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# Preparation of the $Ag_2O_2$-$PbO_2$ Modified Electrode and Its Application towards *Escherichia coli* Fast Counting in Water

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**Abstract:** A novel nano crystalline $Ag_2O_2$-$PbO_2$ film chemically modified electrode (CME) was prepared and the CME was characterized by X-ray diffractometer (XRD) and atomic force microscope (AFM). By chronoamperometry, the nano $Ag_2O_2$-$PbO_2$ CME was used as bioelectrochemical sensor to determine the population of *Escherichia coli* (*E. coli*) in water. Compared with conventional methods, it is found that the technique we used is fast and convenient in counting *E. coli*.

**Keywords:** $Ag_2O_2$-$PbO_2$, modified platinum electrode, *Escherichia coli*, fast counting.

The coliform group has been used extensively as an indicator of water quality and historically led to the public health protection concept. The quantitative determination of total and fecal coliforms, as indicators of fecal pollution, is essential for quality control of water [1]. Approved traditional methods for the coliform detection include multiple-tube fermentation (MTF), membrane filter (MF), plate count and nephelometry[2]. These methods are complicated in operation and time-consuming. The counting of microbial colony on the plate medium is widely used method, but it is time-consuming and the incubation period is too long (from 24 to 48 h) when remedial measures must be taken[3-5].

Nanoparticles (1~100 nm) have many effects, such as quantum size effect, surface effect, minisize effect and macroscopic quantum tunnel effect. So they show many distinctive properties and functions. As an essential species among nano materials, nano oxides play an important role in catalysts and sensors[6, 7].

In this paper, we prepared a novel chemically modified electrode and used to determine the population of *E. coli* by chronoamperometry. Because the oxidation current of *E. coli* on the CME is directly proportional to the number of *E. coli*, the population of *E. coli* in water can be calculated conveniently. In addition, when we used some other electrodes for this purpose, no response could be found. Our nano crystalline $Ag_2O_2$-$PbO_2$ film chemically modified electrode performs higher sensitivity than other single nano material modified electrodes in determination of *E. coli*. The simplicity and quickness of this method made the modified electrode very attractive for applications.

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## Experimental

*Preparation of nano $Ag_2O_2$-$PbO_2$ chemically modified electrode*

All the reagents used were of analytical-reagent grade. Platinum electrode was submerged into solution of acetic acid with 30% hydrogen peroxide (volume ratio is 1:1) for 5 minutes, and then washed thoroughly with doubly distilled water. The electrode was modified in the solution containing silver nitrate, lead nitrate and sodium fluoride under a constant current at 80℃ for 30 minutes in water bath.

*E. coli counting*

The chronoamperometric I-t curve were carried out with a CHI832 electrochemical system (CH Instrument Co. USA). The nano $Ag_2O_2$-$PbO_2$ CME was applied as working electrode at the potential of 0.5 V; a SCE electrode as reference electrode; a platinum electrode as counter electrode. The chronoamperometric I-t curve was performed in 10 mL 0.1 mol/L phosphate buffer solution (pH 7.0 PBS) with the *E.coli* sample added (100 cells once). According to the linear relationships of the response current and the *E. coli* population, working curve can be drawn and the *E. coli* population of sample could be determined. After each determination, if the electrode was kept at a high potential 3 V for 1 min in 0.1 mol/L $Na_2SO_4$, the signal restored to its initial value. It is suggested that the fouling substances of the *E.coli* were demolished on the electrode surface at a high potential, and then the electrodes surface was renewed.

## Results and Discussion

*Characterization of $Ag_2O_2$-$PbO_2$ film*

X-ray data were obtained by using a D8ADVANCE X-ray diffractometer (Bruker Axs company, Germany), based on Cu-Kα radiation. X-ray diffractogram of $Ag_2O_2$-$PbO_2$ film is shown in **Figure 1**.

The diffraction peaks shown in **Figure 1** correspond with those in standard spectrum[8] of β-$PbO_2$ and $Ag_2O_2$. It can be concluded that the modified film consisted of crystalline $Ag_2O_2$-$PbO_2$.

**Figure 1** X-ray diffraction of $Ag_2O_2$-$PbO_2$ film

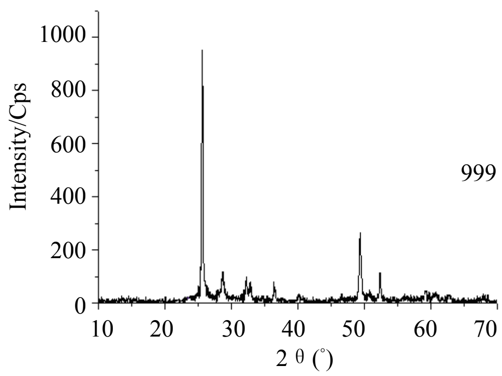
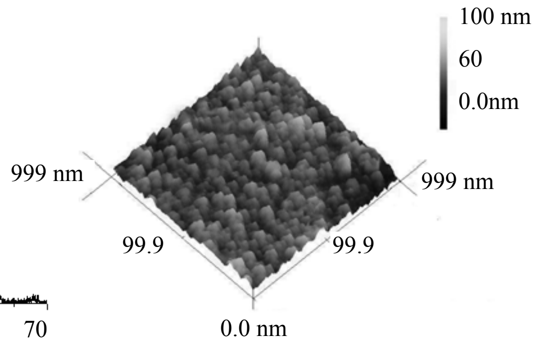



**Figure 2** AFM image of $Ag_2O_2$-$PbO_2$ film



AFM image of $Ag_2O_2$-$PbO_2$ film was recorded using AJ-II atomic force microscope (Shanghai Aijian Nano-technique Science and Technology Co.Ltd). **Figure 2** shows the AFM image of the $Ag_2O_2$-$PbO_2$ film modified on the electrode. The morphology of $Ag_2O_2$-$PbO_2$ film was regular, with good oriented crystals of small size (20-50 nm).

*Reproducibility and stability*

The reproducibility of the current response of *E. coli* at a prepared $Ag_2O_2$-$PbO_2$ CME was determined by the addition of 100 cells in 10 mL, 0.2 mol/L PBS for ten times. The relative standard deviation (R. S. D.) was 1.2%, indicating that the electrode has a good reproducibility. The result stated that this electrode was stable and able to be used repeatedly for a month by activating at a high voltage. It is suitable for long-term operation.

*Mechanism of counting E. coli*

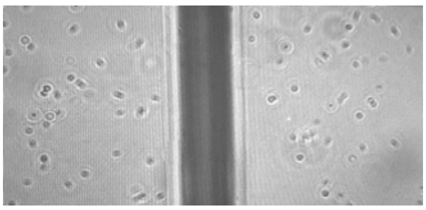
When the working electrode was under a positive potential, the *E. coli* whose surfaces were negative-charged drew close to the surface of the working electrode through electrostatic attraction. Therefore, the basis of our quantification method is that oxidation current of *E. coli* generated on the electrode is related to the population of the bacteria.

**Figure 3** (a, b) shows the pictures of the working electrode and *E. coli* observed with a phase-contrast microscope at 0V and 0.5V, respectively. From these two pictures, we can see *E. coli* are much more close to the electrode at a certain voltage and the number of *E. coli* around the electrode is increasing. It is certified clearly that our assumption on the mechanism of counting *E. coli* is credible. Further studies are proceeding now in our laboratory.

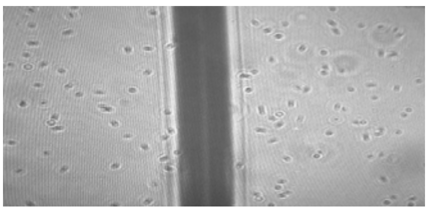
*Working curve of counting E. coli*

**Figure 4** shows chronoamperometric I-t curve in 10 mL, 0.1 mol/L, pH 7.0 PBS with adding 100 cells each time.

**Figure 3** Images observed with phase-contrast microscope I-t curve of 10 mL 0.1 mol/L



(a) at 0V



(b) at 0.5V

**Figure 4** I-t curve of 10 mL 0.1 mol/L pH7.0 PBS with adding 100 cells each time

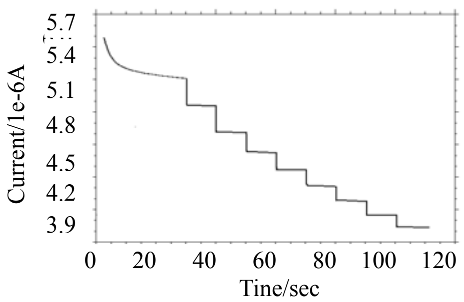
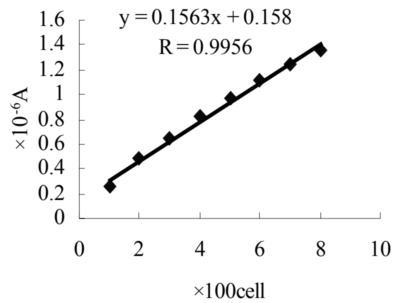



**Figure 5** Working curve




The working curve obtained from **Figure 4** is shown in **Figure 5**. A good correlation was found between the number of *E. coli* and the oxidation current value. According to the oxidation current, the number of *E. coli* in water sample will be obtained. Besides, the current value changed $1.6\times10^{-7}$A, when adding 100 cells into PBS.

## Conclusion

In this paper we described the preparation of a novel nano crystalline $Ag_2O_2$-$PbO_2$ film CME for the first time and made a research of the *E. coli* fast counting. The results showed that the CME was of convenience and good stability. This technique is significantly superior to conventional methods in counting *E. coli* population in water, which has an extremely high research value and broad application prospect.

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