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Determination of Thyroxine with Capillary Electrophoretic Enzyme Immunoassay

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Abstract: A Capillary electrophortic enzyme linked immunoassay with electrochemical detection (CE-EIA-ED) has been developed. The method can be used to determine thyroxine with a limit of 3.8×10^{-9} mol/L.

Keywords: Capillary electrophortic enzyme immunoassay, electrochemical detection, thyroxine.

Capillary electrophoretic immunoassay (CEIA) is a new analytical technique[1,2]. In CEIA, the detection methods were UV absorbance detection[1] and laser induced fluorescence detection (LIF)[2]. However, The UV detection is lack of sensitivity. The LIF is difficult to use due to its high cost, and furthermore, most biological fluids are strongly luminescent when excited by the laser in the blue or green region of the spectrum.

In our present work, a capillary electrophoretic enzyme immunoassay with electrochemical detection (CE-EIA-ED) has been developed and applied to monitoring thyroxine (T4). To our knowledge, the present work is the first report concerning CE-EIA-ED. In the assay, an on-line enzyme catalytic system with catalysis reaction capillary was designed. For detection of T4, the labeled enzyme, horseradish peroxidase can catalyze the reaction of 3,3′,5,5′-tetramethyl-benzidine (TMB(Red)) and H_2O_2. The reaction products are TMB(Ox) and H_2O. TMB(Ox) can be determined with amperometric detection on a carbon fiber microdisk bundle electrode. In the experiment, the immunoassay protocol is a competitive type format. T4 competed with horseradish labeled-T4 (HRP-T4) for a fixed number of anti-T4 binding sites. After incubation, the mixture solution that contains T4, HRP-T4, T4-anti-T4 complex and HRP-T4-anti-T4 complex was introduced into the separation capillary. HRP-T4-anti-T4 complex and HRP-T4 were separated by CE. Both can catalyze the reaction of TMB(Red) and H_2O_2 in the reaction capillary. 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 optimum conditions of the method are 2.0×10^{-3} mol/L H_2O_2 + 5.0×10^{-3} mol/L citrate phosphate (pH 5.0) for the run buffer, 2.0×10^{-4} mol/L TMB(Red) + 20×10^{-3} mol/L citrate phosphate (pH5.0) for the substrate solution, 10 kV

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for the separation voltage, +100 mV for the detection potential. Since the concentration of TMB(Ox) is much higher than those of HRP-T4-anti-T4 complex and HRP-T4 due to the enzyme amplification, the limit of detection (LOD) of CE-EIA-ED should be very low. A typical electropherogram of T4 mixture containing T4 (5 μg/L), HRP-T4 and anti-T4 is shown in **Figure 1**. There are two peaks in the electrophoregram. Peaks 1 and 2 are the signals of HRP-T4-anti-T4 complex and HRP-T4, respectively. With increasing T4, the area of peak 1 decreases and that of peak 2 increases. In this method, the concentration LOD (3σ) of T4 is as low as 3.8×10^{-9} mol/L. The linear range is from 0.5 μg/L to 50 μg/L. In order to verify the method, two T4 controls (25 ± 7.5 μg/L and 100 ± 30 μg/L) were detected. The results are listed in **Table 1**. The concentrations of T4 in the two controls were determined to be 31 μg/L and 112 μg/L, respectively, which agreed with the stated values.

Figure 1 Electropherogram of T4 mixture containing T4 (5 μg/L), HRP-T4 and anti-T4.

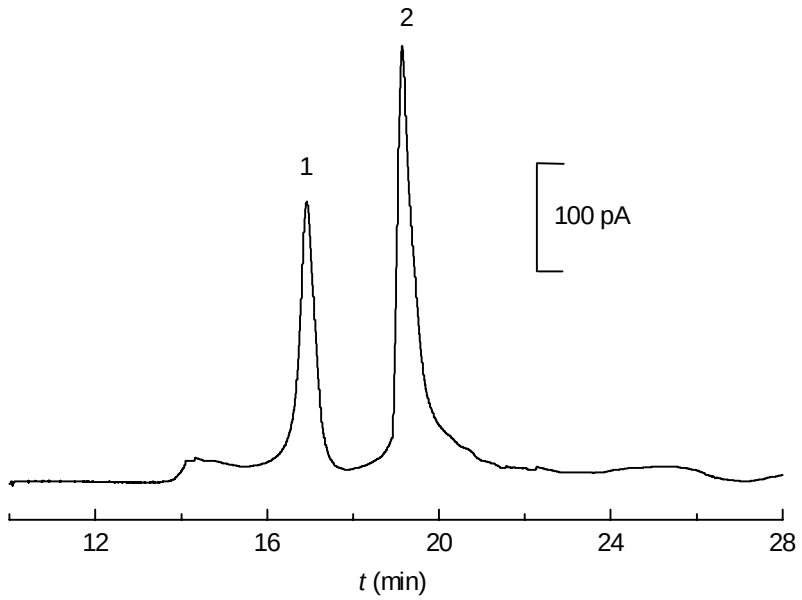

Table 1 Results detected and recovery of T4

sample	determined value (μg/L)	average value (μg/L)	added value (μg/L)	observed value (μg/L)	recovery (%)
I	32	31	50	79	94
	30		150	165	90
II	114	112	150	271	105
	109		250	335	90

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