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Redox cycling-based immunoassay for detection of carcinogenic embryonic antigen

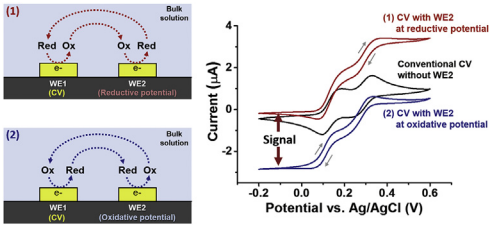
Ga-Yeon Lee [a], Jun-Hee Park [a], Young Wook Chang [a], Sungbo Cho [b], Min-Jung Kang [c], Jae-Chul Pyun [a, *]

[a] Department of Materials Science and Engineering, Yonsei University, 134 Shinchon-dong, Seodaemun-gu, Seoul 120-749, South Korea
[b] Department of Biomedical Engineering, Gachon University, South Korea
[c] Korea Institute of Science and Technology (KIST), Seoul, South Korea

HIGHLIGHTS

- Redox cycling was developed for a highly sensitive immunoassay.
- Interdigitated electrodes (IDEs) were used as the generator and the collector electrodes.
- Redox cycling was optimized by controlling potential and distance of IDEs.
- Redox cycling with IDEs was applied to a commercial immunoassay for CEA.

GRAPHICAL ABSTRACT



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ABSTRACT

Redox cycling based on an interdigitated electrode (IDE) was used as a highly sensitive immunoassay for carcinogenic embryonic antigen (CEA) through the quantification of 3,3′,5,5′-tetramethylbenzidine (TMB). For the redox cycling process, one pair of interdigitated finger electrodes was used as the first working electrode (generator) for cyclic voltammetry of TMB, and another pair of interdigitated finger electrodes was used as the second working electrode (collector) for sequential application of potentials for reduction and oxidation of TMB. The reduction (and oxidation) products of TMB at the collector were supplied to the generator, and following sequential oxidization (and reduction) at the generator, again supplied to the collector. Such redox recycling processes between the generator and collector allowed signal amplification. In this work, the influences of the following factors on the redox cycling of TMB were analyzed: (1) the redox potential at the collector, (2) the gap between the interdigitated finger electrodes, and (3) the scan rate of the generator. The redox potential and electrode gap influences were simulated with COMSOL software and compared with empirical results. At the optimum redox potentials and electrode gap, redox cycling was estimated to be five-fold more sensitive for the quantification of TMB than conventional cyclic voltammetry using one pair of interdigitated finger electrodes as the working electrode. Finally, redox cycling was applied to a commercial immunoassay for CEA, and the sensitivity of redox cycling was three-fold higher than that of conventional cyclic voltammetry using a single set of interdigitated finger electrodes as the working electrode.

Abbreviations: HRP, horseradish peroxidase; TMB, 3,3′,5,5′-tetramethylbenzidine; IDE, interdigitated electrode; WE2, collector; WE1, generator; CV, cyclic voltammogram; CEA, carcinogenic embryonic antigen; DA, reactant diffusion coefficient; DB, product diffusion coefficient.

* Corresponding author.
E-mail address: jcpyun@yonsei.ac.kr (J.-C. Pyun).

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1. Introduction

Immunoassays are widely used for the detection of target analytes based on the highly specific interactions of antibodies with a specific antigen (analyte). Usually, antibodies are immobilized on a solid support and the antigen in a sample binds specifically to the antibodies [1,2]. The amount of antigen remaining is detected using secondary antibodies with the enzyme horseradish peroxidase (HRP), which undergoes a chromogenic reaction with the substrate 3,3′,5,5′-tetramethylbenzidine (TMB). During this reaction, TMB molecules are oxidized in two steps, as shown in Fig. 1(a): TMB (transparent) → ox1-TMB (blue, λ_{max} = 650 nm) → ox2-TMB (yellow, λ_{max} = 490 nm) [3–5]. As this chromogenic reaction involves the oxidation of TMB molecules, quantification of the antigen (analyte) is also possible by using amperometric reduction of oxidized TMB [6–9].

In this work, redox cycling performed with an interdigitated electrode (IDE) was used for highly sensitive detection of the chromogenic reaction of TMB. IDEs have been employed for redox cycling because redox species generated at a finger electrode (generator, WE1) can undergo the reverse reaction at the adjacent finger electrode (collector, WE2) if the potentials of the two electrodes are set at an appropriate level for the reverse reaction [10]. As shown in Fig. 1(b), the redox species undergo reaction at the collector (WE2) and then diffuse back to the generator (WE1), and this redox cycling process can amplify currents at both the generator and collector electrodes [11]. Thus, one molecule only reacts many times if the potentials of both the interdigitated finger electrodes are sufficient for consecutive oxidation and reduction reactions [12]. Additionally, the shape of cyclic voltammogram (CV) was consistent with that of a microelectrode owing to enhanced diffusion during redox cycling [13]. The influence of electrode geometry, such as electrode area and the electrode gap between interdigitated band electrodes, has been well studied during redox cycling [14–18]. In this work, the factors that affect redox cycling of TMB were analyzed by controlling (1) the potential of the collector, (2) the electrode gap between the generator and the collector, and (3) the scanning rate at the generator. Further, the behaviors of the electrodes during redox cycling of TMB were simulated using the commercial simulation software COMSOL®. Finally, redox cycling of TMB was used along with a commercial ELISA assay kit for the detection of the carcinogenic embryonic antigen (CEA), and the sensitivity of the measurements was compared with that of conventional cyclic voltammetry using a single set of interdigitated finger electrodes as the working electrode.

2. Materials and methods

2.1. Materials

TMB, HRP, and other analytical grade chemicals were purchased from Sigma-Aldrich Korea (Seoul, Korea). CEA ELISA kits were purchased from Perfumed Group, Inc. (San Francisco, CA, USA). The photoresist (AZ-GXR601) was purchased from Merck Co.

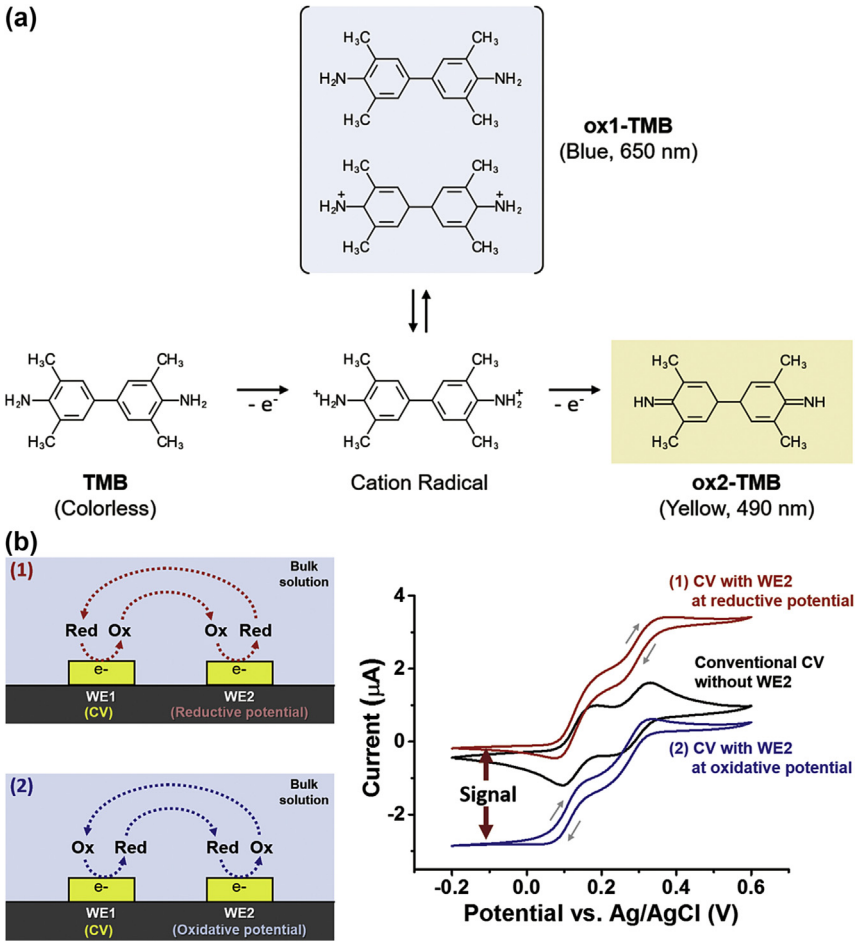

Fig. 1. Redox cycling for the quantification of oxidized-TMB. (a) Redox species of TMB. (b) Redox cycling of TMB with an IDE.