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Article

Voltammetric Studies on the Interaction of Orange G with Proteins. Analytical Applications

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A interação do alaranjado G com proteína foi investigada pela técnica de voltametria. O alaranjado G, em solução tampão Britton-Robinson (B-R), pH 2,0, mostrou um pico voltamétrico irreversível de redução, a –0,17V (*vs*. SCE) utilizando mercúrio como eletrodo de trabalho. A adição de albumina de soro humano (HSA) à solução de alaranjado G, resultou no decréscimo do pico de corrente de redução, aparentemente sem variações dos portenciais de pico e sem o surgimento de novos picos de corrente. Os parâmetros eletroquímicos da solução de alaranjado G, na ausência e presença de HSA, foram calculados e comparados. Os resultados mostraram que não ocorreram variações significantes, indicando que o comportamento eletroquímico da solução reacional utilizando mercúrio como eletrodo de trabalho não variou e que foi formado um biocomplexo supramolecular, eletroquimicamente inativo. O mecanismo de interação foi devido à formação do campo microeletrostático na estrutura da albumina em solução aquosa, causando a interação com o alaranjado G, o que induziu o decréscimo da concentração de equilíbrio do alaranjado G na solução reacional, e o decréscimo do pico de corrente de redução. As condições de interação foram discutidas cuidadosamente. Sob condições ótimas, o decréscimo do pico de corrente foi proporcional à concentração da proteína e posteriormente, usado para a determinação de diferentes tipos de proteínas. As curvas de calibração para a determinação de HSA, albumina de soro bovino (BSA), ovalbumina (OVA), hemoglobina bovina (BHb) e lipase, foram lineares nos intervalos de 4,0~28,0 mg L^{-1}, 4,0~30,0 mg L^{-1}, 2,0~20,0 mg L^{-1}, 2,0~25,0 mg L^{-1} e 2,0~30,0 mg L^{-1}, respectivamente. O limite de detecção foi 3,0 mg L^{-1} para HSA, 3,5 mg L^{-1} para BSA, 1,0 mg L^{-1} para OVA, BHb e lipase. O novo método eletroquímico estabelecido foi aplicado para determinar o teor de albumina em amostras de soro humano sadio e os resultados apresentaram boa concordância com o tradicional método espectrofotométrico Coomassie Brilliant Blue (GBB G-250).

The interaction of orange G with protein was investigated by voltammetric method in this paper. In pH 2.0 Britton-Robinson (B-R) buffer solution orange G displayed an irreversible voltammetric reduction peak at -0.17 V (*vs.* SCE) on mercury working electrode. The addition of human serum albumin (HSA) into the orange G solution resulted in the decrease of the reduction current peak apparently without the changes of peak potentials and no new peaks appeared. The electrochemical parameters of orange G solution in the absence and presence of HSA were calculated and compared. The results showed that there were no significant changes, which indicated that the electrochemical behaviors of reaction solution on the mercury working electrode showed no changes and a supramolecular electrochemical inactive biocomplex was formed. The interaction mechanism was due to the formation of the microelectrostatic field in albumin structure in aqueous solution and caused the interaction with orange G, which induced the decrease of the equilibrium concentration of orange G in the reaction solution, and the decrease of the reductive peak current. The interaction conditions were discussed carefully. Under the optimal conditions the decrease of peak current was proportional to the concentration of protein and further used to the determination of different kinds of proteins. The calibration curves for the determination of HSA, bovine serum albumin (BSA), ovalbumin (OVA), bovine hemoglobin (BHb), lipase were linear over the ranges of 4.0~28.0 mg L^{-1}, 4.0~30.0 mg L^{-1}, 2.0~20.0 mg L^{-1}, 2.0~25.0 mg L^{-1}, 2.0~30.0 mg L^{-1}, respectively. The detection limit was 3.0 mg L^{-1} for HSA, 3.5 mg L^{-1} for BSA, 1.0 mg L^{-1} for OVA, BHb and lipase. The new established electrochemical method was applied to determine the content of albumin in healthy human serum samples and the results were in good agreement with the traditional Coomassie Brilliant Blue (GBB G-250) spectrophotometric method.

Keywords: orange G, human serum albumin, voltammetry, interaction, supramolecule

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Introduction

Recently the interaction of biomacromolecules with small ions and molecules has aroused great interests in chemists and biologists. In order to understand the structure and functions of different biopolymers such as DNA and protein, many methods have been proposed. Protein chemistry is one of the research focus and the interactions of organic compounds with protein have not been explained clearly with Pesavento equation[1] or Scatchard model[2] until now. At the same time the determination of the content of proteins is often a necessary in clinical tests and food analysis. Different kinds of analytical methods including Biuret method, Bradford method,[3] Lowry method,[4] Bromocresol Green[5] among others, had been proposed in the literature. Recently the light scattering technique had been used and successfully applied to the investigation of biological macromolecular complex such as protein and DNA with organic dyes.[6-9]

In this paper electrochemical method was applied to investigate the interaction of orange G with proteins. Compared with other often-used methods such as spectrophotometry, fluorometry and chemiluminicence, electrochemical method is seldom used in studying the protein binding reaction. However, the electrochemical reaction occurs on the electrode/liquid surface and especially suitable for small amount of samples with high sensitivity and low detection limit. Li *et al.*[10,11] had studied the interaction of albumin or hemoglobin with TPPS and 9,10-anthraquinone. The voltammetric studies on interaction of Re (V) complexes with protein had also been reported.[12] Our group had applied alizarin red S and beryllon III as the electrochemical probe for the detection of human serum albumin (HSA) and bovine serum albumin (BSA).[13-15]

Orange G (OG) is an azo dye with the molecular structure shown in Figure 1 and it had been used in protein determination by resonance rayleigh method.[16] In the acidic solution the sulfonic and hydroxyl groups in its structure were in negatively charged and the amino groups of protein were protonated, so OG and protein could easily bind to each other by electrostatic force. It was found that the interaction of OG with proteins could be studied by the voltammetric method according to the changes of peak current of the OG-protein reaction solution. So the electrochemical method was applied to investigate the binding reaction process and further used to establish a sensitive method for protein assay.

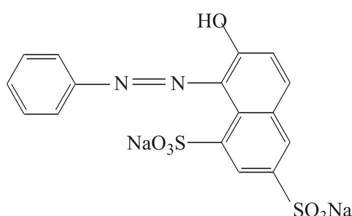

Figure 1. The molecular structure of orange G.

Experimental

Apparatus

Cyclic voltammetric experiments were performed on a DS model 2004 electrochemical analyzer (Shandong Dongsheng Electronic Instrument, China) with a DS-991 hanging mercury drop working electrode (Shandong Dongsheng Electronic Instrument, China), a saturated calomel reference electrode (SCE) and a platinum wire auxiliary electrode. The second order derivative linear sweep voltammetric determination was obtained on a JP-303 polarographic analyzer (Chengdu Apparatus Factory, China) with traditional three-electrodes system composed of a dropping mercury working electrode (DME), a SCE reference electrode and a platinum wire auxiliary electrode, respectively. UV-Visible absorption spectra were recorded by a Cary model 50 probe spectrophotometer (Varian, Australia) with 1 cm path length. The values of pH were measured with a pHS-25 acidimeter (Shanghai Leici Instrument Factory, China). All of the related experiments were carried out at room temperature.

Reagents

Human serum albumin (HSA, 99%, Shanghai Biomedical Products Research Institute), bovine serum albumin (BSA, 99%, Sigma), ovalbumin (OVA, Sigma), lipase (Simga), bovine hemoglobin (BHb, Tianjin Chuanye Biotechnology Co.) were purchased and used as received. The 1.0 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. 1.0×10^{-3} mol L^{-1} orange G solution (OG, Shanghai Xinzhong Chemical Reagent Factory) was used as stock solution and diluted to the working concentration when used. 0.2 mol L^{-1} Britton-Robinson (B-R) buffer solution was used to control the pH of the tested solutions. All other chemicals used were of analytical grade and doubly distilled water was used throughout to prepare to aqueous solutions.

Procedure

Into a 10 mL calibrated flask were added in sequences 0.8 mL of 1.0×10^{-4} mol L^{-1} OG, 2.5 ml of 0.2 mol L^{-1} B-R (pH 2.0) buffer solution and an appropriate amount of HSA solution or human serum samples. The mixture was diluted to the scale with doubly distilled water and mixed homogeneously. After reaction at room temperature for 10 min, the voltammetric curves were recorded to show the electrochemical responses of the reaction system in the potential range from 0 mV to -600 mV. The mixture without the addition of HSA was also measured and used as the reference. Before each measurement the solution was deoxygenated, by bubbling with highly purified nitrogen.

Results and Discussion

UV-Vis absorption spectra of reaction solution

Figure 2 shows the UV-Vis absorption spectra of OG-protein interaction system. In pH 2.0 B-R buffer solution and in the scanning range of 300~700 nm, HSA didn't have any absorption (curve 1) and OG had two absorption peaks at 323 and 480 nm (curve 2). When HSA was mixed with OG, both of the absorption peaks of OG decreased without the movement of maximum absorption wavenumber (curve 3 and 4). The higher the protein concentration, the lower the absorbance value, which indicated that a binding reaction had taken place between OG and HSA.

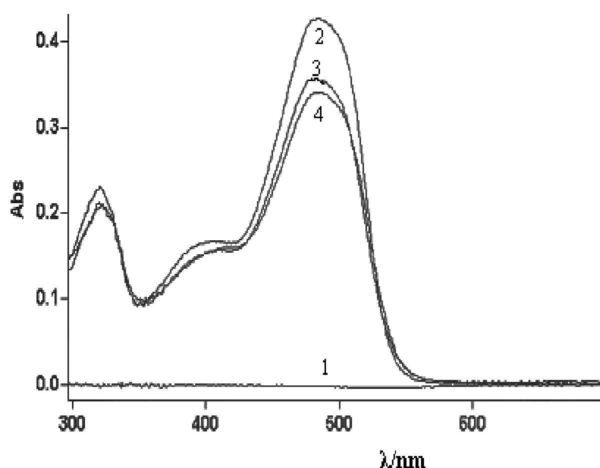

Figure 2. UV-Vis absorption spectra of OG-HSA reaction system. (1) pH 2.0 B-R + 20.0 mg L^{-1} HSA; (2) pH 2.0 B-R + 2.5×10^{-5} mol L^{-1} OG; (3) 2 + 20.0 mg L^{-1} HSA; (4) 2 + 100.0 mg L^{-1} HSA.

Electrochemical behavior of OG

OG is an electrochemical active dye with azo group in its molecular structure. The typical cyclic voltammogram of OG was recorded under the selected conditions and the result was shown in Figure 3. OG had a reductive peak at -0.17 V (*vs.* SCE) and didn't have any oxidative peak (curve 1), which indicated that the electrochemical behavior of OG on Hg electrode was irreversible in pH 2.0 B-R buffer solution. The peak current increased with the increase of the scan rate and the relationship of reductive peak current against the scan rate in the range of 20~250 mV s^{-1} was plotted (results not shown). The results showed that a straight line was got with the linear regression equation as Ip (μA)= -3.86ν–2.02 (n=13, γ=0.991). Multi-sweep cyclic voltammetric studies showed that with the increase of the scan cycle, the reductive peak currents decreased greatly, which was the strong adsorption behaviour of OG on the Hg electrode (curve 2~curve 5). The relationship of the reductive peak potential against pH of buffer solution was investigated and with the increase of pH of buffer solution, the peak potential moved negatively. A linear relationship was obtained in the pH range from 1.5 to 7.0 with the equation as Ep(V) = -0.077pH -0.017 (n=9, γ=0.998), which indicated that there were hydrogen ions participating in the electrode reaction (results not shown). So the reduction of OG on the Hg electrode was irreversible surface adsorption electrode process.

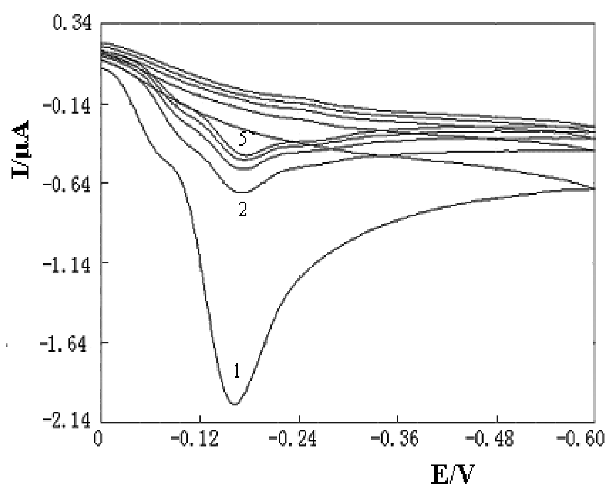

Figure 3. The multi-sweep cyclic voltammogram of OG. pH 2.0 B-R + 0.4×10^{-5} mol L^{-1} OG; scan rate: 100.0 mV s^{-1}.

Cyclic voltammogram of reaction solution

Figure 4 shows the cyclic voltammograms of OG in the absence and presence of different amounts of HSA. Curve 1 was the reductive curve of OG with the reductive peak at -0.17 V (*vs.* SCE). On the addition of HSA, the reductive peak current of OG decreased greatly without the shift of peak potential and no new reductive peaks appeared (curve

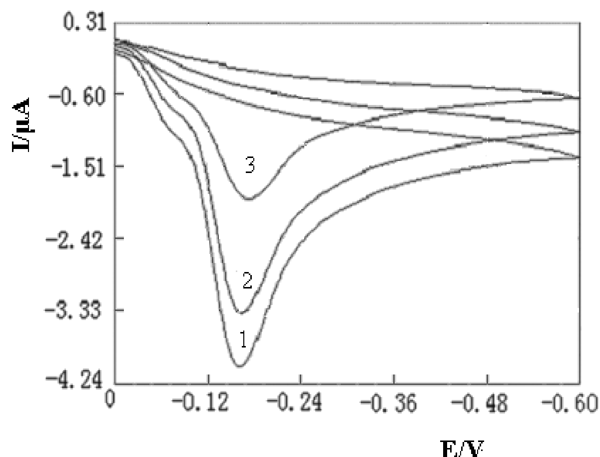

Figure 4. Cyclic voltammograms of OG-HSA binding reaction system. (1) 0.8×10^{-5} mol L^{-1} OG in pH 2.0 B-R buffer; (2) 1 + 3.0 mg L^{-1} HSA; (3) 1 + 5.0 mg L^{-1} HSA, scan rate: 100.0 mV s^{-1}.

2 and 3). The more HSA added, the greater the peak current decreased. The results also indicated that there were binding reaction happened in the reaction solution and the electrode process was also irreversible.

As for the reason of the decrease of the reductive peak current after the reaction of OG with HSA, three factors may be considered: (*i*) the competitive adsorption between the OG and HSA on the mercury electrode; (*ii*) the formation of electrochemical active complex and the changes of electrochemical parameters; (*iii*) the formation of electro-inactive complex without the changes of electrochemical parameters. The competitive adsorption factor can be excluded by the following investigation: (*i*) the UV-Vis absorption spectrophotometric results proved that an interaction existed between OG and HSA, which resulted in the decrease of the absorbance of OG in the presence of HSA and no changes in its absorption wavelength; (*ii*) the peak current of OG did not disappear completely with the increase of the concentration of protein, which was not the character of competitive adsorption. Li and co-workers[17-19] have studied the interaction of some electro-active small molecules such as 9,10-anthraquinone, rutin with bio-molecules such as hemoglobin, albumin and DNA. The results also 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 area of electrode, so the competitive absorption between small molecule with protein can hardly exist. The electrochemical parameters of OG-HSA reaction solution were calculated with the following method and compared to distinct the electrochemical activity of the formed biocomplex.

The reductive reaction of OG-HSA interaction solution had the characteristics of the strong adsorption behavior and the irreversible electrode process. So Laviron's equation [20,21] maybe used to evaluate the kinetic constants of electrode reaction in the absence and presence of protein.

$$Ep = E^0 + RT/(\alpha nF)[\ln [(RTk_s)/(\alpha nF)] - \ln \nu]$$

Where α is the electron transfer coefficient, k_s the standard rate constant of the surface reaction, ν the scan rate, E^0 the formal potential and n the electron transfer number.

According to above equation, if the E^0 is known, Ep is in linear with lnν and the αn value can be calculated from the slope and k_s from the intercept. The E^0 value can be deduced from the intercept of Ep *vs.* ν plot on the ordinate by extrapolating the line to ν=0, when ν was approached to zero, then Ep was approached to E^0. Based on this principle, the electrochemical parameters of OG-HSA reaction solution were calculated respectively.

The plot of Ep of OG *vs.* ln ν was shown in curve l of Figure 5, which was a well-defined straight line. From the slope, the αn values of OG can be determined, and from the intercepts, the k_s values can be calculated, if the value of E^0 was known. The E^0 values of OG can be determined from curve 1 of Figure 6 on the ordinate by extrapolating the line to ν=0.

Parameters of the OG-HSA reaction solution were also calculated with the same method and the results were also got from curve 2 of Figure 5 and curve 2 of Figure 6. All the results of these two reaction solutions were listed in Table 1. It can be seen that the electrochemical parameters of the reaction system such as the values of αn and k_s did not change obviously, so OG interacting with HSA formed an electro-inactive complex, which could not be reduced on the Hg electrode. In the presence of HSA, the equilibrium concentration of free OG in solution decreased, and resulted in the decrease of the reductive peak current.

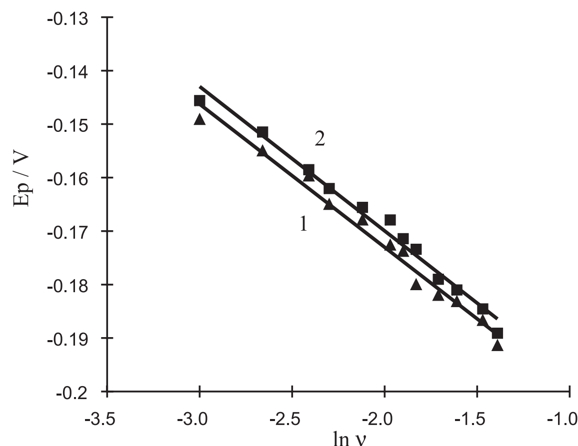

Figure 5. Semilogarithmic dependence of the peak potential Ep on potential scan rate (ln ν). (1) pH 2.0 B-R buffer + 0.8×10^{-5} mol L^{-1} OG; (2) 1 + 3.0 mg L^{-1} HSA.

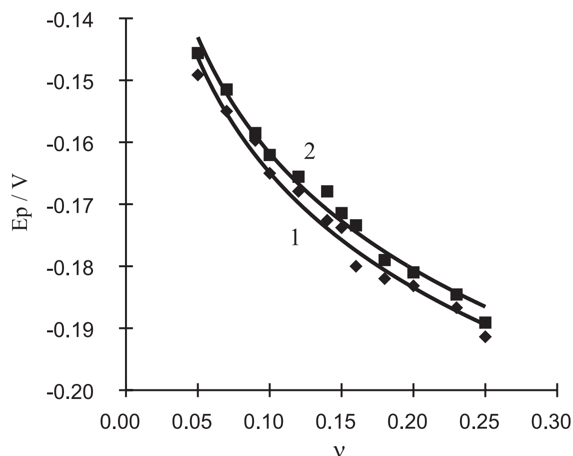

Figure 6. Dependence of the peak potential Ep on the potential scan rate (ν). (1) pH 2.0 B-R buffer + 0.8×10^{-5} mol L^{-1} OG; (2) 1 + 3.0 mg L^{-1} HSA.

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

Parameters	OG	OG-HSA
E^0(V)	-0.142	-0.139
α n	0.958	0.951
k_s (s^{-1})	1.606	1.578

Measurement of stoichiometry of HSA-OG complex

According with Li and Min,[22] the composition and the equilibrium constant can be calculated based on the changes of peak current. It was proposed that a single complex of HSA-mOG was formed.

$$\text{HSA} + \text{mOG} \leftrightarrow \text{HSA-mOG} \quad (1)$$

The equilibrium constant β_s was deduced as follows:

$$\beta_s = \frac{[\text{HSA - mOG}]}{[\text{HSA}][\text{OG}]^m} \quad (2)$$

Because of:

$$\Delta I_{max} = kC_{HSA} \quad (3)$$

$$\Delta I = k[\text{HSA-mOG}] \quad (4)$$

$$[\text{HSA}] + [\text{HSA-mOG}] = C_{HSA} \quad (5)$$

Therefore:

$$\Delta I_{max} - \Delta I = k(C_{HSA} - [\text{HSA-mOG}]) = k[\text{HSA}] \quad (6)$$

Introducing equations (2), (4) and (6) gave:

$$1/\Delta I = 1/\Delta I_{max} + (1/\beta_s \Delta I_{max})(1/[\text{OG}]^m) \quad (7)$$

$$\text{or } \log [\Delta I/(\Delta I_{max} - \Delta I)] = \log \beta_s + m \log [\text{OG}] \quad (8)$$

Where ΔI was the peak current difference in the absence and presence of HSA, ΔI_{max} corresponded to the obtained value when the concentration of OG was much higher than that of HSA. C_{HSA}, [HSA], [HSA-mOG] were corresponding to the total, free and bound concentration of protein in the solution, respectively.

For minimizing the reading error the second order derivative linear sweep polarographic peaks were used. In Figure 7, curve a was the relationship of peak current with the concentration of OG (Ip''_a), curve b represented the change of peak current after the addition of 20.0 mg L^{-1} HSA on varying the concentration of OG (Ip''_b), and curve c was plotted using the differences between curve a and curve b, which represented the relationship between $\Delta Ip''(Ip''_a - Ip''_b)$ and the concentration of OG. From the equation (8) the relation of $\log[\Delta I/(\Delta I_{max} - \Delta I)]$ with log[OG] was calculated and plotted in Figure 8.

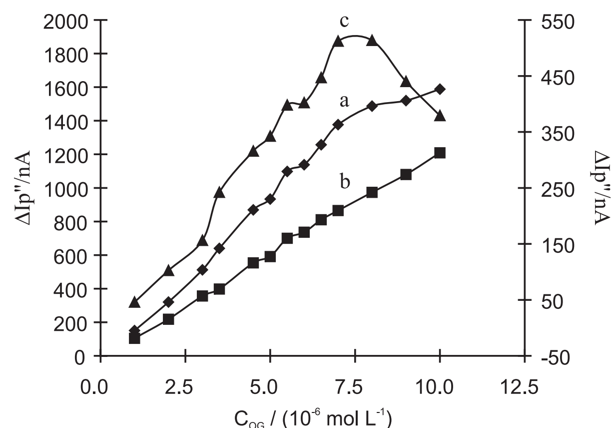

Figure 7. Relationship between Ip″ and C_{OG} (a, b), ΔIp″ and C_{OG} (c). (a) C_{HSA}=0; (b) C_{HSA}=20.0 mg L^{-1}; (c) $\Delta Ip'' = Ip''_a - Ip''_b$.

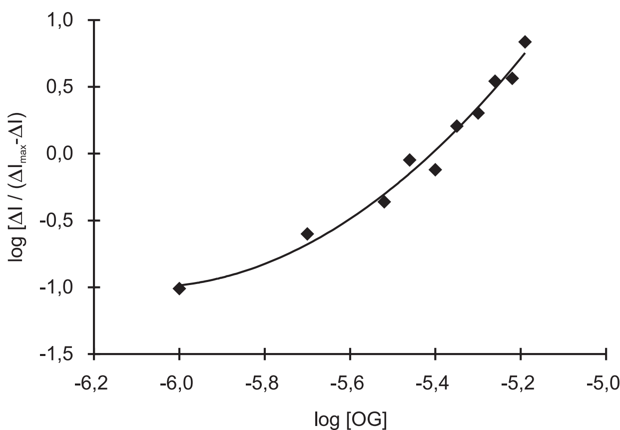

Figure 8. Relationship between log [ΔI / (ΔI_{max}-ΔI)] and log [OG].

If protein and OG formed a single complex, then the plot $\log[\Delta I/(\Delta I_{max} - \Delta I)]$ *vs* log[OG] became linear with the slope of m (equation 8). However, Figure 8 showed a non-

linear relation, which implied that HSA and OG formed multi-complexes by steps. If the curve of Figure 8 was divided into two sectors, two lines with the slopes of 1.02 and 4.10 can be obtained, respectively. Two values of m (m=1 and m=4) accounted for two kinds of complex formed. The intercepts of the two sectors were 5.15 and 22.1, respectively. Thus the equilibrium constants were calculated as $\beta_1=1.41\times10^5$ and $\beta_2= 1.26\times10^{22}$.

According to the formula given by Yatsimirskii and Budalin,[23]

$$\Delta I= \frac{\Delta I_1\beta_1X+\Delta I_2\beta_2X^2+\Delta I_3\beta_3X^3+\cdots+\Delta I_n\beta_nX^n}{1+\beta_1X+\beta_2X^2+\beta_3X^3+\cdots+\beta_nX^n} \quad (9)$$

I stood for the total decrease of Ip, ΔI_1, ΔI_2......ΔI_n for the decrease of Ip in each step, X for the equilibrium concentration of OG.

Let $f= \Delta I/X$, and introduced it in equation (9), we obtained

$$f=\frac{\Delta I_1\beta_1+\Delta I_2\beta_2X+\Delta I_3\beta_3X^2+\cdots+\Delta I_n\beta_nX^{n-1}}{1+\beta_1X+\beta_2X^2+\beta_3X^3+\cdots+\beta_nX^n} \quad (10)$$

Since there were mainly two kinds of complexes formed in the above experiment, equation (10) could be simplified to:

$$f=\frac{\Delta I_1\beta_1+\Delta I_4\beta_4X^3}{1+\beta_1X+\beta_4X^4} \quad (11)$$

$$\lim_{x\to0} f = \Delta I_1\beta_1= \mathrm{b} \quad (12)$$

$$\lim_{x\to0}\frac{df}{dx}= -\Delta I_1\beta_1^{\ 2}= -\mathrm{a} \quad (13)$$

$$\beta_1= -\frac{\mathrm{a}}{\mathrm{b}}$$

Read ΔI from curve c in Figure 7 and look for the corresponding equilibrium concentration of OG from curve b and a, and calculate every ΔI and f, plot f *vs.* X curve (Figure 9). When X approached zero the tangent of the curve intersects the f axis at β_1 with a slope of a. Thus, the association constant β can be calculated as $\beta_1= 4.0\times10^5$, which was close to the result from Figure 8 ($\beta_1=1.41\times10^5$).

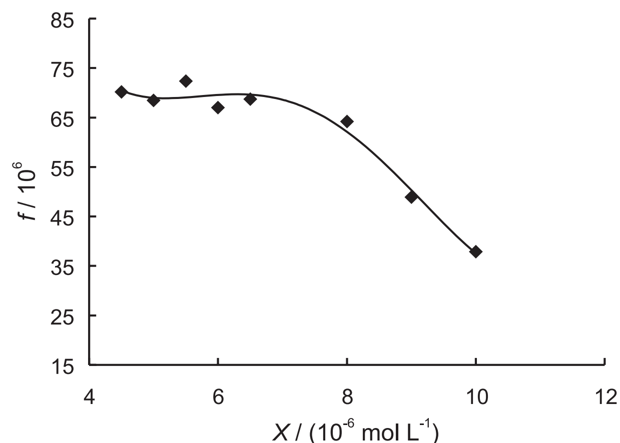

Figure 9. Relationship between f and X.

Analytical application. 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 1.5~4.0, the results showed that at pH 2.0 the difference of peak current reached its maximum (Figure 10), so pH 2.0 B-R buffer solution was used throughout in this experiment. Different buffers such as B-R, HOAc-NaOAc, NH_3-NH_4Cl were tested and in B-R buffer solution the response was the maximum. So the pH 2.0 B-R buffer was recommended in this paper.

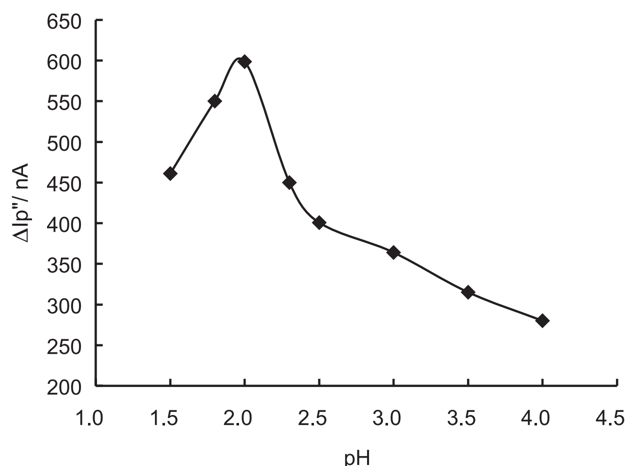

Figure 10. The influence of pH on the peak current; 0.8×10^{-5} mol L^{-1} OG + 20.0 mg L^{-1} HSA in different pH B-R buffer solution.

After they were mixed, the difference of peak currents reached the maximum after reaction for about 5 min and remained unchanged for at least 2 hours. Therefore, the system was suitable for the routine measurements. The effect of the reaction temperature on the interaction was tested at 15 °C, 25 °C, 30 °C and 37 °C, respectively. The results showed that there were no obviously differences among them. So the temperature had little influence on the binding reaction and room temperature was used throughout. Different adding orders of OG, HSA and B-R buffer were tested and the results showed that the best addition sequences were OG, B-R buffer and protein. This result indicated that the electronic coupling made OG bind to HSA.

The scan rate and the standing time of the mercury drop of the instrument for the assay were studied. The peak current increased with the increase of potential scan rate within 100~1000 mV s^{-1} and the mercury drop standing time from 5 to 22 s. When the dropping mercury time was more than 22 s, the mercury drop would fall down

automatically. But after 18 s, the shape of the voltammetric peak wasn't good, so the scan rate and the standing time were selected as 800 mV s^{-1} and 18 s, respectively.

Interference of coexisting substances

The interference of coexisting substances such as metal ions, amino acids, glucose et al on the determination were tested. The results were presented in Table 2. It can be seen that most substances didn't interfere with the proposed assay.

Different kinds of surfactants such as sodium dodecylsulfate (SDS), Tween-20, β-CD were added into the reaction solution and the results showed that they could greatly influence the changes of peak current, which indicated that it could compete with the binding reaction.

The effect of ionic strength was investigated by the addition of 0.10~0.60 mol L^{-1} NaCl into the mixed solution and the results proved to have significant effects on the interaction. The difference of peak current decreased with the increase of salt concentration, which indicated that the interaction of OG with HSA was mainly caused by electrostatic attraction. The electrostatic shielding effect reduced the binding between the dye and HSA, which was unbeneficial to the formation of OG-HSA complex and resulted in a decrease of signal.

Standard regression equation for different proteins

Different proteins were determined by this method under the optimal conditions and the standard linear regression equations for various proteins were listed in Table 3. The results showed that different proteins had different responses and the sensitivities were enough for routine sample determination.

Sample determination

The proposed method was further applied to determine the albumin content in healthy human serum sample according to the general procedure and the results were listed in Table 4. The results were closed to that of the traditional Coomassie Brilliant Blue G-250 (CBB G-250) spectrophotometric method. Therefore this new electrochemical detection method was reliable, practical and reproducible.

Table 2. Effect of foreign substances on the determination of 10.0 mg L^{-1} HSA

Coexisting Substance	Concentration (mg L^{-1})	Relative error (%)	Coexisting substance	Concentration (μmol L^{-1})	Relative error (%)
L-Lysine	10.0	4.94	Mg^{2+}	10.0	-0.22
L-Arginine	10.0	-0.90	Zn^{2+}	10.0	-1.20
L-Tryptophan	10.0	-3.58	Co^{2+}	10.0	1.73
L-Lencine	10.0	1.24	Cu^{2+}	10.0	-2.40
L-Valine	10.0	0.49	Cd^{2+}	10.0	0.84
L-Glutamine	10.0	2.39	Pb^{2+}	10.0	-1.20
Citric acid	10.0	-4.43	Fe^{3+}	10.0	-0.64
Glucose	10.0	0.80	Ni^{2+}	10.0	0.14

Table 3. Analytical parameters for the determination of different proteins

Protein	Linear range (mg L^{-1})	Linear regression equation	Detection limit (mg L^{-1})	Correlation coefficient (γ)
HSA	4.0~28.0	ΔIp″ (nA) = 15.93 C + 85.02	3.0	0.993
BSA	4.0~30.0	ΔIp″ (nA) = 8.26 C -12.80	3.5	0.994
BHb	2.0~25.0	ΔIp″ (nA) = 12.32 C + 61.78	1.0	0.988
OVA	2.0~20.0	ΔIp″ (nA) = 15.10 C + 65.32	1.0	0.996
Lipase	2.0~30.0	ΔIp″ (nA) = 11.37 C-23.64	1.0	0.991

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

Table 4. The results for the determination of HSA in human serum samples

Sample	This method (g L^{-1})	RSD (%, n=5)	Recovery (%)	CBB G-250 method (g L^{-1})	RSD (%, n=5)
1	130.24	1.19	91.5	133.16	2.25
2	72.16	4.89	103.4	73.12	0.97
3	97.12	2.87	104.4	100.68	1.77

Conclusions

In this paper the voltammetric method is used for the investigation of the interaction of OG with HSA and further used for the HSA determination. In the selected pH 2.0 acidic buffer solution the lysine, arginine and other amino acid residues in the HSA (iso-electric point p*I*=4.7) molecular chains are positively charged, while the OG species are negatively charged. So OG is attracted to HSA by electrostatic attraction firstly. Other weak responses such as ionic, van der waals and hydrogen bonding can enhance the binding interaction to form an electro-inactive supramolecular complex, which results in the decrease of free concentration of OG in the reaction solution and the decrease of the reductive peak current of the reaction solution. The electrochemical behaviours of OG and its interaction with HSA are carefully studied. Based on the decrease of peak current, a new electrochemical method is established for the determination of different kinds of proteins such as HSA, BSA, OVA, BHb and lipase. The method is simple, convenient, sensitive and is applied to the human serum samples determination with satisfactory results.

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