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Research Article

IN VIVO SEPTICEMIA POISONING EFFECT BY USING VOLTAMMETRIC CARBON FIBER MICRO ELECTRODE

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ABSTRACT

In vivo assay of Escherichia Coli (EC) was searched at poisoned vascular organ using carbon fiber micro electrode systems. A diagnostic instrument was used with our systems of bio electronic workstation-2, and detection was searched using a handmade micro working electrode, whose probe was made by copper immobilization on a carbon fiber micro sensor; also, two electrode were used to counter and reference probe fiber instead of expensive Ag/AgCl standard and Pt counter electrode. Under final condition, cyclic peak potential appeared at a 0.2 v anodic. Square wave stripping working range was to 0.1-0.8 mg/l EC, linear equation of y=0.0027x+1.35, and R^2=0.97. The developed probe was applied to non-treated in vivo vascular using health and septicemia poisoned flog by EC injection, and a fast 210 sec accumulation stripping time. Diagnostic detection limit attained to micro range. Developed results can be applied to in vivo vascular at real and non-treated organs.

INTRODUCTION

Poisoned in vivo Septicemia is related to bloody diarrhea and hemorrhagic colitis [1], which can be kidney failure and nerve damage [2]. A number of diagnostic EC recognitions have been considered as PCR amplification [3], plasmon resonance immuno assay [4], multiplex PCR method [5] and others. Here PCR amplifications require complicated electrophoresis, gel separation, and expensive spectrometric detection techniques demand [6,7]. However, voltammetric method [8] is more simple [9] and inexpensive instrumental systems [10] with a sensitive detection limit [11]. For this reason, faster stripping real detection [12] has been developed for our study, such as Papilloma Virus 58 DNA [13], human hepatitis B virus [14], Helicobacter pylori DNA [15], and carcinoembryonic antigen tumor markers [16]. Here method is a simple, inexpensive [17] and low detection ranges, here sensitivity was improved by immobilization [18] on a working electrode and is more convenient and renewable, which yielded lower working limits than those of common PCR methods. The final results can be used in medicinal detection for septicemia or poisoning in vivo vascular direct.

Experimental Procedure

Diagnostic System: The cognitic instruments were carried out using with our Bioelectronic-2 circuits, which were made by the author's institution. System designing was fabricated into a computerized handheld voltammetric size, similar to a standard cellular phone. Scanning Potential windows from 2.0 V to -2.0 V range, amplified working current can detect to $10^{-5\sim6}$A. Cyclic and square wave stripping voltammetric behavior, it was connected to handmade sensor probe.

Sensor preparation; The EC sensor of working counter and reference probe were made by Copper immobilize on a 0.3 um diameter, 50 mm long wire type carbon fiber micro electrode by 1000 mg/L Cu(II) standard coat using cyclic electrolyte 40th repeated immobilization. All experiments were performed at room temperature without removing the oxygen and using seawater electrolyte instead of an expensive regent solution.

Reagents, bacteria, and procedure: All the reagents were of standard grade from Merck. Voltammetry was performed on an open circuit. Highly purified water was used (three times distilled) 18 M/cm of the Milli Q Ultra-pure Water Systems. For the reaction potential, Cyclic Voltammetry was performed from 2.0 V initial, -2.0 V switching potential, 0.5 V scan rate. E. coli was obtained from our research center at the University. All cultures were performed on Tryptic Soy Agar slants and plates. Cultures for the ECs were grown for 20 hours at 37C with aeration and serially diluted ten-fold in sterile 10 mmol/l phosphate buffered saline using a pH of 7.0. The number of CFU was counted for $3\times10^2\sim4\times10^2$ CFU/1ml.

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RESULTS AND DISCUSSION

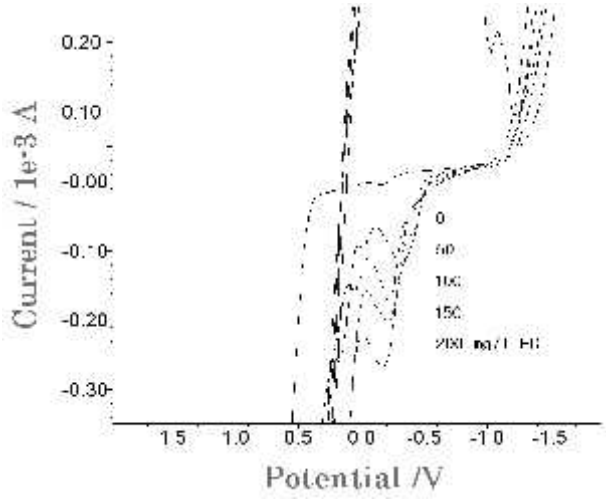

Fig1(A)

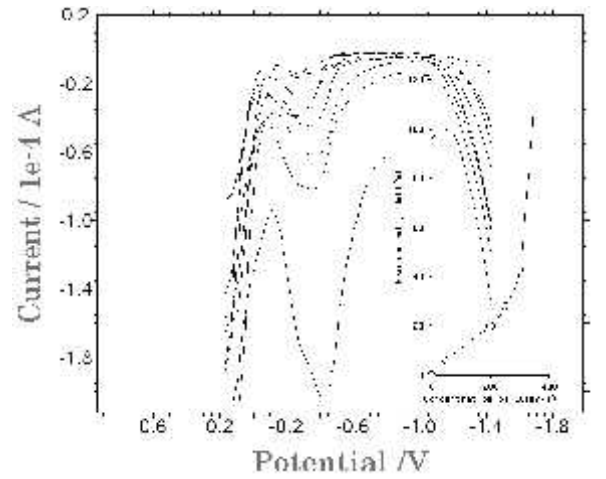

Fig1(B)

Figure 1(A) Cyclic concentration effects for the EC. The first curve represents the saline electrolyte blank, then 0, 50, 150, 200, 250, 300, and 350 mg EC added, a -2.0 V initial potential, a 0.4 V switching potential, with a 0.1 mV/sec scan rate by oxidation start. **(B)** the anodic stripping voltammetry in 0, 50, 150, 200, 250, 300, and 350 mg EC variation using in the electrolyte blank,.

EC peak detection was conducted using cyclic and stripping voltammetric scan with a wide potential window from 0.4 V to -2.0 V switching, 0.1 mV/sec scan rate, oxidation start performed. Saline solution was used as a blank electrolyte. While blank was not representing any signals, EC showed a variety of continuing peaks, as is shown in Fig. 1(A). In this diagram, reaction voltammogram is showing two peaks during oxidation scan, which were obtained with -0.2 V direction and -0.6 V reduction with 50 mg added. Here, peak is 5.67×10^{-5}A, then 7.04×10^{-5}A, 9.31×10^{-5}A, 6.97×10^{-5}A, 6.38×10^{-5}A, and 5.93×10^{-5}A; peak current was oscillated, however reduction peak was not. Then, more sequential stripping was examined by the same methods with 50-350 mg EC added. Fig. 1(B) shows results from real voltammograms anodic range, -2.0 V initial, 2.0 V switching potential, and accumulation time with 0.0 sec applied; here, peaks were of 5.88×10^{-5}A, 15.69×10^{-5}A, 19.91×10^{-5}A, 27.87×10^{-5}A, 42.78×10^{-5}A, and 111.7×10^{-5}A. A linear curve was inserted, whose peak potential is -0.4 V, which can be obtained for high ranges. Under these conditions, more sensitive stripping parameters were examined using fixed strength by 350 mg EC added anodic scan.

SW diagnostic parameters

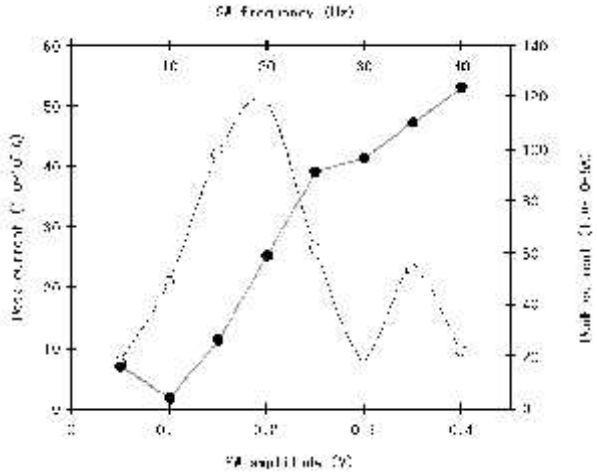

Fig.2

Figure 2(A) Determining optimum conditions by gradually changing amplitude from 0.05 V to 0.4 V (white ball) and frequency from 0.00 HZ to 40 HZ (black ball). Throughout the voltammograms, the highest peak current obtained while changing amplitude was 50x10-6A at amplitude 0.2 V, and frequency was 40 HZ at 120x10-5A when 10 mg EC was added.

In the 10-mg/EC constant, the optimum SW diagnostic parameters of amplitude and frequency were sought via EC probe variations for 0.05 V, 0.1 V, 0.15 V, 0.2 V, 0.25 V, 0.3 V, 0.35 V, and 0.4 V eight points (X scale). Amplitude scan studied by anodic. Fig. 2(A) shows the raw voltammograms peak current (Y scale), which was obtained to 7.78×10^{-6}A, 21.05×10^{-6}A, 42.1×10^{-6}A, 50.65×10^{-6}A, 26.48×10^{-6}A, 8.73×10^{-6}A, 22.98×10^{-6}A, and 9.52×10^{-6}A. where are quickly increased when weak current appeared; 0.2 V is very sensitive at this amplitude, SW frequency variation was performed to 5, 10, 15, 20, 25, 30, 35, and 40 Hz. Here, results are shows in Fig. 2(B), where black ball continues to increase for 15.91×10^{-6}A, 3.78×10^{-6}A, 26.14×10^{-6}A, 59.00×10^{-6}A, 90.99×10^{-6}A, 96.31×10^{-6}A, 110.2×10^{-6}A, and 123.2×10^{-6}A. Optimum frequency is 40 Hz; thus, 0.2 V amplitude and 40 Hz frequency were fixed under these conditions, and the SW accumulation potential and times were sought. The results of 210 sec accumulation time and -2.0 V initial potential were obtained. Under these conditions, the diagnostic linear working ranges were examined.

Diagnostic working range and statistics

Under the conditions, analytical linear ranges were examined using cyclic and stripping scan. Fig. 3(A) shows results for cyclic scan with -0.6 V initial, 0.6 V switching potential, a scan rate of 0.5 V/sec; added concentrations were varied from 0.1~0.7 mg/l EC and first voltammogram is a blank solution. that is simple, not any reaction peak appeared, while next peak is 0.1 ml add, so 0.1 V reduction peak obtained with 8.26×10^{-5}A, then 19.62×10^{-5}A, 29.77×10^{-5}A, 35.3×10^{-5}A and 40.8×10^{-5}A. Here, linear equation was y=0.0607x-1.9714, R^2=0.9864; only oxidation peak appeared, where working results can be applicable to ex vivo vascular and in vivo poisoning assays.

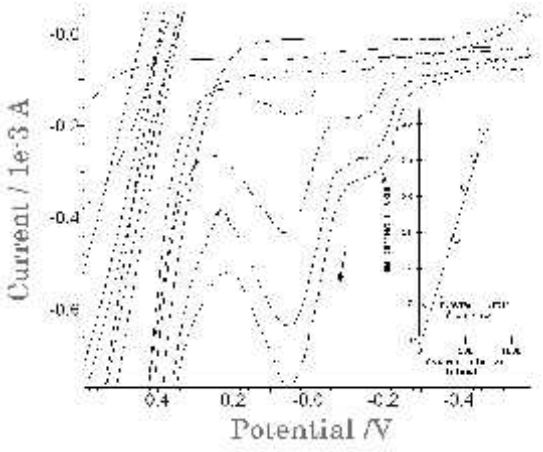

Fig3(A)

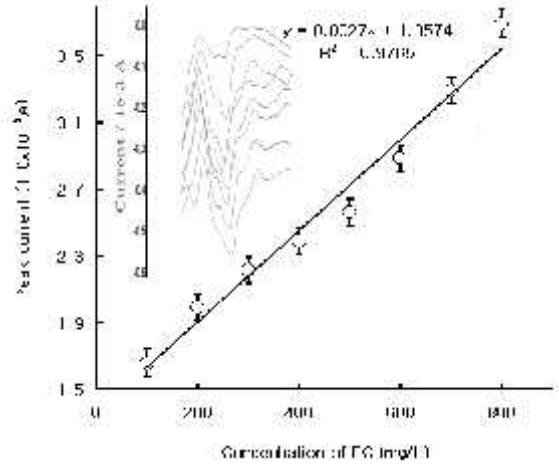

Fig3(B)

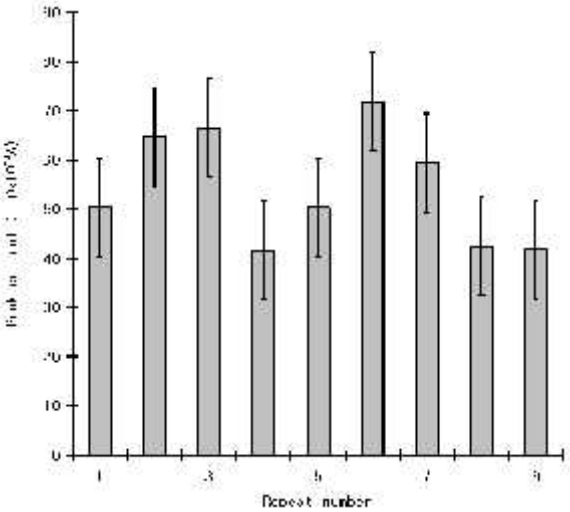

Fig3(C)

Figure 3(A): The square wave concentration effect of 0, 0.2, 0.4, 0.5, 0.6, and 0.7 mg/l EC added, 60 sec accumulation time, anodic window for -0.6 V initial, 0.6 V final potential, by optimum parameters. **Figure (B):** The statistic repeated SW detection at 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, and 0.8 mg/l EC constant, SW anodic 60 sec accumulation, with optimum conditions. **Figure (C):** 15th repeated SW voltammetry statistics by 10 mg/l EC constant.

Thus, a more sensitive method of stripping accumulation techniques was examined to oxidation scan, sequential add is shown in Fig. 3(B). Also, the first curve is linear and did not obtain any reaction peak, file next of 0.1 mg/l EC is -0.2 V cathodic appeared to 1.66×10^{-5}A, then continued addition was obtained at 1.99×10^{-5}A, 2.27×10^{-5}A, 2.38×10^{-5}A, 2.56×10^{-5}A, 2.88×10^{-5}A, 3.29×10^{-5}A, 3.69×10^{-5}A, where are better sensitive then cyclic voltammetry. Here, stripping can be used for in vivo vascular and organic diagnosis. More advanced statistics for repeated application was performed at the same conditions by cyclic scan using electrolyte blank and 10 mg/l EC standard. Here, results show for probe stability, accuracy and repeated precisions, Fig. 3(C) is a histogram, oxidation peak obtained to 15th repeated voltammogram, while shows 9th, each peak are 50.38×10^{-6}A, 64.7×10^{-6}A, 41.5×10^{-6}A, 50.25×10^{-6}A, 71.84×10^{-6}A, 59.35×10^{-6}A, 42.32×10^{-6}A, and 41.82×10^{-6}A, relative error percentage is shown insert gram, where is varied to 15.0×10^{-5}A range, The peak became sensitive. Respectively. Stripping cathodic show that there were EC traces, which results can be applied to any diagnostics.

In vivo vascular application

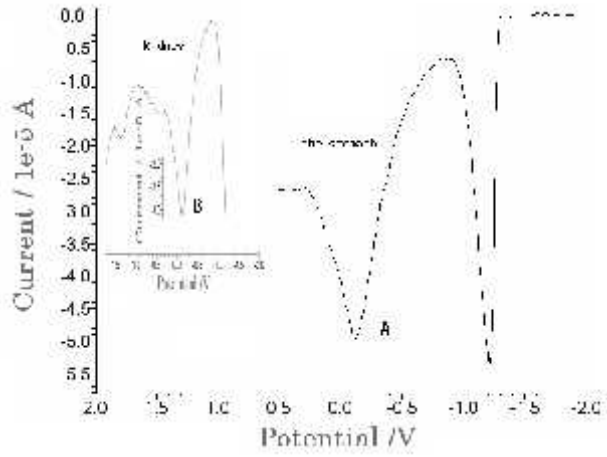

Fig.4

Figure 4: Stripping voltammetric EC detection for poisoned in vivo vascular of frog stomach (A) and a kidney (B) using SW 40 Hz frequency, 0.2 V amplitute, 210 sec accumulation time, --2.0 V initial potential

The developed results were applied to frogs by weight 60 g, real time assay in *in vivo* vascular under anesthesia conditions. Needle type working probe was inserted into the stomach (A), kidney (B), and other vascular organs. The open tissue was cemented with a tooth binder, all prove connected to a 0.05 mm enamel coated copper wire with an electrochemical work station system; then, SW anodic stripping was performed. Here, results are shown in Fig. 4, with peak A is poisoned signal by 50 mg EC injection; at this figure, a large peak appeared, anodic at -0.2 V potential for 5.0×10^{-5}A current high. Here, results can be applied to ex vivo or in vivo poisoning detection. Moreover, other kidney sites show the results of Fig. 4 insert B, where 5.0×10^{-5}A peak was obtained, and the peak became sharper. Results show that there can be accumulated in animal bodies in *in vivo* vascular.

CONCLUSION

Recognition of Escherichia Coli was searched by cyclic and stripping voltammetry, and diagnostic SW optimum parameters were obtained at 40 Hz frequency, 0.2 V amplitude, 210 sec accumulation time, and -2.0 V initial potential. Under these conditions, the detection limit was attained to 80 µg/L EC, in

which the developed probe was applied to in vivo poisoned vascular by EC injection. It was found that the living tissue and the real organ. The developed results of this study can be applied to in vivo fluid or medicinal assay.

References

1. Maysaa El Sayed Zaki, Hala El Adrosy, Diagnosis of Shiga toxin producing Escherichia coli infection, contribution of genetic amplification technique, *Microbes and Infection* 9 (2007) 200-203.
2. E. Cummins, P. Nally, F. Butler, G. Duffy, S. O'Brien, Development and validation of a probabilistic second order exposure assessment model for Escherichia coli O157:H7 contamination of beef trimmings from Irish meat plants, *Meat Science* 79 (2008) 139-154.
3. Y. Li, A. Mustapha, Development of a polymerase chain reaction assay to detect enteric bacteria in ground beef, *Food Microbiology* 21 (2004) 369-375.
4. Anand Subramanian, Joseph Irudayaraj, Thomas Ryan, A mixed self assembled monolayer based surface plasmon immunosensor for detection of E. coli O157:H7. *Biosensors and Bioelectronics* 21 (2006) 998-1006.
5. Vijay K. Sharma, Evelyn A. Dean-Nystrom, Detection of enterohemorrhagic Escherichia coli O157:H7 by using a multiplex real-time PCR assay for genes encoding intimin and Shiga toxins, *Veterinary Microbiology* 93 (2003) 247-260.
6. Jay L.E. Ellingson, Jeff J. Koziczkowski, Jennifer L. Anderson, Steve A. Carlson, Vijay K. Sharma, Rapid PCR detection of enterohemorrhagic Escherichia coli (EHEC) in bovine food products and feces, *Molecular and Cellular Probes* 19 (2005) 213-217.
7. Shaw Jye Wu, Alex Chan, Clarence I. Kado, Detection of PCR amplicons from bacterial pathogens using microsphere agglutination, *Journal of Microbiological Methods* 56 (2004) 395- 400.
8. Suw Young Ly, Diagnosis of Diabetes in a Diabetic Patient's Urine and Blood Using a Combination Electrode with a Ubiquitous Handheld Analyzer, *Current Analytical Chemistry*, 2009, 5, 59-64.
9. Suw Young Ly, Sang Su Song, Sung kuk Kim, Young Sam Jung, Chang Hyun Lee, Determination of Ge(IV) in rice in a mercury-coated glassy carbon electrode in the presence of catechol, *Food Chemistry* 95 (2006) 337-343.
10. Suw Young Ly, Young Sam Jung, Sung Kuk Kim, Hyun Kuy Lee, Trace Analysis of Lead and Copper Ions in Fish Tissue Using Paste Electrodes, Analytical Letters, 2007. 40: 2683-2692.
11. Suw Young Ly, Sang Su Song, Sung kuk Kim, Young Sam Jung, Chang Hyun Lee, Determination of Ge (IV) in rice in a mercury-coated glassy carbon electrode in the presence of catechol, *Food Chemistry* 95 (2006) 337–43
12. Suw Young Ly, Hwa Jin Heo, Min Jung Kim, Real Time Analysis of Neurotransmitters in the Brain Using a Micro Electrode System, *Current Neurovascular Research*, 2010, 7, 32-38
13. Suw Young Ly, WoonN Won Jun, Sung Kuk Kim, Myun HoonN Kim, Square-Wave Stripping Voltammetric Analysis of Human Papilloma Virus 58 DNA on Carbon Fiber Microelectrode, *Asian Journal of Chemistry*, Vol. 21, No. 5 (2009), 3509-3519
14. Suw Young Lya, Nam Sun Cho, Diagnosis of human hepatitis B virus in non-treated blood by the bovine IgG DNA-linked carbon nanotube biosensor, *Journal of Clinical Virology* 44 (2009) 43–47
15. Suw Young Ly, So Dam Yoo, Seung Kyu Chun, Detection of Helicobacter pylori DNA in preliminary stage gastric cancer cells, Pathology, 2012, 44, 251–254.
16. Suw Young Ly, Jung Eun Kim, Woo Yeon Moon, Diagnostic assay of carcinoembryonic antigen tumor markers using a fluorine immobilized biosensor with handmade voltammetric circuit, *European Journal of Cancer Prevention* 2011, 20, 58-62
17. Suw Young Ly, Determination of Epinephrine in Carp Brain Cells by Square Wave Anodic Stripping Voltammetry with Carbon Nanotubes, *Analytical Letters*, 45: 1197–203, 2012
18. Suw Young Ly, Kyung Lee, Hyung Tae Kang, Hai Soo Yoo, Investigation of Trace Cobalt in Glass Beads From an Archaeological Tomb, *IEEE Sensors Journal*, 6, 2011, 1325-1328

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