Electronic supplement Information (ESI)

Cost-effective paper-based electrochemical immunosensor using a label-free assay for sensitive detection of ferritin

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1.1 Electrochemical condition of DPV

To achieve the optimal condition for ferritin detection, various parameter of DPV condition include modulation amplitude, modulation time, step potential, and standby potential were optimized. The discussion of the optimal conditions are as follows:

The effect of modulation amplitude was varied in the range from 10 to 250 mV and keep other parameters constant (Fig. S1a). It was observed that the current response gradually increases with increasing amplitude until 250 mV. However, the resolution of peak shape decreased consequently. Therefore, a 150 mV of modulation amplitude was chosen. Afterwards, the effect of step and standby potential were studied. The step potential was evaluated from 10 to 100 mV (Fig. S1b). For the standby potential was studied in the range from 0 to 7 V as displayed in Fig. S1c. In all cases, the current response decreases with increasing step and standby potential. In order to obtain a high sensitivity and resolution of peak shape, a 10 mV of step potential and 0 V of standby potential was selected. Additionally, the modulation time was studied in the range from 10 to 100 ms as can be seen in Fig. S1d. The similar trend was achieved from modulation time. The current response increase with increasing the modulation time until 100 ms. The results showed that the modulation time at 75 ms was the best condition in term of current response and resolution of peak. Hence, a 75 ms of modulation time were chosen for further studied.

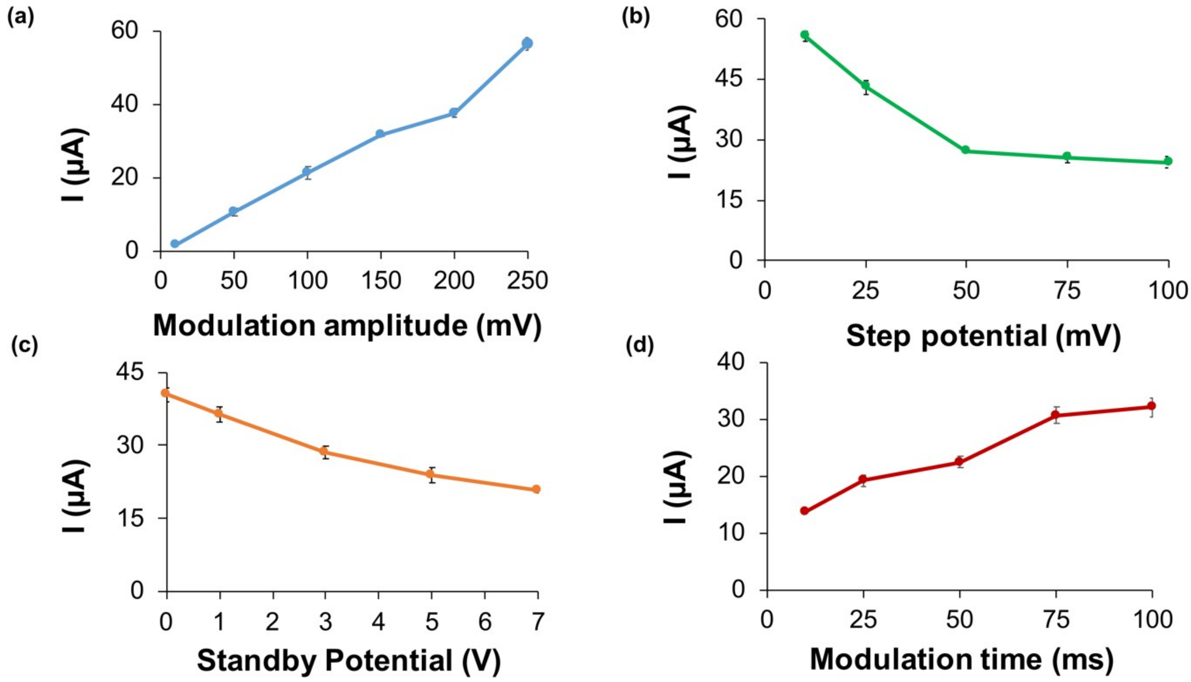


Fig. S1. Each electrochemical condition of DPV technique including; (a) the modulation amplitude, (b) step potential, (c) standby potential, and (d) modulation time. The optimal conditions were as follow: a modulation amplitude, step potential, standby potential, and modulation time were 150 mV, 10 mV, 0 V, and 75 ms.

1.2 Inkjet printing parameters

1.2.1 The effect of GO:NMP ratio and number of GO layer

The effect of GO:NMP ratio on the electrochemical response of the modified electrode was investigated [1]. Briefly, GO was prepared by sonicating the mixture of GO and NMP at different ratio. A 5 mM $[Fe(CN)_6]^{3-/4-}$ in 0.1 M KNO_3 was used as a redox solution. The current response of redox solution was obtained as shown in Fig. S2a. It can be seen that the current responses increased with increasing of GO:NMP ratio from 5:5 to 7:3 %v/v and decreased when the ratio of GO: NMP was over 7:3 %v/v. Above 7:3 %v/v of GO: NMP ratio, the current response exhibited poor anodic, cathodic peak currents and also provided high capacitive current. It is due to the agglomeration of graphene within GO solution and a thicker GO films decelerated electron transfer at the electrode surface [2, 3]. Therefore, an optimal ratio of GO: NMP was found to be 7:3 %v/v.

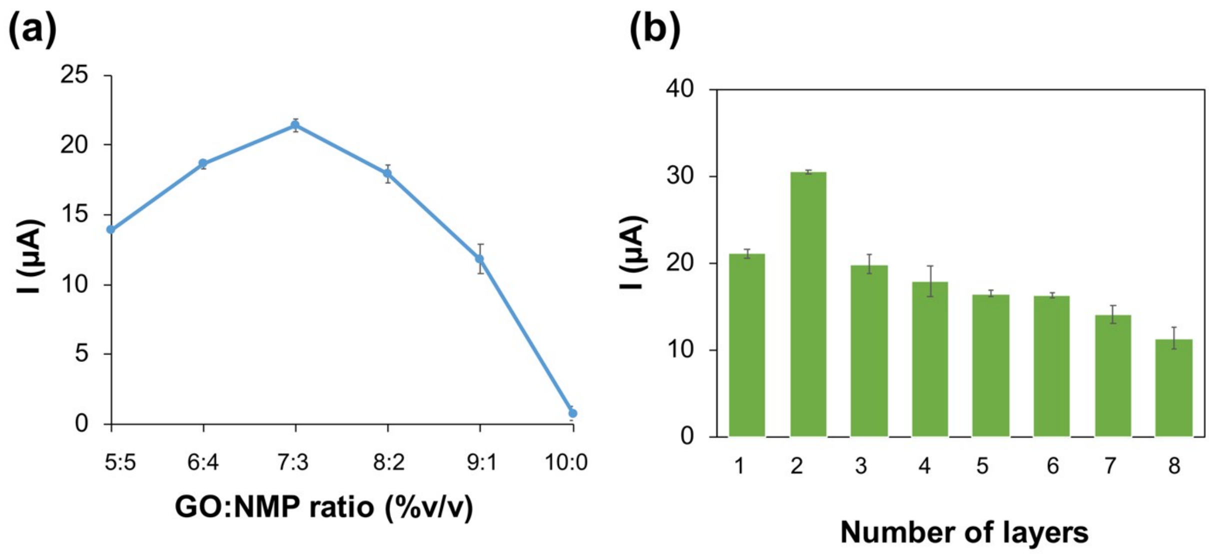
The number of GO printing layer is another key parameter which affects a loading amount of antibody and an electrochemical behavior of the immunosensor. As illustrated in Fig. S2b, a similar trend can be clearly observed, as in case of GO:NMP ratio. The current response of redox solution tends to increase with an increase of GO printing layers. In contrast, the response slightly reduced over 2 printing layers. It is obvious that the aggregation of excess graphene stack on the modified electrode which can cause a decrease in current response [2].

1.2.2 The effect of drop spacing

Drop spacing was evaluated to provide the homogenous pattern of GO on the electrode surface. Drop spacing is spot size of solution or fluid on the substrate. The range of drop spacing was examined from 10 to 100 µm (Fig. S2c). If the drop spacing is too low, the GO ink droplets are connected with each other and affect to a spread in a large amount of GO on the electrode surface. On the other hand, if the drop spacing is too large, the dot of GO ink was printed on the electrode surface, leading to a low amount of GO and provide a low current response. Therefore, the 25 µm of drop spacing was used to print the GO from the cartridge.

1.2.3 Apply voltage of inkjet printing

The voltage of piezoelectric inkjet was further studied. The voltage was applied in the range from 20 to 40 V (Fig. S2d). If the voltage is too low, the drop of GO was applied at a low amount on the substrate surface. However, if the voltage is high, a splashing of ink will occur when the drops print on the electrode surface. Therefore, the suitable voltage is 40 V, cause them provides high sensitivity.



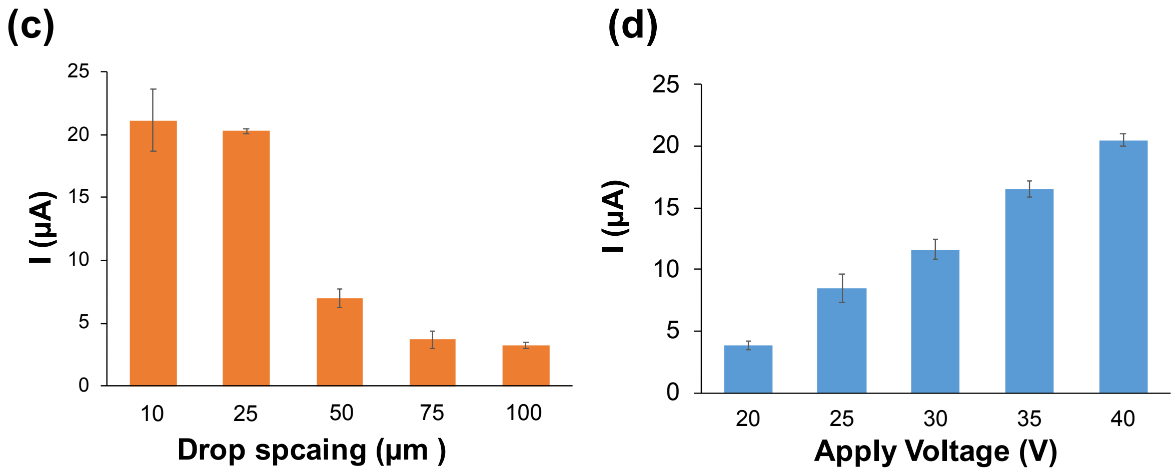


Fig. S2. Optimization of the variable parameters: (a) The effect of GO:NMP ratio, (b) number of GO layer modified on the SPGE, (C) the effect of drop spacing, (d) and applied voltage of inkjet printing, using DPV technique in 5.0 mM $[Fe(CN)_6]^{3-/4-}$ as a redox probe.

1.3 Electrochemical characterization

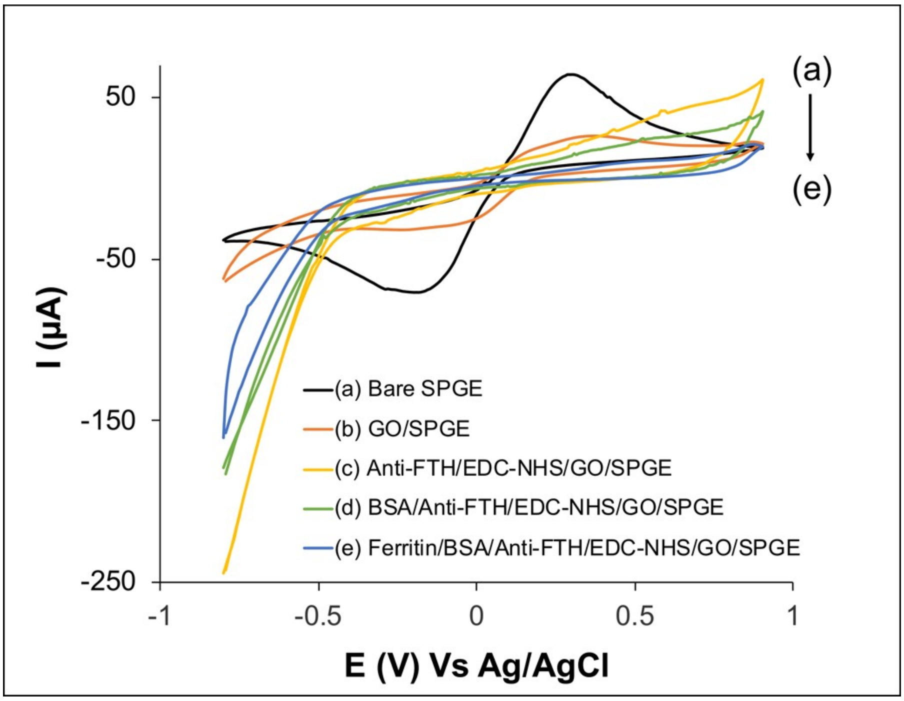


Fig. S3. The cyclic voltammogram of stepwise modification on the modified GO/SPGE electrode was performed in the potential between −0.8 and 0.9 V vs Ag/AgCl at a scan rate of 50 mV s^{-1}.

1.4 Calculation of heterogeneous electron-transfer constant

Additionally, to confirm the improvement on the R_{ct} value from semiconductor properties of GO and the shielding effect of immunocomplexes on the electrode surface. The relationship between the R_{ct} and the heterogeneous electron-transfer constant (K_{et}) according to the equation (1) was employed to calculate the K_{et} value [4, 5].

$$K_{et} = \frac{RT}{n^2 F^2 R_{et} A C_{redox}} \qquad (1)$$

Where R is ideal gas constant (J K^{-1} mol^{-1}), T is temperature (K), n is a number of electron transfer, F is the faraday constant, R_{ct} value is obtained from the Nyquist plot, A is the geometrical area of the electrode surface (cm^2), and C_{redox} is the concentration of $[Fe(CN)_6]^{3-/4-}$ redox solution (5×10^{-3} M). From the calculation, the K_{et} values of 2.80×10^{-4} cm s^{-1}, 3.26×10^{-5} cm s^{-1}, 2.62×10^{-5} cm s^{-1}, 2.42×10^{-5} cm s^{-1}, and 1.79×10^{-5} cm s^{-1} were calculated for the bare SPGE, GO/SPGE, Anti-FTH/EDC-NHS/GO/SPGE, BSA/Anti-FTH/EDC-NHS/GO/SPGE, and ferritin/BSA/Anti-FTH/EDC-NHS/GO/SPGE, respectively. The decrease in K_{et} value was caused by the shielding effect of immunocomplex on the electrode surface. The evidence here indicates that the successful modification of antibody and antigen complex on the electrode surface.

1.5 Storage stability of the developed immunosensor

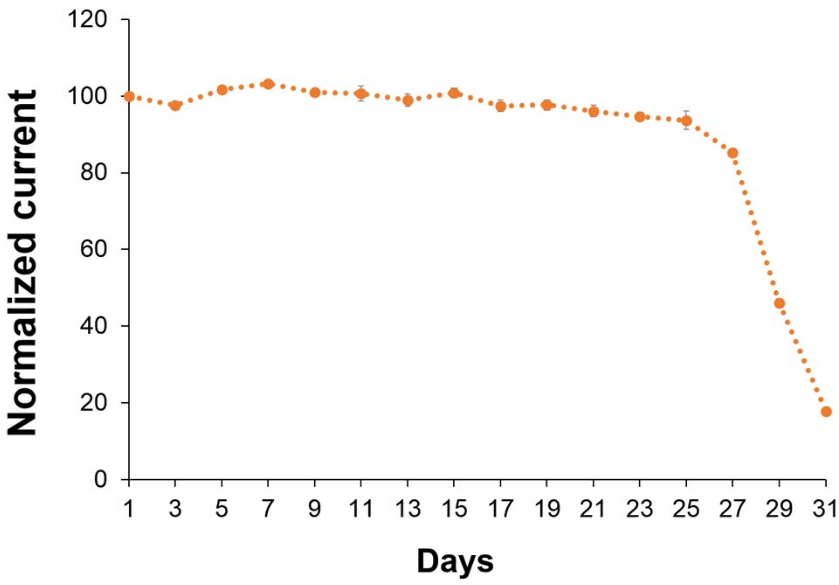


Fig. S4. The storage stability of the electrochemical paper-based immunosensor.

References

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