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# Clinical In Vivo Bio Assay of Glucose in Human Skin by a Tattoo Film Carbon Nano Tube Sensor

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**Abstract** : In vivo assay of glucose detection was described using a skin tattoo film electrode (STF), and the probe was made from carbon nano tube paste modification film paper. Here in the square-wave stripping anodic working range obtained of 20-100 $mgL^{-1}$ within an accumulation time of 0 seconds only in sea water electrolyte solutions of pH 7.0. The relative standard deviations of 50 mg glucose that were observed of 0.14 % (n=12), respectively, using optimum stripping accumulation of 30 sec, the low detection limit (S/N) was pegged at 15.8 mg/L. The developed results can be applied to the detect of in vivo skin sensing in real time. Which confirms the results are usable for in vitro or vivo diagnostic clinical analysis.

*Keywords : Glucose, tattoo film, carbon nano tube, paste, film electrode*

## 1. Introduction

Under human physiological conditions, in vivo glucose ion is the major activity of energy factors for the brain control and other body systems; however, concentrated high level conditions are related to heart failure, [1] Ischemic disease risk [2] and diabetes mellitus coronary effects [3], therefore, in vivo analysis is particularly important in medical treatment and disease control. Sensitive detection methods are developed for this purpose such as common type colorimetric detection [4] paper based on the micro fluidic devices assay [5], fluorescence spectroscopy detection[6], high performance liquid chromatography electrospray ionization mass spectrometry [7], ion chromatography coupled mass spectrometry using pulsed amperometric detection [8] and the infrared spectrometric micro dialysis method. [9] All of these methods of experimental technics are performed only in electrolyte solutions; moreover, complicated spectric and separation methods require expensive systems. Recently, more advanced detection methods have been developed for this domain. Voltammetric adsorption stripping methods are simple [10,11], very sensitive and provide fast [12] results usable for diagnostics. In addition, more sophistic working electrodes appeared for these purpose - such as infrared photo diode electrodes [13], DNA immobilized carbon nano tube mixed paste electrodes [14],

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a copper immobilized on a graphite carbon electrode [15] and bovine IgG immobilized onto a DNA linked carbon nanotube electrode. [16] These can be applicable for diagnostic control [17]. However, they should be used only in laboratory conditions and are not applicable for in vivo direct detection. This study was developed for more simplified voltammetric in vivo [18] and vitro tattoo film probes using printing paper electrodes, which was optimized on the human skin surface in a very short accumulation time and easily detected the flued ions [19,20]. It can be usable for analytical bioassay and other materials requiring skin sensing and tattoo film modified diagnostics.

## 2. Methodology

### 2.1. Instrumental apparatus, reagents and working probe

The diagnostic instrumental circuits were carried out using a bioelectronic-2 system made by the authors' institution. The system was designed to be as small as a computerized handheld voltammetry, similar to a cellular phone. The scanning potential windows had was 2.0 to -2.0 V, and the current was detected during $10^{5\sim9}$A. The handmade working counter and reference probe was made with 0.2 mm film paper. The paste film was made by a ratio of 30: 30: 40 % using nafion, carbon nanotube and the mineral oil was mixed, that of paste was coated on the with 0.1 mm thick film paper. All electrolyte solutions were prepared with double-distilled water (18-M ohm $cm^{-1}$). Other reagents were obtained from Aldrich and were diluted as needed, while the other experimental parameters were maintained at optimal conditions. All the experiments were performed at a room temperature of 25°C without removing oxygen.

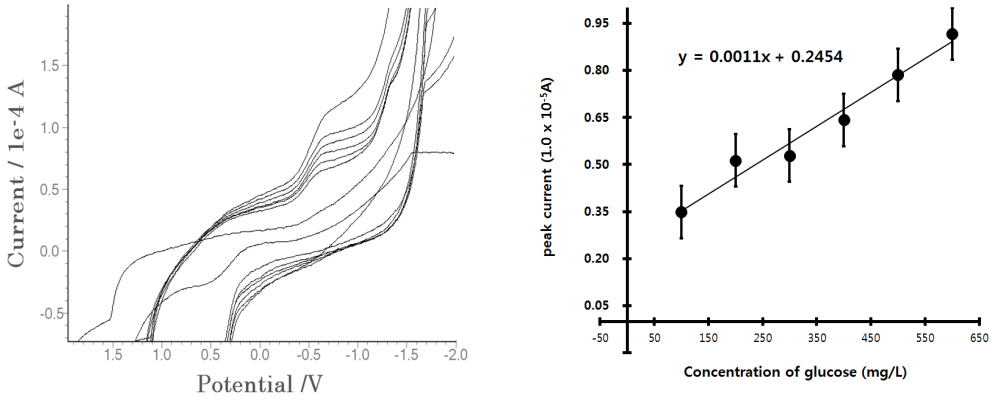
### 2.2. Experimental procedure

Electrolyte solutions were tested by acid and base 0.01 mol standard; however, in vivo requires bio friendly electrolyte. Thus, more satisfied solutions of seawater electrolyte was used from Korea, 2000 m under water from Dokdo Islands using these conditions. The following voltammetry was performed to cyclic voltmmetry, stripping anodic and the cathodic accumula process.

## 3. Results

### 3.1. Cyclic voltammetric comparison with conventional electrodes

In 10 ml electrolyte solution, 50 mg/L glucose standard was spiked, then the cyclic redox scan was searched during -2.0 V initial to 2.0 V switching potential by 0.5 V/Sec scan rate using graphite three electrode systems and STF. Here in oxidation, the peak appeared at -0.1V peak potential, for the anodic only while the cathodic was not obtained. Under these conditions, the concentration effect tested from 100 to 600 mg/L glucose standard sequencial add can be obtained for linear increasing equation of fig1. (B). Which the first current obtained for $5.0 \times 10^{-5}$A and then the continued statistics equation appeared. These are more sensitive then common type electrodes, thus more sensitive and amplified stripping voltammograms were examined using anodic and cathodic methods by 0 sec accumulation. In these conditions, conventional graphite electrodes and the developed film electrode were compared using the same para conditions, which show that the results of the peak ratio and the real voltammogram are identical conditions for the 10 $mgL^{-1}$ glucose examined. All optimized diagnostic parameters were used. Both electrodes showed the same peak potential, that appeared at 0.0 V, but the STF is better then the conventional graphite electrodes as it responded with significantly increased peak signals of the same potential,




(A) (B)

Fig. 1. (A) Cyclic effect for 7 point variation during 0, 100, 200, 300, 400, 500 and 600 mg/L add by −2.0 v initial potential, 2.0 v switching potential, 0.5 V scan rate in 10 ml electrolyte using STF three electrode systems. (B) Cyclic linear working equation

and a half-width of very narrow signals was obtained.

### 3.2. Stripping effect on various parameters in electrolyte solution

In the electrolyte conditions, voltammetric parameters of the electrolyte solution's pH, accumulation time, and stripping para conditions were examined (not shown here). First, the hydrogen ionic activity of the acid and base solution were examined from pH 2.0 to 8.0 pH variation stripping peaks current in a 50 $mgL^{-1}$ glucose constant. The hydrogen ionic activity was changed by using 0.1 M HCl and 0.1 M NaOH add. The glucose ionic force increased quickly from 3.3 to 6.0 at a pH of 6.7 when the anodic peak reached the maximum level. The peak's shape and width narrowed, and the maximum peak was decreased. Beyond this rate, the activity of the peak current linearly decreased. There was no linear response. Herein, the 6.7 pH level could be used for optimum conditions. Then the other effect of accumulation times were searched from 10, 20, 30, 40, 50, 60, 70, 80, 100, and 120 seconds, in which the signal was linearly increased with the same ratios. Thus, the optimum time was fixed to 60 sec accumulation. Under this condition, other parameters of amplification, square frequency and stripping potential windows were examined, and the results were applied to in vitro assay (not shown here)

### 3.3. SW electrode comparison using graphite and tattoo film printing electrodes

Values of 50 mg glucose add, common type graphite and tattoo film electrode were compared in a cyclic scan with −2.0 ~ 2.0v potential windows using sea water electrolyte 10 ml constant. However, both peak currents appeared at the same peak potential at −0.1 V during the oxidation peak current obtained to $3.5 \times 10^{-5}$A graphite and $4.7 \times 10^{-5}$A tattoo film electrode. More of the same peak current shows that the peak signal of the tattoo was very deep and much sharper then graphite. Therefore, the sequential spiking of 100, 150, 200 and 250 glucose add, and both curves displayed linear increases. Also the slop ratio of the film tattoo demonstrated a positive and bigger increase.

### 3.4. Statistic results for in vivo and vitro application

Under optimum conditions, statistics in vivo applications were examined using healthy people's urine electrolyte, 10 ml flued. Fig 2 (a) is the result for standard addition methods. The fist curve is electrolyte blank and then the repeated standard addition by adding anodic voltammogram to health urine. It shows the first peak is the sharp width obtained by $0.14 \times 10^{-3}$A. It appeared at 0.1 V potential windows. The next curve is glucose standard spike by 10 m/L. In these conditions, the same peak potential was obtained with $0.18 \times 10^{-3}$A, which is the sharp width that appeared. The next curve is 20 and 30 mg/L glucose added. The statistic equation obtained, fig 2(b), is the linear curve for fig 2(a) and the statistic addition curve is stabilized and can be usable for diabatic patients analysis.

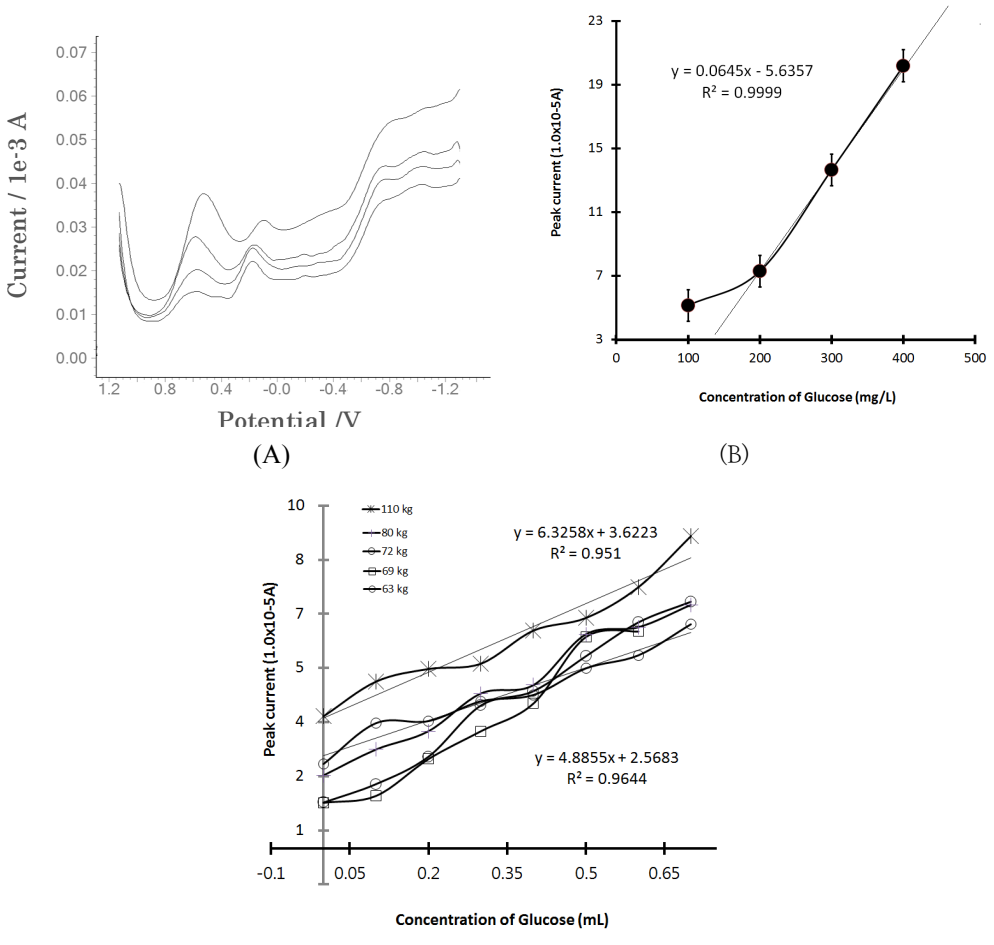



Fig. 2. (A) Varying glucose assay of urine 10 mL electrolyte by health flued, stripping −2.0 V anodic 0 Sec accumulation time. The first curve is urine electrolyte, then 10, 20 and 30 mg/L glucose standard add, oxidation, using film electrode. (B) Linear equation of standard addition method using (a). (C) Five different people's standard addition method using (a) for healthy males aged 62 69 72 80 and 110 kg wight

Then fig 2(c) shows the statistic application results for three linear curves have different weights for the female urine test. All curves are standard addition by the same method during urine stripping, and then glucose 10, 20' and 30 spike added are sequentially increased. The waking equation is stable; however, the weight high concentration females was obtained

Under optimum conditions, health and real patients diagnostic assay was performed on the face. Fig 3(a) is in vitro detection on the skin surface and the histogram shows repeated statistics for the 3th applications. The first three parts were $0.04 \times 10^{-5}$A, obtained from a healthy-faced skin. That is a very weak current obtained. However, the next three peaks of patient results increased in a better fashion. That is $0.22 \times 10^{-5}$A appeared with the same peak high, which is five times better then that of health current, where experimentlal real voltammogram and face

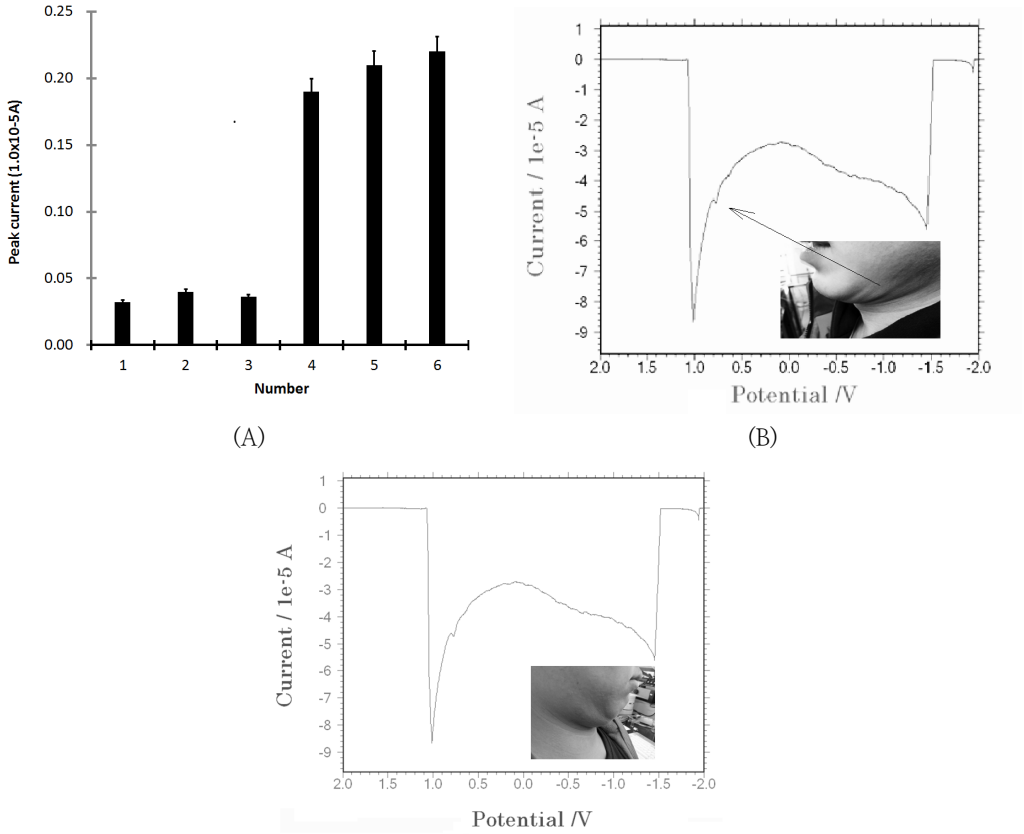



Fig. 3. (A) Applications of in vitro detection by on skin surface probe, (a): health and diabatic patients detection using optimum para conditions in stripping anodic peak highs repeated three times on the skin's surface. (B) Stripping anodic voltammogram on the skin from the right of the face. (C) The same conditions of (b) for the left of the face. voltammogram was performed to 0 sec accumulation with oxidation and reduction scan. Other experimental parameters in Fig. 3 were held constant.

picture is shown in fig3 (b). The shown photo is the right obesity and voltammogram responding at 0.6 V peak potential during an anodic scan. The repeated other fat side of the left is shown at fig3 (c). The peak high shows the same results of $0.22 \times 10^{-3}$A peak potential. 0.6 V anodic was obtained. Under these condition, other skin on the neck and the back of the hand were examined and the same peak potential was obtained for 0.6 V. The final applications of film electrode can be used for in vitro and in vivo disgnostoc analysis.

## 4. Conclusions

The method of the STF modified skin film electrode system was made by using tattoo painting fabrication. Under sea water electrolyte, optimum analytical parameters were examined with scanning potential of 2.0 V ~ −2.0 V windows, a cyclic and stripping anodic linear working curve was obtained to 50 ~50 0 mg/L Glucose at an accumulation time of only 30 seconds. The optimum stripping oxidation anodic scan used for 5.0 V/sec amplitude, a 0.5 V scan rate, demonstrated $1.0 \times 10^{-5}$A increment potential. In these conditions, the diagnostic detection limit attained to 35 mg/L here, means the results can be applied to real detection on skin from the face. Analytical applications were then performed on the healthy Patient's urine, skin surface and other in vivo direct detection. That of the developed tattoo probe can be usable for vital analysis and in other fields requiring skin sensing and in vivo diagnostic analysis.

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