# Simultaneous Diagnostic Assay of Catechol and Caffeine Using an *in vivo* Implanted Neuro Sensor

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Catechol and caffeine were simultaneously analyzed with a bismuth-immobilized carbon nanotube paste electrode (BPE) using square wave (SW) stripping voltammetry. Optimum analytical conditions were determined. Simultaneous working ranges of 100-1,500 $mgL^{-1}$ for caffeine and 5-75 $mgL^{-1}$ for catechol were obtained. In the separated cell systems, a working range of 0.1-2.1 $mgL^{-1}$ catechol with a correlation coefficient of 0.9935, and a working range of 10-210 $mgL^{-1}$ caffeine with a correlation coefficient of 0.9921 were obtained. A detection limit (S/N) of 0.15 $mgL^{-1}$ ($7.7 \times 10^{-7}$ M) and a detection limit of 0.02 $mgL^{-1}$ ($1.82 \times 10^{-7}$ M), respectively, manifested for catechol and caffeine. It was found that three macro-type electrode systems could be implanted in fish and rat neuro cells. For both ions, the ion currents were observed. The physiological impulse conditions and the neuronal thinking current were also obtained.

**Key Words :** Catechol, Caffeine, Brain, Voltammetry

## Introduction

Caffeine and ethanol are commonly used as psychoactive dietary components,[1] clinical central stimulants,[2] diuretics and analgesics, and also for the treatment of brain disorders such as vascular headaches and Parkinson's disease.[3] Moreover, catechol ions are related to various mental functions, behavior,[4] breast cancer, schizophrenia,[5] Parkinson's disease, and high blood pressure.[6] Thus, in *in-vivo* diagnosis, the traces of these assays are essential for the proper functioning of a body's central nervous system and for physiological control. Furthermore, the redox activities of these materials have been associated with several neurodegenerative disorders. For these reasons, *in vivo* direct assays are very important in treating neuroblastoma, heart failure,[7] cancer, Parkinson and behavior controls.[4-6]

Various analytical methods that have low detection limits (DL) have been used for both ions, but all these methods were performed under laboratory conditions and are thus not usable in *in vivo* direct analysis. These methods are HPLC with the UV detection method (DL: 0.3 $mgL^{-1}$ caffeine),[8] capillary zone electrophoresis (DL: 1.9 $mgL^{-1}$ caffeine),[9] microchannel electrophoresis with the electrochemical detection method (DL: 4 uM caffeine),[10] solid-phase Fourier transform Raman spectrometry (DL: 18 $mgL^{-1}$ caffeine),[11] high-performance liquid chromatography assay (DL: 0.3 $mgL^{-1}$ caffeine),[12] the capillary gas chromatography method,[13] capillary electrochromatography,[14] GC and HPLC quantification,[15] gas chromatography-ion trap tandem mass spectrometry,[16] flow injection mass spectrometry,[17] and the capillary high-performance liquid chromatography/electrospray mass spectrometry system.[18] These techniques are applied only in in vitro conditions and are not applicable in *in vivo* direct assay. Moreover, catechol was studied in various analytical methods such as the capillary electrophoresis microchip with chemiluminescence method (DL: 10.0 uM catechol),[19] the voltammetric conducting polymer electrode (DL: 10 ppb catechol),[20] the amperometric detectors for liquid chromatographic method (DL: 1.5 $uML^{-1}$ catechol),[21] and the electrochemical detectors in flow injection HPLC method (DL: 0.5 $uML^{-1}$ catechol).[22] These methods were also applied only under laboratory conditions and required a complicated design, pre-treatment techniques, and expensive instruments. Moreover, these techniques are not applicable in *in vivo* or tissue assays, so simpler and lower-cost methods are needed. Voltammetric methods are inexpensive and useful for this purpose.[23,24] Related methods such as the anodic conductive diamond electrode,[25] the glass membrane pH electrode (DL: 0.6 $mgL^{-1}$ caffeine)[26] and others are studied in terms of sensitive working ranges, but these are still not applicable in *in vivo* direct assay. This study was first carried out for an *in-vivo* direct assay with an implanted BPE (bismuth-immobilized carbon nanotube paste electrode). The BPE sensor was prepared by using a graphite carbon nanotube (CN), which is usable in electrode sensor systems owing to its mechanical and catalytic capabilities.[27] Its CN powder was used with bismuth[28] immobilized on a carbon paste electrode.[29] Optimum analytical conditions were obtained, and the sensor was found to be more sensitive than other common methods. The sensor was interfaced with an electrochemical workstation and the neuron of a fish brain or a rat brain to gain a neuronal current. Initially, the method can be applied in real-time live direct assay. It is also applicable to organ monitoring, *in-vivo*

diagnosis, and other methods that require physiological interface control.

## Experimental Systems, Reagents, Electrodes, and Animals

The analytical system used was the CHI electrochemical workstation 660A in Cordova, (CH Instruments, Inc., Tennison, Austin, USA). The reagent-grade catechol (MW: 110.11 g/mol), caffeine (MW: 194.19 gmol$^{-1}$), bismuth (1,000 mgL$^{-1}$) and other reagents were from Sigma. The carbon nanotubes, having an outside diameter of 8 nm, an inside diameter of 2-5 nm, and a length of 0.5-200 nm, from Nanostructured & Amorphous Materials, Inc. were prepared overnight *via* catalytic CVD (metal-catalyzed chemical vapor deposition) prior to use by magnetic stirring in a 2 M nitric acid solution, and then washed with pure water. The BPE working electrode was prepared with a paste composed of a mixed carbon nanotube and bismuth (standard) with a ratio of 4:4.2 by % (w/w) in reagent-grade mineral oil (New Jersey, USA 1-800-01, Acro). The mixed paste was inserted into a plastic syringe needle with a diameter of 2 mm and with a copper wire connected to the electric system. The CN electrode was made with a carbon nanotube and mineral oil at a ratio of 4:2% (w/w) with hand mixing. The glassy carbon electrodes were BAS stationary voltammetric electrodes MF-2012 with a 3.0-mm diameter. The Ag/AgCl/KCl reference electrodes were prepared with a chloride-coated 0.2 mm silver wire, and a 0.2 mm platinum wire was used as the auxiliary electrode. A 120-gram fish and a 250-gram male rat from the authors' laboratory were used. An electrolyte solution of 0.1 M $H_3PO_4$ was prepared without removing the oxygen. All the experiments were performed at room temperature.

## Results and Discussion

**Electrode Comparison by Voltammetry.** In cyclic voltammetry, analytical peak potential is determined using a high concentration of caffeine and catechol spiked with a glassy carbon electrode. Figure 1(a) shows the results. In the blank solution, no signal was obtained, and thus, two ions were spiked at 10-20 mgL$^{-1}$. At the anodic scan, two oxidation peaks were obtained at 0.6 V (catechol) and 1.6 V (caffeine). As higher ranges were spiked from 30 to 80 mgL$^{-1}$, the peaks linearly increased, whereas no reduction peak appeared. Under these conditions, a usable working range of y = 0.0761 x −0.24 and a precision of $R^2$ = 0.9957 for catechol and y = 0.0352 x −0.1895 and a precision of $R^2$ = 0.9972 for caffeine were obtained. Catechol turned out to be two times more sensitive than caffeine. Under this condition, electrode comparisons were performed with the common-type glassy carbon and the non-treated CN-type electrode. Figure 1(b) shows the results. In the inset, the voltammogram is the 130 mgL$^{-1}$ spike. In this condition, the glassy carbon electrode was obtained with a current of only 4.3 × 10$^{-6}$ A for caffeine and a peak of 0.01 × 10$^{-6}$ A for

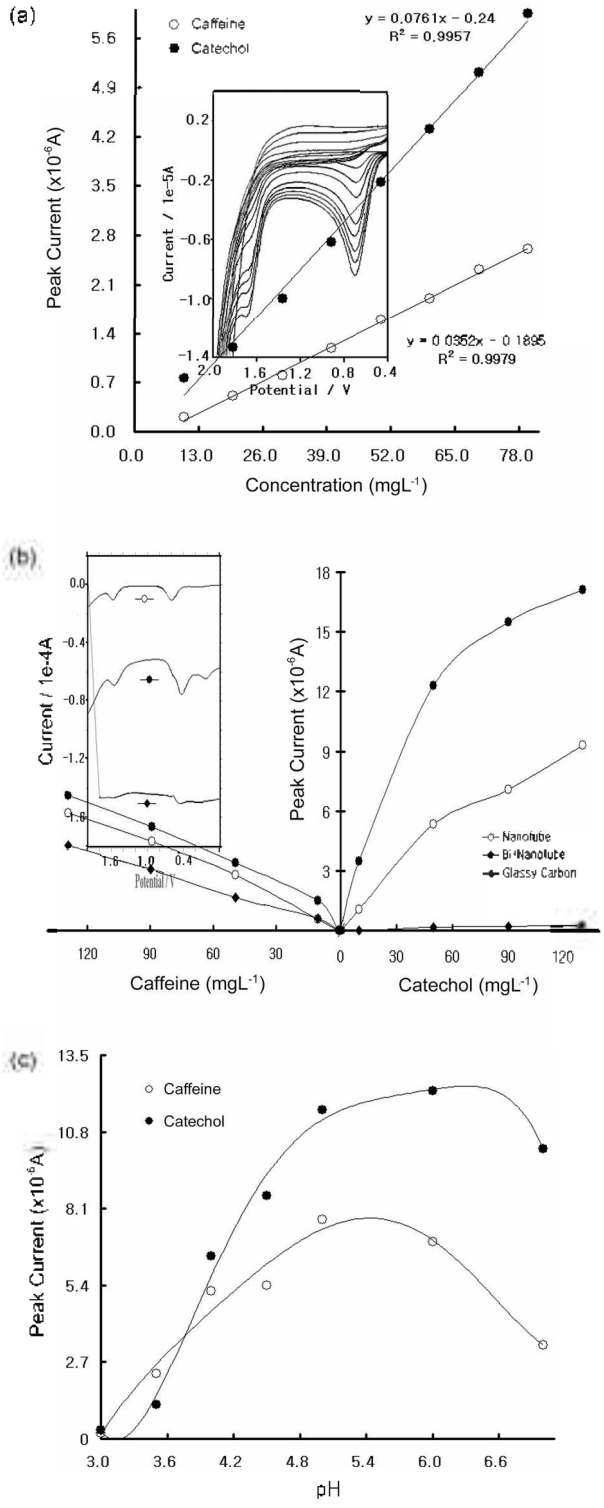


**Figure 1**. (a) Simultaneous variations for the 0-, 10-, 20-, 30-, 40-, 50-, 60-, 70- and 80 mgL$^{-1}$ caffeine and catechol spikes. The potentials were scanned between 0.0 V initial potential and 2.0 V switching potential with a scan rate of 3.5 V/s for CV using glassy carbon electrode. (b) Electrode comparison of BPE, common-type CN, and the glassy carbon electrode method in 0-, 10-, 50-, 90- and 130 mgL$^{-1}$ caffeine and catechol spikes. (c) Electrolyte pH variations of 3, 3.5, 4.0, 4.5, 5.0, 6.0 and 7.0 pH using BPE and 80 mgL$^{-1}$ catechol and caffeine spikes in a 0.1 M $H_3PO_4$ electrolyte solution. Other optimum parameters were used.

catechol. Also, the non-treated CN electrode showed a 5.9 × $10^{-6}$ A caffeine peak and a 9.3 × $10^{-6}$ A catechol peak. The BPE yielded a big 6.76 × $10^{-6}$ A caffeine current and a 17.1 × $10^{-6}$ A catechol current, however, and was much more sensitive than the other two electrodes. Thus, more sensitive parameters were obtained with BPE. Figure 1(c) shows the electrolyte pH variations. The electrolyte pH was controlled with the 0.1 M HCl and 0.1 M NaOH spikes to pH 5.0, a peak current of 7.72 × $10^{-6}$ A caffeine, a sensitive 11.57 × $10^{-6}$ A catechol current, and a narrow peak. In these conditions, the SW stripping parameters were examined.

**Optimization of BPE with Stripping Voltammetry.** Figure 2(a) illustrates the stripping peak current in a 150 $mgL^{-1}$ caffeine spike and a 70 $mgL^{-1}$ catechol spike for variation of the anodic accumulation potential. In this figure, catechol quickly decreased, but the peak width remained unaltered. On the other hand, the caffeine peaks continually and sharply increased, plus the optimum conditions were fixed at the −0.6 V accumulation potential, where the peak heights of 2.997 × $10^{-6}$ A for caffeine and 2.929 × $10^{-6}$ A for catechol appeared. This manifested as the best condition. At this potential, the SW stripping voltammetric amplitude variations were examined. Figure 2(b) shows the results at the −0.6 V accumulation potential. Other conditions were set for the optimum parameters, and both peaks continually increased and yielded an optimum amplitude of 0.3 V for 4.03 × $10^{-6}$ A caffeine and 43.8 × $10^{-6}$ A catechol. The amplitude of the catechol peak current was 10 times bigger than that of the caffeine peak current. Figure 2(c) shows the resulting SW accumulation times. At 0-60 sec, the peak sensitively increased for 12.68 × $10^{-6}$ A caffeine and 9.92 × $10^{-6}$ A catechol, then decreased. Thus, the optimum conditions were a −0.6 V accumulation potential, a 0.3 V amplitude, and a 30 sec accumulation time. Other parameters were fixed as in the conditions shown in Figure 3. Under these parameters, the analytical interference, working ranges, and *in-vivo* application were examined through stripping voltammetry.

**Analytical Interference, Working Range, Statistics and *in vivo* Application.** Under optimum conditions, the interference analogy ions were examined. Figure 3(a) shows the results. In the solutions with 20.0 $mgL^{-1}$ caffeine and catechol, other metal and neuro transmitter ions of [Cd(II), Ca(II), Zn(II), Ba(II), Co(II), Cu(II), Ge(IV), Cr(III), Fe(II), Hg(II), Ni(III), Ag(I), Pt(IV), glycine, dopamine, epinephrine, histidine and glucose] were spiked, the concentrations of which were all 200 $mgL^{-1}$. The caffeine peaks resulted in the following percentages: −4.38%, −6.78%, 10.27%, 3.49 %, −35.55%, −53.27%, −25.23%, 26.52%, −19.90%, −17.63 %, −13.76%, 12.4%, −26.82%, 64.74%, 5.05%, 12.22%, −10.89% and 3.70%. The catechol peaks resulted in percentages as follows: 33.19%, 6.13%, 13.06%, 7.56%, 5.13%, −6.87%, 0.09%, −17.54%, 16.38%, 4.90%, −21.76 %, 12.64%, 134.0%, 30.35%, 16.43%, 45.88%, 14.35% and 5.07%. Under these conditions, Pt and glycine strongly interfered, but other ions only weakly interfered. In Figure 3(b), the anodic working ranges were examined with

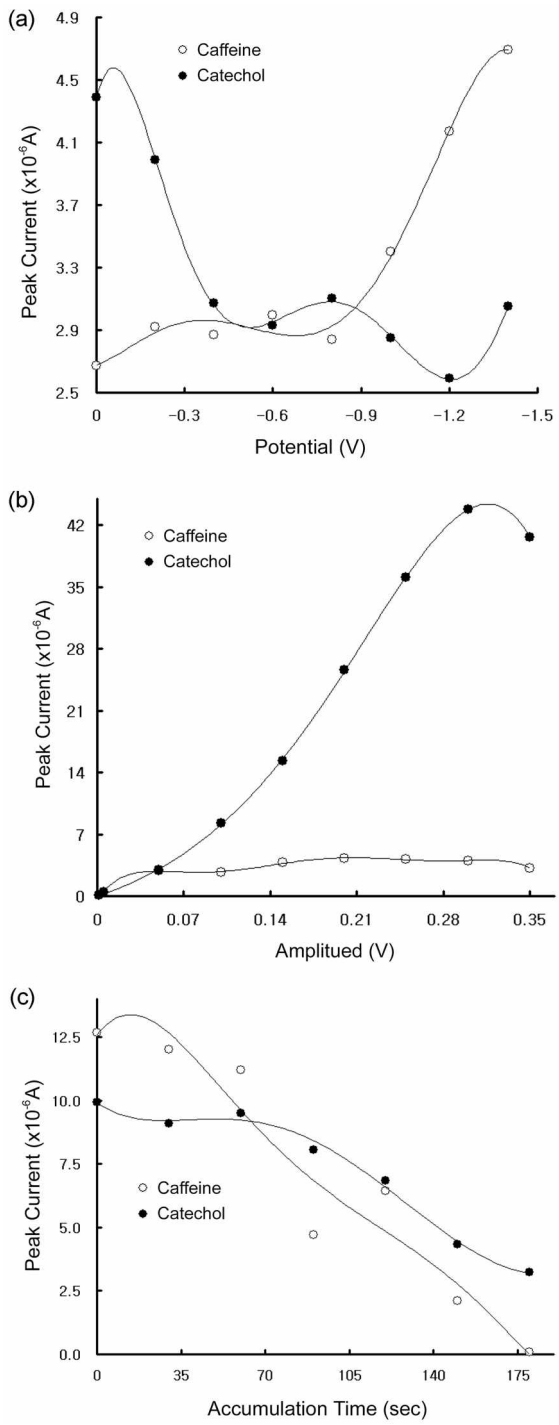


**Figure 2.** (a): Variations of 0 ~ −1.4 V SW accumulation potentials. (b): SW amplitude variations of 0.001 V-0.35 V. (c): Variations of 0-180 s SW accumulation time using a simultaneous 150 $mgL^{-1}$ caffeine and 70 $mgL^{-1}$ catechol spike in a 0.1 M $H_3PO_4$ electrolyte solution. Other parameters were fixed for the optimum conditions.

simultaneous spiking at a high concentration. Both peaks increased linearly, the peak width was narrow, and the sensitivity of caffeine was $\Delta x/\Delta y$ = 6.7961 and of catechol, $\Delta x/\Delta y$ = 5.7313, in which $\Delta x$ = the catechol and caffeine concentration ($mgL^{-1}$) and $\Delta y$ = the peak current (1.0 × $10^{-6}$ A). This working range is usable in any analytical assay. The

results are applicable in pharmacy, but *in vivo* conditions require a variation of low detection limits. Thus, more sensitive working ranges were examined. At each electrolyte cell, more sensitive conditions were obtained. Figure 3(c) shows the detection limits (DL) (S/N = 3) of 0.4 $mgL^{-1}$ caffeine and 0.06 $mgL^{-1}$ catechol, calculated as in Equation (1).

$$DL = ks_B/b, \quad (1)$$

in which DL is the detection limit, $k$ is a constant value of 3 according to IUPAC (S/N = signal noise ratio), $s_B$ is the standard deviation of the blank signals, and $b$ is the slop of the calibration plot.[30] Based on the calculation, catechol was 10 times more sensitive than caffeine, but their slop ratios were similar. These results are applicable in any living cell system, so a diagnostic application was performed in *in vivo* neuro conditions.

Analyses were carried out on the implanted *in vivo* fish brain and rat brain cells under real conditions. Under anesthetic conditions, the macro-type BPE was inserted deep (7 mm) into the fish's brain using a micro-hand drill. Then a 0.2 mm-diameter wire-type platinum counter electrode and a 0.2 mm-diameter chloride-coated Ag/AgCl wire-type reference electrode were implanted into the fish's chest. All the cables were interfaced with the electrochemical workstation, and the open tissues were closed using a dental adhesive.

Using the same method, the rat implantation was performed. The fish specimen was transferred to a basin. In this bowl, 500 $mgL^{-1}$ caffeine and standard catechol were spiked. The next day, both ions were absorbed into the body system. Under living conditions, SW stripping was performed with a −0.6 V accumulation potential, a 30 mV SW amplitude, a 40 Hz SW frequency, a 3.0 mV step potential,

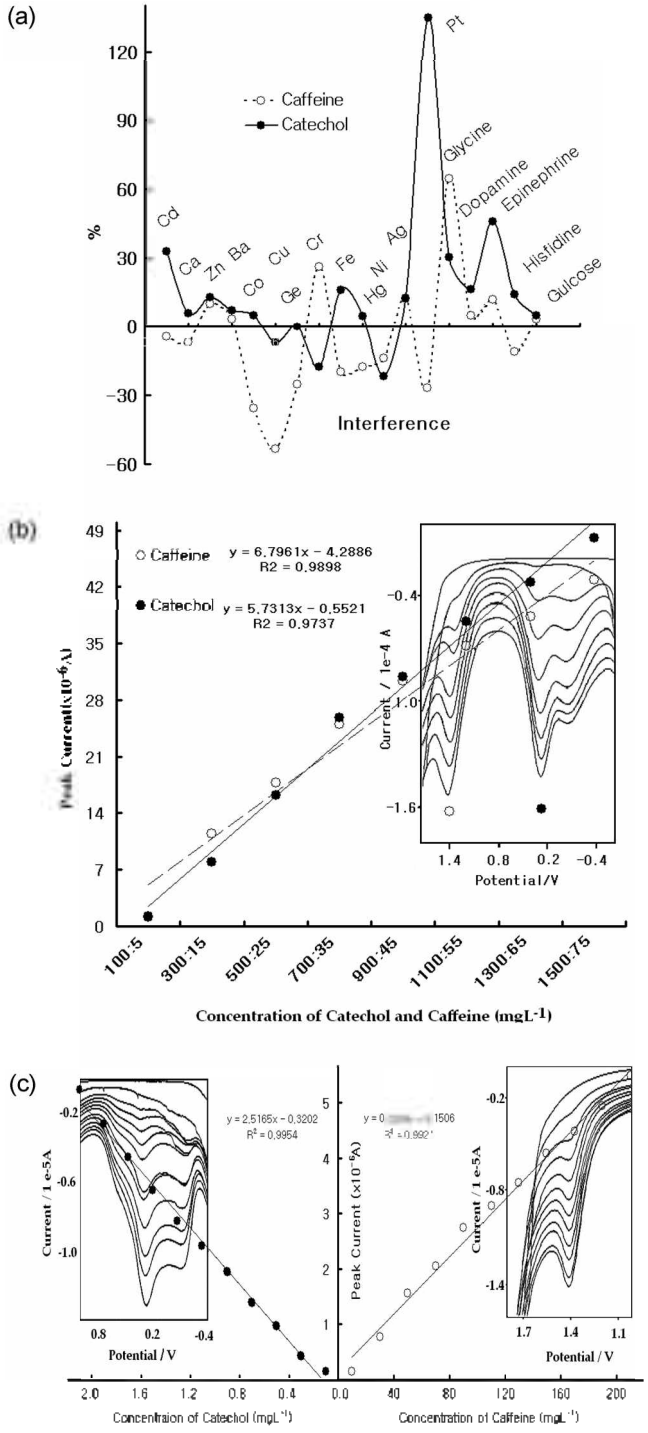


**Figure 3**. (A): Interference test. (B): High working range of 100:5-1,500:75 $mgL^{-1}$ caffeine and catechol at simultaneous addition. (C): Low working range of 10-210 $mgL^{-1}$ caffeine and another cell of the 0.1-2.1 $mgL^{-1}$ catechol spike, in a 0.1 M $NH_4H_2PO_4$ solution with a pH of 5.0, a −0.6 V initial potential, a 0.3 V amplitude, a 40 Hz frequency, a 0.003 V increment potential, and a 0 sec accumulation time. Optimum conditions were set for other parameters.

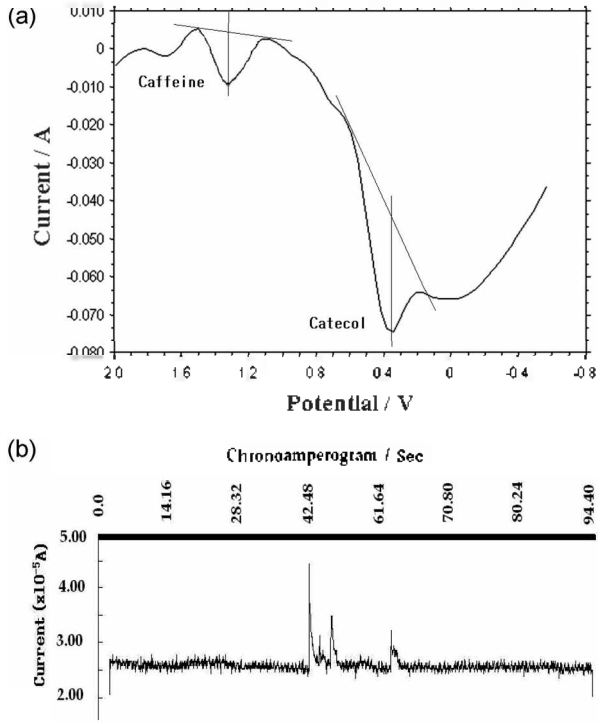


**Figure 4**. (A): SW stripping voltammograms of caffeine and catechol ions in 500 $mgL^{-1}$ caffeine and catechol contained in a bowl fish using BPE implanted in a living fish brain. (B): Chronoamperometry using BPE implanted in a living rat brain. Other parameters were used for optimum conditions.

and a 30 seconds accumulation time. Figure 4(a) shows the two peaks with a $1.25 \times 10^{-2}$ A peak height at 1.3 V for caffeine and with a $2.369 \times 10^{-2}$ A peak height at 0.4 V for catechol (for healthy humans and rats plasma the standard is $375 \pm 57$ pgmL$^{-1}$, $475 \pm 52$ pgmL$^{-1}$).[31] Thus, they can be assayed directly *in vivo* ($1.78 \sim 2.90 \times 10^{-8}$ molL$^{-1}$ in rat brain),[32] and can be used for neural diagnosis and medicinal treatment. More advanced methods were performed in the rat brain with the implanted BPE macro-sensor. In the interfacing circuits, 0.4 V was applied to catechol, and chronoamperometry was performed. Figure 4(b) shows the resulting physiological current. Within 0-350 sec, the rat was not moved and only slight noise signals were obtained. Within 410-455 and 570 sec, a physiological impulse was supplied to the rat body skin using a bristle tip, producing a sensitive sharp current and a repeated identical peak current under the same conditions. Thus, physiological neural signals (EEG: electroencephalogram)[33] can be detected, and the methods presented in this study can be applied to brain diagnosis,[34] neural contro.[35,36] and *in vivo* signal.[37]

## Conclusion

A low catechol and caffeine concentration was assayed using a voltammetric BPE sensor. The optimum SW conditions were found to be as follows: 0.3 V amplitude, 40 Hz frequency, −0.6 V accumulation potential, 3 mV increment potential, 30 sec deposition time, and 5.0 pH electrolyte strength. The method was shown to be more sensitive than the previous DL[8-12,19-22,26] and could be attained at low working ranges for *in vivo* diagnosis. Tree electrode systems can be implanted into the fish and rat brain cell. In this circuit, physiological neuro-currents were obtained in real time as well as in *in vivo* conditions. The method can also be applied to pharmaceuticals and *in vivo* drugs in addition to in vitro monitoring.

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