Chinese Chemical Letters Vol. 13, No. 11, pp 1090 – 1092, 2002
http://www.imm.ac.cn/journal/ccl.html

Electrochemical Enzyme Immunoassay of Tumor Marker CA15-3 with Capillary Electrophoresis

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Abstract: Tumor marker CA15-3 was determined by using capillary electrophoretic enzyme immunoassay with electrochemical detection (CE-EIA-ED). The method can be used to detect CA15-3 with a limit of 0.024 U/mL.

Keywords: Capillary electrophoretic enzyme immunoassay, electrochemical detection, tumor marker, CA15-3.

CA15-3 is a circulating antigen that is relatively specific for human breast tissue. CA15-3 is significantly more sensitive than carcinoembryonic antigen in the evaluation of patients with both primary and metastatic breast cancer. CA15-3 levels are often measured by using radioimmunoassay and enzyme immunoassay (EIA) in clinic. These methods are carried out manually. Tedious processes, slow reaction, poor reproducibility are their major problems. Capillary electrophoretic immunoassay (CEIA) is a new analytical technique[1,2]. There was no report about the determination of CA15-3 by using CEIA.

In the present work, a capillary electrophoretic enzyme immunoassay with electrochemical detection (CE-EIA-ED) has been developed and applied to monitoring CA15-3 in serum. In the assay, the immunoassay protocol is a non-competitive format. A reaction capillary following the separation capillary, which is similar to the post-column reactor described in Ref. 3, was used. An excess amount of anti-CA15-3 of labeled horseradish peroxidase (monoclonal antibody, Ab*) was added to a standard solution or a sample containing CA15-3 (Ag) to ensure the completion of the immuno-reaction between Ag and Ab*. The formation of complex, Ag-Ab*, was quantitative and directly dependent on the amount of CA15-3. After the solution containing Ab* and Ag-Ab* was injected to the poly(vinyl alcohol)-coated fused-silica separation capillary, Ab* and Ag-Ab* were separated by CE. Then, both eluted into the poly(vinyl alcohol)-coated fused-silica reaction capillary and catalyzed the reaction of the enzyme substrate 3,3,5,5-tetramethyl-benzidine (TMB(Red)) and H_2O_2. The reaction product TMB(Ox) was determined with amperometric detection on a carbon fiber microdisk bundle electrode at the outlet of the reaction capillary. The procedure of electrochemical detection used here was similar to our previous description[4]. The optimum conditions of the method

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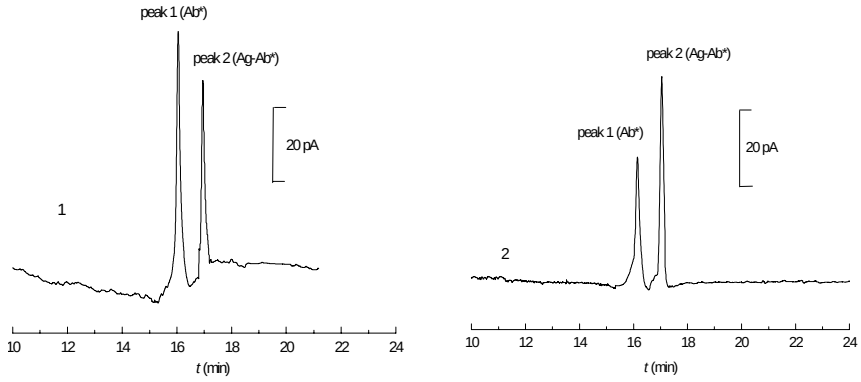
were 2.0×10^{-3} mol/L H_2O_2 + 1.0×10^{-2} mol/L borate (pH 7.4) for the running buffer, 2.0×10^{-4} mol/L TMB(Red) + 2.0×10^{-3} mol/L citrate phosphate (pH 5.0) for the substrate solution, 1.6 mg/L for Ab* concentration, 20 kV for the separation voltage, 0.00 V (*vs*. saturated calomel electrode) for the detection potential. Since the concentration of TMB(Ox) was much higher than those of Ab* and Ag-Ab* due to the enzyme amplification, the limit of detection (LOD) of CE-EIA-ED was very low.

The typical electropherograms of the solution containing the Ab* with 20.8 and 41.7 U/mL Ag are shown in **Figure 1**. Two peaks (peak 1 and 2) appeared at *ca*. 16 and 17 min, respectively. With increasing Ag, the area of peak 1 decreased and the area of peak 2 increased. Therefore, peaks 1 and 2 are the peak of Ab* and the peak of Ag-Ab*, respectively, according to the principle expected for a non-competitive immunoassay. In this method, the limit of detection (3σ) of CA15-3 was as low as 0.024 U/mL. The linear range was from 0.20 U/mL to 41.7 U/mL. In order to verify the method, two breast cancer serum samples were detected. The results are listed in **Table 1**. The concentrations of CA15-3 in the two serum samples obtained by the standard addition method were 67.3 and 52.6 U/mL, respectively, which agree with the values (69.3 U/mL and 50.0 U/mL) detected by the Qilu Hospital, Shandong University using EIA. The recovery of the method was between 92% and 104%.

Table 1 Results detected and recovery of CA15-3

Serum sample	Determined value (U/mL)	Average value (U/mL)	Value by EIA (U/mL)	Added Value (U/mL)	Observed value (U/mL)	Recovery (%)
1	68.1			40.0	108	100
	67.0	67.3	69.3	50.0	115	96
	66.9			60.0	129	104
2	52.5			40.0	90.2	94
	51.4	52.6	50.0	50.0	97.5	92
	53.8			60.0	112	97

Figure 1 Electropherograms of the solution containing 1.6 mg/L Ab* with (1) 20.8 and (2) 41.7 U/mL CA15-3. Separation capillary, 25 cm×50 μm ID, reaction capillary, 5 cm×50 μm ID



Acknowledgment

This project was supported by the National Natural Science Foundation of China, the Natural Science Foundation of Shandong Province and the State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences.

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Received 31 January, 2002