Diagnostic Assay of Chromium (VI) in the Ex Vivo Fluid of the Urine of a Smoker Using a Fluorine-Doped Handmade Sensor

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A voltammetric diagnosis of a chromium (VI) ion was investigated using a fluorine-doped graphite pencil electrode. Square wave (SW) stripping working conditions were attained at a high range of $0.051\text{--}0.45\text{ mg L}^{-1}$ and a microrange of $0.05\text{--}0.4\text{ }\mu\text{g L}^{-1}$ in a $0.1\text{ M NH}_4\text{H}_2\text{PO}_4$ electrolyte solution, at a relative standard deviation of 1.68% (RSD, $n = 15$), using $10.0\text{ }\mu\text{g L}^{-1}$ Cr(VI). A fast experimental time was used only for the 120 sec SW

accumulation time. An analytical detection-limit (DL) of $0.008\text{ }\mu\text{g L}^{-1}$ was attained. DL appeared to be more sensitive than common voltammetric and spectrophotometric assays. The developed sensor was applied to tap water and the urine of a smoker. It was found that the methods can be applicable for in vivo fluid or medicinal diagnosis. J. Clin. Lab. Anal. 23:82–87, 2009. © 2009 Wiley-Liss, Inc.

Key words: chromium; mercury; voltammetry; urine; tissue

INTRODUCTION

In biological ecosystems, chromium is widespread in soil (1), industry (2), pigments or chromium plates, cigarettes, drinking water (3), and cement (4). Concentrated in vivo chromium absorptions are associated with various diseases, such as fibroproliferative disease, airway hypersensitivity (5,6), lung cancer (7–9), nasal cancer, and various other types of cancer (10). Recently, various low analytical detection methods have been considered, such as electrothermal atomic absorption spectrometry (detection limit, DL, $0.13\text{ }\mu\text{g L}^{-1}$) (11,12), atomic absorption spectrometry (DL, $0.81\text{ }\mu\text{L}^{-1}$) (13), inductively coupled plasma mass spectrometry [DL, 8.30 pg mL^{-1} (Cr(VI))] (14), and flame atomic absorption spectrometry (DL, around $0.18\text{ }\mu\text{g L}^{-1}$) (15). Some of these techniques approached sensitive analytical DLs but had high DLs, owing to which they are unusable for in vivo diagnosis. Moreover, these instruments are complicated, time-consuming, and expensive. Recently, simpler and lower-cost voltammetric analytical methods have been developed, such as those using the bismuth-coated glassy-carbon electrode [DL, 0.3 nM Cr(VI) and after a 2-min accumulation time] (16), the carbon paste modified electrode (DL, about $5.0 \times 10^{-8}\text{ M}$) (17), the refreshable mercury film silver-based electrode (DL, 0.19 nM) (18), and the stannum film electrode (DL, $2.0\text{ }\mu\text{g L}^{-1}$) (19), as well as ion transfer voltammetry [DL, $4.8\text{ }\mu\text{M}$ of Cr(VI) or 0.25 ppm] (20); humic acid

voltammetry (DL, $3 \times 10^{-11}\text{ mol}^{-1}$) (21); catalytic adsorptive stripping voltammetry (DL, $0.18\text{ }\mu\text{g L}^{-1}$ for Cr) (22); catalytic cathodic stripping voltammetry (DL, 0.1 nM) (23); and the pyridine-functionalized sol–gel film method (DL, 4.6 ppb) (24). Such methods have high DLs, however, and thus, cannot be used in in vivo direct assay. As such, a more sensitive handmade sensor with the new type of chemically modified electrodes of fluorine doped onto a graphite pencil electrode was considered, which is also less expensive, more convenient, and renewable. Previous studies have compared this electrode with other common electrodes (25). Moreover, the fluorinated graphite carbon structure (-CF-) includes large electron capacities (26), hydrophilic or hydrophobic electric reaction (27), and strong electrodepletion (28), which are useful in redox voltammetry and analytical sensor techniques. This study compares peak current with other common electrodes and obtained a more sensitive peak current for the results. Optimum analytical conditions were examined, and the final working conditions appeared very sensitive

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within the nano-level ranges. This method yielded lower DLs than those of common voltammetric and photometric methods. Thus, an analytical application was performed on the urine of a human smoker and tap water. The developed technique can be used in medicinal diagnostic assays.

MATERIALS AND METHODS

Systems, Reagents, Electrode Preparation, and Experimental Procedure

Analytical measurement systems were used with the new system of Bioelectronics-1, which was constructed at the authors' institute. The first version is a computerized handheld voltammetric sensor with a ± 2.4 V potential range, a 2 mA current range, a 10-pA measuring current, and a 5 in $\times$ 4 in $\times$ 1 in instrument size, and which uses a rechargeable battery or external power and has a USB port interface with a PC. The instrument is as big as a typical cellular phone and can be used in bioassay and sensor techniques for individual and laboratory applications. A chromium standard solution ($1,000 \text{ mg L}^{-1}$) was obtained from Jumsei Chemical (Tokyo, Japan). The $1,000 \mu\text{g L}^{-1}$ Cr standard solution was prepared by diluting 0.1 mL of $1,000 \text{ mg L}^{-1}$ Cr(VI) into 100 mL of pure water. A $1,000 \text{ mg L}^{-1}$ standard solution was also prepared using the same method. All the chemicals that were used were of analytical-grade purity. Highly purified water was prepared through three-time distillation using $18 \text{ M}\Omega \text{ cm}^{-1}$ of the Milli-Q Ultra-pure Water System (ICD4, SMT Lab, Seoul, Korea). The urine sample was obtained from our Research Center. A pencil-type graphite carbon electrode (PE) with a diameter of 0.5 mm and a length of 60 mm (Type 6H, Pentel Co. Ltd., Tokyo, Japan) was used as the working electrode (25). A fluorine-doped graphite pencil electrode (FPE) was coated with the 40% HF solution at the PE surface (1.5 mm diameter, 5 mm long), and a copper wire was connected to the electrical system. A 0.2 mm-diameter $\times$ 50 mm-long (63.05 mm^2) chloride-coated silver wire was used on the Ag/AgCl(s)/KCl (3 M) reference electrode, and a 0.2 mm-diameter $\times$ 50 mm-long platinum wire was used as the auxiliary electrode. The three-electrode system was immersed in a 10 mL-concentration HF solution. In this solution, -CF- immobilization was performed using a 10-cycle scan with a 1.0 V initial potential, a -1.0 V switching potential, and a 0.5 V sec^{-1} scan rate. As such, the -CF- structure can be stabilized and used in voltammetry. All the experiments were performed at room temperature, without removing oxygen. The electrolyte solution composed of 0.1 M $\text{NH}_4\text{H}_2\text{PO}_4$ with a pH of 3.6 was found to be the most suitable electrolyte solution. Then the peak potentials of the FPE and PE were compared. The FPE was obtained for the sensitive cyclic voltammetric peak current from the electrocatalytic

reaction of $[\text{C}_x^{-1}(\text{H-F})_n]n + \text{Cr(VI)} \leftrightarrow \text{CrO}$ (29). Also, the stripping voltammetry showed that the sensor is very sensitive and can thus be used in biological in vivo analysis.

RESULTS AND DISCUSSION

First, the voltammetric peak potential was determined for the CV variation with anodic and cathodic scans using a high-concentration Cr ion. Figure 1A shows the CV effects using PE and the variations for the standard $0\text{--}40 \text{ mg L}^{-1}$ Cr(VI) spike. In the oxidation scan, a 0.2 V peak potential was obtained from 2.5×10^{-6} to

Fig. 1. (A) Cyclic voltammetry for Cr(VI) variations. The first curve is blank and with 10, 20, 30, and 40 mg L^{-1} Cr(VI) ion spikes at the optimum conditions of -1.0 V initial potential and 1.0 V switching potential, at a 0.1 V sec^{-1} scan rate in the PE $0.1 \text{ M NH}_4\text{H}_2\text{PO}_4$ electrolyte solution. (B) Comparison of the PE and FPE at the conditions in (A).

5.15×10^{-6} A, the peak width of which was wide and slowly increased. Also, a reduction peak current appeared for the 0.17 V potential (30), although it was very small, and is thus not usable for analysis. At these conditions, the SW concentration effects were examined at the $5\text{--}40\text{ mg L}^{-1}$ Cr(VI) spike with nine various points. In this scan, three separate peak potentials of -0.6 , -0.1 , and 0.3 V were obtained, but 0.3 V is very big and the current ranges were linear from 6.10×10^{-6} to 12.56×10^{-6} A and increased and 0.3 V is sensitive and usable for trace analysis via square wave (SW) stripping. Thus, the -CF- doping structure (FPE) was compared with the common pencil electrode (PE). Figure 1B shows the Cr ion variations at the $40\text{--}100\text{ mg L}^{-1}$ Cr(VI) spike and their peak increments with the FPE and PE in the same electrolyte cell systems. The FPE varied, however, for 1.65×10^{-5} to 4.08×10^{-5} A, and the PE for 4.44×10^{-5} to 7.61×10^{-5} A in this figure is big and its peak width is broad, whereas the FPE peak appears sensitive and its peak width, very sharp. Thus, the FPE was used for SW stripping for the trace analysis. The optimized SW conditions were examined with the FPE electrode.

SW Optimizations of FPE

Using FPE, the SW amplitude variations that ranged from 0.021 to 0.035 V (eight points) were studied by maintaining the $0.5\text{ }\mu\text{g L}^{-1}$ Cr(VI) spike. Figure 2A shows the resulting raw voltammograms and peak current. The peak high increased from 5.02×10^{-6} to 9.25×10^{-6} A, and the maximum peak high appeared at a sharp 35 mV amplitude. Thus, 35 mV was used in the other experiments. At this point, the SW accumulation times were studied from 0 to 240 sec . The results are shown in Figure 2B. Here, two separate peaks appeared at -0.1 and 0.3 V, but the 0.3 V was very sensitive, and was varied from 0.76×10^{-6} to 21.65×10^{-6} A and linearly increased and 240 sec accounts for significant time savings for the experiment. Thus, 240 sec was used in the other experiments. At these conditions, the SW initial potential, SW frequency, and SW final potential were examined. Optimum parameters of -1.0 V accumulation potential, 4 mV incremental potential, 35 mV amplitude, 15 Hz frequency, and 240 sec deposition time were obtained, and a pH 4.0 electrolyte solution was generated (not shown here). With these results, usable analytical working ranges and diagnostic statistics were examined with the FPE.

Analytical Working Range and Statistics

Optimum SW conditions were used for the analytical working ranges, and various ranges were examined using anodic stripping. The final results were obtained in milli-

Fig. 2. (A) Variations of the SW amplitude range for 0.021, 0.023, 0.025, 0.027, 0.029, 0.031, 0.033, and 0.035 V. (B) SW accumulation time variations of 0, 30, 60, 90, 120, 150, 180, 210, 240, 300, 350, and 400 sec for the $0.5\text{ }\mu\text{g L}^{-1}$ Cr ion spike in the $0.1\text{ M NH}_4\text{H}_2\text{PO}_4$ electrolyte solution. With a 15 Hz SW frequency, 4 mV incremental potential, -1.0 V accumulation potential, and 60 sec accumulation time, the electrolyte pH strength was 3.5 pH .

and micrograms. Figure 3A shows that for $0\text{--}0.45\text{ mg L}^{-1}$ with 10 points, the peak current increased from 3.47×10^{-6} to 38.09×10^{-6} A, the peak width was narrow, and $\Delta x/\Delta y = 92.793$ was sensitive. A precision of $R^2 = 0.987$ was obtained and the experiment time was fast at 120 sec accumulation, which show that results can be used for high concentration ranges. More sensitive working ranges were examined, however, using a 150 sec accumulation time, and the results are shown in Figure 3B, in which the peak currents are linear from 3.3×10^{-5} to 14.97×10^{-5} A and the slope sensitive is $\Delta x/\Delta y = 3.713$. With these parameters, the SW stripping

working ranges were $0.05\text{--}4.0\ \mu\text{g L}^{-1}$, a very low range, which makes medicinal usable for any in vivo application. Under such conditions, the statistical precision was examined with the replicated 10th determination of the $0.45\ \text{mg L}^{-1}$ Cr(VI) standard spike, from which 0.401% of RSD was obtained. These results are highly reproducible and sensitive, making suitable for in vivo urine and in vivo tissue analysis. Thus, the electrode stability was examined with the $2.0\ \text{mg L}^{-1}$ Cr(VI) standard spike. Statistical peak currents were found for 5.06% RSD ($n = 5$) when the sensor was used for 1 or 2 weeks. Under these

conditions, the analytical DL was calculated as $0.008\ \mu\text{g L}^{-1}$ using the blank electrolyte solution with a three times signal-to-noise ratio.

In Vivo Applications

The analytical application was performed first with a $2\ \mu\text{g L}^{-1}$ Cr(VI) fixed known standard by a five-point spike, and the final result was $2.13 \pm 0.07\ \mu\text{g L}^{-1}$, which precision is usable for biological assay (not shown here). Thus, city tap water was examined as drinking water. Figure 4A shows the results of the standard addition method in the range of microgram additions. The first

Fig. 3. (A) Analytical working ranges of 0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, and $0.45\ \text{mg L}^{-1}$ Cr ion spike using FPE within 120 sec. (B) Working ranges of 0, 0.05, 0.1, 0.15, 0.2, 0.25, and $0.4\ \mu\text{g L}^{-1}$ spikes, within 150 sec in a $0.1\ \text{M NH}_4\text{H}_2\text{PO}_4$ electrolyte solution with a pH of 4.0, an SW amplitude of 35 mV, an SW frequency of 15 Hz, an incremental potential of 35 mV, and an accumulation potential of $-1.0\ \text{V}$.

Fig. 4. Determinations of Cr(VI) with the standard addition method. (A) The first curve is a blank electrolyte in $0.1\ \text{mL}$ tap water, then in $0.5, 1.0, 1.5, 2.0,$ and $2.5\ \text{mL}$ standard $10\ \mu\text{g L}^{-1}$ Cr(VI) spikes. (B) In the in vivo fluid test, the linear curve represents the smoker's (—■—) and the nonsmoker's urine (—●—) with standard addition curves. The first curve is blank and the next shows the $1\ \text{mL}$ urine sample spike and the $0.5, 2.0, 2.5,$ and $3.0\ \mu\text{g L}^{-1}$ Cr(VI) standard spikes. The other conditions are as those shown in Figure 3.

curve represents the blank electrolyte solution, which was linear and not noised; then the nontreated 1 mL tap water was spiked and obtained a 1.13×10^{-6} A peak current, which is chromium's peak potential. Thus, the $10 \mu\text{g L}^{-1}$ Cr(VI) standard was added with 0.5–2.6 mL five points, and the peak currents linearly increased from 2.35×10^{-6} to 8.49×10^{-6} A, the linear equation was $y = 3.133x + 1.32$, and the precision was $R^2 = 0.961$. Replication was performed with five times and yielded $2.7 \pm 0.08 \mu\text{g L}^{-1}$. The results are usable for human urine and medicinal diagnosis. Thus, urine samples for 25-year-old smokers and healthy nonsmokers were examined. Their SW voltammetric comparison is shown in Figure 4B. The nonsmoker curves are from 0.31×10^{-6} to 2.74×10^{-6} A and $\Delta x/\Delta y = 1.024$, and $0.08 \mu\text{g L}^{-1}$ was obtained, the initial peak of which was small. The smoker curves were from 1.02×10^{-6} to 5.76×10^{-6} A, and $0.32 \mu\text{g L}^{-1}$ was obtained, the initial peak of which was three times that of the nonsmokers. The smoker sensitivity was $\Delta x/\Delta y = 1.959$. It was significant and sharp. The developed technique can be applied to urine tests for patients, specifically for in vivo fluid diagnosis of live organs.

CONCLUSION

The novel fluorine-doped FPE sensor described herein is for the diagnosis of trace chromium ion in in vivo fluid. The optimum conditions of pH 4.0 electrolyte solution, -1.0 V accumulation potential, 4 mV incremental potential, 35 mV amplitude, 15 Hz frequency, and 240 sec deposition time and a fast experiment time were obtained. Moreover, the developed sensor approached the DL of $0.008 \mu\text{g L}^{-1}$, which is lower than that of the common spectrophotometric (12–15) and voltammetric methods (19,20,22,26). Thus, developed method can be used for any in vivo or in vitro diagnostic application. Here, stripping voltammetry was applied to the in vivo urine of a smoker and tap water. Its precision was sensitive. It can also be applied in other fields that require medicinal diagnosis of humans.

REFERENCES

1. Bousquet B, Sirven J-B, Canioni L. Towards quantitative laser-induced breakdown spectroscopy analysis of soil samples. *Spectrochim Acta Part B* 2007;62:1582–1589.
2. Barbosa RMT, de Almeida A-AF, Mielke MS, Loguercio LL, Mangabeira PAO, Gomes FP. A physiological analysis of *Genipa americana* L.: A potential phytoremediator tree for chromium polluted watersheds. *Environ Exp Bot* 2007;61:264–271.
3. Uddin AN, Burns FJ, Rossman TG, Chen H, Kluz T, Costa M. Dietary chromium and nickel enhance UV-carcinogenesis in skin of hairless mice. *Toxicol Appl Pharmacol* 2007;221:329–338.
4. Chen CJ, Shih TS, Chang HY, et al. The total body burden of chromium associated with skin disease and smoking among cement workers. *Sci Total Environ* 2008;391:76–81.
5. O'Hara KA, Klei LR, Barchowsky A. Selective activation of Src family kinases and JNK by low levels of chromium (VI). *Toxicol Appl Pharmacol* 2003;190:214–223.
6. Uddin AN, Burns FJ, Rossman TG, Chen H, Kluz T, Costa M. Dietary chromium and nickel enhance UV-carcinogenesis in skin of hairless mice. *Toxicol Appl Pharmacol* 2007;221:329–338.
7. Martin BD, Schoenhard JA, Hwang J-M, Sugden KD. Ascorbate is a pro-oxidant in chromium-treated human lung cells. *Mutat Res* 2006;610:74–84.
8. Kondo K, Takahashi Y, Hirose Y, et al. The reduced expression and aberrant methylation of p16INK4a in chromate workers with lung cancer. *Lung Cancer* 2006;53:295–302.
9. Martin BD, Schoenhard JA, Hwang J-M, Sugden KD. Ascorbate is a pro-oxidant in chromium-treated human lung cells. *Mutat Res* 2006;610:74–84.
10. Sato H, Murai K, Kanda T, Mimura R, Hiratsuka Y. Association of chromium exposure with multiple primary cancers in the nasal cavity. *Auris Nasus Larynx* 2003;30:93–96.
11. Huskova L, Bobrowski A, Sramkova J, Krolicka A, Vytras K. Catalytic adsorptive stripping voltammetry versus electrothermal atomic absorption spectrometry in the determination of trace cobalt and chromium in human urine. *Talanta* 2005;66:999–1004.
12. Vilar Farinas M, Garcya JR, Martyn SG, Crecente RMP, Latorre CH. Determination of Cr and Ni in Orujo spirit samples by ETAAS using different chemical modifiers. *Food Chem* 2008;110:177–866.
13. Elci L, Kartal AA, Soylak M. Solid phase extraction method for the determination of iron, lead and chromium by atomic absorption spectrometry using Amberlite XAD-2000 column in various water samples. *J Hazard Mater* 2008;153:454–461.
14. Zhang N, Suleiman JS, He M, Hu B. Chromium(III)-imprinted silica gel for speciation analysis of chromium in environmental water samples with ICP-MS detection. *Talanta* 2008;75:536–543.
15. Kiran K, Kumar KS, Prasad B, Suvardhan K, Babu LR, Janardhanam K. Speciation determination of chromium(III) and (VI) using preconcentration cloud point extraction with flame atomic absorption spectrometry (FAAS). *J Hazard Mater* 2008;150:582–586.
16. Lin L, Lawrence NS, Thongngamdee S, Wang J, Lin Y. Catalytic adsorptive stripping determination of trace chromium (VI) at the bismuth film electrode. *Talanta* 2005;65:144–148.
17. Svancara I, Foret P, Vytras K. A study on the determination of chromium as chromate at a carbon paste electrode modified with surfactants. *Talanta* 2004;64:844–852.
18. Bas B. Refreshable mercury film silver based electrode for determination of chromium(VI) using catalytic adsorptive stripping voltammetry. *Anal Chim Acta* 2006;570:195–201.
19. Zhu WW, Li NB, Luo HQ. Simultaneous determination of chromium(III) and cadmium(II) by differential pulse anodic stripping voltammetry on a stannum film electrode. *Talanta* 2007;72:1733–1737.
20. O'Mahony AM, Scanlon MD, Berduque A, et al. Voltammetry of chromium(VI) at the liquidliquid interface. *Electrochem Commun* 2005;7:976–982.
21. Korolczuk M. Voltammetric determination of traces of Cr(VI) in the presence of Cr(III) and humic acid. *Anal Chim Acta* 2000;414:165–171.
22. Husakova L, Bobrowski A, Sramkova J, Królicka A, Vytras K. Catalytic adsorptive stripping voltammetry versus electrothermal atomic absorption spectrometry in the determination of trace cobalt and chromium in human urine. *Talanta* 2005;66:999–1004.

23. Li Y, Xue H. Determination of Cr(III) and Cr(VI) species in natural waters by catalytic cathodic stripping voltammetry. *Anal Chim Acta* 2001;448:121–134.
24. Carrington NA, Yong L, Xue Z-L. Electrochemical deposition of sol-gel films for enhanced chromium(VI) determination in aqueous solutions. *Anal Chim Acta* 2006;572:17–24.
25. Ly SY, Jung YS, Kim MH, Han IK, Jung WW, Kim HS. Determination of caffeine using a simple graphite pencil electrode with square-wave anodic stripping voltammetry. *Microchim Acta* 2004;146:207–213.
26. Matsumoto K, Li J, Ohzawa Y, Nakajima T, Mazej Z, Zemva B. Surface structure and electrochemical characteristics of natural graphite fluorinated by ClF_3 . *J Fluorine Chem* 2006;127:1383–1389.
27. Yonezawa S, Yamasaki M, Takashima M. Surface fluorination of the cathode active materials for lithium secondary battery. *J Fluorine Chem* 2004;125:1657–1661.
28. Weglikowska UD, Skakalova V, Meyer J, Cech J, Mueller BG, Roth S. Effect of fluorination on electrical properties of single walled carbon nanotubes and C60 peapods in networks. *Curr Appl Phys* 2007;7:42–46.
29. Mazej Z. Primoz Benkic, copper(I) hexafluoroantimonate—an example of a compound with Cu in a solely fluorine environment. *J Fluorine Chem* 2005;126:803–808.
30. Carrington NA, Yong L, Xue Z-L. Electrochemical deposition of sol-gel films for enhanced chromium(VI) determination in aqueous solutions. *Anal Chim Acta* 2006;572:17–24.