Thermodynamic and kinetic analysis of the interaction between hepatitis B surface antibody and antigen on a gold electrode modified with cysteamine and colloidal gold via electrochemistry

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Abstract

Hepatitis B surface antibody (HBsAb) was immobilized to the surface of a gold electrode modified with cysteamine and colloidal gold as matrices to detect hepatitis B surface antigen (HBsAg). Differential pulse voltammetry (DPV) method was used for the investigation of the specific interaction between the immobilized HBsAb and HBsAg in solution, which was followed as a change of peak current in DPV with time. With the modified gold electrode, the differences in affinity of HBsAb with HBsAg at the temperatures of 37 and 40 °C were easily distinguished and the kinetic rate constants ($k_{ass}$ and $k_{diss}$) and kinetic affinity constant $K$ were determined from the curves of current versus time. In addition, the thermodynamic constants, $\Delta G$, $\Delta H$ and $\Delta S$, of the interaction at 37 °C were calculated, which were $-56.65$, $-64.54$ and $-25.45$ kJ mol$^{-1}$, respectively.

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1. Introduction

The extremely high selectivity and affinity of hepatitis B surface antibody (HBsAb) molecules to hepatitis B surface antigen (HBsAg) have been widely exploited for analytical purpose mainly as enzyme-linked immunosorbent assays (ELISAs) in the clinical diagnostics. Recently the immunochemical interactions may be directly detected by a change in potentiometer difference (Wang et al., 1998), current (Darain et al., 2003), resistance (Pei et al., 2001), heat (Miura et al., 2003), and frequency (Tang et al., 2004). But all the methods studied the specific interaction between HBsAb and HBsAg for analytical purposes, the thermodynamic and kinetic analysis of the interaction has never been reported. With the development of immunoassay, the kinetics of most molecular interactions is now routinely accessible. It is well known that an antibody of high affinity is more important than that of low affinity in immune pathology. Therefore, the characterization of the kinetics and thermodynamics of biospecific interactions is increasingly important for developing an understanding of the molecular basis of such events as adhesion and viral infection and may ultimately aid in the rational design of antagonists such interactions.

The self-assembled monolayer (SAM) film, which has made great progress in fabrication and characterization, was a new means for controlling the chemical nature of the electrode–solution interface in the past 20 years. The SAM film of organosulphur compounds on gold surfaces comprised a wide field of potential applications due to their versatility in modifying surfaces in a controllable manner. The ability to control surface properties at the molecular level made the SAMs excellent platform for the study of the interactions between manmade surface and biological systems. The use of colloidal particles as versatile and efficient templates for the immobilization of biomolecules has been recognized since the early 1980s (Rembaum and Dreyer, 1980). Colloidal gold has a very large surface area and good bio-compatibility. Biosensors based on enzyme-covered nano-gold have been reported (Cai et al., 2001; Xiao et al., 1999; Zhao et al., 1992). In this experiment, a novel route to immobilize antibody onto an electrode surface with cysteamine (Doron et al., 1995) and Au colloid of 14 nm was
provided. Self-assembly of colloidal Au onto the SAM film of cysteamine modified gold electrode could result in larger electrode surface area and easier attachment of antibody. And the recognition kinetics of an immobilized monolayer of antibody on Au colloid by an antigen was monitored by the differential pulse voltammetry (DPV) method.

The DPV method is an effective and simple electrochemical method to probe the interfacial properties of modified electrode. The adsorption and dynamics of charge transfer at the modified electrode interface can be obviously probed by the change of the current of DPV. In this paper, DPV was used to investigate the interaction between HBsAb and HBsAg. The binding of HBsAg in solution could be monitored on-line based on the decrease of the current of DPV. The current–time (i–t) curves were used to calculate the kinetic affinity constant for the interaction between the analyte and the ligand. The obtained quantitative information on the kinetics of binding is vital to understand the immune pathology of hepatitis B on a molecular level.

2. Experimental

2.1. Materials

All chemicals and solvents were commercially available and were used as received. HBsAb and HBsAg (E.C. 1.1.3.4, 1.28 μg ml⁻¹) were purchased from Kehua Bioengineering Company (Shanghai, China). Cysteamine (Cys) and bovine serum albumin (BSA, 96–99%) were obtained from Sigma Company (Shanghai, China). Glutaraldehyde (Glu, 50%) was obtained from Kelong Chemical Engineering Company (Shanghai, China). Glutaraldehyde (Glu, 50%) was obtained from Kelong Chemical Engineering Company (St. Louis, MO, USA). Glutaraldehyde (Glu, 50%) was obtained from Kelong Chemical Engineering Company (Chengdu, China). Trisodium citrate, HAuCl₄, K₃Fe(CN)₆, K₄Fe(CN)₆ and other chemicals were of analytical-reagent grade and were used without further purification. Doubly distilled water was used throughout. The standard HBsAb solution was prepared daily with 0.1 mol l⁻¹ phosphate buffer solution (PBS, pH 7.4) and stored at 4 °C.

2.2. Apparatus

All electrochemical measurements were carried out with a CHI 660B electrochemistry work station (Shanghai CH Instruments, China). The determination of gold nanoparticle size was carried out on a N5 submicron particle size analyzer (Beckman Coulter) and a transmission electron microscopy (TEM) (H600, Hitachi Instrument, Japan). The electrochemical cell consisted of three electrodes where the HBsAb modified gold electrode (d = 2 mm) acted as the working electrode, Pt as the counter electrode, and an Ag/AgCl electrode as the reference electrode. A model 501A thermostat (Shanghai, China) was used to control the incubating temperature.

2.3. Preparation and characterization of colloidal gold

All glassware used in these preparations were thoroughly cleaned in aqua regia (3 parts HCl + 1 part HNO₃), and dried prior to use in air. Au colloids were prepared according to Frens (1973) with slight modification. First, 1.0 ml of 0.01% HAuCl₄ was brought to a boil of doubly distilled water with vigorous stirring in a 100 ml round-bottom flask. Then, 2.5 ml of 1% sodium citrate was added rapidly to the vortex of the solution, which resulted in a color change from pale yellow to burgundy. Boiling was continued for 10 min, the heating mantle was then removed, and stirring was continued until the solution reached room temperature. The particle size was 14 nm, which was determined with a N5 submicron particle size analyzer and a transmission electron microscopy.

2.4. Antibody immobilization

A gold electrode was polished to a mirror-like surface with 0.3 and 0.05 μm Al₂O₃ powder on micro cloth pads, and then rinsed with water. Subsequently, it was immersed in a Piranha solution (a mixed solution of 30% H₂O₂ and concentrated H₂SO₄ with a volume ratio of 1:3) for 30 min, and then rinsed ultrasonically with water and absolute ethanol for 3 min, respectively. The electrode was then cycled between 0 and +1.6 V at 100 mV s⁻¹ in 0.1 mol l⁻¹ H₂SO₄ solution until a stable cyclic voltammogram was obtained. The cleaned electrode was modified with a cysteamine aqueous solution (0.02 mol l⁻¹) for about 12 h. After the electrode was thoroughly rinsed with water to remove adsorbed cysteamine, it was soaked in a glutaraldehyde solution (12%, v/v) for about 1 h and rinsed with water. Then the electrode was placed into 0.02 mol l⁻¹ cysteamine solution for approximately 12 h and rinsed with water again. Following that, it was immersed in 1.0 ml of prepared gold colloids for 10 h to form a nano-Au monolayer. The resulting electrode was immersed in 1.0 ml of HBsAb solution diluted by PBS (pH 7.4) (1:4, v/v) for more than 12 h and then thoroughly rinsed with PBS (pH 7.4) to remove the weakly absorbed antibodies subsequently. Eventually a 10 mg ml⁻¹ BSA solution was used to block non-specific sites on the electrode surface. Finally, the electrode was washed again with PBS (pH 7.4) and stored at 4 °C when not in use. The schematic illustration of the stepwise self-assembly procedure is shown in Scheme 1.

2.5. Electrochemical measurement

The electrochemical characteristics of the modified electrode were characterized by using CV during the self-assembled process. Electrochemical experiments were performed in a
conventional electrochemical cell containing a three-electrode system by sweeping the potential between −0.2 and +0.6 V with a sweeping rate of 0.1 V s\(^{-1}\). Impedance measurements and DPV measurements were performed in the presence of a 5.0 mmol l\(^{-1}\) Fe(CN)\(_6^{4-}/3-\) solution as a redox probe in PBS (containing 0.1 mol l\(^{-1}\) KCl, pH 7.4). Impedance measurements were performed at the frequency range from 0.05 to 10\(^5\) Hz at the formal potential of 220 mV. DPV measurements were performed by sweeping the potential between −0.2 and +0.5 V with an amplitude of 0.05 V, and all evaluations were based on the peak current of DPV.

3. Results and discussion

3.1. Cyclic voltammetry characteristics on the modified electrode surface

CV is an effective method for probing the feature of surface-modified electrode and testing the kinetic barrier of the interface because the electron transfer between solution species and the electrode must occur by tunneling either through the barrier or through the defects in the barrier. Therefore, CV was chosen as a marker to investigate the changes of electrode behavior after each assembly step. Fig. 1 shows the results of the CVs of the bare gold electrode and different modified electrodes during the self-assembled process in the presence of redox probe, Fe(CN)\(_6^{4-}/3-\), measured at the formal potential. As expected, ferricyanide exhibited reversible behavior on the bare gold electrode (Fig. 1a) with a peak-to-peak separation (\(\Delta E_p\)) of 75 mV at a scan rate of 0.1 V s\(^{-1}\). After the electrode was immersed stepwisely in cysteamine, glutaraldehyde and cysteamine solutions, the voltammetric response of the ferricyanide redox couple at each modified electrode decreased (Fig. 1b–d). The reason why the peak current gradually decreased from a to d was that with the gradual increase of packing density on the electrode, the barrier increased for the electron transfer between the electrode surface and the electroactive species in solution. Comparison of Fig. 1d and e showed that the peak currents increased after nano-Au modification. The reason is that nanometer-sized gold colloids play an important role similar to a conducting wire or electron-conducting tunnel, which makes it easier for the electrons transfer to take place. So we can know that the nano-Au was modified on the gold electrode. However, the peak current decreased (Fig. 1f) after the electrode was immersed in HBsAb solution, which indicated that HBsAb had been immobilized on the electrode surface. Subsequently, the modified immunosensor was blocked with a protein, BSA, and a further decrease of the peak current was observed, which might be contributed to the adsorption of BSA (Fig. 1g). In the end, after the HBsAb modified electrode was immersed in a 12.8 ng ml\(^{-1}\) HBsAg solution (pH 7.4) for 30 min, the peak current almost disappeared, illustrating that there was a reaction of the specific binding of hepatitis B surface antibody–antigen on the modified electrode (Fig. 1h).

3.2. Impedance characteristics of antibody–antigen binding

Electrochemical impedance spectroscopy (EIS) is an effective method to probe the interfacial properties of modified electrode, understand chemical transformations (Brillas et al., 1997; Janek et al., 1998) and characterize immuno-complexes (Kharitonov et al., 2001; Alfonta et al., 2001). In EIS, the semicircle diameter equals the electron-transfer resistance (\(R_{et}\)). Fig. 2 illustrates the results of impedance spectroscopy on different electrodes in the presence of a 5.0 mmol l\(^{-1}\) Fe(CN)\(_6^{4-}/3-\) solution as a redox probe in PBS (containing 0.1 mol l\(^{-1}\) KCl, pH 7.4) with a scan rate of 0.1 V s\(^{-1}\). The inset shows the equivalent circuit used to model impedance data.
immobilization of HBsAg, pH 7.4). As shown in Fig. 2, an obvious semicircle was observed at the HBsAb modified electrode (Fig. 2b) in contrast to that at the bare gold electrode (Fig. 2a). The reason is that there is a stepwise self-assembly of cysteamine, gold colloids, HBsAb and BSA on the gold electrode. A further increase of the semicircle can be found after the specific binding of HBsAb–HBsAg on the modified gold electrode (Fig. 2c). That is because a lot of immuno-complexes were associated on the modified electrode, the modified gold electrode (Fig. 2c), suggesting that HBsAg was adsorbed on the surface of the HBsAb modified electrode successfully.

The equivalent circuit for an electrode undergoing heterogeneous electron transfer is usually described on the basis of the model by Randles as shown in the inset of Fig. 2. The impedance data were fitted by the EvoCRT software. The circuit includes: (1) the electrolyte resistance, $R_s$; (2) double-layer capacitance, $C_{dl}$; (3) electron transfer resistance, $R_{et}$; (4) Warburg element, $Z_w$. We used this equivalent circuit to fit the impedance spectroscopy and determined $C_{dl}$ and $R_{et}$. The calculation results showed that $R_{et}$ of the bare gold electrode and the HBsAb modified electrode was 148.5 and 731.4 kΩ, respectively; after immobilization of HBsAg, $R_{et}$ increased to 5158.0 kΩ. Compared with $R_{et}$ of the bare gold electrode, $R_{et}$ of the HBsAb modified electrode clearly increased due to step-wise assembly on the electrode surface, generating a tightly packed film and introducing a barrier to the interfacial electron transfer. The $R_{et}$ value in the last process was very large compared with earlier steps, which meant that Faradaic impedance spectroscopy could be applied for biorecognition (Mandal et al., 2003) and the immobilization of HBsAg was very obvious.

### 3.3. Effect of the incubation time of HBsAb modified electrode on DPV response of the HBsAg solution

DPV using the conventional three-electrode system was employed to detect HBsAg in samples. In the incubation solution, when an analyte antigen reaches an antibody at an electrode surface, it will take some time for the contacting species to form immuno-complexes. Fig. 3 shows the relationship between incubation time and amperometric response of the HBsAb modified electrode at 37°C. The immunoreaction on the electrode surface reached equilibrium after approximately 25–35 min. And the experimental results showed that the immunoreactions could also reach equilibrium at about 30 min at other temperatures. Therefore, 30 min was chosen as the optimum incubation time in the following experiment.

### 3.4. Effect of the incubation temperature of HBsAb modified electrode on DPV response of the HBsAg solution

Fig. 4 shows the effect of incubation temperature on DPV response for 12.8 ng ml$^{-1}$ HBsAg at the temperature range from 23 to 40°C in a 5.0 mmol l$^{-1}$ Fe(CN)$_6^{3−/4−}$ solution (0.1 mol l$^{-1}$ KCl + pH 7.4 PBS). As shown in Fig. 4, the current increased with the increasing temperature up to 37°C, and then it decreased at 40°C. It is well known that the optimal temperature of immunoreaction is 37°C because most immunoreactions exhibit optimal binding at this temperature. This immunoreaction also supports this phenomenon. And the temperature is much closer to the physical condition. Therefore, the temperature of 37°C was selected in the following experiment.

### 3.5. Kinetic analysis for the affinity binding of HBsAg

#### 3.5.1. Basic principle of kinetic analysis

The reaction between the immobilized compound (B) and molecule in solution (A) was often assumed to follow pseudofirst order kinetics (Guilbault and Hock, 1995). For the reversible interaction, $A + B ⇔ AB$, the formation rate of the product, $AB$, 

![Fig. 3. DPV curves of HBsAb/nano-Au/Cys/Glu/Cys-modified gold electrode blocked with BSA immersed in 5.12 × 10$^{-10}$ mol l$^{-1}$ HBsAg solution every 5 min from 0 to 60 min (1–13) in 5.0 mmol l$^{-1}$ Fe(CN)$_6^{3−/4−}$ solution (0.1 mol l$^{-1}$ KCl + pH 7.4 PBS). The inset of the top left corner shows the effect of the incubation time on DPV response of the HBsAg solution. The inset of the top right corner shows 3D DPVs of different times of the modified electrode in 12.8 ng ml$^{-1}$ HBsAg solution.](image)

![Fig. 4. Influence of temperature on the DPV response of the HBsAb modified electrode in a 5.0 mmol l$^{-1}$ Fe(CN)$_6^{3−/4−}$ solution (0.1 mol l$^{-1}$ KCl + pH 7.4 PBS). Scan rate, 100 mV s$^{-1}$.](image)
at time $t$ may be written as:

$$\frac{d[AB]}{dt} = k_{ass} \times [A] \times [B] - k_{diss} \times [AB]$$

(1)

where $k_{ass}$ is the association rate constant and $k_{diss}$ is the dissociation rate constant. After some reaction time, $t$, $[B] = [B]_0 - [AB]$, and substituting it into Eq. (1) gives:

$$\frac{d[AB]}{dt} = k_{ass} \times [A] \times ([B]_0 - [AB]) - k_{diss} \times [AB]$$

(2)

where $B_0$ is the concentration of B at $t = 0$.

Considering the decrease of current $\Delta i$ is directly proportional to the attached mass and using $\Delta i_{fm}$ as the current change after a complete saturation of the surface of the modified electrode with A, the concentration of free B is directly proportional to ($\Delta i_{fm} \sim \Delta i$), and the concentration of the complex AB to $\Delta i$. Eq. (2) can thus be expressed as:

$$\frac{d[\Delta i]}{dt} = k_{ass} \times (\Delta i_{fm} - \Delta i) \times C - k_{diss} \times \Delta i$$

(3)

thus transformed as:

$$\frac{d[\Delta i]}{dt} = -(k_{ass} \times C + k_{diss}) \times \Delta i + k_{ass} \times \Delta i_{fm} \times C$$

(4)

where $C$ is the concentration of free A, hold constant in a mass of solution.

For the determination of the constants, $k_{ass}$ and $k_{diss}$, from the experimental $i$ versus $t$ curves, the derivatives $d(\Delta i)/dt$ must be plotted versus the corresponding current change $\Delta i$. A straight line characterized by a slope SL and intercept INT should be obtained according to Eq. (4). The parameter SL is related to the kinetic constants:

$$SL = -(k_{ass} \times C + k_{diss})$$

(5)

By measuring the binding curves ($i$–$t$) determined for several concentrations $C$, the parameters $k_{ass}$ and $k_{diss}$ could be obtained. Kinetic affinity constant $K$ for the AB complex can be obtained as a ratio:

$$K = \frac{k_{ass}}{k_{diss}}$$

(6)

3.5.2. Calculation of kinetic affinity constant

According to the basic principle of kinetic analysis, the individual binding curves for several concentrations of HBsAg with immobilized HBsAb were obtained by the DPV method. The current of the HBsAb modified electrode was determined for five times parallel experimentation. The relative standard deviation (R.S.D.) was 5.8%. So it is assumed that only one binding site of HBsAg interacts with the immobilized HBsAb for the stability. The $M_w = 25000 \text{ g mol}^{-1}$ for HBsAg was used (Zhou et al., 1990).

In our experiment, the immunoreaction reached equilibrium after about 30 min at 37°C. Before the immunoreaction on the electrode surface reached the equilibrium, $i$–$t$ curves were recorded at different concentrations of HBsAg and were transformed to the dependencies of $d(\Delta i)/dt$ on $\Delta i$ at 37°C. As shown in Table 1, derivatives $d(\Delta i)/dt$ were calculated using the linear regression, and the correlation coefficient ($r$) and the parameter SL were also obtained. A similar data handling method has been reported (Liu et al., 2003). Based on Eq. (5) described above, the SL value was plotted against the concentration of HBsAg in Fig. 5. The linear regression equation for the DPV method is $-SL = 0.01127 + 3.9446 \times 10^7 C$ (SL: min$^{-1}$, C: mol l$^{-1}$) with a correlation coefficient of 0.9962 at 37°C. Therefore, $k_{ass}$ and $k_{diss}$ could be calculated from the slope and intercept of the linear fit, and obtained as:

$$k_{ass} = 3.9446 \times 10^7 \text{ mol}^{-1} \text{l} \text{min}^{-1}$$

$$k_{diss} = 0.01127 \text{ min}^{-1}$$

Simultaneously, kinetic affinity constant can be calculated:

$$K = \frac{k_{ass}}{k_{diss}} = 3.50 \times 10^9 \text{ mol}^{-1}$$

The $K$ value is logical for an antibody–antigen reaction (typically in the range of $10^8$ to $10^{12}$ mol$^{-1}$ for antibodies), and it implies that the HBsAb–HBsAg binding reaction is very easy at 37°C which is closer to the physical condition. It is important

### Table 1

<table>
<thead>
<tr>
<th>Concentration (10$^{-10}$ mol l$^{-1}$)</th>
<th>Linear regression equation</th>
<th>$r$</th>
<th>SL (min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.024</td>
<td>$-d(\Delta i)/dt = -0.3714 + 0.01388 \times \Delta i$</td>
<td>0.9872</td>
<td>0.01388</td>
</tr>
<tr>
<td>3.072</td>
<td>$-d(\Delta i)/dt = -0.5875 + 0.02490 \times \Delta i$</td>
<td>0.9959</td>
<td>0.02490</td>
</tr>
<tr>
<td>5.120</td>
<td>$-d(\Delta i)/dt = -0.8461 + 0.03136 \times \Delta i$</td>
<td>0.9828</td>
<td>0.03136</td>
</tr>
<tr>
<td>7.168</td>
<td>$-d(\Delta i)/dt = -1.0740 + 0.04048 \times \Delta i$</td>
<td>0.9757</td>
<td>0.04048</td>
</tr>
<tr>
<td>10.24</td>
<td>$-d(\Delta i)/dt = -1.1536 + 0.05075 \times \Delta i$</td>
<td>0.9759</td>
<td>0.05075</td>
</tr>
</tbody>
</table>
that HBsAb has a high affinity constant in immune pathology, which makes for the cure of hepatitis.

3.6. Thermodynamic analysis for the affinity binding of HBsAg

Understanding the interaction of two biological macro-molecules requires not only detailed knowledge of the structure of the complex, but also a functional characterization. The function activity can be described by the kinetic rate constants and equilibrium constant of the interaction as well as by the thermodynamic parameters of the interaction. The thermodynamic parameters of the interaction between HBsAg and HBsAb were determined by measuring the temperature dependence of the ratio of its kinetic association and dissociation rate constants. The changes in enthalpy (\(\Delta H\)) and entropy (\(\Delta S\)) are related to the energy change of binding (\(\Delta G\)) and to the kinetic affinity constant (\(K\)):

\[
\Delta G = \Delta H - T\Delta S = -RT \ln K
\]  

(7)

Based on Eq. (6) calculated above, the \(K\) value was obtained as \(3.50 \times 10^9\) mol\(^{-1}\) at 37 °C. In addition, The \(K\) value of 40 °C could be calculated as \(2.77 \times 10^9\) mol\(^{-1}\) with the same method. Subsequently, \(\Delta G\) of the reaction can also be obtained:

\[
\Delta G (310 K) = -RT \ln K = -56.65 \text{kJ mol}^{-1}
\]

\[
\Delta G (313 K) = -RT \ln K = -56.57 \text{kJ mol}^{-1}
\]

Meanwhile, \(\Delta H\) can be calculated according to the following equation:

\[
\Delta H = \frac{R(\ln K_2 - \ln K_1)}{(1/T_1 - 1/T_2)}
\]  

(8)

where \(K_1\) and \(K_2\) are the kinetic affinity constants of 37 and 40 °C, respectively. Since \(K_1\) and \(K_2\) have been calculated above, \(\Delta H\) could be obtained as \(-64.54\) kJ mol\(^{-1}\).

According to Eq. (7), it is now possible to calculate the entropy change, \(\Delta S\), of the immunoreaction from \(\Delta G\) and \(\Delta H\). Therefore, \(\Delta S\) was calculated as \(-25.45\) kJ mol\(^{-1}\) at 37 °C.

The thermodynamic parameters, \(\Delta G\) and \(\Delta H\), are the functions of temperature. In our experiment, the \(\Delta G\) value of \(-56.65\) kJ mol\(^{-1}\) measured at 37 °C by the DPV method implied that the association was easier than the dissociation in the binding reaction. Compared to the value of \(\Delta G\) measured at 40 °C, the value of \(\Delta G\) at 37 °C was much better. So the temperature of 37 °C was more suitable for the immunoreaction. The value of \(\Delta S\) at 37 °C (\(-25.45\) kJ mol\(^{-1}\) < 0) showed that the immuno-complex was increased, and the state of confusion was decreased, and the system became to be in order.

4. Conclusion

This study represents the first attempt to determine the thermodynamic parameters of HBsAb–HBsAg interaction by measuring the temperature dependence of the ratio of its kinetic association and dissociation constants using biosensor technology by DPV. The present results suggest that DPV is a very sensitive technique for the real-time investigation of the immunoreaction between the immobilized HBsAb and HBsAg in solution. The self-assembled process of colloidal gold enhances the specific surface and surface free energy of the modified electrode. A comparative study of the effect of incubation temperature on DPV response indicates that the HBsAb–HBsAg binding reaction is very easy at 37 °C which is closer to our physical condition. Using the proposed method, the information on the kinetics of binding between antibody and antigen can be obtained, which is vital to understand the immune pathology of hepatitis B on a molecular level. The acquisition of thermodynamic and kinetic constants is of benefit to the pathology of hepatitis B, and provides valuable insights into the cure of hepatitis B in clinic.

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