

A disposable two-throughput electrochemical immunosensor chip for simultaneous multianalyte determination of tumor markers

Jie Wu, Zhijie Zhang, Zhifeng Fu, Huangxian Ju*

Key Laboratory of Analytical Chemistry for Life Science (Education Ministry of China), Department of Chemistry, Nanjing University, Nanjing 210093, China

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Abstract

A disposable two-throughput immunosensor array was proposed for simultaneous electrochemical determination of tumor markers. The low-cost immunosensor array was fabricated simply using cellulose acetate membrane to co-immobilize thionine as a mediator and two kinds of antigens on two carbon electrodes of a screen-printed chip, respectively. With two simultaneous competitive immunoreactions the corresponding horseradish peroxidase (HRP) labeled antibodies were captured on the membranes, respectively, on which the immobilized thionine shuttled electrons between HRP and the electrodes for enzymatic reduction of H_2O_2 to produce detectable signals. The electrochemical and electronic cross-talks between the electrodes could be avoided, which was beneficial to the miniaturization of the array without considering the distance between immunosensors. Under optimal conditions the immunosensor array could be used for fast simultaneous electrochemical detection of CA 19-9 and CA 125 with the limits of detection of 0.2 and 0.4 U/ml, respectively. The serum samples from clinic were assayed with the proposed method and the results were in acceptable agreement with the reference values. The proposed method for preparation of immunosensor array could be conveniently used for fabrication of disposable electrochemical biochip with high throughput and possessed the potential of mass production and commercialization. © 2007 Elsevier B.V. All rights reserved.

Keywords: Bioanalysis; Biosensors; Immunosensor; Electrochemistry; Multianalyte determination; Biochip; Tumor marker; CA 19-9; CA 125

1. Introduction

In recent years, biochip technology has attracted considerable interest due to the need of massive parallel detection for both clinical diagnostics and study of chemical biology and/or life science. The fabrication of biochips based on microarrays has also made great progress. However, they have not been extensively applied in clinic due to the high cost for biochip preparation. On the other hand, the diagnostic value of a single tumor marker measurement is limited because most markers are not specific. To improve the diagnostic value the accurate multi-analyte test of combinations of tumor markers has become more and more important for the screening and diagnosis of a certain type of tumors, which makes the multianalyte assays and sensor arrays for tumor markers be becoming an interesting and promising research field. Thus, tremendous efforts are focusing on the development of low-cost multianalyte immunoassay meth-

ods and convenient preparation of immunosensor arrays. Using a general screen-printed technique and simple immobilization method this work proposed a low-cost immunosensor array for fast multianalyte electrochemical immunoassay of tumor markers.

Simultaneous multianalyte immunoassays (SMIAs) are a promising analytical method in protein analysis with the advantages of short analysis time, simplified analytical procedure, decreased sampling volume, improved test efficiency and reduced cost as compared to parallel single-analyte assays (Brecht and Abuknesha, 1995; Diaz-Gonzalez et al., 2005). Usually SMIAs include multiple label assays (Kricka, 1992; Masseyeff et al., 1997; Liu et al., 2004), spatially resolved methods (Ding et al., 1999; Christodoulides et al., 2002; Mastichiadis et al., 2002; Taitt et al., 2002), separation based techniques (Chen and Evangelista, 1994; Haake et al., 2000) and sequential detection (Gonzalez-Martinez et al., 2001). Multi-label methods and spatially resolved assay systems have been well developed (Brecht and Abuknesha, 1995). However, the multi-label assays need a compromise in assay conditions for different labels, thus the assay formats based on spatially separated test zones,

* Corresponding author. Tel.: +86 25 83593593; fax: +86 25 83593593.
E-mail address: hxju@nju.edu.cn (H. Ju).

particularly optical SMIAs relying on fluorescence emission and optical reflectance (Cho and Bright, 2002; Cho et al., 2002; Joos et al., 2002), have gained more considerations recently. Although optical SMIAAs are highly sensitive, they need expensive array detector, for example, charge-coupled device (CCD) camera for optical detection (Rowe et al., 1999; Delehanty and Ligler, 2002; Barry and Soloviev, 2004; Knecht et al., 2004). Thus, assays based on alternative detection strategies have also been developed. One of the developing spatially resolved SMIAAs is electrochemical immunoassay. It is an effective analytical technique with a most peculiar feature in miniaturization for high-throughput applications and low cost of the entire assay system (Yao et al., 1995; Yu et al., 2004).

Many attempts have been made towards development of electrochemical sensor array for SMIAAs (Meyerhoff et al., 1995; Bordes et al., 1997; Ronkainen-Matsuno et al., 2002; Kojima et al., 2003; Seidel and Gauglitz, 2003; de Prada et al., 2004; Dill et al., 2004; Liang et al., 2004; Paitan et al., 2004; Wilson, 2005; Wilson and Nie, 2006a,b). Although the miniaturization of electrochemical sensor array can reduce both the incubation time and the cost for an assay because of the low volume of the immunoassay reaction chamber and the use of a disposable sample carrier (Ronkainen-Matsuno et al., 2002; Seidel and Gauglitz, 2003), cross-talk is the main trouble in such system (Ding et al., 1999). For example, at a pioneer electrochemical immunosensor array prepared with a plasma-polymerized siloxane film to immobilize capture antibodies the cross-talk caused by the diffusion of enzymatic product, hydrogen peroxide, to surrounding working electrodes depends upon the distance between electrodes and the time required for the measurements (Kojima et al., 2003). Wilson (2005) and Wilson and Nie (2006a,b) excluded the electrochemical cross-talk by controlling the distance between adjacent electrodes larger than the diffusion distance of enzymatic product. This work suggested one method to avoid completely the electrochemical cross-talk by immobilizing simply an electron-transfer mediator on individual immunosensor to shuttle electrons, which produced detectable signals without interference resulted from the diffusion of enzymatic product, thus was advantageous to the further miniaturization of electrochemical sensor array.

Compared with the fabrication of immunosensor array reported previously (Kojima et al., 2003; Dill et al., 2004; Wilson, 2005; Wilson and Nie, 2006a,b), this work used a screen-printed technique to prepare the substrate electrode array. This technique was much simpler than the methods of microelectronics and photolithography for preparation of electrochemical immunosensor array (Kojima et al., 2003; Dill et al., 2004; Wilson, 2005; Wilson and Nie, 2006a,b) and decreased the cost of the array fabrication. It enabled easy production of very flexible configurations of electrode array devices with low cost and good disposability and portability. Using two important tumor markers, carbohydrate antigen 19-9 (CA 19-9) and carcinoma antigen 125 (CA 125) as models a novel immunosensor array for SMIAAs was developed. The consideration in interval of detecting two analytes and the separate distance between neighboring electrodes was unnecessary, which was beneficial to rapid detection and the miniaturization of multianalyte detection device.

The proposed method could be conveniently used for low-cost fabrication of electrochemical biochips with mass production and high analyte-throughput.

2. Materials and methods

2.1. Reagents

CA 19-9 and CA 125 enzyme-linked immunoabsorbent assay (ELISA) kits were all purchased from CanAg Diagnostics AB (Sweden). The CA 19-9 ELISA kits consisted of a series of CA 19-9 standard solutions with different concentrations from 0 to 240 U/ml and a stock solution of horseradish peroxidase (HRP)-labeled CA 19-9 monoclonal antibody. The CA 125 ELISA kits consisted of a series of CA 125 standard solutions with different concentrations from 0 to 500 U/ml and a stock solution of HRP-labeled CA 125 monoclonal antibody. Bovine serum albumin (BSA) was purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). The dilution solution for the enzyme conjugate contained 0.04% BSA and 1.0 mM ethylenediamine tetraacetic acid (EDTA) and 0.1 M phosphate buffered saline (PBS). Thionine, cellulose acetate (CA, approximately 40% of acetate) and H₂O₂ (analytical reagent grade) were from Shanghai Biochemical Reagent Company (China). All other reagents were of analytical reagent grade and used without further purification. 0.1 M PBS of various pHs were prepared by mixing the stock solutions of NaH₂PO₄ and Na₂HPO₄, and then adjusting the pH with 0.1 M NaOH and H₃PO₄. Doubly distilled water was used throughout the experiments. Serum specimens provided by Jiangsu Institute of Cancer Prevention and Cure were stored at 4 °C.

2.2. Instrumentation

Electrochemical measurements were both performed on a CHI 730 electrochemical analyzer (CHI Co., USA) in a three-electrode configuration and an eDAQ manufactures e-corder system with a QuadStat (eDAQ Co., Australia) in a four-electrode configuration. For simultaneous dual-analyte detection on the CHI 730, the two working electrodes were connected to a single potentiostat with a switch. Scanning electron micrographs of bare screen-printed carbon electrode (SPCE), CA membrane modified SPCE and the immunosensors were obtained with a JSM-5610LV scanning electron microscope (JEOL, Japan). The reference values of CA 19-9 and CA 125 in sera were obtained with an automation electrochemiluminescent analyzer (Elecsys 2010, Roche, Switzerland).

2.3. Preparation of immunosensors array

The dual-analyte immunosensor array was composed of two thionine/antigen modified graphite working electrodes, W1 and W2 (2 mm in diameter, 2 mm edge-to-edge separation) (Fig. 1A), one graphite auxiliary electrode, and one Ag/AgCl reference electrode. Both working electrodes shared the same reference and auxiliary electrodes. The four-electrode array was fabricated according to the steps reported previously (Yu et al.,

2004). The insulating layer printed around the working area constituted a reservoir of the electrochemical cell with a volume of $50 \mu\text{l}$.

As shown in Fig. 1B, $0.5 \mu\text{l}$ of 1% CA solution, prepared by dissolving 1.0 g CA in a mixture of 50 ml acetone and 50 ml of cyclohexanone, was dropped on two graphite working electrodes, respectively, and dried in a silica gel desiccator to form CA membrane on two electrodes. Three microliters mixture containing 0.5 mM thionine and CA 19-9 or CA 125 standard solution (1:1, v/v) was then dropped on each CA membrane coated working electrode for co-immobilization of thionine and antigens to obtain thionine/antigen modified SPCEs after incubation at room temperature for 50 min in a water atmosphere to avoid evaporation of solvent. The excess mixtures were removed from the sensors by thoroughly rinsing with doubly distilled water.

2.4. Analysis procedure

The SMIA was based on the competitive immunoreactions to capture corresponding HRP-labeled antibodies and enzymatic reduction of H_2O_2 by the labeled HRP with the immobilized thionine as mediator to shuttle electrons between the HRP and the electrodes (Fig. 1B). The sensor array composed of thionine/CA 19-9 modified W1 and thionine/CA 125 modified W2 was firstly incubated with $50 \mu\text{l}$ mixture of diluted HRP-labeled anti-CA 19-9, CA 19-9, diluted HRP-labeled anti-CA 125 and

CA 125 solutions, or two HRP-labeled antibodies with sample for 1 h at room temperature in a water atmosphere to avoid evaporation of solvent. After the residual was removed with doubly distilled water, $50 \mu\text{l}$ anaerobic pH 7.0 PBS with or without 4.5 mM H_2O_2 was transferred onto the sensor array, and the electrochemical responses from W1 and W2 were recorded using cyclic voltammetry, chronoamperometry and differential pulse voltammetry (DPV) in nitrogen atmosphere. The chronoamperometric measurements were performed with the potential of -450 mV (versus Ag/AgCl), and the DPV measurements were performed from -100 to -650 mV with pulse amplitude of 50 mV and pulse width of 50 ms.

3. Results and discussion

3.1. Morphologies of immunosensor

The surface morphology of an immunosensor is a vital factor affecting the performance of the sensor. As shown in Fig. 1C, the surface of bare SPCE was inhomogeneous and uneven due to the randomly distributed carbon particles (Darain et al., 2005), which resulted in the difficulty in producing screen-printed carbon electrodes of equal roughness. So it showed unrepeatability for immunoassays. The micrograph of CA membrane modified SPCE displayed a three-dimensional uniform porous structure with the aperture of $0.9 \mu\text{m}$ (Fig. 1D). This uniform open structure provided excellent reproducibility and a signifi-

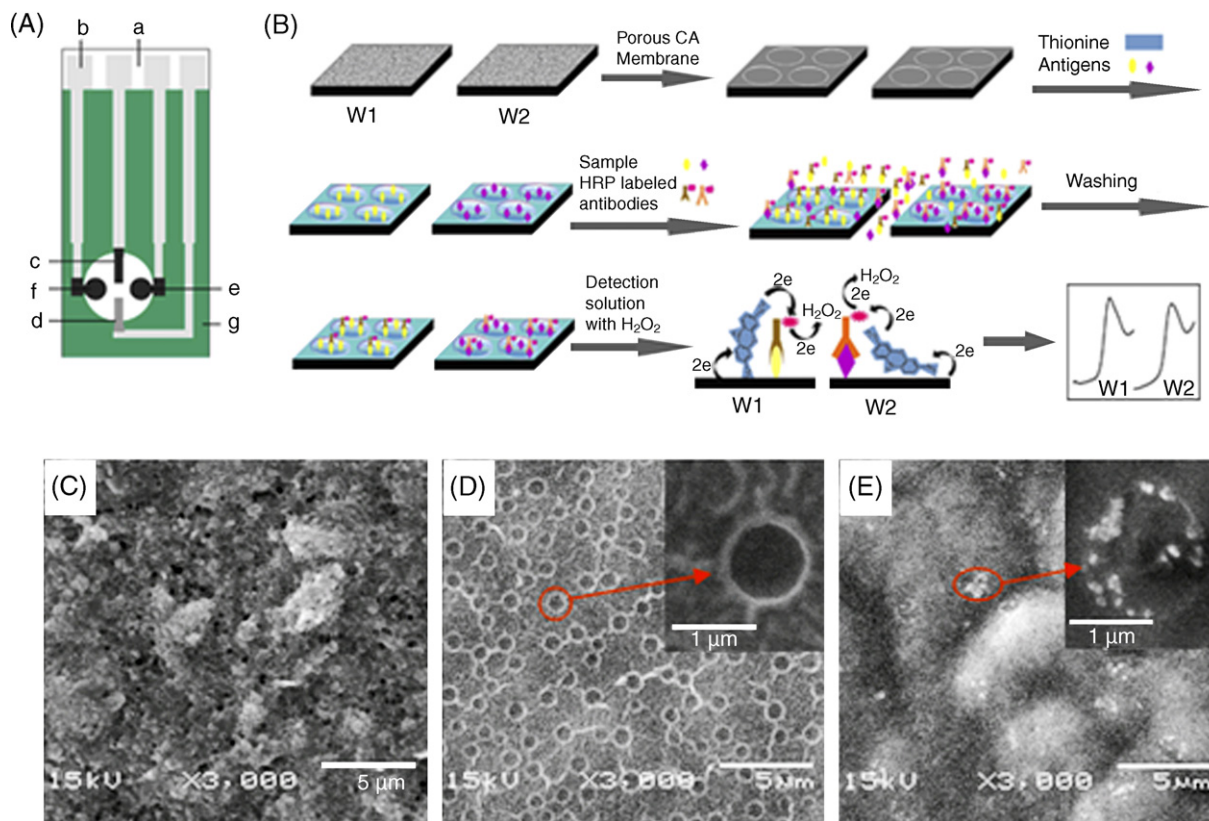


Fig. 1. Schematic diagrams of (A) four-electrode SPCE system and (B) preparation of immunosensor array and SMIA procedure and SEM images of (C) bare, (D) CA membrane modified and (E) thionine/antigen co-immobilized CA membrane modified SPCE at two different magnifications. (a) Nylon sheet, (b) silver ink, (c) graphite auxiliary electrode, (d) Ag/AgCl reference electrode, (e) W1, (f) W2 and (g) insulating dielectric.

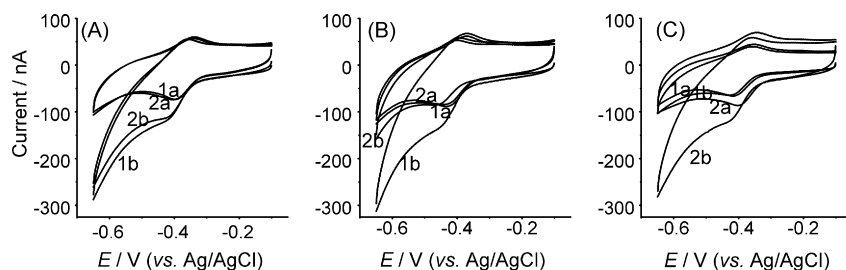


Fig. 2. Simultaneous cyclic voltammetric responses of W1 and W2 in pH 7.0 PBS without (1a and 2a) and with (1b and 2b) 4.5 mM H_2O_2 after incubated in 50 μl mixture containing 1:60 diluted HRP-labeled anti-CA 19-9 and 1:50 diluted HRP-labeled anti-CA 125 (A), 50 μl 1:60 diluted HRP-labeled anti-CA 19-9 (B) and 50 μl 1:50 diluted HRP-labeled anti-CA 125 (C).

cant increase of effective electrode surface for antigen loading. After the thionine and antigen were adsorbed on the CA membrane matrix, the uniform open structure was retained and the three-dimensional frame of the porous structure was weakened (Fig. 1E). The aggregates of trapped biomolecules with a regular distribution in the pore were also observed. The presence of CA membrane played an important role in immobilization of thionine and antigen. The regular open structure assured good reproducibility for sensor preparation, free binding of the enzyme conjugates with the immobilized antigens, and easy access of H_2O_2 molecules to the conjugated enzyme to produce sensitive amperometric responses.

3.2. Evaluation of cross-reactivity and cross-talk behavior

Cross-reactivity and cross-talk are two important factors that must be considered in SMIA. For excellent SMIA, the immunosensor array must exclude the cross-reactivity between analytes and noncognate antibodies and the cross-talk between neighboring electrodes (Wilson and Nie, 2006a). On this principle, experiments were performed to examine the cross-reactivity and cross-talk, respectively.

After the electrodes, W1 and W2, were modified with thionine/CA 19-9 and thionine/CA 125, respectively, they were incubated with 50 μl mixture of 1:60 diluted HRP-labeled anti-CA 19-9 and 1:50 diluted HRP-labeled anti-CA 125 (Fig. 2A), 50 μl 1:60 diluted HRP-labeled anti-CA 19-9 (Fig. 2B) or 1:50 diluted HRP-labeled anti-CA 125 (Fig. 2C) for 1 h at room temperature. The pair of redox peaks of thionine on W1 (curve 1a) and W2 (curve 2a) could be observed with the same peak current in pH 7.0 PBS, respectively, W2 used for Fig. 2C showed a slightly larger background, indicating good fabrication reproducibility of sensor array.

Upon addition of 4.5 mM H_2O_2 into the PBS, the reduction peak currents increased greatly and the oxidation peak currents decreased on both W1 and W2, at the same time all the reduction peak potential shifted slightly to more negative value (curves 1b and 2b in Fig. 2A), showing an obvious electrocatalytic process on the two electrodes. When the sensor array was incubated in 1:60 diluted HRP-labeled anti-CA 19-9 solution or 1:50 diluted HRP-labeled anti-CA 125 solution, the electrocatalytic process was only observed on one electrode, W1 (curve 1b in Fig. 2B) or W2 (curve 2b in Fig. 2C). No obvious change was observed on W2 (curve 2b in Fig. 2B) or W1 (curve

1b in Fig. 2C). These experiments demonstrated that no cross-reactivity occurred between reagents and that the nonspecific binding was minimal.

The mediator thionine was immobilized on the electrodes during the fabrication of immunosensor array instead of in the detection solution, thus no diffusion of electroactive product occurred, and theoretically the electrochemical cross-talk between the electrodes could be completely avoided. Experimental results validated this opinion. Because thionine was immobilized on the electrodes by adsorption, the stability of thionine was firstly examined. The cyclic voltammograms of immunosensor were recorded in pH 7.0 PBS at 5, 10, 20, 30, 40 and 50 mV/s, respectively. Both the anodic and cathodic peak currents are proportional to the scan rate, indicating a surface-controlled electrode process and invariable surface coverage of thionine. The continuous cyclic sweeps at 5, 10, 20, 30, 40 and 50 mV/s were also applied to search the leakage of thionine from the surface of the electrode, respectively. The cyclic voltammograms recorded at different times showed the same peak currents and potentials (Fig. 3A). All the experiments indicated the leakage of thionine from the surface of the electrode was negligible. After the sensor array composed of thionine/CA 19-9 modified W1 and thionine modified W2 was incubated in 50 μl 1:60 diluted HRP-labeled anti-CA 19-9, their amperometric responses to H_2O_2 at -450 mV (versus Ag/AgCl) were recorded simultaneously (Fig. 3B). Upon addition of 4.5 mM H_2O_2 the W1 showed a prominent amperometric response (curve a, Fig. 3B), conversely, very small change was observed at the W2 (curve b, Fig. 3B). The signal change at the W2 was also observed at a thionine modified electrode without the incubation process (curve c, Fig. 3B), thus the signal change resulted from the direct reduction of H_2O_2 at -450 mV. Furthermore, the response of the W2 could be stable for 40 min, indicating no interference occurred between W1 and W2 in the measurement period.

In electronics when one electrode or wire acts as a transmitter and the others as receiver, it was also called “cross-talk”. Fig. 4C shows the cyclic voltammograms of CA 19-9 immunosensor (W1) recorded in single-channel (curves a and b) and dual-channel potentiostat formats (curve c) after the array was incubated. When CA 125 immunosensor (W2) worked the W1 showed the same response to H_2O_2 as that obtained in single-channel format. Thus, this immunosensor array could also avoid electronic cross-talk.

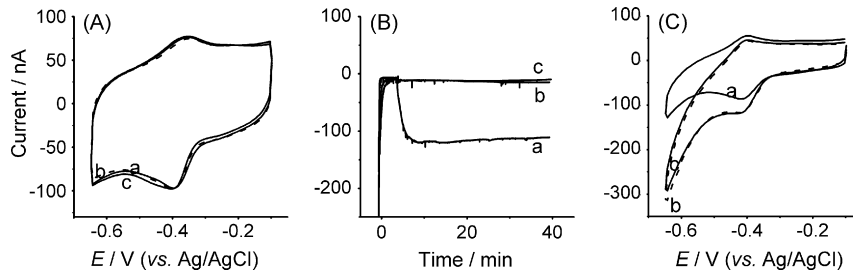


Fig. 3. (A) Stability of the immobilized thionine recorded for continuous cyclic sweep in pH 7.0 PBS at 0 min (a), 40 min (b) and 90 min (c); (B) simultaneous amperometric responses to 4.5 mM H_2O_2 in pH 7.0 PBS at -450 mV after CA19-9/thionine modified W1 (a) and thionine modified W2 (b) were incubated in 1:60 diluted HRP-labeled anti-CA 19-9 solution and amperometric response of the thionine modified electrode to 4.5 mM H_2O_2 without incubation (c); (C) cyclic voltammograms of CA 19-9 immunosensor in pH 7.0 PBS without (a) and with 4.5 mM H_2O_2 (b and c) recorded in single-channel (a and b) and dual-channel potentiostat formats (c) after the array was incubated. Scan rate: 50 mV/s.

3.3. Optimization of immunosensor preparation

The DPV responses of the sensors mainly depended on the surface immobilization of thionine and antigens, and the surface binding of HRP-labeled antibodies. So the concentrations of thionine and antigens used for preparation of immunosensor arrays and their adsorption time on the CA membrane were firstly optimized. The results showed these sensor arrays produced the best responses and analytical performances when they were prepared with a mixture of 0.5 mM thionine and 240 U/ml CA 19-9 or 500 U/ml CA 125 solutions with a volume ratio of 1:1, respectively. At those concentrations of thionine and antigens, all the original and the detection signals increased with an increasing adsorption time, and reached the plateau values at 50 min, which was selected as the optimal adsorption time for immobilization of thionine and antigens.

3.4. Optimization of immunoassay procedure

The performance of an amperometric immunosensor is usually affected by the incubation time, the working concentration of HRP-labeled antibody, the pH value and H_2O_2 concentration in detection solution. Fig. 4A showed the effects of incubation time on DPV responses of CA 19-9 and CA 125 detection systems. With an increasing incubation time both the DPV responses of the trapped HRP to thionine- H_2O_2 system increased. In the CA 19-9 detection system, the response reached the maximum at 40 min, while the response reached the maximum at 1 h in the CA 125 assay. So 1 h was chosen as the optimal incubation time for the SMIA.

The effects of dilutions of HRP-labeled CA 19-9 antibody and HRP-labeled CA 125 antibody in the incubation solutions on the amperometric responses in CA 19-9 and CA 125 assays were shown in Fig. 4B. With the decreasing dilution or increasing concentration of enzyme-labeled antibody the DPV signals increased and trended to the plateau values at the dilutions of 1:60 for HRP-labeled CA 19-9 antibody in CA 19-9 system and 1:50 for HRP-labeled CA 125 antibody in CA 125 assay, which indicated all of the specific sites of the immobilized antigen were occupied. Thus, 1:60 dilution of HRP-labeled anti-CA 19-9 and 1:50 dilution of HRP-labeled anti-CA 125 were chosen as the working enzyme-labeled antibody solutions in this work.

The acidity of the detection solution affected greatly the catalytic activity of the enzyme. Fig. 4C showed the effects of pH on the current responses of the CA 19-9 and CA 125 sensors in 0.1 M PBS containing 4.5 mM H_2O_2 . The maximum response occurred around pH 7.0 in CA 125 assay, while the CA 19-9 system showed a maximum plateau in the pH range of 6.4–7.0. Thus, pH 7.0 was selected as the optimal pH value for the SMIA.

The DPV responses of the CA 19-9 and CA 125 immunosensor array to H_2O_2 were shown in Fig. 4D. Both the CA 19-9 and CA 125 sensors showed the maximum responses at the H_2O_2 concentration of 4.5 mM. So, the optimal concentration of H_2O_2 in SMIA was 4.5 mM.

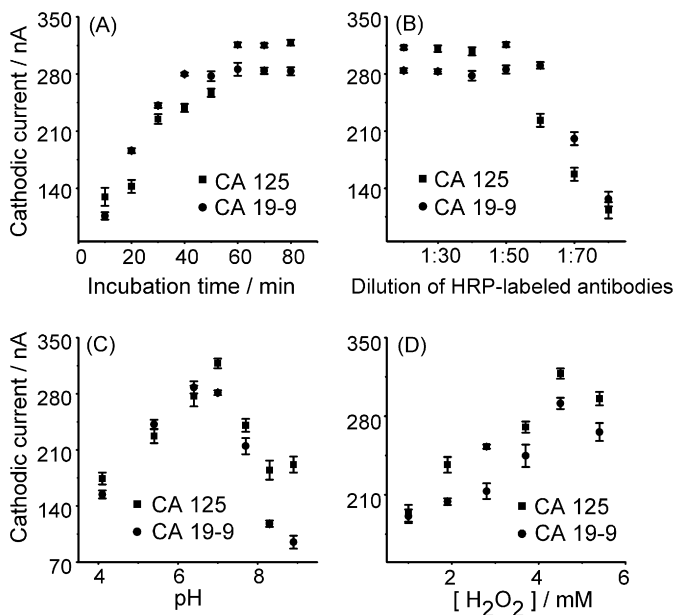


Fig. 4. Dependences of DPV response of immunosensor to single CA 19-9 or CA 125 on incubation time (A), working concentration of HRP-labeled antibody in incubation solution (B), pH of detection solution (C) and H_2O_2 concentration (D).

3.5. Simultaneous detection of CA 19-9 and CA 125

The detection of the tumor markers was based on a competitive assay configuration. Here, the SMIA were performed on

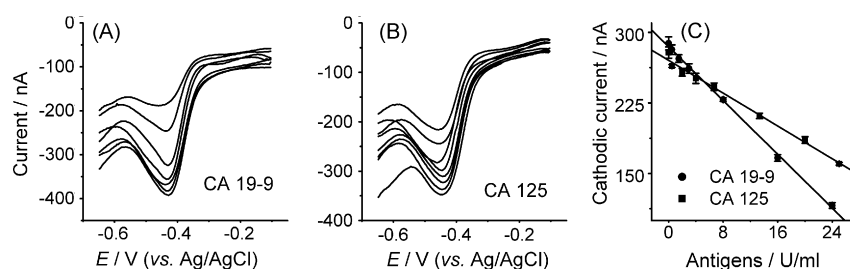


Fig. 5. Differential pulse voltammograms (A and B) and calibration curves (C) for simultaneous immunoassay of CA 19-9 and CA 125.

a single-channel potentiostat with a sequential detection format without interval between two assays and the CA 125 and CA 19-9 immunosensor array, which was prepared by co-immobilizing thionine/CA 19-9 on W1 and thionine/CA 125 on W2. During the incubation process the CA 19-9 and CA 125 in incubation solution competed with the immobilized CA 19-9 on W1 and CA 125 on W2 to bind the limited binding sites of the HRP-labeled CA 19-9 antibody and HRP-labeled CA 125 antibody mixed in the solution, respectively. As expected for a competitive mechanism, with the increasing CA 19-9 and CA 125 concentrations both the catalytic DPV peak currents of the modified W1 and W2 decreased. The decreases in peak currents were proportional to CA 19-9 and CA 125 concentrations, respectively. The dose-response curves obtained from CA 19-9 and CA 125 sensors decreased linearly in the ranges of 0–24 U/ml with a correlation coefficient of 0.9992 and 0 to 25 U/ml with a correlation coefficient of 0.9953, respectively (Fig. 5). The limits of detection of the sensor array, taken as the concentrations equivalent to a 10% decrease in signal, were 0.2 and 0.4 U/ml for CA19-9 and CA125, respectively.

3.6. Application in serum analysis

Ten microliters of serum samples were mixed with 50 μ l diluted solution containing HRP-labeled CA 19-9 antibody and HRP-labeled CA 125 antibody for competitive immunoassay. Thus the detectable linear ranges for CA 19-9 and CA 125 in practical samples were 0–144 and 0–150 U/ml. The ranges expected for CA 125 in the serum sample of patient with ovarian or breast cancer and CA 19-9 in the serum sample of patient with pancreatic cancer are more than 35 U/ml, respectively. Thus, the suggested method was suitable for diagnosis purpose. The precision of simultaneous detection of CA 19-9 and CA 125 by the proposed sensor array was examined by comparing the results obtained from five sera with this method and the commercial electrochemiluminescent single-analyte test performed in Jiangsu Institute of Cancer Prevention and Cure. The results were listed in Table 1. The CA 19-9 and CA 125 levels obtained using present assay system were in acceptable agreement with those using the electrochemiluminescent method. The significant discrepancies between two methods might result from the dilution process. Thus, the proposed dual-analyte immunosensor array and the immunoassay system could be anticipant to detect simultaneously CA 19-9 and CA 125 levels in clinical diagnosis.

Table 1

Assay results of clinical sera using the proposed and reference methods

Sample	CA 19-9 concentration (U/ml)		CA 125 concentration (U/ml)	
	Proposed method	Reference method	Proposed method	Reference method
1	11.9	11.1	12.1	10.4
2	5.7	4.2	10.4	12.3
3	6.1	5.2	3.9	2.2
4	14.5	14.4	15.4	14.5
5	5.9	6.8	5.1	6.5

3.7. Precision, reproducibility, stability of the immunosensor

The inter-assay precision of the immunosensor array was evaluated using five immunosensor chips. The coefficients of variation for inter-assay on this method were 6.4 and 7.0% for 8 and 16 U/ml CA 19-9, and 4.3 and 3.0% for 6.7 and 13.3 U/ml CA 125, indicating acceptable precision and fabrication reproducibility. The immunosensor arrays were stored in dry air at 4 °C. The DPV responses were 94.1 and 97.2% of initial responses for CA 19-9 and CA 125 after a storage period of 30 days, respectively. Thus, the storage stability of the immunosensor arrays was acceptable, and the proposed array was suitable for clinical diagnostics.

4. Conclusions

A disposable electrochemical immunosensor array has been designed for simultaneous multianalyte determination. The array can be prepared by co-immobilizing thionine and different antigens on CA membrane modified SPCEs. The array fabrication is simple, low-cost, mass-producible and reproducible, and the concept of mediator immobilization allows the individual immunoassay to be performed at each electrode without any interference within reported signals. Both electrochemical and electronic cross-talks between adjacent immunosensors can be completely excluded. The effects of both interval of multianalyte analyses and the separate distance between neighboring electrodes are neglectable, which is beneficial to design of smaller and cheaper device with more electrodes and immunosensors in the future. The proposed sensor array possesses satisfied stability and acceptable veracity compared to commercial ELISA, showing the potential for practical detection of multi-tumor markers in clinic serum samples.

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