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Carbon microelectrode arrays for multiplexed potentiostat performing electrochemical cyclic voltammetry

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Abstract. Glassy carbon microelectrode arrays (2x2, 3x3 and 4x4) were fabricated by carbonizing photoresist at high temperature (900 °C). These arrays are meant to work as part of a traditional electrochemical cell (working and counter parts). A reference electrode is still needed to complete the electrochemical cell. The 2x2 arrays have four working electrodes and the 4x4 arrays have 16 working electrodes. Many different shapes can be arranged, either 2D or 3D shapes. The microelectrode array size, in the order of μ-meters, makes them useful for miniaturization of biosensor devices. The microelectrodes are 2D and have circular shape. Dimensions for the working electrodes are 67 μ-meters in diameter for each one. Distance between center of each microelectrode and another one is 280 μ-meters.
An application of such microelectrodes would be a combination of carbon microelectrode arrays and a potentiostat device. This arrangement can be implemented to provide a simple, yet useful option for electrochemical analyses. A potentiostat could run for example, electrochemical cyclic voltammetry redox experiments using this type of carbon microelectrode arrays. The potentiostat circuit is used for electrochemical experiments that follow redox reaction processes like the reduction of ferricyanide ion to ferrocyanide. Results for those experiments using both, standard-platinum and carbon, microelectrodes show the same reduction potential of 0 mV vs Platinum in Cyclic Voltammetry (CV) electrochemical experiments.

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Abstract: Se construyeron arreglos de microelectrodos (2x2, 3x3 y 4x4) de carbón vítreo por carbonización (pirólisis) a altas temperaturas (900 °C). Estos arreglos fueron diseñados para trabajar como parte de una celda electroquímica tradicional (como electrodos de trabajo y auxiliar). Un electrodo de referencia sigue siendo necesario para completar la celda electroquímica. Los arreglos de 2x2 tienen cuatro electrodos de trabajo y los arreglos de 4x4 tienen 16 electrodos de trabajo. Se pueden diseñar muchas formas diferentes, ya sea en formas 2D o en 3D. El tamaño del arreglo de los microelectrodos, en el orden de micrómetros, los hace útiles para la miniaturización de dispositivos biosensores. Los microelectrodos son en 2D y tienen forma circular. Las dimensiones para los electrodos de trabajo son de 67 micrómetros en diámetro para cada uno. La distancia entre el centro de dos microelectrodos contiguos es de 280 micrómetros. Una aplicación para tales microelectrodos puede ser una combinación de arreglos de microelectrodos de carbón y un potenciostato. Este arreglo puede ser implementado para proveer de una simple, pero útil opción para análisis electroquímicos. Un potenciostato podría correr por ejemplo, experimentos redox con voltametrías cíclicas electroquímicas utilizando este tipo de arreglos de microelectrodos de carbón. El circuito del potenciostato se utilizó para experimentos electroquímicos que utilizan la reacción redox de ion ferricianido a ferrocianido. Los resultados de estos experimentos, utilizando microelectrodos estándares tanto de platino como de carbón, muestran el mismo potencial de reducción en experimentos electroquímicos de voltametría cíclica (CV).

Introduction

The development of microfabrication technologies has benefited numerous fields, including environmental, clinical, pharmaceutical, biochemical and biotechnological applications. There is a growing interest on the development of techniques that can be used in microscale applications to perform analytical

assays and electrochemical sensor design [1]. One important application of microtechnology is the characterization and probing of living organisms, such as cells, in order to access information regarding their activity, type and morphology. Carbon microelectrode arrays for a multiplexed potentiostat have the potential to be used in these types of applications since it allows individual processing of current coming from the electrochemical redox reactions at each element of the array allowing the acquisition of large volume of data in shorter times and with statistical value. The electrical properties and mechanical stability of glassy carbon electrodes make them useful for biosensor applications. Also, glassy carbon electrodes have the advantage of being chemically inert. An example of these applications is discussed in reference [12]. Turon-Teixidor et al. were able to place single cells of an immortalized motor neuron cell line (NSC34) on top of carbon electrodes. Since this type of cells generate action potentials and show complex patterns of electrical activity, a method for individual stimulation is necessary to further understand their behavior as a network. A multiplexed potentiostat coupled with a recording system would serve this purpose. Other cell lines are also being considered. For instance in reference [2] is suggested that it is possible to influence the differentiation of stem cells by using electric and magnetic fields. Once a single cell placement is achieved (like in the case of NSC-34 cells), carbon microelectrode arrays used over a multiplexed potentiostat will make this study a reality.

The field of DNA detection will also benefit from the development of a microelectrode arrays with the multiplexed potentiostat instrumentation. So far, detection in DNA arrays has been performed either by optical detection equipment or using enzyme based DNA arrays [11]. Optical DNA arrays present major problems that prevent this procedure to be used in hospitals, such as very high cost. Enzyme based DNA arrays have the disadvantage of crosstalk between spots, however they are a low cost solution due to the inexpensive detection strategies and are relatively easy to miniaturize. A microelectrode array in conjunction with a multiplexed potentiostat would enhance the efficiency of DNA microarrays producing a larger use of this technology in hospital environments and general purpose laboratories.

Recently, an electrochemical array microsystem with an integrated potentiostat has been proposed by Zhang *et al.* [15]. His 3x3 biosensor array based in CMOS covers the need of a protein based biomimetic sensor. This method shows a promising alternative for an assortment of electrochemical current based sensors. Moreover, additional characteristics include the use of a load amplifier and an integrator based on switching capacitors.

Other proposed solutions have been published in several papers; nevertheless, none of those meet the modularity feature which allows the flexibility to increase the array size as needed. Table 1 summarizes the most relevant publications. Concerning commercial products, several multiplexed potentiostats are readily available from industrial manufacturers such as Gamry Instruments (Warminster, PA) and Princeton Applied Research (Oak Rigde, TN). Such devices are computer controlled, have a limited channel number and are specially large in size making them inadequate for biomedical applications. Examples of these systems are *MultEchem 8 Electrochemistry System* that allows using 8 independent potentiostats installed all at the same time on industrial computers. Other examples are the VMP3 potentiostat from Princeton Applied Research and the Model *1000 Series Multi-Potentiostat* from IJ Cambria Scientific/CH Instruments.

The present paper demonstrates the feasibility of developing efficient carbon microelectrode arrays for multiplexed potentiostat technology that will allow performing individual addressing of cells using single array activation. This carbon microelectrodes array is to keep a modularity approach in order to further integrate additional microelectrodes to the system. The paper is divided in six sections: 1) Introduction, 2) Multiplexed potentiostat description that illustrates the development of the instrumentation, 3) Potentiostat testing describes some of the electrode and device verifications achieved to implement the potentiostat, 4) Cyclic Voltammetry tests that provide a direct evaluation of the carbon microelectrode results and 6) Conclusions which provide the consolidation of this contribution.

Multiplexed Potentiostat Circuit

For an electrochemical experiment, it is desirable to use a three electrode configuration. Such arrangements include one or several working electrode(s) (WE), an auxiliary electrode (counter electrode or AE), and a reference electrode (RE). This arrangement will be called an electrochemical cell. The potentiostat circuit

will enforce an applied voltage to electrodes in the electrochemical cell, and will sense the response current to such applied voltage. A useful technique that requires this type of arrangement is cyclic voltammetry (CV). With CV we can obtain information about the analyte under interrogation.

Cyclic voltammetry requires sweeping the current flowing from the AE to the WE as the voltage is varied in the WE at a specified sweep rate in volts per second. Measurements that need to be sampled or that are given at a minimum frequency will limit the sweep rate of the experiment. If the frequency resulting from the current measurement is low, then the sampling frequency is not high enough to get a minimum resolution in the voltammograms to provide reliable information about the reaction.

Using the specifications of measurement times and sweep rates provided in different contributions of the technical literature [3-5,7-10,13], the best performance according to simulations is obtained using a high sensitivity current to voltage (I to V) converter scheme. The design of an individual potentiostat system will be carried out in two stages which are the control and the current sensing stage. Figure 1 illustrates a block diagram of the complete system. Notice however that normally, a single working electrode is used. With the proposed microelectrode arrays, the number of available working electrodes in a single electrochemical cell will increase.

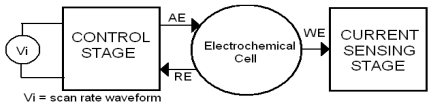

Fig 1: Single-cell potentiostat block diagram

Due to the number of working electrodes now available, a multiplexed potentiostat circuit is necessary. The multiplexed potentiostat provides a way to select individual points of measurement over an array of electrodes arranged in a matrix fashion. For multiplexing purposes, the number of *AEs* needed is one. The function of the *AE* is to convey the current from (or to) the *WE*. In the case of a multiplexed potentiostat, if only one *AE* is used, the current flowing will be the sum of all the currents flowing through the *WEs*. Therefore, as the individual potentiostat system is able to measure currents at the *WE*, only one *AE* is needed. Figure 2 shows a block diagram of the multiplexed potentiostat system. This figure illustrates the different stages of the design: control stage, sensitivity stages, MUX stage and the electrochemical cell.

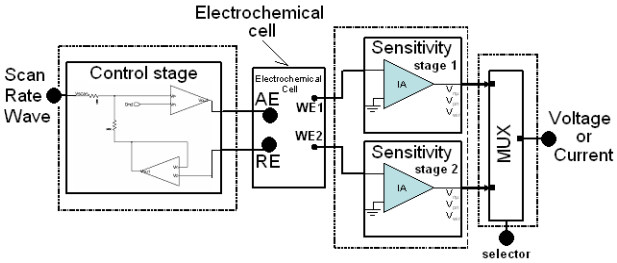

Fig 2: Block diagram of the multiplexed potentiostat system

After completing the multiplexed potentiostat circuit, it is required its testing which will be described in the next section.

Potentiostat testing

Some experiments are required to test the potentiostat in measuring currents ranging from picoamperes to microamperes. First, the experiments were performed with a simple potentiostat (1 *WE*, 1 *RE*, 1 *AE*). In a future work, the experiments will be performed with a multiplexed potentiostat using a 2x2 microelectrodes array (4 *WE*, 1 *RE*, 1 *AE*).

Microelectrodes

To prevent the effect of the voltage loss in the solution that is caused by the redox current, a third electrode was located nearby the working electrode; therefore, a three electrodes system was selected. The electrodes used for these experiments are made of carbon that comes from pyrolysis of epoxy resins (photoresist in this case) at 900 °C.

Table 2 shows the specifications of the microelectrodes geometry that were used and Table 3 shows the specifications of the microelectrodes array that will be used. Figure 3 shows the 2x2, 3x3 and 4x4 microelectrode arrays fabricated. Also in figure 3, there is an example of how the microelectrode arrays and a container (cuvette) for the analyte get together.

Redox reaction

One of the most common redox reactions used to characterize the system by means of a reversible cyclic voltammetric response is the reduction of the ferricyanide ion to ferrocyanide [6]. One of its disadvantages is that a passivating layer of Prussian blue ($KFe^{II}[Fe^{III}(CN)_6]$) may deposit on the electrode surface during the experiment. Equation 1 shows the redox equation of the reaction.

$$[Fe(CN)_6]^{3-} + \bar{e} \rightleftharpoons [Fe(CN)_6]^{4-} \quad (1)$$

Table 2: Geometry specifications of the microelectrodes

Shape	Cylindrical
Diameter	67 µm (active area in contact with analyte)
Height	10µm (flexible according to needs up to 200 µm)
Static/Rotating	Static

Table 3: Specifications of the microelectrodes array cell design

#of electrodes	1 RE, 1 (up to 2) AE, 4 WE
Square area	600 x 600 μm^2
O ring diameter	1 cm
Distance between WEs	280 µm (center to center)
WEs	67 µm diameter
RE	Pseudo – reference
AE	Platinum Filament

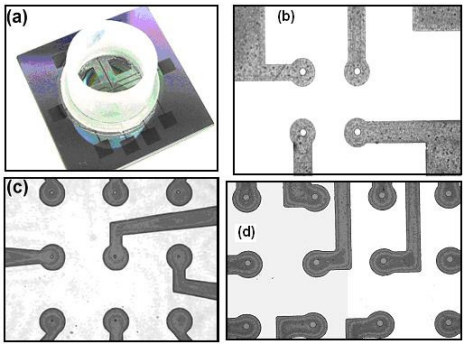

Fig 3: C-MEMS electrodes. (a) Circuit and cuvette (upper-left), (b) Microcircuit array 2x2 (upper-right), (c) Microcircuit array 3x3 (under-left), (d) Microcircuit array 4x4 (under-right)

Table 4 shows the specifications for the solution to be used, for more detailed data about the ferricyanide ion to ferrocyanide refer to reference [14].

Table 4: Specifications of the reaction to be used in the CV experiments

Total Solution Volume	around 0.5 cm^3
Redox reaction	ferricyanide ion to ferrocyanide
Electron transfer reaction	$Fe(CN)_6^{3-} + e^- \rightarrow Fe(CN)_6^{4-}$ (5mM)
Supporting electrolyte	KNO_3 (0.1M)

Tests and results

First an experiment with a potentiostat circuit and an electrochemical cell, with standard (platinum) microelectrode, was performed. Figure 4 shows the CV curve resulted from this experiment and table 5 shows the details for this experiment. Labels inside figures indicate current peaks and their respective voltages.

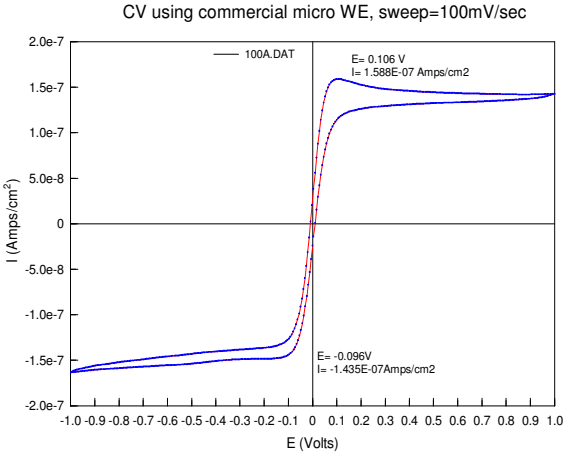

Fig 4: CV experiment with standard platinum electrodes

Table 5: Details for experiment with standard platinum microelectrodes

WE	Platinum microelectrode
AE	Platinum electrode
RE	Platinum,pseudo reference
Slew Rate	100 mV/s
V1	1000 mV
V2	-1000 mV
Start point	0 V

Other experiments with the carbon microelectrode arrays were conducted to compare them against figure 4 CV graph. Only one WE at a time has been used in each experiment. A different WE was used for each experiment. The results for the carbon microelectrode arrays are depicted in figures 5 and 6. Table 6 and 7 contain the details for those experiments. In both experiments, carbon microelectrodes were used for WE and AE; and a platinum electrode was used for pseudo-reference.

Given the results of figures 5 and 6 we can observe that they have a close potential for its peak currents (redox currents) as in figure 4 for its respective peak currents, especially in figure 6 where the error is about 6.25%. This potential is set at 0 V vs the platinum pseudoreference electrode. Also, it can be observed that a CV like curve shows in each experiment as expected. However, some differences between them are the amplitude of their corresponding oxidation and reduction current peaks. Due to the scale of the arrays these differences were expected. However, a better sigmoid CV shape (similar to the one shown in figure 4) is desired as an ideal result for the experiments.
Causes of the results obtained are: geometry of the array, capacitive currents and inherent resistance of the current path.

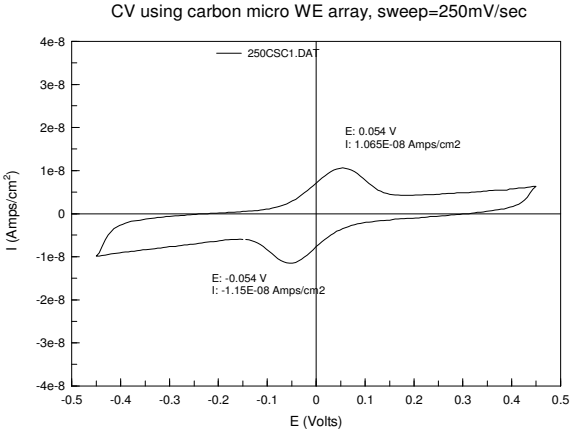

Fig 5: Cyclic Voltammetry of experiment #01.

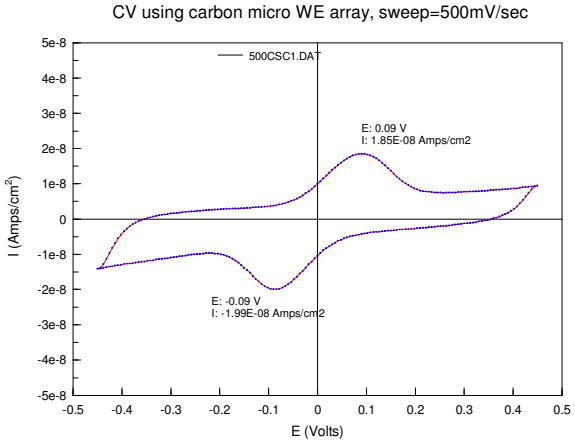

Fig. 6: Cyclic Voltammetry of experiment #02

Table 6: Details for experiment #01 using carbon microelectrodes

WE	Carbon microelectrode
AE	Carbon microelectrode
RE	Platinum Pseudoelectrode
Slew Rate	250 mV/s
V1	500 mV
V2	-500 mV
Start point	0 V
Number of cycles / run	3

Table 7: Details for experiment #02 using carbon microelectrodes

WE	Carbon microelectrode
AE	Carbon microelectrode
RE	Platinum Pseudoelectrode
Slew Rate	500 mV/s
V1	0.5 V
V2	-0.5 V
Start point	0 V
Number of cycles / run	3

Figures 7 and 8 were taken to the carbon microarrays using an AFM (Atomic Force Microscrope). In figure 7 we observe a portion of a working electrode (WE) area (darker semicircle in the left part of the 3D image). As expected from the design, a total high of 200 nm-250 nm for the protective layer covering the glassy carbon electrodes is observed. This layer covers the conductive surface of the glassy carbon portions that are not supposed to be in contact with the analyte. At this

resolution, the rugosity of this layer is observed. In the bottom of figure 7, 2D images are shown for the area inspected. These views help us detect if there are relevant failures in the layers. The small circles over the surface are due mainly because of air bubbles; however no relevant depth can be observed in these small circles. A failure in the protective layer would add microelectrode contact area. More electrode contact area would mean more current than expected, and depending on the geometry, other phenomena like the capacitive current presented in CV results.

Figure 8 shows a scan made in another portion of the same carbon microelectrode area; this scan was performed to search for failures in the protective layer that lie over the glassy carbon structure. The darker circle shown in figure 8 represents a well on the layer. This well was not designed to be there. If the depth of this well is such that makes contact with the glassy carbon at ground level, then it could account in some extent for part of the undesired current in the results shown in figures 5 and 6. The presence of several of these wells could add significant undesired currents in the results. Further scans using the AFM are programmed to find out the depth of this type of well. Also, ongoing work is being done to perform other CV experiments with different geometries and redox reactions than the ones shown in this work.

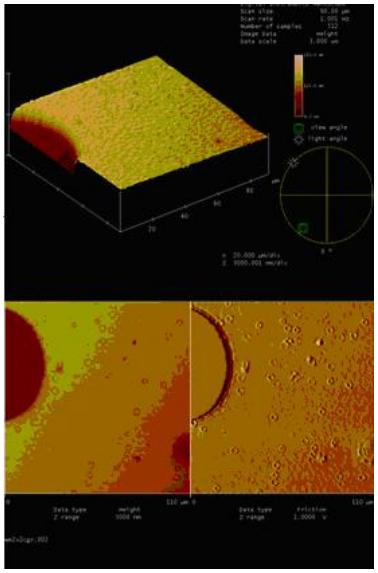

Fig. 7: 3D and 2D image of a working electrode from the microarray.

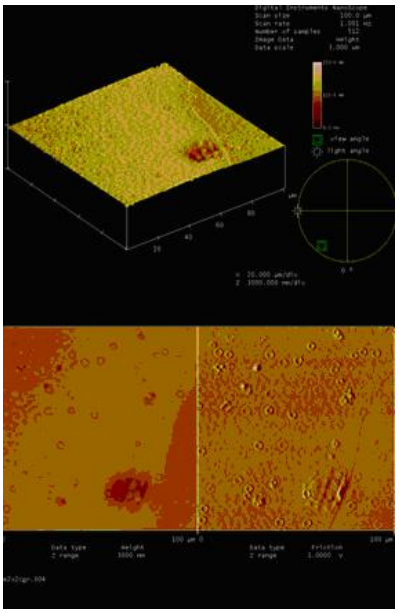

Fig. 8: 3D and 2D image of a defect on the protective surface.

Conclusions

Feasibility of the use of carbon microelectrode arrays to run electrochemical CV experiments, in combination with potentiostat circuits, has been presented. Standard platinum microelectrodes were used as a reference experiment to validate the CV results. For the ferricyanide ion to ferrocyanide Redox reaction the CV experiments show typical curves with values of -20 nA/cm^2 to 18.5 nA/cm^2 using sweep voltages of 250 mV/sec and 500 mV/sec. This shows that the carbon microelectrode arrays can be used in the construction of electrochemical sensor devices but considering the additional capacitance generated at the terminal connections. Moreover, microscopic images, taken from an atomic force microscope, show fabricated electrode defects that can cause small differences in the CV curve due to malformations in the process. Also, rapid electrochemical CV analyses and individual point monitoring or manipulation are possible using carbon microelectrodes. Further improvements are needed on the geometry of the microarray to get improved results, as well as the materials and process used to fabricate the structures.

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