Real-time Assay of Toxic Lead in *In Vivo* Living Plant Tissue

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A method of detecting lead was developed using square wave anodic stripping voltammetry (SWASV) with DNA-carbon nanotube paste electrode (CNTPE). The results indicated a sensitive oxidation peak current of lead on the DNA-CNTPE. The curves were obtained within a concentration range of $50 \, \text{ngL}^{-1}$-$20 \, \text{mgL}^{-1}$ with preconcentration time of 100, 200, and 400 sec at the concentration of mgL^{-1}, μgL^{-1}, and ngL^{-1}, respectively. The observed relative standard deviation was 0.101% (n = 12) in the lead concentration of $30.0 \, \mu\text{gL}^{-1}$ under optimum conditions. The low detection limit (S/N) was pegged at $8 \, \text{ngL}^{-1}$ (2.6×10^{-8} M). Results showed that the developed method can be used in real-time assay *in vivo* without requiring any pretreatment and pharmaceutical samples, and food samples, as well as other materials requiring water source contamination analyses.

Key words: DNA-CNTPE, Lead anodic stripping voltammetry, Low concentration, *In vivo*, Plant, Tap water

INTRODUCTION

Lead is an important toxicological heavy metal that may pose a health risk to humans. Exposure to lead can cause the metal to enter the blood stream and transmitted to children (1). Lead exposure can cause encephalopathy, kidney damage (2), brain and lung cancers (3), can be found in foods and drinks or environmental water source (2), and induce mutations (4). Lead analysis is particularly important in the biological matrix and clinical treatments not only in human erythrocytes and lymphocyte membranes (5), serum (6), blood, DNA (7), but also play a role in the violent behavior of young children (8), because lead is strongly

toxic and poisonous. Lead is also present in vegetables (9), ground water, soil (10), food, atmospheric air (11), plants (12), and so on. Various analytical techniques have been developed to study lead content in food, pesticide, and snow (13). Some of the more specialized continuous flow methods that have been developed include the capillary electrophoresis detection method (14), high-throughput screening (HTS), high performance liquid chromatography (HPLC) analysis technique (15), the micellar electrokinetic capillary chromatography (MECK) method (16), and the batch injection analysis (BIA) (17). All these methods involve complicated pretreatment techniques and expensive instruments. Attempts to simplify such methods have resulted in the development of methods such as the energy-dispersive polypyrrole-coated mercury film electrode (18), lead (II)-selective electrode (19), and nonmagnetic metallic electro-encephalography (EEG) electrodes (20). Nonetheless, these methods use specific properties and modified working electrode systems that are still complicated. These electrochemical methods are widely used in monitoring lead in various substances around human beings and more developed methods are expended for low detection range. For example, lead (II)-selective electrode can achieve a low detection limit of 5.0×10^{-7} M (19). The screen-printed carbon electrode ($2.5 \, \text{ngmL}^{-1}$) (21), bismuth-coated screen-printed electrode ($0.3 \, \mu\text{gL}^{-1}$) (22), carbon fiber disk ultramicroelectrode

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$(0.8\,\mu gL^{-1})$ (23), carbon paste electrode $(1.00 \times 10^{-8}\,M)$ (24), and the hanging mercury dropped electrode $(20\,ngmL^{-1})$ (17) achieve very sensitive detection ranges. However, they still pose problems that make biological, environmental, and other sensitive analyses insufficient because of low concentration and interference from several kinds of electroactive lead. In this study, the stripping voltammetric technique, which manifests a faster response, better cost efficiency, and sensitive preconcentration techniques, was used along with a simpler DNA-CNTPE. The DNA property was searched in various papers with biological attraction (25), catalytic effects (26) and interaction structure with metal elements (27). Also, the carbon nanotube property is specific in the biosensor (28), which combined with the DNA. The method achieved lower detection ranges compared to results obtained using other complicated modified methods. The objective of this study is to find real-time continuous detecting reagents at infinitesimal concentrations without causing any destruction and injury to subjects, and allows direct analysis of living cells. The proposed method can be used to assay for toxicological heavy metals and pharmaceuticals, food samples, and other materials requiring lead analysis.

MATERIALS AND METHODS

Apparatus and reagents. All voltammetric measurements were carried out using a CHI660A instruments electrochemical workstation (CH Instruments Inc., Cordova, TN, USA). A three-electrode system was used to monitor the SWASV signal. DNA-CNTPE was used as the working electrode and with saturated Ag/AgCl as the reference electrode $(3\,molL^{-1}KCl)$. Platinum wire was used as the auxiliary electrode. The working electrode was made of paste, which is a mixture of nanotube (from Nanostructured & Amorphous Materials, Inc.) and DNA (double-stranded and prepared from calf thymus sigma) (in the ratio of 1 : 1) with mineral oil. The small amount of mixed paste was inserted into a plastic needle-type capillary tube with a 1.5 mm diameter 5 Cm in length, using a 0.5-mm diameter copper wire connected to the measurement system. All systems were subjected to a room temperature of (24 ± 2) °C. The reagent solution was prepared from doubly distilled water $(18\,MOhm.Cm^{-1})$. The lead standard solution and other reagents were obtained from Aldrich and diluted as needed.

Experimental procedure. Several electrolyte solutions of acid, base, and buffer (all in 0.1 M) were initially examined in search for a possible supporting electrolyte. Ammonium phosphoric acid solution was found to be the most suitable medium, yielding the best peak separation from the background currents. The effect of the concentration of phosphoric acid was studied within the range of 0.01~0.3 M. A concentration of 0.1 M phosphoric acid solution with a

pH of 4.6~4.8 was found to be most suitable. Square wave anodic stripping voltammograms were tested for optimum conditions. Lead accumulated on the DNA-CNTPE at −1.2~1.0 V generally. Lead peak currents were found in stripping voltammograms at −0.4 V. This finding was consistent with those of previous studies. It was also found to be not necessary to carry out electrode cleaning for every measurement. In the results, all experiments could be performed in an open circuit.

Experimental optimization of the various SW parameters and electrode comparison. The unit "mgL^{-1}," commonly used to detect the presence of even a limited amount of lead, was used in this study for the same purpose (Fig. 1A). The presence of lead was indicated by the CV signals of its various concentrations. In the CV graph, it is shown that the peak current correctly and clearly appeared at −0.4 V during oxidation while the peak potential of reduction moved and presented at around −0.5 V, not −0.4 V. Both peak currents that were attained after different lead concentrations were prepared were close to a direct proportion graph, as shown in Fig. 1(A). The growth of the signal during oxidation, however, is greater than that during reduction. It was then determined that lead could be detected and the response on DNA-CNTPE during oxidation is more sensitive than the response during reduction. Thus, only the oxidation signal was analyzed in this paper using SWASV. The next experiment is electrode comparison between the DNA-CNTPE and a nanotube electrode. As shown in two SW graphs in Fig. 1B, there were many noises on the nanotube electrode, but the definite peak current on DNA-CNTPE was found to be around −0.4 V. As shown in the bigger graph in the same figure, the currents from DNA-CNTPE grew regularly and visually with 4.95, 11.65, $18.49 \times 10^{-6}\,A$, and that is close to a direct proportional graph. Whereas, the currents from the nanotube electrode grew similar to that from the DNA-CNTPE. The peak currents on the nanotube electrode were 0, 0321, $0.36 \times 10^{-6}\,A$. Through this experiment, DNA-CNTPE was determined to be an effective electrode for the detection of lead. Fig. 1C illustrates the hydrogen ionic activity of the SWASV peak current in various pH values within the range of 2.55~6.5 with 0.1 M ammonium phosphoric acid solution as the electrolyte solution. Although there were three relatively high current peaks (initial three pH with 25.16, 17.26, $15.09 \times 10^{-5}\,A$) in the SW graph in Fig. 1C, they could not be said as the peaks for lead because they appeared at other potentials and not at −0.4 V. Moreover, pH 3.92 had the highest current, $2.74 \times 10^{-5}\,A$, recognized as the optimum pH for lead as shown in the bigger graph in Fig. 1C. At that time, the parameters were primary figures except for the accumulation time of 100 sec. As shown in Fig. 1D, the first frequency, 300 Hz, had the highest peak current $(2.18 \times 10^{-5}\,A)$. After 300 Hz, peak current
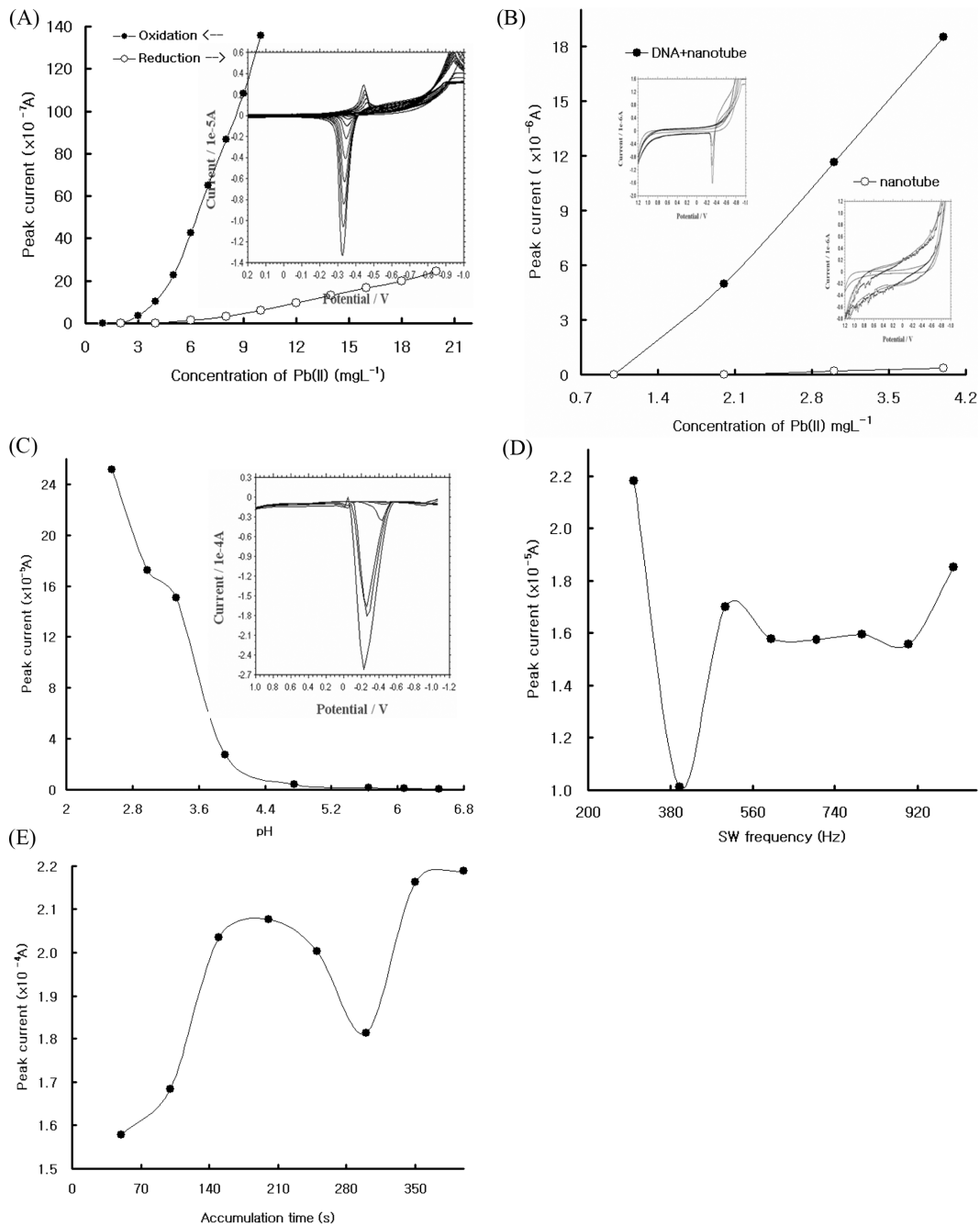
Fig. 1. (A) various concentration effects of 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 mgL^{-1} using CV; (B) comparison between DNA-CNTPE and nanotube electrode with 2, 4, and 6 mgL^{-1} Pb(II); (C) SW signals when pH was varied in 2.55, 2.98, 3.33, 3.92, 4.75, 5.65, 6.08, and 6.5 pH; (D) SW signals from diverse frequency in 300, 400, 500, 600, 700, 800, 900, and 1000 Hz; (E) various accumulation time in 50, 100, 150, 200, 250, 300, 350, and 400 sec using SWASV.

decreased quickly until it reached 400 Hz. Aside from that variation, there was almost no change of peak current. In the SW graph, the clear peak current appeared at around −0.4 V on DNA-CNTPE, although the figure is not shown in this paper. Thus, 300 Hz was chosen as the optimum frequency. The parameter for this test was pH 3.92, and other parameters were not specified. Fig. 1E shows the effect of

varying square wave accumulation time (50~400 sec) on DNA-CNTPE when oxidation progressed. At first, peak current increased quickly till 200 sec. After that, there was a decline between 200 and 300 sec, and peak current started to increase until 400 sec. The highest peak current (2.18 × 10^{-4} A) appeared at 400 sec as shown in Fig. 1E. In the SW graph, the peak current also existed at around −0.4 V.

For this reason, 400 sec was chosen as an optimum parameter for detecting lead. The following parameters were considered: pH of 3.92, frequency of 300 Hz, and accumulation time of 400 sec. Except for them, other parameters were not managed. In this study, the optimum conditions were determined: pH of 3.92, frequency of 300 Hz, accumulation time of 400 sec, amplitude of 0.15 V, increment of 0.03 V, and initial potential of −1.1 V. They are detected in that order. The detection of other parameters except frequency, accumulation time, and pH were also performed (data not shown).

RESULTS

SWASV peak current from various concentrations on DAN-CNTPE with the units, 'mgL^{-1}'; 'µgL^{-1}' and 'ngL^{-1}' and interference-test.   Fig. 2A illustrates the effects of varying lead concentrations expressed in the unit "mgL^{-1}." The width of the lead peak narrowed following a 100-sec deposition of raw voltammograms, as shown in the SW graph. The peak currents were detected following various concentrations. Those peak currents increased gradually at the range of $1.67\text{-}119.7 \times 10^{-6}$ A. Thus, the line in the bigger graph is close to the direct proportion graph in Fig. 2A. It was determined that the response of DNA-CNTPE from a lead concentration expressed in the unit "mgL^{-1}" is sensitive, as shown in Fig. 2A. After determining the sensitiveness of DNA-CNTPE as expressed in the unit "mgL^{-1}," the response of the DNA-CNTPE was checked at the level of unit "µgL^{-1}." The result is shown in Fig. 2B. The deposition time was 200 sec. In the SW graph, the peak current clearly appeared at around −0.4 V, and it regularly grew henceforth. The line in the bigger graph is also close to a straight line because each signal increased gradually at the range of $0\text{-}10.32 \times 10^{-6}$ A. Lead was also detected in a lower "ngL^{-1}" concentration in the subsequent examination. The last concentration examination was carried out in the concentration of "ngL^{-1}." The deposition time was 400 sec. It is recognized through Fig. 2C that the response of DNA-CNTPE in the concentration of "ngL^{-1}" is also sensitive as well as those in concentrations of "mgL^{-1}" and "µgL^{-1}." The peak currents appeared between $7.26\text{-}96 \times 10^{-7}$ A and an almost linear graph could be given through those data as shown in Fig. 2C. In the result, the response in this concentration was also effective. These detections were performed in turn of the units, "mgL^{-1}", "µgL^{-1}" and "ngL^{-1}" as indicated in Fig. 2. The unit "µgL^{-1}" is used to examine the detection limits of DNA-CNTPE in other papers. In this paper, however, the unit, "ngL^{-1}" was also used to examine the detection limits of DNA-CNTPE as well as "µgL^{-1}," and this study proved that the sensitivity of DNA-CNTPE is effective even in the concentration of "ngL^{-1}." After all the concentration tests, analogy interference tests were carried out (data not shown). Deposition time for this test was 100 sec, and reagents used were Pb,
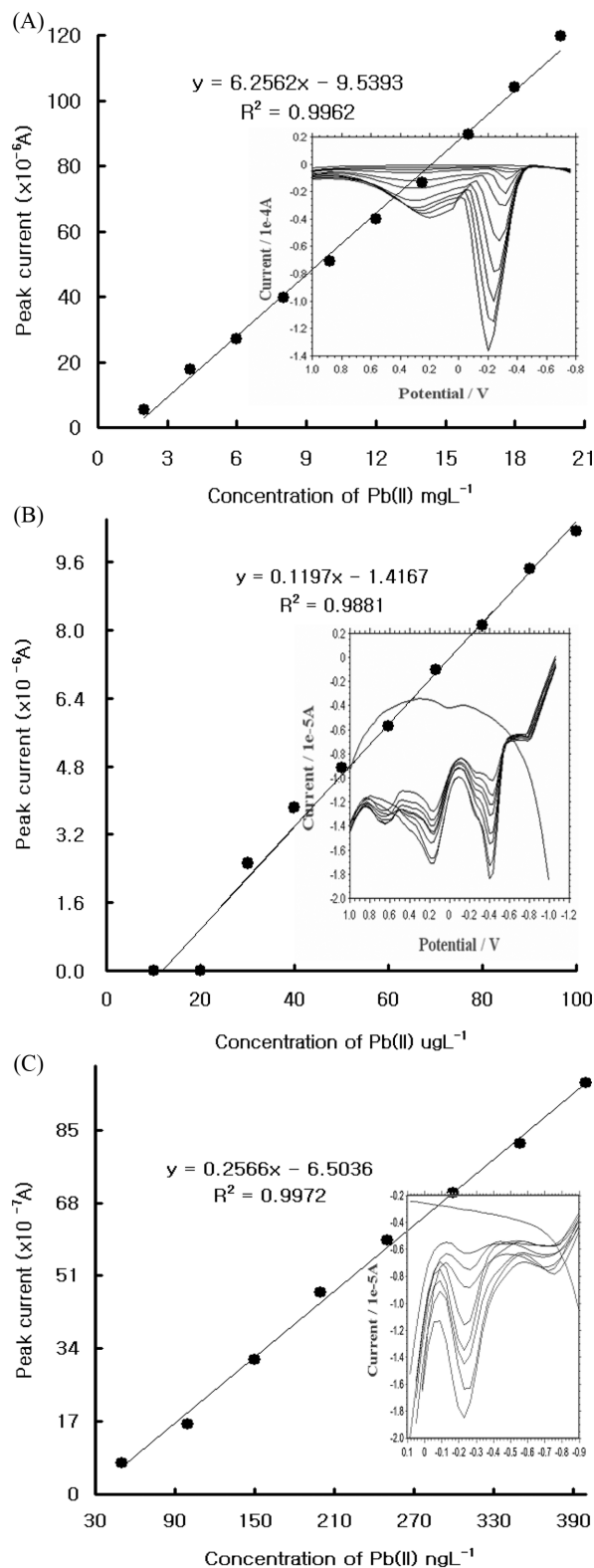

Fig. 2. (A) SWASV signals from various concentrations of 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 mgL^{-1} with 100sec; (B) diverse concentrations of 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µgL^{-1} with 200 sec; (C) change in concentrations of 50, 100, 150, 200, 250, 300, 350, and 400 ngL^{-1} with 400 sec using SWASV.
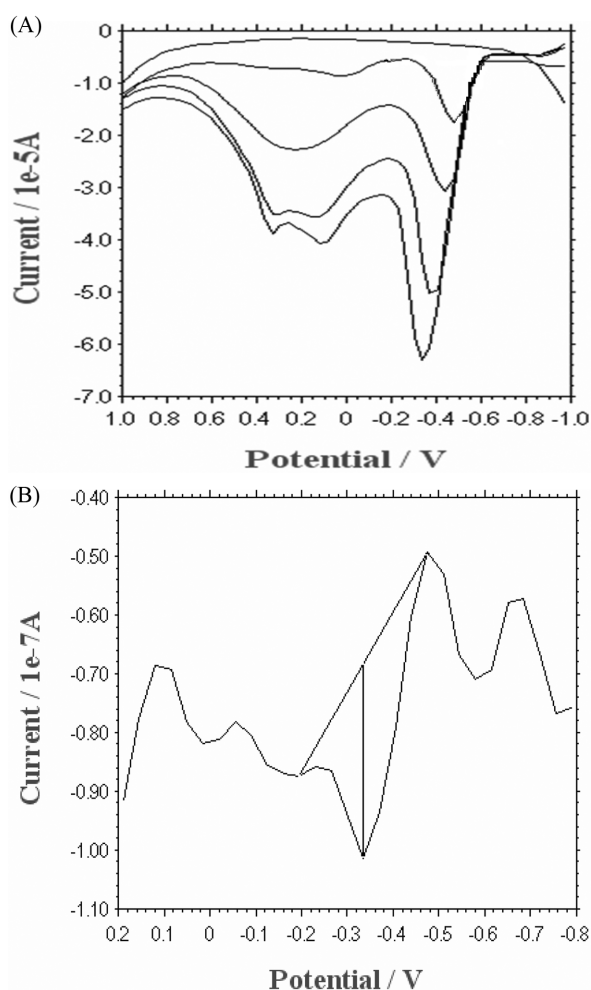
(A)

(B)

Fig. 3. (A) the result of application using 2 ml pond water with 300 sec with various lead concentrations of 1 mgL^{-1}, 2 mgL^{-1}, and 3 mgL^{-1} spike; (B) the result of application using a living plant's tissue raised in that pond water.

Ba, Cr, Co, Hg, Ge, Bi, and Cd. Pb and other reagents are in the ratio of 1 to 10 (mgL^{-1}). Each of them influenced the concentration of lead as 100%, 144.93%, −94.71%, −96.84%, −92.12%, −92.95%, −90.54%, and −8.5%. Ba had the strongest effect on lead with 28.2 × 10^{-6} A. The presence of other ions was also effectively corrected using standard addition methods.

DISCUSSION

Before the experiments shown in Fig. 3A and Fig. 3B, the statistics of detections of lead were carried out. At first, detection was performed in 0.1 M ammonium phosphoric electrolyte acid without any lead with a deposition time of 300 sec as blank. After that, 1000 μgL^{-1}-lead solution of 10 μgL^{-1} was spiked, and lead was examined 15 times with a deposition time of 300 sec. These statistics were gathered to prove that the response of DNA-CNTPE was effective

through RSD. The peak current signal clearly appeared at −0.4 V and grew closely and in an orderly way. According to this experiment, RSD was 0.02. The last experiments were applications of this study. The first application was carried out using 2 mL pond water. Lead solution of 1 mgL^{-1}, 2 mgL^{-1}, and 3 mgL^{-1} were spiked in prepared pond water and the peak currents were checked if they were of the same strength. As shown in Fig. 3A, the peak current, which is comparatively close together and linear, appeared at −0.4 V. With the use of standard addition methods, it can be concluded that there is 0.585 μgL^{-1} lead per 2 ml pond water. The next application was performed using a living plant's tissue. Lead solution of 1000 mgL^{-1} was spiked in 0.05 ml of the previously described water, where the living plant had been raised, in a 500 mL-erlenmayer flask. After about 1 or 2 days, 0.1 ml lead solution (total 0.15 ml lead solution) was spiked in the same water. The living plant, which had been left alone in that spiked water, was implanted by three electrodes and detected in real time to find whether the peak current was present in the same place or not. Those three electrodes were Cl-plate Ag electrode as the reference electrode, Pt electrode as count electrode, and DNA-CNTPE as working electrode. Fig. 3B shows that there was lead in that living plant by the position of the peak current, −0.4 V, although it could not be accurately determined how much lead was there. The experiment shown in Fig. 3A was conducted using quantitative and qualitative analysis. This experiment, however, was performed using only qualitative analysis.

Low lead concentration was detected by using SWASV and DNA-CNTPE. The electrode response was found to be linearly related to lead concentrations ranging from 20 ngL^{-1} to 20 mgL^{-1} at 400 sec ("ngL^{-1}" as the unit), 200 sec ("μgL^{-1}" as the unit) and 100 sec ("mgL^{-1}" as the unit) accumulation time. Lead ionic activity reached the maximum peak at a pH of 3.92, and various interference ions were corrected by using standard addition methods. Ba, Cr, Co, Hg, Ge, and Bi were observed to cause strong interference. The method used in this study had a lower detection limit of 0.8 ngL^{-1} (S/N = 3), could continuously detect lead in real time without having to destroy living subjects, can conduct infinitesimal investigations at the level of mgL^{-1} and ngL^{-1} as well as mgL^{-1}, and can replace extremely expensive and complex devices such as GC-mass and ICP-MASS that detect substances only with the destruction or causing damage on subjects by many kinds of preprocessing. With the given advantages, it can be used to assay biological materials, pharmaceuticals, food, and drugs. It can also be applied in other fields that require lead analysis.

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