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Electrochemical Impedance Spectroscopy of Colloidal Gold Nanoparticles in Chromatography Paper for Immunochromatographic Assay

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Abstract

We propose an impedimetric detection principle applicable for the quantitative immunochromatographic assay which uses gold nanoparticles as a label. The solution resistance due to the ion transport is shown to change depending on gold nanoparticle concentrations in chromatography paper. It demonstrates the possibilities of electrochemical immunochromatography.

1. Introduction

Immunochromatographic assays are simple and rapid methods which display results by revealing color lines (Fig. 1) [1]. While such assays are qualitative, electrochemical detection can enable quantitative analyses to avoid false negative results due to low concentration of analytes [2, 3, 4]. This paper describes the electrochemical detection of gold nanoparticles (AuNPs) in chromatography paper (ChrPr) used as labels in the immunochromatography.

2. Experimental

We adopted part of experimental methods in our preveous work [5]. A 10 mL of AuNPs suspension (Cat. No. 753688, SIGMA-ALDRICH) with 0.1 mM phosphate buffer solution (PBS) is left in the refrigerator (3 ^{o}C) so that the AuNPs will settle at the bottom of the vial. The solution is separated into two parts: 1 mL of high concentration AuNPs ($40*10^9$ mL^{-1}), and 9 mL without AuNPs. These two solutions are further used to prepare AuNPs suspensions with a concentration of 2.0 and $8.0*10^9$ mL^{-1}. Electrode chip is fabricated in our lab and is based on the print circuit board (PCB) covered by polyvinyl chloride plate (PVC). Working electrode (WE) and counter electrode (CE) are made of carbon paste (Cat. No. 001010, BAS), and reference electrode (RE) is made of silver/silver chloride ink (Cat. No. 011464, BAS) as shown in Fig. 2. During experiments, a sample of AuNPs solution (23 μL) is dropped onto ChrPr (Cat. No. 3001-861, Whatman) fixed on the electrode chip as shown in Fig. 3. Impedance measurements are then performed with ALS/CH Instruments Electrochemical Analyzer Model 610DR (BAS). After each measurement, the electrode chip and the fixture are washed twice with PBS (0.1 mM, pH 7.0). The temperature of AuNPs solutions is kept around 3 ^{o}C during the experiments.

3. Results and discussion

The measured impedance and phase dependence on frequency in Fig. 4 exhibits parasitic capacitance (C_p, 100 kHz – 10 kHz) due to the wiring, solution resistance (R_{sol}, 10 kHz – 100 Hz), and electric double layer capacitance (C_{dl}, 100 Hz – 1 Hz). We focus on the difference of R_{sol}, since C_{dl} dependence on AuNPs concentration is not straightforward. The use of R_{sol} is appropriate in this case because AuNPs spread uniformly in a solution. The R_{sol} dependence on frequency shown in Fig. 5 exhibits the increase due to that of the AuNPs concentration. A plausible explanation for this is that the ion transport is obstructed because AuNPs occupy some portion of the pores in the cellulose matrix of ChrPr. As shown in Fig. 6, normal probability plot of R_{sol} at 1221 Hz, where the phase angle is close to 0°, indicates that the data follow a normal distribution. Our method shows distinct change in R_{sol} for different AuNP concentration which brings colorimetric change of ChrPr shown in Fig. 7. In future, our method can be potentially used for immunochromatography with better sensivity and accuracy as illustrated in Fig. 8.

4. Conclusions

The detection of AuNPs in ChrPr by electrochemical impedance spectroscopy in terms of R_{sol} at 1221 Hz is successfully demonstrated regardless of the results of coloring ChrPr. This study is a first step toward quantitative immunochromatographic assay.

References

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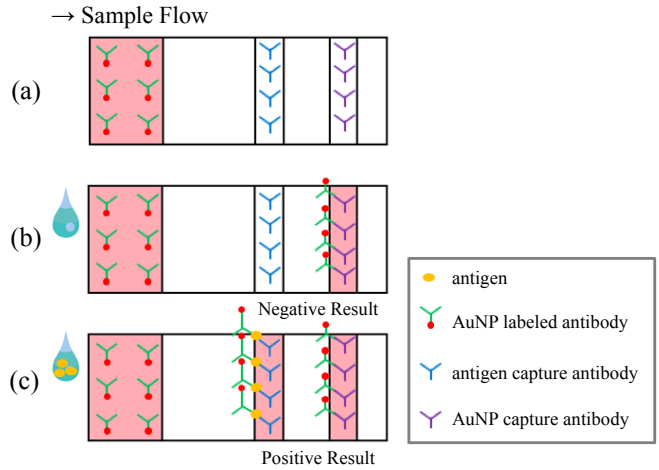
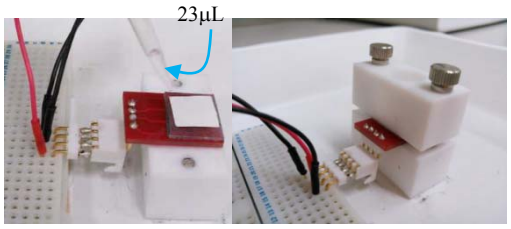


Fig. 1 Schematic description of the immunoassay principle: (a) before dropping a sample, (b) dropping a sample without antigen (negative), (c) dropping a sample with antigen (positive).



(a) (b)

Fig. 3 Photograph of the measurement setup. (a) sample dropping onto the chromatography paper, (b) electrode held by a fixture.

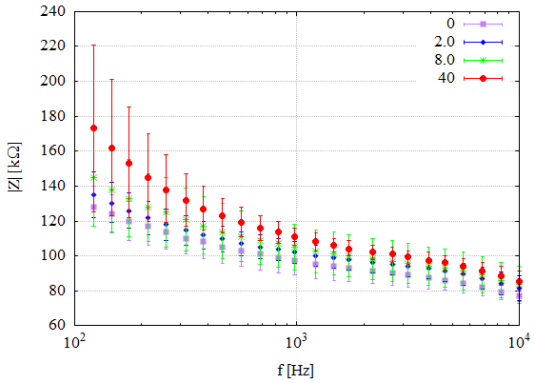


Fig. 5 Average and standard deviation values of R_{sol} for various AuNPs concentrations calculated at each frequency point ranging from 100 Hz to 10 kHz shown in Fig. 4.

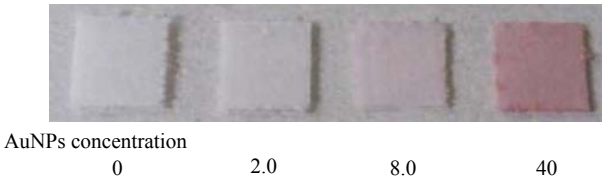


Fig. 7 Photograph of chromatography paper after solutions with various AuNPs concentrations are dropped.

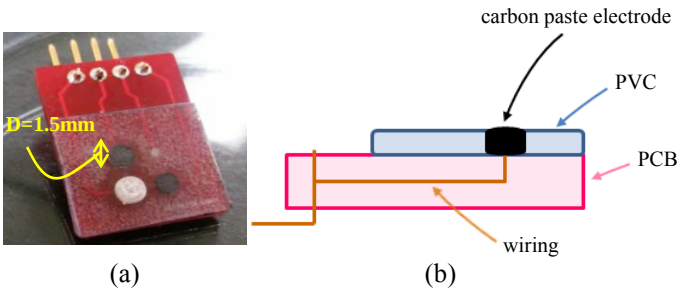


Fig. 2 Electrode chip used in experiments: (a) photograph of the chip based on PCB, (b) cross sectional view of the chip showing the electrical connection.

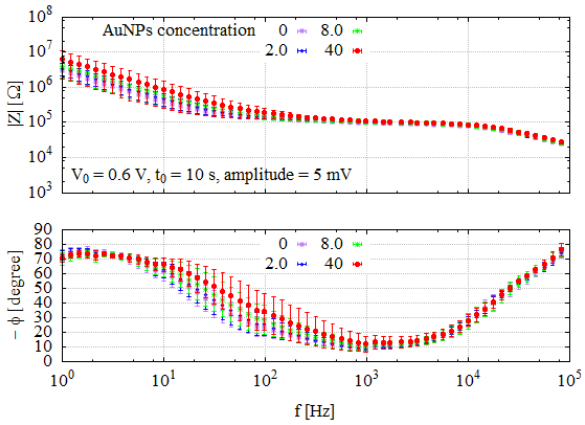


Fig. 4 Bode diagram showing the average and standard deviation values of impedance for various AuNPs concentrations [*10^9 mL^{-1}]: 0, 2.0, 8.0, and 40 (t_0 means waiting time and V_0 means DC, and amplitude means AC).

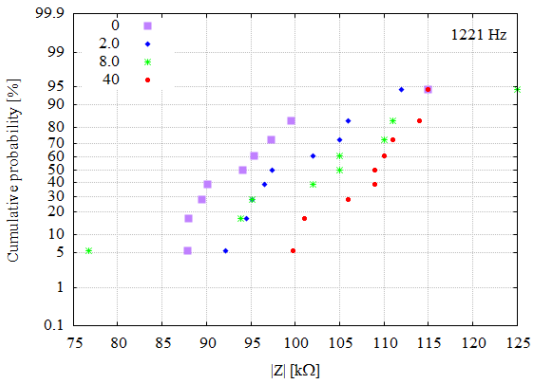


Fig. 6 Normal probability plot of impedance regarding R_{sol} at a representative frequency of 1221 Hz for various AuNPs concentrations shown in Fig. 5.

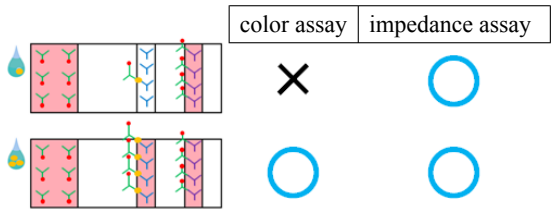


Fig. 8 Schematic diagram illustrating the possibilities of electrochemical immunoassay based on our preliminary results.