily applicable for the labeling of nucleic acids and aptamers. The proposed detection method is also suitable for automation and is potentially applicable to onsite analysis combined with the automated sample preparation system.

ASSOCIATED CONTENT

Supporting Information

Experimental details including materials and preparation of Ag nanoparticle-labeled antibody. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone: +81 761 51 1661. Fax: +81 761 51 1665. E-mail: yztakamura@jaist.ac.jp.

ACKNOWLEDGMENTS

The authors would like to thank J. Minami for her experimental assistance throughout the investigation.

REFERENCES

(1) Careri, M.; Elviri, L.; Mangia, A. *Anal. Bioanal. Chem.* **2009**, *393*, 57−61.
(2) Tholey, A.; Schaumlöffel, D. *Trends Anal. Chem.* **2010**, *29*, 339−408.
(3) Jiménez, M. S.; Gómez, M. T.; Beloa, E.; Laborda, F.; Castillo, J. *Int. J. Mass Spectrom.* **2011**, in press.
(4) Careri, M.; Elviri, L.; Mangia, A.; Mucchino, C. *Anal. Bioanal. Chem.* **2007**, *387*, 1851−1854.
(5) Lou, X.; Zhang, G.; Herrera, I.; Kinach, R.; Ornatsky, O.; Baranow, V.; Nitz, M.; Winnik, M. A. *Angew. Chem., Int. Ed.* **2007**, *46*, 6111−6114.
(6) Razumienko, E.; Ornatsky, O.; Kinach, R.; Milyavsky, M.; Lechman, E.; Baranov, V.; Winnik, M. A.; Tanner, S. D. *J. Immunol. Methods* **2008**, *336*, 56−63.
(7) Liu, R.; Xing, Z.; Lv, Y.; Zhang, S.; Zhang, X. *Talanta* **2010**, *83*, 48−54.
(8) Zhao, Q.; Lu, X.; Li, F.; Yuan, C.-G.; Li, X.-F.; Le, X. C. *Anal. Chem.* **2009**, *81*, 7484−7489.
(9) Li, F.; Zhao, Q.; Wang, C.; Lu, X.; Li, X.-F.; Le, X. C. *Anal. Chem.* **2010**, *82*, 3399−3403.
(10) Kerr, S. L.; Sharp, B. *Chem. Commun.* **2007**, 4537−4539.
(11) Hsu, I.-H.; Chen, W.-H.; Wu, T.-K.; Sun, Y.-C. *J. Chromatogr., A* **2011**, *1218*, 1795−1801.
(12) Iiduka, A.; Morita, Y.; Tamiya, E.; Takamura, Y. Micro Total Analysis Systems 2004; The Royal Society of Chemistry, Cambridge, U.K., 2004; Vol. *1*, pp 423−425.
(13) Kitano, A.; Iiduka, A.; Yamamoto, T.; Ukita, Y.; Tamiya, E.; Takamura, Y. *Anal. Chem.* **2011**, *83*, 9424−9430.

(14) Banno, M.; Tamiya, E.; Takamura, Y. *Anal. Chim. Acta* **2009**, *634*, 153−157.
(15) Hao, H.; Li, H.; Iong, Y.; Zhang, L.; Zhao, X.; Xu, D.; Chen, H.-Y. *J. Electroanal. Chem.* **2011**, *656*, 50−54.
(16) Farah, A. A.; Alvarez-Puebla, R. A.; Fenniri, H. *J. Colloid Interface Sci.* **2008**, *319*, 572−576.
(17) Yeh, C.-H.; Chen, W.-T.; Lin, H.-P.; Chang, T.-C.; Lin, Y.-C. *Talanta* **2010**, *83*, 55−60.
(18) Chikae, M.; Idegami, K.; Nagatani, N.; Tamiya, E.; Takamura, Y. *Electrochemistry* **2010**, *7* (8), 748−753.
(19) Idegami, K.; Chikae, M.; Kerman, K.; Nagatani, N.; Yuhi, T.; Endo, T.; Tamiya, E. *Electroanalysis* **2008**, *20*, 14−21.
(20) Baranov, V.; Quinn, Z.; Baraura, R.; Tanner, S. *Anal. Chem.* **2002**, *74*, 1629−1636.
(21) Gao, X.; Ye, Y.; Liu, S. *Anal. Biochem.* **2011**, *417*, 1−16.
(22) Cho, I.-H.; Seo, S.-M.; Paek, E.-H.; Paek, S.-H. *J. Chromatogr., B* **2010**, *878*, 271−277.
(23) Liao., K.-T.; Huang, H.-J. *Anal. Chim. Acta* **2005**, *538*, 159−164.
(24) Chen, Z.-P.; Peng, Z.-F.; Jiang, J.-H.; Zhang, X.-B.; Shen, G.-L.; Yu, R.-Q. *Sens. Actuators, B* **2008**, *129*, 146−151.
(25) Luo, Y.; Mao, X.; Peng, Z.-F.; Jiang, J.-H.; Shen, G.-L.; Yu, R.-Q. *Talanta* **2008**, *74*, 1642−1648.

dx.doi.org/10.1021/ac202782b | *Anal. Chem.* 2012, 84, 1210−1213