

# A Linear Sweep Voltammetric Determination of Proteins With Thorin

Wei Sun\*, Kui Jiao, Junying Han, Changzhi Zhao

College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology,  
Qingdao 266042 P.R.China

E-mail: sunwei\_1975@public.qd.sd.cn

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## Abstract

In pH 3.0 acidic buffer solution, 2-(2-hydroxy-3,6-disulfo-1-naphthyl)-azo-phenylarsenic acid (thorin) can interact with protein to form a supramolecular complex. The interaction of thorin with human serum albumin (HSA) was studied in solution by cyclic voltammetry on mercury electrode. In the presence of HSA, the reductive peak current of thorin at  $-0.49$  V (vs.SCE) was decreased apparently without the changes of peak potentials and no new peaks appeared. The electrochemical parameters of the thorin-HSA interaction solution were calculated and compared with that of thorin solution, the results showed that there were no obvious differences in the two sets of parameters. So the formed biosupramolecular complex was electrochemical inactive and couldn't take place redox reaction on the mercury electrode. The binding reaction resulted in the decrease of the free concentration of thorin in the reaction solution, and the decrease of the reductive peak current of thorin. The binding constant and the binding ratio of thorin-HSA were calculated as  $1.15 \times 10^9$  and 2:3, respectively. Based on the decrease of peak current, a sensitive protein assay method was proposed with second order derivative linear sweep voltammetry. The optimal conditions such as the effect of pH, the concentration of thorin, reaction time and temperature, ionic strength et al on the binding reaction had been carefully studied. The interference of coexisting substances was checked. Under the selected conditions, the decrease of the reductive peak current of thorin was in proportion to the concentration of  $1.0 \sim 12.0$  mg L<sup>-1</sup> for HSA,  $0.1 \sim 16.0$  mg L<sup>-1</sup> for bovine serum albumin (BSA),  $1.0 \sim 20.0$  mg L<sup>-1</sup> for oval albumin (OVA) and  $0.4 \sim 18.0$  mg L<sup>-1</sup> for lipase et al. This new electrochemical method was further applied to the determination of human serum samples and the results were in good agreement with the traditional Commassie Brilliant Blue G-250 (CBB G-250) spectrophotometric method.

**Key words:** protein, thorin, linear sweep voltammetry, interaction

## 1. Introduction

Protein determination is of great importance in clinical test and bioanalytical chemistry. Dye-protein interactions have aroused great interests in protein chemistry and supramolecular chemistry. Many dyes have been used for this purpose with different analytical methods. Among them spectrophotometric method such as Bradford,<sup>1</sup> Lowry,<sup>2</sup> Bromocresol green,<sup>3</sup> Biuret et al were the most widely used and further applied to protein assay. Human serum albumin (HSA) has often been used as a model protein for many diverse physicochemical investigations, which has conformational adaptability and thus binds to a great deal of substances with high affinity. So it is important to know the interaction mechanism of albumin with other compounds.

Many procedures have been used to describe dye-protein interactions including spectrophotometry,<sup>4-6</sup>

fluorometry,<sup>7-8</sup> light scattering techniques<sup>9-12</sup> and electroanalytical chemistry.<sup>13-16</sup> In this paper electrochemical method was used to investigate the binding reaction between thorin with HSA. Thorin is an azo dye and most frequently used as spectral analytical reagent with the structure shown in Figure 1. It had been used in rayleigh light scattering spectroscopy for the microdetermination of proteins.<sup>17</sup> The interaction of protein with thorin were carefully studied by the electrochemical method. The electrochemical parameters of the interaction solution was calculated and compared. The results showed that an electrochemical inactive complex was formed and resulted in the decrease of the free concentration of thorin in the solution, and the decrease of the peak current. With the proposed method, different kinds of proteins and the total albumin in human serum samples were determined with satisfactory results.

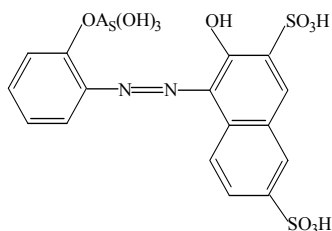


Figure 1. The molecular structure of thorin

## 2. Results and Discussion

### 2.1. Cyclic Voltammograms of Interaction Solution

Figure 2 was the cyclic voltammograms of thorin solution in the absence (curve 1) and presence of HSA (curve 2) on the hanging mercury drop electrode (HMDE). Thorin is an electrochemical active dye with azo group in its molecule and it can be easily reduced on the mercury electrode. Curve 1 showed that thorin had a reductive peak at  $-0.49$  V (vs. SCE) without any oxidative peaks, which indicated that the electrochemical process of thorin on the mercury electrode was irreversible in pH 3.0 B-R buffer solution. Curve 2 showed the cyclic voltammogram of thorin-HSA reaction solution. After the addition of HSA, the peak current decreased apparently without the changes of peak potential, which indicated that HSA could interact with thorin to form a supramolecular complex.

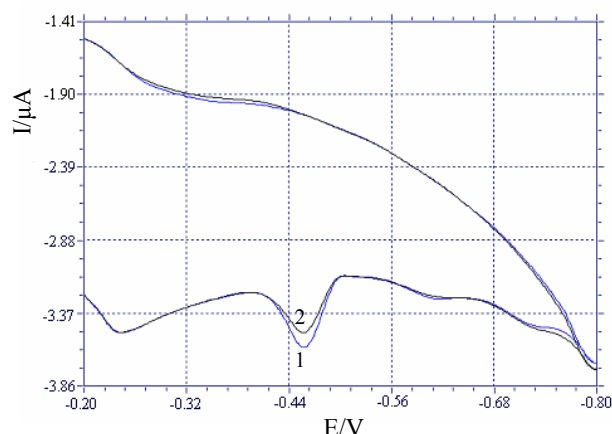


Figure 2. Cyclic voltammograms of thorin and its mixture with HSA  $1.15 \times 10^{-5}$  mol L<sup>-1</sup> thorin in pH 3.0 B-R buffer;  $2.1 + 3.0$  mg L<sup>-1</sup> HSA, scan rate:  $200.0$  mV s<sup>-1</sup>

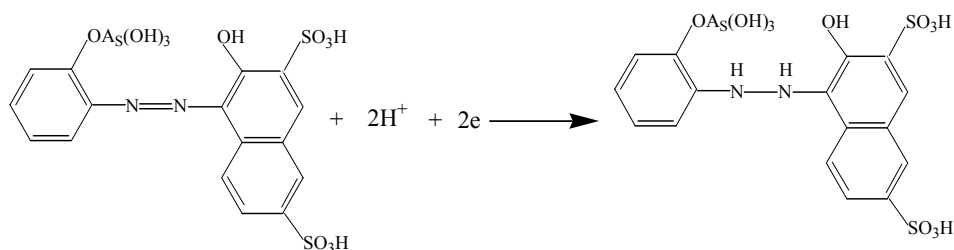


Figure 3. The proposed mechanism of electrode process of thorin

### 2.2. Electrochemical Behavior of Thorin

The electrochemical behavior of thorin on hanging mercury drop electrode was carefully studied in B-R buffer solution. The reductive peak current increased with the increase of the scan rate and the plot of peak current with the scan rate in the range of  $100 \sim 900$  mV s<sup>-1</sup> was a line with the linear regression equation as  $I_p(\text{nA}) = 3.16v(\text{mV s}^{-1}) + 1251.5$  ( $n=9$ ,  $\gamma=0.996$ ). The peak current against the square root of the scan rate was an upward curve, which indicated that the electrode reaction of thorin was controlled by the adsorption process. The reductive peak potential moved negatively against the increase of the pH of the buffer solution and a good linear relation was got in the pH range from 2.0 to 6.0 with a linear regression equation as  $E_p(\text{V}) = -0.071\text{pH} - 0.028$  ( $n=9$ ,  $\gamma=0.994$ ). The result indicated that hydrogen ion was participated in the electrode process. According to Li's report,<sup>18</sup> the electrochemical reduction of azo dyes often suffered from the two electrons with two hydrogen ions process to form a hydroazo product, so the proposed mechanism of the electrode process of thorin was given in Figure 3.

### 2.3. Electrochemical Parameters of Thorin-HSA Interaction s System

As shown in Figure 2, significant decrease of the peak current of thorin was observed without the change of peak potential after the addition of HSA into thorin solution. As for the reason accounted for the noticeable decrease of peak current, there maybe several possible explanations. First, the competitive adsorption between thorin and HSA on the mercury electrode may exist. Second, the interaction of HSA with thorin forms an electroactive complex and results in the changes of the electrochemical parameters. Third, the interaction of HSA with thorin forms an electroinactive complex, which hinders the electron transfer between thorin and the mercury electrode, the electrochemical parameters don't change in this case. As for the competitive adsorption between HSA and thorin, it was relatively trivial under the conditions of low concentration analytes, large electrode areas and short accumulated time. Li et al had studied the competitive adsorption effect of many electroactive small molecules such as 9,10-anthraquinone, rutin,

tetraphenylporphyrin tetrasulfonate (TPPS) with biomolecules such as hemoglobin, albumin and antibody on the mercury electrode.<sup>19-21</sup> The results showed that in such lower concentration of protein and shorter accumulated time, the coverage of electrode surface was only accounted for about 10% of the total electrode area, so the competitive absorption between small molecule and protein could hardly exist.

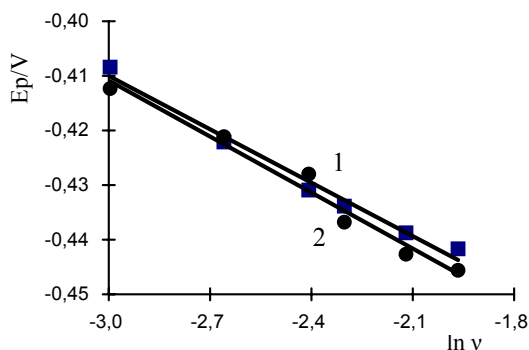
In order to investigate the binding mechanism, the electrochemical parameters of thorin, either in the absence or presence of HSA, were calculated and compared.

Because of the strong adsorption behavior and the irreversible electrode reduction process of thorin, the following Laviron's equation<sup>22,23</sup> may be used to calculate the electrochemical parameters on the mercury electrode.

$$E_p = E^0 + RT/(anF) \{ \ln [(RTk_s)/(anF)] - \ln v \}$$

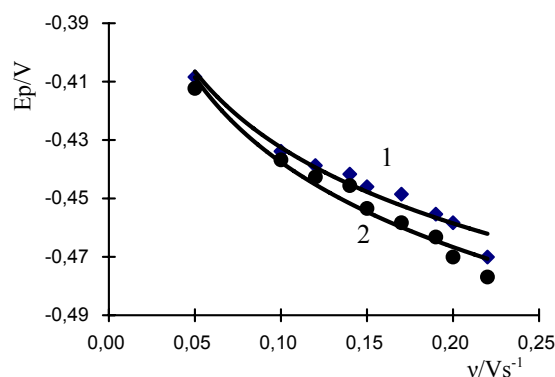
Where  $\alpha$  is the electron transfer coefficient,  $k_s$  the standard rate constant of the surface reaction,  $v$  the scan rate and  $E^0$  the formal potential.

According to above equation, if the  $E^0$  is known,  $E_p$  is in linear with  $\ln v$  and the  $an$  value can be calculated from its slope and  $k_s$  from the intercept. The  $E^0$  value can be deduced from the intercept of  $E_p$  vs.  $v$  plot on the ordinate by extrapolating the line to  $v=0$ . Based on this method, the electrochemical parameters were calculated for thorin solution and its mixture with HSA.



**Figure 4.** Semilogarithmic dependence of the peak potential  $E_p$  on the potential scan rate ( $\ln v$ ) 1.pH 3.0 B-R buffer+  $0.9 \times 10^{-5}$  mol L<sup>-1</sup> thorin; 2.1 +3.0 mg L<sup>-1</sup> HSA

The plot of  $E_p$  vs.  $\ln v$  of thorin solution was shown in curve 1 of Figure 4, which was a well-defined straight line. From the slope, the  $an$  values of thorin can be determined, and from the intercepts, the  $k_s$  values can be calculated, if the value of  $E^0$  was known. The values  $E^0$  of thorin can be determined from curve 1 of Figure 5. The intercepts of the  $E_p$  vs.  $v$  plots of thorin in Figure 5 on the ordinate by extrapolating the line to  $v=0$  represent the  $E^0$  values of thorin.



**Figure 5.** Dependence of the peak potential  $E_p$  on the potential scan rate ( $v$ ) 1.pH 3.0 B-R buffer+ $0.9 \times 10^{-5}$  mol L<sup>-1</sup> thorin; 2.1 +3.0 mg L<sup>-1</sup> HSA.

The electrochemical behavior thorin in the presence of HSA was also an irreversible processes, so the Laviron's equation was used to calculate the electrochemical parameters of the thorin-HSA reaction solution. With the same method, the results were got from curve 2 of Figure 4 and curve 2 of Figure 5. All the results were listed and compared in Table 1. It can be seen that the values of  $an$  and  $k_s$  of thorin in the absence and presence of HSA had not significant changes. So the presence of HSA didn't change the electrode reduction kinetics of thorin on the mercury electrode. The interaction of thorin with HSA formed an electro-inactive complex, which could not be reduced on the mercury electrode. In the presence of HSA, the equilibrium concentration of free thorin in solution decreased, which resulted in the decrease of the peak current.

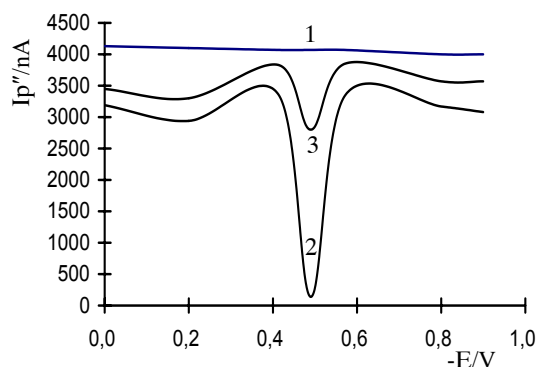
**Table 1.** The electrochemical parameters of thorin in the absence and presence of HSA

Parameters	Thorin solution	Thorin-HSA solution
$E^0$ (V)	-0.3968	-0.3972
$an$	0.751	0.788
$k_s$ (s <sup>-1</sup> )	0.967	1.031

#### 2.4. Second Order Derivative Linear Sweep Voltammogram

In order to improve the sensitivity of detection, the second order derivative linear sweep voltammetric method was used in the following analytical procedure with a JP 303 polarographic analyzer. Compared with the traditional normal linear sweep voltammetry, it had a well-defined peak shape curve with higher sensitivity. Figure 6 showed the typical second order derivative linear sweep voltammogram of thorin-HSA interaction solution. There was no electrochemical response of B-R on the mercury electrode (curve 1) and thorin had a sensitive peak shape voltammetric reductive wave at  $-0.49$  V (vs. SCE) in pH 3.0 B-R buffer (curve 2). Curve

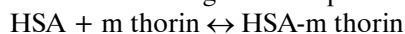
3 was the voltammogram of the mixture of thorin with HSA. Owing to the addition of HSA, an electroinactive complex was formed and resulted in the decrease of peak current greatly, which can be further used for the sensitive protein detection.



**Figure 6.** Second order derivative linear sweep voltammograms of thorin-HSA reaction system 1. pH 3.0 B-R buffer; 2. 1 +  $0.9 \times 10^{-5}$  mol L<sup>-1</sup> thorin; 3. 2 + 10.0 mg L<sup>-1</sup> HSA. Scan rate: 900 mV s<sup>-1</sup>.

## 2.5. Measurement of Stoichiometry of HSA-thorin Complex

According to a method proposed by Li,<sup>23</sup> which discussed the interaction of tetraphenylporphyrin tetrasulfonate with albumin, the binding ratio (*m*) and the equilibrium constant ( $\beta_s$ ) for thorin-HSA complex could be calculated based on the changes of the reductive peak current. It was assumed that thorin and HSA formed a single biocomplex.



The equilibrium constant was deduced as follows:

$$\beta_s = \frac{[\text{HSA-}m\text{thorin}]}{[\text{HSA}][\text{thorin}]^m} \quad (1)$$

Because of:

$$\Delta I_{\max} = kC_{\text{HSA}} \quad (2)$$

$$\Delta I = k[\text{HSA-}m \text{ thorin}] \quad (3)$$

$$[\text{HSA}] + [\text{HSA-}m \text{ thorin}] = C_{\text{HSA}} \quad (4)$$

Therefore:

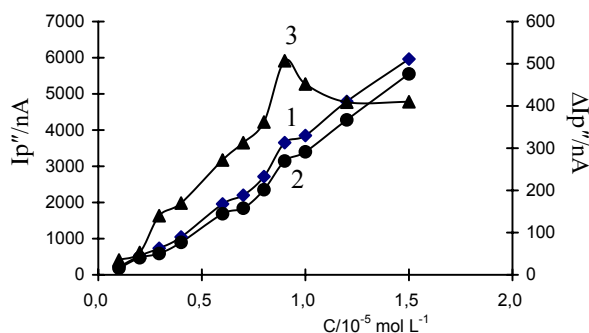
$$\Delta I_{\max} - \Delta I = k(C_{\text{HSA}} - [\text{HSA-}m \text{ thorin}]) = k[\text{HSA}] \quad (5)$$

Introducing equations (1), (3) and (5) gave:

$$\lg[\Delta I/(\Delta I_{\max} - \Delta I)] = \lg\beta_s + m \lg[\text{thorin}] \quad (6)$$

Where  $\Delta I$  was the difference of peak current in the presence and absence of HSA,  $\Delta I_{\max}$  corresponded to the obtained value when the concentration of thorin was extremely higher than that of HSA.  $C_{\text{HSA}}$ ,  $[\text{HSA}]$ ,  $[\text{HSA-}m\text{thorin}]$  were corresponding to the total, free and bound concentration of protein in the solution, respectively.

To improve the accuracy the values of second order derivative linear sweep voltammetric peak current were used. In Figure 7 curve 1 was the relationship of  $I_p''$  with the concentration of thorin, curve 2 represented the change of peak current after the addition of 3.0 mg L<sup>-1</sup> HSA on varying the concentration of thorin, and curve 3 was the differences between curve 1 and curve 2, which represented the relationship between  $\Delta I_p''(I_p''_1 - I_p''_2)$  and the concentration of thorin. From the equation (6) the relation of  $\lg[\Delta I/(\Delta I_{\max} - \Delta I)]$  with  $\lg[\text{thorin}]$  was calculated and plotted with a linear regression equation as  $\lg[\Delta I/(\Delta I_{\max} - \Delta I)] = 9.06 + 1.5 \lg[\text{thorin}]$ . From the slope and the intercept  $m = 1.5$  and  $\beta_s = 1.15 \times 10^9$  were deduced, which indicated that a stable 2:3 complex of 2HSA-3thorin was formed in the desired concentration range of thorin with respect to the concentration of HSA.

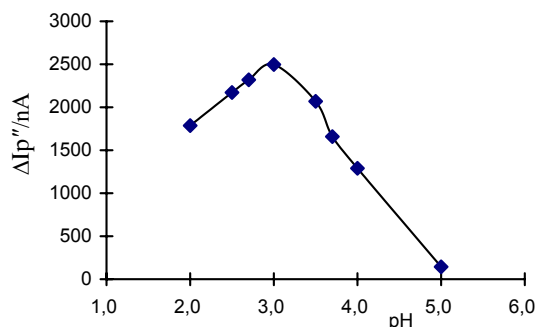


**Figure 7.** Relationship between  $I_p''$  and  $C_{\text{thorin}}$  (1, 2),  $\Delta I_p''$  and  $C_{\text{thorin}}$  (3) 1.  $C_{\text{HSA}} = 0$ ; 2.  $C_{\text{HSA}} = 3.0$  mg L<sup>-1</sup>; 3.  $\Delta I_p'' = I_p''_1 - I_p''_2$ .

## 2.6. Optimal of Reaction Conditions

The pH of buffer solution greatly influenced the binding reaction and the optimal reaction pH was selected in the pH range of 2.0~5.0 with the result shown in Figure 8. It can be seen that at pH 3.0 the difference of peak current reached its maximum, so pH 3.0 B-R buffer solution was used in this experiment. In pH 3.0 acidic buffer solution the amino acid residues in the HSA (isoelectric point  $pI = 4.7$ ) molecular chains were in positively charged and the thorin species were in negatively charged, so it was possible for them to combine together by electrostatic attraction to form a supramolecular biocomplex. Different buffers such as B-R, HOAc-NaOAc,  $\text{NH}_3\text{-NH}_4\text{Cl}$  were tested and in B-R buffer solution the response was the maximum.

The reaction time was selected and the differences of peak currents reached the maximum for about 5 min and remained unchanged for at least 2 hours. Therefore, the system was stable for routine measurement. The effect of the reaction temperature on the interaction was investigated at 15°C, 25°C and 37°C, respectively. The results showed that there were no obvious differences among them. So the temperature had little influence



**Figure 8.** The influence of pH on the peak current  $0.9 \times 10^{-5} \text{ mol L}^{-1}$  thorin +  $10.0 \text{ mg L}^{-1}$  HSA in different pH of B-R buffer solution

on the binding reaction and  $25^\circ\text{C}$  was used throughout. Different adding sequences of thorin, HSA and B-R buffer were tested and the results showed that the best addition sequences were thorin, B-R buffer and proteins. This result also indicated that the electronic coupling made thorin bind to HSA.

The scan rate and the dropping mercury standing time of the JP 303 polarographic analyzer were studied. The peak current increased with the increase of potential scan rate within  $100\sim 1000 \text{ mV s}^{-1}$  and the dropping mercury standing time from 3 to 20 s. When the standing time was more than 20 s, the mercury drop would fall down. But after 18 s, the shape of the reductive wave wasn't good, so the scan rate and the standing time were selected as  $900 \text{ mV s}^{-1}$  and 16 s, respectively.

## 2.7. Interference of Coexisting Substances

The effects of coexisting substances such as metal ions, amino acids and carbohydrates on the determination were tested by premixing HSA with the interference substances. Table 2 showed that the commonly observed metal ions in blood such as  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cu}^{2+}$  *et al* can be allowed with higher concentrations. But the addition of some surfactants such as sodium dodecylsulfate (SDS), Tween-20,  $\beta$ -cyclodextrin ( $\beta$ -CD) can greatly influence the value of  $\Delta\text{Ip}''$ , which indicated that it could greatly influence the interaction, or they could be greatly adsorbed on the surface of mercury electrode.

The effect of ionic strength on the interaction was also examined by adding  $0.10\sim 0.50 \text{ mol L}^{-1}$  NaCl in the mixed solution and the results proved to have significant influences on the interaction. The  $\Delta\text{Ip}''$  decreased with the increase of salt concentration, which indicated that the interaction of thorin with HSA was mainly caused by electrostatic attraction. The electrostatic shielding effect of the charges on binding reaction with the increase of  $\text{Na}^+$  concentration was unbeneficial to the formation of thorin-HSA complex.

## 2.8. Working Curves of Different Proteins

Under the optimal conditions different kinds of proteins such as HSA, BSA, BHb, OVA, lipase were determined by this method. The difference of peak current was proportional to the concentration of proteins with the linear regression equations listed in Table 3. The results showed that different proteins had

**Table 2.** Effect of interference substances on the determination of  $10.0 \text{ mg L}^{-1}$  HSA

Coexisting substance	Concentration ( $\text{mg L}^{-1}$ )	Relative error (%)	Coexisting substance	Concentration ( $\mu\text{mol L}^{-1}$ )	Relative error (%)
L-Alanine	1.0	2.55	$\text{Mn}^{2+}$	20.0	1.83
L-Valine	1.0	-3.50	$\text{Ca}^{2+}$	20.0	2.42
L-Serine	1.0	4.68	$\text{Co}^{2+}$	20.0	2.29
L-Glutamic acid	1.0	0.50	$\text{Cu}^{2+}$	20.0	-0.50
L-Arginine	1.0	2.02	$\text{Ni}^{2+}$	20.0	2.36
Glucose	1.0	2.39	$\text{Mg}^{2+}$	20.0	2.26

**Table 3.** Analytical parameters for the determination of different proteins

Protein	Linear range ( $\text{mg L}^{-1}$ )	Linear regression equation	Detection limit ( $\text{mg L}^{-1}$ )	Correlation coefficient ( $\gamma$ )
HSA	1.0~12.0	$\Delta\text{Ip}''(\text{nA})=234.81 \text{ C}(\text{mg L}^{-1}) - 86.50$	0.47	0.995
BSA	0.1~16.0	$\Delta\text{Ip}''(\text{nA})=161.79 \text{ C}(\text{mg L}^{-1})+459.48$	0.10	0.995
OVA	1.0~20.0	$\Delta\text{Ip}''(\text{nA})=108.82 \text{ C}(\text{mg L}^{-1})+20.54$	1.0	0.996
BHb	1.0~20.0	$\Delta\text{Ip}''(\text{nA})=91.66 \text{ C}(\text{mg L}^{-1})+455.11$	1.0	0.997
Lipase	0.4~18.0	$\Delta\text{Ip}''(\text{nA})=54.29 \text{ C}(\text{mg L}^{-1})+204.19$	0.40	0.992

\*HSA (Human serum albumin), BSA (Bovine serum albumin), BHb (Bovine hemoglobin), OVA (Oval albumin).

different responses and the sensitivities were enough for routine sample determination. The detection limit of this assay is given by  $3S_0/S$ , where 3 is the factor at the 99% confidential level,  $S_0$  the standard deviation of the blank measurements without HSA ( $n=5$ ) and  $S$  the slope of the calibration curves.

## 2.9. Sample Determination

The proposed method was further applied to determine the albumin content in healthy human serum samples and the results were listed in Table 4. The results were close to those obtained by the Coomassie Brilliant Blue G-250 (CBB G-250) spectrophotometric method. Therefore this new electrochemical detection method was reliable, practicable and reproducible. The recovery of the samples had also been examined with the results between 92.5% and 105.0%.

**Table 4.** The results for the determination of HSA in human serum samples ( $n=5$ )

Sample	This method ( $\text{g L}^{-1}$ )	RSD (%)	Recovery (%)	CBB G-250 method ( $\text{g L}^{-1}$ )	RSD (%)
1	64.30	0.47	105.0	66.20	1.91
2	68.60	1.90	92.5	69.20	2.24
3	64.90	2.01	100.8	65.10	1.38

## 3. Conclusions

In summary the electrochemical behaviors of thorin and its interaction with HSA were studied on the mercury electrode by cyclic voltammetry in this paper. The electrochemical parameters of thorin-HSA interaction system were calculated and compared, the results indicated that an electrochemical inactive supramolecular complex was formed. The interaction of thorin with HSA caused the decrease of free concentration of thorin in the solution and further decrease of the reductive peak current of thorin. Based on this result, a second order derivative linear sweep voltammetric method for the determination of different kinds of proteins were established and successfully applied to the determination of healthy human serum samples.

## 4. Experimental

### 4.1. Apparatus

Cyclic voltammetric experiments were carried out using a DS model 2004 electro-chemical analyzer (Shandong Dongsheng Electronic Instrument, China) with a DS-991 hanging mercury drop electrode (HMDE, Shandong Dongsheng Electronic Instrument, China), a saturated calomel reference electrode (SCE) and a platinum wire auxiliary electrode. All the analytical

results were got with second order derivative linear sweep voltammetry on a model JP-303 polarographic analyzer (Chengdu Apparatus Factory, China), with a traditional three-electrode system composed of a dropping mercury electrode (DME) as working electrode, a saturated calomel reference electrode (SCE) and a platinum wire auxiliary electrode. The values of buffer pH were measured with a PHS-25 acidimeter (Shanghai Leici Instrument Factory, China). All the experiments were carried out at 25°C.

### 4.2. Reagents

Human serum albumin (HSA, 99%, Shanghai Biomedical Products Research Institute), bovine serum albumin (BSA, 99%, Sigma), oval albumin (OVA, Sigma), Lipase (Sigma), bovine hemoglobin (BHb, Tianjin Chuanye Biotechnology, Shanghai Institute of Biochemistry) were used as received. The  $1.0 \text{ g L}^{-1}$  stock solution of different proteins were prepared by directly dissolving them in doubly distilled water from all-quartz still and stored at 4°C. The working solutions were obtained by diluting the stock solution with water.  $1.0 \times 10^{-3} \text{ mol L}^{-1}$  thorin solution (Shanghai Xinzhong Chemical Reagent Factory) was used as the stock solution and diluted to the working concentration when used.  $0.2 \text{ mol L}^{-1}$  Britton-Robinson (B-R) buffer solution was used to control the pH of the tested solutions. The Coomassie Brilliant Blue G-250 (CBB G-250, Shanghai Chemical Reagent Company) solution for spectrophotometric determination of protein was prepared according to the common procedure. Fresh human serum samples were kindly provided by the Hospital of Qingdao University of Science and Technology. All other reagents used were of analytical reagents grade and doubly distilled water was used throughout.

### 4.3. Procedure

Into a 10 mL volumetric flask were added in sequence 0.9 mL of  $1.0 \times 10^{-4} \text{ mol L}^{-1}$  thorin, 2.0 mL of  $0.2 \text{ mol L}^{-1}$  B-R (pH 3.0) buffer solution and an appropriate amount of HSA standard solution or sample solution. The mixture was diluted to 10 mL with doubly distilled water and mixed homogeneously. After reaction at 25°C for 10 min, the voltammetric curves were recorded to show the electrochemical responses of the reaction system in the potential range from  $-0.2$  to  $-0.8 \text{ V}$ . The mixture without the addition of HSA was used as the reference and the differences were used for protein analysis.

## 5. Acknowledgements

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## Povzetek

2-(2-hidroksi-3,6-disulfo-1-naphthil)-azo-fenilarzenova kislina (torin) tvori s proteini v kisli puferni raztopini (pH=3) supramolekularne komplekse. V tem delu smo z metodo ciklične voltometrije proučevali interakcije med torinom in človeškim serumskim albuminom (HSA). Na nastanek kompleksa lahko sklepamo iz zmanjšane koncentracije torina v raztopini in zmanjšanja pika redukativnega toka torina, vendar je nastali kompleks elektrokemično neaktiven. Ugotovili smo, da se torin in HSA vežeta v razmerju 2:3, konstanta vezanja pa znaša  $1.15 \cdot 10^9$ . Na osnovi opazovanja zmanjšanja pika redukativnega toka smo razvili občutljivo metodo za določanje proteinov s pomočjo linearne "sweep" voltometrije, ki smo jo uporabili za določanje HSA v vzorcih. Rezultati so se dobro ujemali z običajno metodo spektrofotometrijsko metodo CBB G-250.