Extended Abstracts of the 2013 International Conference on Solid State Devices and Materials, Fukuoka, 2013, pp368-369

Electrochemical Impedance Spectroscopy of Aqueous Solution in Chromatography Paper and Its Application to Immunochromatography

Shohei Iwahara, Masashi Miki, and Shigeyasu Uno

Department of Electrical Systems, Graduate School of Science and Engineering, Ritsumeikan University

1-1-1 Noji-Higashi, Kusatsu, Shiga, 525-8577, Japan

Phone: +81-77-559-4278 E-mail: suno@fc.ritsumei.ac.jp

Abstract

Electrochemical detection of latex beads in chromatography paper by impedance spectroscopy is demonstrated. Existence of latex beads can be detected as absolute impedance value at 10kHz, which could be exploited for quantitative immunochromatographic assay.

1. Introduction

Immunochromatography is one of the rapid bioassay used for pregnancy and influenza test [1]. Sample solution is dropped onto chromatography paper supporting antibodies labeled by colored latex beads or metal particles (Fig. 1(a)) [2, 3], and transferred by capillary flow to reach immobilized antibodies at the detection area (Fig. 1(b)). The labeled antibodies are captured at the detection area with the help of antigens in the sample solution, or otherwise arrive at the control area. Existence of antigen is then detected by color line development at the detection area due to label agglutination (Fig. 1(c)). Although this method is well established, its qualitative nature of detection method could lead to erroneous results. In this work, we address this problem by electrochemically measuring latex beads within chromatography paper for quantitative immunoassay.

2. Experimental method

The electrochemical electrodes were formed on the layered polyvinyl chloride (PVC) plate sandwiching brass plates as metal contacts (Fig. 2). Carbon paste was used to form the working electrode (WE) and counter electrode (CE), and Ag/AgCl ink was used for the reference electrode (RE). In the first experiment, 100mM phosphate buffer solution (PBS) was dropped directly onto the electrodes. Next, chromatography paper was fixed by a PVC plate onto the electrodes, and PBS solution was dropped near the edge of the chromatography paper, being transferred up to the electrodes by capillary flow (Fig. 3). Finally, PBS with or without latex beads with 3μm diameter were prepared (Fig. 4). Those solutions were dropped onto chromatography paper, and then the paper was fixed on the electrodes by PVC plate. Electrochemical impedance measurement was performed by ALS/CH Electrochemical Analyzer Model 610DR.

3. Results and discussion

Fig. 5 shows comparison between impedance spectroscopy with and without chromatography paper on the electrodes, where the low frequency component (f < 100 Hz) represents electric double-layer capacitance and the high frequency component (f > 100 Hz) represents solution resistance due to ion conduction. As shown in the figure, chromatography paper does not affect double-layer capacitance, while it has great impact on ion conduction resistance. This is due to the fact that the cellulose matrix of the paper reduces effective cross section area for ion conduction. Fig. 6 shows comparison between results from solutions with (WLB) and without (WoLB) latex beads, where the same measurements were repeated several times. Fig. 7 shows zoom-up of the results in Fig. 6 ranging from 1kHz to 100kHz, and Fig. 8 shows magnitude of impedance taken at 10kHz, plotted for repeated measurements. Here, the data was sorted according to its impedance value. Figs. 7 and 8 clearly show that the impedance of solution with latex beads is larger than that without beads, and the median values are 1802Ω and 1600Ω, respectively (12.6% increase). This increase occurs because latex beads occupy some portion of pores in the cellulose matrix, impeding ion conduction through chromatography paper. This result indicates that the existence of latex beads in the chromatography paper can be detected by impedance measurement performed at around 10kHz, which can be used as a quantitative measure of antigen existence in the immunochromatographic assay. From another point of view, latex beads can be regarded as living cells in physiological fluids, so this method could also be used as a living cell counter.

4. Conclusion

Electrochemical detection of latex beads in chromatography paper by impedance spectroscopy has successfully been demonstrated. This could open the way to quantitative immunochromatographic assay.

References

[1] J. Sherma, B. Fried, Handbook of Thin-Layer Chromatography, CRC Press, New York, USA, (2003)

[2] Y. Hsu et. al., US Patent No. 4703017 (1987).

[3] S. Birnbaum et. al., Anal. Biochem. 206, p. 168 (1992).

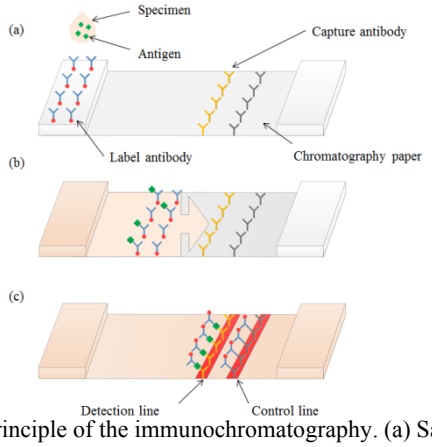

Fig.1 The principle of the immunochromatography. (a) Sample solution is dropped onto chromatography paper supporting antibodies labeled by colored latex beads or metal particles, (b) Transferred by capillary flow to reach immobilized antibodies at the detection area, (c) The labeled antibodies are captured at the detection area with the help of antigens in the sample solution.

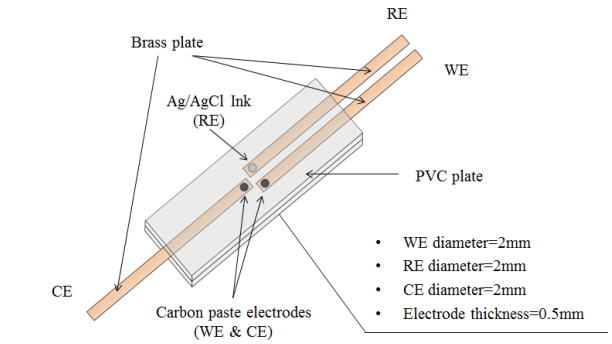

Fig.2 Schematic diagram of the electrochemical electrodes formed on the layered polyvinyl chloride (PVC) plate sandwiching brass plzates as metal contacts. WE: working electrode, CE: counter electrode, RE: reference electrode.

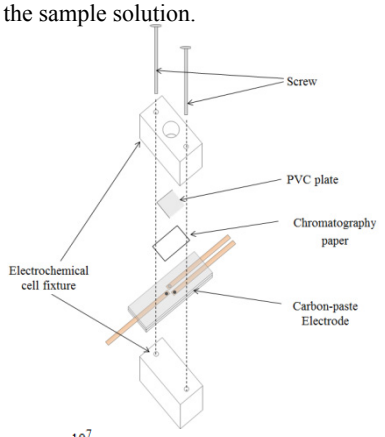

Fig.3 Schematic diagram of electrodes, chromatography paper, and electorochemical cell fixture.

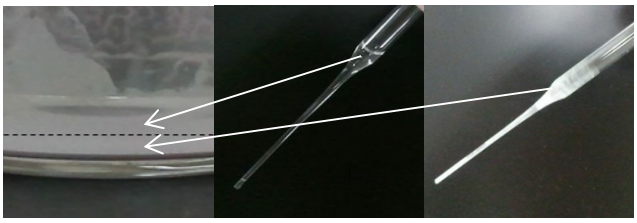

Fig.4 Photographs of phosphate buffer solution with or without latex beads with 3μm diameter.

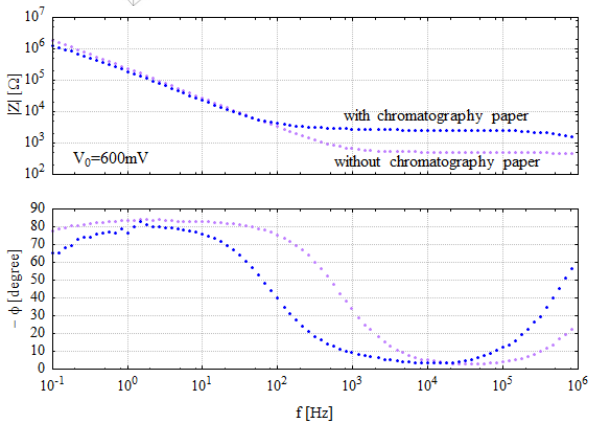

Fig.5 Comparison between impedance spectroscopy with and without chromatography paper on the electrodes.

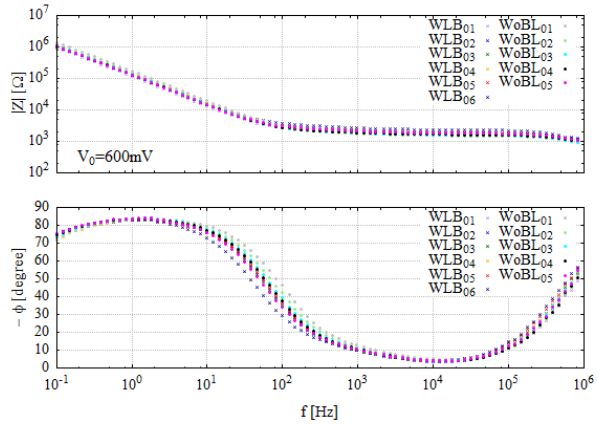

Fig.6 Comparison between results from solutions with (WLB) and without (WoLB) latex beads.

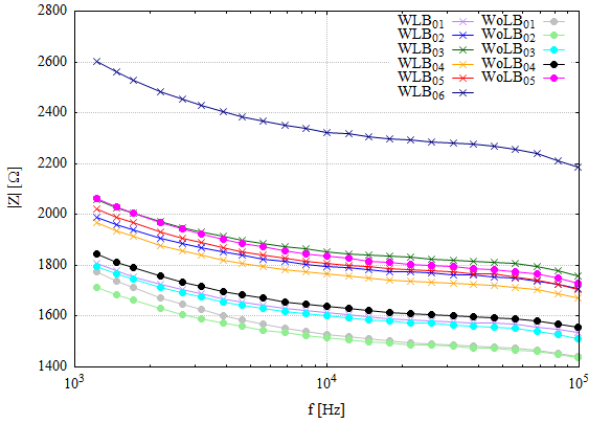

Fig.7 Zoom-up of the results in Fig. 6 ranging from 1kHz to 100kHz.

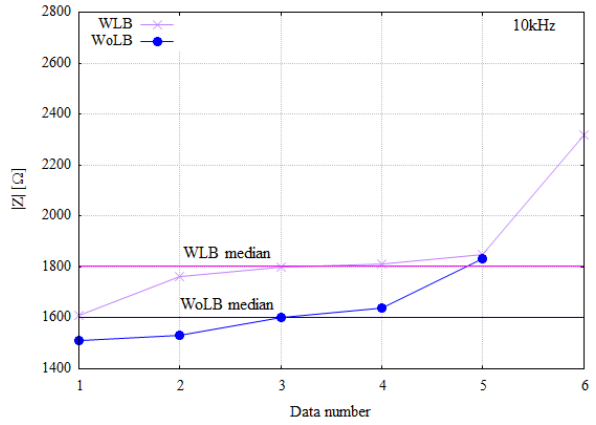

Fig.8 Magnitude of impedance taken at 10kHz, plotted for repeated measurements.