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An Antimony Oxide pH Electrode for Tissue Culture Medium

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Abstract: A new approach named "caterpillar melt method" was developed to prepare wire type antimony oxide electrode for pH measurement in agar medium for tissue culture. A micro antimony wire was prepared from melt of the metal with the help of a glass capillary and the surface of the wire was oxidized in nitrate melt to obtain an antimony oxide electrode. Characterization results showed that the oxide layer is dense and uniform, with high physical and chemical stability. The electrode has a fast and stable response toward pH change for aqueous solutions. The potential of the antimony electrode has a linear relationship with the pH of the solution (R^2=1.00) with a sensitivity of 54.1mV/pH. The electrode works well and is more stable in agar medium during tissue culture for pH monitoring.

Key words: pH measurement; antimony oxide; pH electrode; tissue culture media

1 Introduction

Some endangered plants are hard to propagate in natural environment or in greenhouse by seed, inarch, or other methods. A new approach to solve such a problem is tissue culture, in which a small part of tissure from the plant is taken and put in an aseptic system with enough nutriment including needed ions and organic compounds, and illumination under an appropriate temperature. The whole method is based on the theory of almightiness of frond, which indicates that a cell from any tissue of the plant can differentiate into all types of histolytic and turn into a new whole plant eventually.

We usually choose agar to hold the nutriment. The pH value of the media has a great impact on the growing of the plant[1]. It is necessary to record the pH of the agar media, and even monitor pH change during the whole growing process. Requirements for the measurement system include fast response, stability, reproducibility, small size, and ruggedness. Furthermore, in order to keep the media aseptic, the electrodes need to be treated at high temperature prior to use.

Micro wire type metal oxide electrode is one of the candidates for such applications[2,3]. Antimony oxide has a long history as a pH sensing material, and much cheaper compared with the metals of platinum family. However, the reported SbO_x electrodes are not suitable for long term applications due to the drift of potential response and limited pH range. In addition, the electrode performance was dependent on the degree of polishing and the low reproducibility also limits its application[4]. As a result antimony oxide is now only used in cases where the sample solution contains F^- and where no high accuracy is required. On the other hand, metal antimony is very hard and crisp to make into its wire mechanically; and there is no commercially available antimony wire. To improve the pH sensing performance of antimony oxide electrode, a "caterpillar melt method" was developed and coupled with "nitrate melt oxidation" to prepare wire type SbO_x electrodes for tissue culture applications.

2 Experimental

2.1 Fabrication of the antiomony oxide pH electrode

Metal antimony lumps (National Medicine Group Shanghai Chemical Reagent Company, China) were heated to 750℃ till melt. Then the hot liquid metal was sucked into a glass capillary (Instruments Factory, Southwest University of Medical Sciences of China) with an inner diameter of 1mm. After the metal was cooled down, the glass at the surface was washed away by HF solution to release the antimony wire inside. And the obtained silver bright antimony wires were cleaned in 1 mol/L HCl, isopropyl alcohol and de-ionized water each ultrosonically. Here, the diameter of the wire is determined by the inner diameter of the glass capillary as required by paticular applications.

To oxidize the surface of the metal, the wires were cut into 3mm long each and were positioned in an alumina crucible and covered with KNO_3 powder. The

oxidation of the antimony wire was performed at 500℃ in a furnace for 2 hrs under ambient atmosphere. When the crucible was cooled down to room temperature, the solid KNO_3 was dissolved and the oxidized wire was rinsed with de-ionized water many times to wash attached soluble compounds away. At last, the oxidized wire was dried at 120℃ overnight. The antimony electrode was characterized by SEM (Scanning Electronic Microscope, XL30-ESEM), AFM (Atomic Force Microscope, CSPM 3000s), and XRD (X-Ray Diffraction, Bruker D8 Advance).

2.2 pH measurement of the antimony pH electrode

To calibrate the fabricated Sb/SbO_x electrode, open circuit potential of the electrode was measured against a commercial Ag/AgCl reference electrode in pH buffer solutions. The measured signal was recoreded every 10 sec by a Data Acquisition/Switch Unit (HP 34970A) connected to a personal computer. A universal multi-ion buffer solution with the composition of 0.01 mol/L H_3PO_4-H_3BO_3-CH_3COOH and 0.1M KCl was served as the base solution. pH change of the solution was realized by adding 0.1 mol/L KOH step by step. The solution was magnetically stirred throughout the measurement and a previously calibrated glass pH electrode (pH 211 Microprocessor pH Meter, Hanna Instruments) was used to monitor the pH of the solution.

For the agar system measurement, the electrodes were used as prepared. Agar powder was added into standard solutions with their pH value previously determined. After the solution was boiled and cooled down, the Sb/SbO_x electrode and a wire type Ag/AgCl reference electrode[5] were inserted into the jelly like gel to measure the potential difference, to obtain a working curve of potential vs. pH. The micro reference electrode was fabricated by inserting a silver wire into 0.1 mol/L $FeCl_3$ solution for 24 hrs to oxidize the surface into AgCl. Then, the pH value of an unkown medium was determined by measuring the potential difference between the working and the reference electrode in the sample. Both electrodes were stable and can be kept in the agar medium to monitor its pH change as long as needed.

3 Results and Discussion

3.1 Charactorization of the electrode

The results of SEM illustrates that the oxide layer is dense and uniform, and Fig. 1 shows that it is about 4 μm thick under the current fabrication condition. In Fig. 1, the upper layer is the oxide of antimony (part A), which has a differet appearence compared with the metal below (part B). The AFM diagram (Fig. 2) illuminates that the maximum difference on the surface is less than 120nm, which indicates that the surface is relatively flat considering the thickness of the oxide layer. The nitrate melt oxidation is an effective way to oxidize the antimony wire surface.

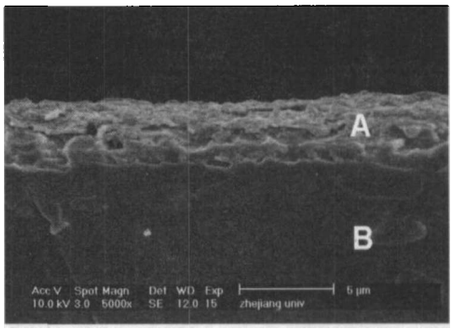

Fig.1 Cross section of the antimony oxide electrode shown by SEM: (A) oxide layer, (B) metal antimony

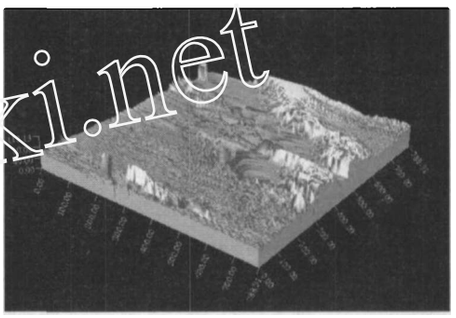

Fig.2 The surface of the antimony oxide electrode shown by AFM (unit: nm)

From XRD results of the oxide powder scrapped from the electrode surface, it can be determined that the oxide layer is Sb_2O_3. The oxidation state (+3) of the oxide is confirmed from the results of CV and the apparent standard potential ($E^{\circ\prime}$=–38.4V vs. Ag/AgCl) obtained from the calibration curve (Fig. 4) of the electrode potential vs. pH.

If the antimony wire is oxidized at a temperature over 580℃, some white powders would be formed on its surface. XRD result shows that the powder is Sb_6O_{13}. Therefore 500℃ was selected as the oxidation temperature for all later fabrications.

3.2 pH sensing performance

The antimony electrodes demonstrated excellent pH sensing performance in aqueous solutions. The 90% response time was less than one second for all the pH changes (Fig.3). Besides, the fabrication method is quite reproducible, with different electrodes giving almost the same response results.

Figure 4 shows that the potential of the electrode and the pH value of the solution had a great linear relationship in the measured pH range of 2~12, which is sufficient for most bio systems. The E value of the plot in the figure is the average of the potentials in Fig. 3.

The slope of the line in Fig. 4 is 54.1 mV per pH unit. This result matches the reported sensitivity for antimony oxide pH electrodes[5].

$$E = 54.1\text{pH} - 38.4 \quad (1)$$

$$R^2 = 1.00 \quad (2)$$

The linear relationship will not be disturbed by the organics added in, but the slope of the line varies with different ingredients. That means the sensoring system can be a good candidate for pH measuring for most biolabs.

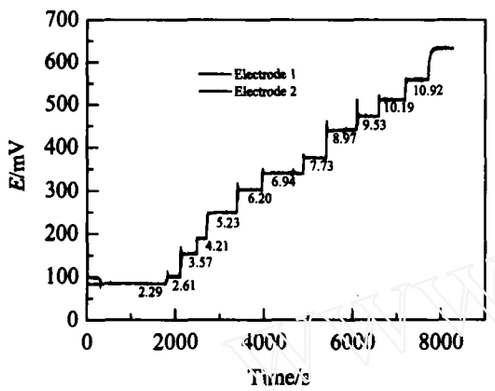

Fig.3 The potential change of the fabricated antimony oxide pH electrodes in a multi-ion-solution. pH values indicated at each step were measured by a glass pH electrode

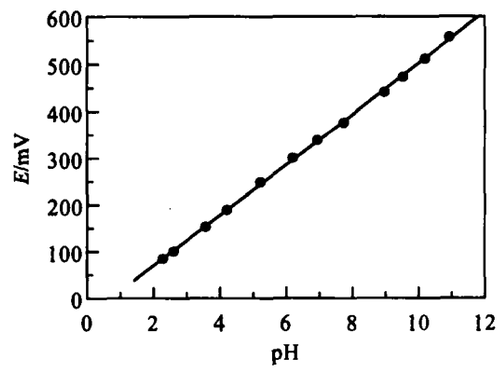

Fig.4 Calibration curve of the potential of the Sb_2O_3 electrode in pH buffers

The electrode also worked well in agar medium. An unkown medium's pH can be calculated by measuring the potential difference with the electrode. Because the electrode can be treated at high temperature, it can be used to monitor the pH change during the whole process of tissue culture, to keep the growing enviornment aceptic.

The measured potential of the electrodes was more stable in agar media, since the system is less fluid than the liquid solution.

4 Conclusions

The "caterpillar melt method" provides an effective way to make antimony wires with enough physical and chemical stability. Besides, this method is ready to make wires of other metals with appropriate melting points.

The micro Sb_2O_3/Sb electrode fabricated by the method in this study can work well as a pH sensor in most bio tissue culture media. It has the advantages of convenient measurement, stable and fast-response and reproducible sensitivity.

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