# Voltammetric albumin quantification based on its interaction with carminic acid

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Abstract: In a weak acidic Britton-Robinson (B-R) buffer of pH 4.0, human serum albumin (HSA) can react rapidly with carminic acid (CAA) to form a supramolecular complex. Microelectrostatic fields in the molecular structure of HSA cause the electrostatic binding reaction of the positively charged CAA with the negatively charged HSA. CAA has a sensitive, second order derivative linear sweep voltammetric reductive peak at -0.54 V (vs. SCE) on a dropping mercury electrode (DME). After the addition of HSA to a CAA solution, the reductive peak current of CAA decreased without a shift of the peak potential. Based on the decrease of the peak current, a sensitive voltammetric method for the determination of microamounts of the protein is proposed. The conditions for the binding reaction and the voltammetric detection were optimized. Under the optimal conditions, the decrease of the voltammetric detection were optimized. Under the optimal conditions, the decrease of the voltammetric peak current was in proportion to the quantity of HSA in the range of 2.0 to 70.0 mg l<sup>-1</sup>, with a linear regression equation  $\Delta I_n$ " (nA) = 11.74 C (mg l<sup>-1</sup>)-12.33 and a detection limit of 2.0 mg l<sup>-1</sup> ( $3\sigma$ ). The method was further applied to the detection of different kinds of proteins, such as bovine serum albumin (BSA), oval albunim (OVA), lipase, etc. The established method was further applied to the determination of the albumin content in human serum samples and the results obtained by this method were in good agreement with the traditional Coomassie Brilliant Blue G-250 (CBB G-250) spectrophotometric method. The binding mechanism is discussed and the binding ratio was calculated from the voltammetric data.

Keywords: carminic acid, human serum albumin, interaction, linear sweep voltammetry.

# INTRODUCTION

Proteins are important biomolecules in biochemistry and the interactions of proteins with various molecules such as drugs, dyes and metal complexes, have aroused great interest because of their importance in the explanation of the structure and function of proteins. Protein assay is also widely employed in clinical tests, food analysis

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and biochemistry. Many analytical methods, such as spectrophotometry, fluorometry and the light scattering technique, have been continuously developed for the determination of the concentration of a protein. <sup>1-6</sup> Most of them were based on the interaction of a chromophore with the protein. Different binding models have been proposed to elucidate the interaction between biological macromolecules and organic substances. Scatchard<sup>7</sup> treated the reaction of small ions with albumin as a combination of the ions with specific sites in the macromolecule. Pesavento<sup>8</sup> proposed a two-phase distribution model to interpret the binding of T-azo-R with bovine serum albumin (BSA) in acidic solution. Recently Gao<sup>9–11</sup> proposed a microelectric field model to explain the interaction of macromolecues with organic dyes. In aqueous solution, complex spatial structures such as winding, folds, coils and other arrangements of a protein can result in a lot holes, gullies and/or helical grooves. Many secondary bonds, such as van der Waals bonds, salt bonds, hydrogen bonds and other polar bonds, are formed. Hence, the protonization of the amino group in polar amino acids would form a positive electrostatic film and the carboxyl groups a negative one, which results in the formation of a microelectrostatic field, which could attract cations, such as spectral probes or charged stains, leading to the formation of a complex. The existence of micro electric fields is the basis of the interaction of a chromophore with a protein. Human serum albumin (HSA) is the most abundant plasma protein in serum and is involved in many physiological functions, such as transportation, modulation, inactivation of metabolites and drug binding. Based on the interaction of dyes with albumin, many quantitative methods for the determination of albumin have also been proposed. 12-14

The electrochemical method is a useful tool to determine the electrochemical redox response of biomolecules in biological samples. As the electrochemical reaction occurs at electrode/liquid surface, it is especially suitable for small amounts of sample and has the advantages of being simple, reliable and practicable with low detection limits and a wide dynamic range. The electrochemical behaviors of proteins have been widely reported in the litarature, for example, the electrochemical reduction process of the disulfide bond of insulin and albumin, as well as the catalytical hydrogen wave of proteins in the presence of metal ions, 15-19 Luo et al., 20 reported polarographic results of the Co(II)–HSA complex in the present of a guanidine modifier, which were further used for protein analysis. Song et al., 21–23 studied a new type of kinetic wave, termed parallel catalytic hydrogen wave, of HSA and BSA in the presence of different kinds of oxidants, such as KIO<sub>3</sub>, H<sub>2</sub>O<sub>2</sub> or potassium persulfate. However, compared with other analytical methods, there are seldom reports on electrochemical investigation of protein-dye interactions. Li<sup>24,25</sup> et al., studied the interaction of albumin and hemoglobin with 9,10-anthraquinone and tetraphenylporphyrin tetrasulfonate (TPPS). The interaction of Re(V) complexes with proteins has also been reported.<sup>26</sup> The application of alizarin red S, beryllon III and amaranth as electrochemical probes for the detection of HSA by linear sweep voltammetry has also been reported.<sup>27–29</sup> In this paper the results of a study of the interaction of carminic acid with proteins using the electrochemical method is reported for the first time. The method was further used for protein assay.

Carminic acid (CAA) is an anthraquinone dye with the molecular structure shown in Fig. 1. It is often used as an additive in foods and drinks. Hence, the interaction of carminic acid with albumin is important to explain its binding mechanism. In this study, the linear sweep voltammetric method was used to investigate the interaction of CAA with HSA and further used for the determination of various proteins. CAA has a sensitive linear sweep voltammetric reductive peak at -0.54 V ( $\nu s$ . SCE) on a dropping mercury working electrode (DME). After the addition of HSA to a CAA soltuion, the reduction peak current of CAA decreased markedly. The changes of the cathodic current signal of CAA were monitored in the presence and absence of a solution-phase protein. Under the optimal conditions, the decrease of the peak current was proportional to the concentration of different proteins. This new method was satisfactorily applied for the detection of the albumin content in human serum samples.

### EXPERIMENTAL

#### Apparatus

The second order derivative linear sweep voltammetric measurements were performed 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 the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum wire electrode as the auxiliary electrode. The UV-Visible absorption spectra were recorded using a Cary model 50 probe spectrophotometer (Varian, Australia). The pH values were measured with a pHs-25 acidimeter (Shanghai Leici Instrument Factory, China).

#### Reagents

Human serum albumin (HSA, Sigma), bovine serum albumin (BSA, Beijing Shuangxuan Microorganism Bio-products Company), bovine hemoglobin (BHb, Tianjin Chuanye Bio-chemical Products Company), horseradish peroxidase (HRP, Shanghai Lizhu Dongfeng Biological Company) and oval albumin (OVA, Sigma) were used without further purification. Stock solutions of the different proteins were prepared by dissolving them in doubly distilled water from an all-quartz still and stored at 0–4 °C.

Carminic acid (CAA) was purchased from Chroma (Schmid GmbH & Co). A 1.0 × 10<sup>-3</sup> mol l<sup>-1</sup> CAA solution was prepared by dissolving 0.1200 g CAA in 250 ml water. A 0.2 mol l<sup>-1</sup> Britton–Robinson (B–R) buffer solution was used to control the pH of the interaction solution. Coomassie Brilliant Blue G-250 (CBB G-250) was purchased from Shanghai Chemical Reagent Company and used for the traditional, spectrophotometric method of protein analysis according to a common procedure.<sup>30</sup>

All the other employed reagents were of analytical reagent grade and doubly distilled water from an all-quartz still was used throughout. All the experiments were carried out at 25  $\pm$  2 °C, except when otherwise stated.

#### Procedure

To a 10 ml colorimetric tube were added consequently 2.0 ml of pH 4.0 B–R buffer solution, 1.8 ml of  $1.0 \times 10^{-4}$  mol  $1^{-1}$  CAA solution and different concentration of HSA solution. The mixture was then diluted to 10 ml with water and mixed thoroughly. A blank solution without the addition of HSA was prepared in the same way. After 20 min for the reaction to occur, the second order derivative linear sweep voltammetric curve was recorded in the potential range between -0.10 and -0.90 V (vs. SCE) and the peak current of CAA at -0.54 V (vs. SCE) was measured. The current difference for the determinatio ( $\Delta I_p$ " =  $I_p$ 0"  $-I_p$ ") is the difference between the peak current of the blank ( $I_p$ 0") and the protein solution ( $I_p$ ").

For the determination of the albumin concentration in blood samples, healthy human sera, which were kindly provided by the Hospital of Qingdao University of Science and Technology, were diluted 4000 fold with water and used in the above procedure. The concentration of albumin in the human sera samples was obtained from the calibration graph of HSA. The results of the spectrophotometric determination of the albumin concentration in the human serum samples were obtained by the CBB G-250 method<sup>30</sup> with measurement of the absorbance at 595 nm.

#### RESULTS AND DISCUSSION

# Absorption spectra

The absorption spectra of CAA, HSA and their mixtures are shown in Fig. 2 interaction system. In pH 4.0 B–R buffer solution, CAA showed absorption peaks at 495 nm and 520 nm (curve 1), while HSA had no absorption peaks from 300 nm to 700 nm (curve 4). On adding HSA, the peak absorbance of CAA at 495 nm decreased. The more HSA was added, the greater the absorbance value decreased, which indicated that CAA interacted with HSA to form a complex (curve 2 and 3).

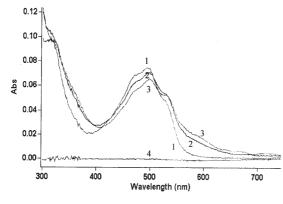
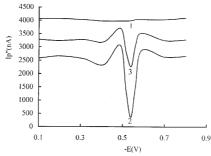


Fig. 2. Abosrption spectra of the CAA–HSA reaction solutions.

1. pH 4.0 B–R buffer+1.8×10<sup>-5</sup> mol l<sup>-1</sup> CAA; 2. pH 4.0 B–R buffer+1.8×10<sup>-5</sup> mol l<sup>-1</sup> CAA + 100.0 mg l<sup>-1</sup> HSA; 3. pH 4.0 B–R buffer+1.8×10<sup>-5</sup> mol l<sup>-1</sup> CAA + 200.0 mg l<sup>-1</sup> HSA; 4. pH 4.0 B–R buffer + 100.0 mol l<sup>-1</sup> HSA

Linear sweep voltammogram of CAA-HSA interaction solution

In order to improve the sensitivity of the determination, second order derivative linear sweep voltammetry was used as the peaks were sharper compared to those obtained by traditional normal linear sweep voltammetry. Typical voltammograms of this reaction system are shown in Fig. 3. Curve 1 is the voltammogram of the B–R buffer solution and no voltammetric peaks were registered. Curve 2 is the voltammogram of the CAA solution, which shows a well-defined second order derivative linear sweep voltammetric reduction peak at –0.54 V (vs. SCE), which was due to the electrochemical reduction of the quinone group in the molecular structure of CAA. Curve 3 is the voltammogram of CAA solution with HSA. After the addition of HSA to the CAA solution, the second order derivative linear sweep voltammetric reduction peak current of the CAA decreased significantly, without a shift of the peak potential, which indicates that an interaction of CAA with HSA occurred.



350 300 250 200 150 1.5 2.5 3.5 pH on the difference of

Fig. 3. Second order derivative linear sweep voltammograms of the CAA– HSA reaction solution. 1. pH 4.0 B–R buffer; 2. pH 4.0 B–R buffer+1.8×10<sup>-5</sup> mol l<sup>-1</sup> CAA; 3. pH 4.0 B–R buffer+1.8×10<sup>-5</sup> mol l<sup>-1</sup> CAA + 25.0 mg l<sup>-1</sup> HSA; scan rate: 600 mV s<sup>-1</sup>.

the peak current.  $2.0\times10^{-5}$  mol l<sup>-1</sup> CAA + 25.0 mg l<sup>-1</sup> HSA in different pH B–R buffer.

The reason for the decrease of the reduction peak current after reaction of CAA with HSA may be due to either a competitive adsorption between CAA and HSA on the mercury electrode or to the interaction of CAA with HSA to form a biosupramolecular complex. Li *et al.*, studied the interaction of electro-active small molecules, such as 9,10-anthraquinone, rutin, tetraphenylporphyrin tetrasulfonate (TPPS) with biomolecules, such as hemoglobin, albumin and antibody.<sup>24,25</sup> The results showed that at low concentrations of protein and short accumulated time, the coverage of the electrode surface was only about 10 % of the total electrode area. Hence, competitive adsorption between the small molecule and the protein could hardly exist. Since CAA is negatively charged under acidic conditions, while the HSA molecular chains are positively charged, CAA and HSA could be easily electrostatically bound together to form a supramolecular biocomplex. The formation of the complex results in a decrease in the CAA concentration in the reaction solution, and hence the reduction peak current of CAA decreased. The dif-

ference between the peak current of CAA and CAA–HSA solution was further used to determine the concentration of HSA. The peak potential of CAA is at -0.54 V (vs. SCE) and it was not disturbed by the polarographic reduction wave of the oxygen in the aqueous solution, which often appears at -0.20 V. Hence, it was unnecessary to deaerate the reaction solution and so the proposed method has the advantage of easily handle without the trouble of bubbling the solutions.

### Optimization of the reaction conditions

The influence of pH on the interaction of CAA and HSA was investigated and the result are shown in Fig. 4. It can be seen that  $\Delta I_p$ " reached its maximum at pH 4.0. Hence, this pH of B–R buffer solution was chosen for the assay. The optimal volume of B–R buffer solution to be present in 10 ml final solution was examined and when the volume of buffer solution was 2.0 ml, the difference of the peak current ( $\Delta I_p$ ") reached a maximum. Hence, 2.0 ml of pH 4.0 B–R buffer solution was chosen for this study.

The difference in the magnitude of the peak current reached a maximum about 15 min after the solutions of CAA and HSA had been mixed and remained unchanged for at least 2 h. Therefore, there was enough time for routine measurements. The effect of the reaction temperature was tested at 20 °C, 25 °C and 37 °C, and the results showed that there was no obvious difference between them. Hence, the temperature had little influence on the binding reaction and 25 °C was used throughout.

The effect of changing the mixing order of CAA, HSA and B–R buffer was tested and the results showed that the best addition sequence was CAA, B–R buffer and protein. This result indicates that CAA binds to HSA by electronic coupling.

The instrumental conditions, *i.e.*, the standing time of the dropping mercury electrode and the potential scan rate for the assay, were studied. The peak current increased with increasing standing time of the dropping mercury in the range from 3 to 20 s and increasing scan rate in the range from 300 to 800 mV s<sup>-1</sup>. Hence, a dropping mercury standing time of 18 s and a potential scan rate of 600 mV s<sup>-1</sup> were selected.

Discussion of interaction mechanism and determination of the stoichiometry of the HSA-mCAA complex

Generally speaking, there are two kinds of reaction modes between small molecules and a protein. One is electrostatic binding and the other hydrophobic interaction. In pH 4.0 acidic solution the lysine, arginine and other amino acid residues in the HSA molecular chain (isoelectric point pI = 4.7) are positively charged. At the same time the winding, folds, coils and other arrangements of HSA lead to many holes, gullies and helix grooves, which have a high charge density in their vicinity due to the proximity of many secondary bonds, such as salt bonds and hydrogen bonds. The formed microelectrostatic field of HSA can attract negatively

charged organic compound, such as the CAA species in the buffer solution. Hence, it is possible that CAA interacts with HSA by electrostatic attraction and other weak responses, such as ionic, van der Waals and hydrogen bonding, to form a supramolecular complex.

According to a method proposed by Li, $^{31}$  the stoichiometry of HSA-CAA complex can be calculated on the basis of the changes of the peak current, assuming that HSA and CAA form a single complex of stoichiometry HSA-mCAA:

$$HSA + mCAA \rightleftharpoons HSA - mCAA$$

The equilibrium constant  $\beta_s$  is given by:

$$\beta_{\rm S} = \frac{[{\rm HSA} - m{\rm CAA}]}{[{\rm HSA}][{\rm CAA}]^m} \tag{1}$$

where [HSA], [CAA] and [HSA–*m*CAA] correspond to the concentration of HSA, CAA and complex respectively.

In addition

$$\Delta I_{\text{max}} = k c_{\text{HSA}} \tag{2}$$

$$\Delta I = k[\text{HSA-}m\text{CAA}] \tag{3}$$

$$[HSA] + [HSA - mCAA] = c_{HSA}$$
 (4)

Where  $\Delta I$  is the peak current difference in the presence and absence of HSA,  $\Delta I_{\rm max}$  corresponds to the peak current value when the concentration of CAA is much higher than that of HSA.  $c_{\rm HSA}$  the concentration of bound protein in the solution and k is a constant.

Therefore:

$$\Delta I_{\text{max}} - \Delta I = k(c_{\text{HSA}} - [\text{HSA} - m\text{CAA}] = k[\text{HSA}]$$
 (5)

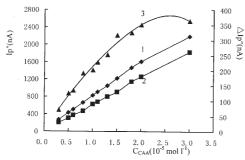
Introducing Eqs. (2), (4) and (5) into Eq. (1) and rearranging gives:

$$\lg \left[ \Delta I / (\Delta I_{\text{max}} - \Delta I) \right] = \lg \beta_{\text{S}} + m \lg \left[ \text{CAA} \right]$$
 (6)

According to Eq. (6), the composition of the supramolecular complex and the equilibrium constant can be calculated.

In Fig. 5 curve 1, the relationship between the measured peak current  $I_p$ ", and the concentration of CAA in the absence of HSA is shown. Curve 2 represents the current  $I_p$ " after the addition of 25.0 mg l<sup>-1</sup> HSA to various concentrations of CAA. Curve 3 was plotted from the differences ( $\Delta I_p$ ") between curve 1 and 2 ( $I_p$ ",  $I_p$ ", and the concentration of CAA.  $I_{max}$  was experimentally obtained as 350 nA,  $I_{max}$  was read from curve 3 of Fig. 5 and then the relationship lg [ $I_p$ ( $I_p$ ( $I_p$ )] vs. lg [CAA] was plotted. The resulting linear plots shown in Fig. 6. The line has a

slope of 2 (*i.e.*, m=2) and an intercept of  $\lg \beta_s = 11.00$ , which indicated that a stable 1:2 complex of HSA–2CAA was formed in the studied concentration range of CAA with respect to the concentration of HSA and the binding constant  $\beta_s$  was calculated as  $1.00 \times 10^{11}$ .



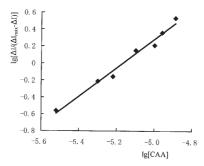


Fig. 5. The plot of  $I_{\rm p}$ " against  $c_{\rm CAA}$  (1,2),  $\Delta I_{\rm p}$ " with  $c_{\rm CAA}$  (3) 1.  $c_{\rm HSA}$  = 0; 2.  $c_{\rm HSA}$  = 25.0 mg l<sup>-1</sup>; 3.  $\Delta I_{\rm p}$ " =  $I_{\rm p}$ "<sub>1</sub> -  $I_{\rm p}$ "<sub>2</sub>.

Fig. 6. The plot of  $\lg \left[\Delta I/(\Delta I_{\rm max} - \Delta I)\right]$  against  $\lg \left[{\rm CAA}\right]$ .

Equation (6) can also be rearranged to:

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

The relationship of  $1/\Delta I$  vs.  $1/[\text{CAA}]^m$  could be regressed by iterative programming. The initial value of  $\Delta I_{\text{max}}$  was given by the experimental data as 350 nA. It was assumed that CAA and HSA formed a single complex and that m was 1, 2 or 3, from the plot of the curve  $1/\Delta I$  against  $1/[\text{CAA}]^m$ ,  $\beta_s$  and  $\Delta I_{\text{max}}$  were obtained and results are shown in Table I. If the calculated value  $\Delta I_{\text{max}}$  is near to the experimental value, then the assumed m was correct. Obviously, the composition of the supramolecular complex was HSA: CAA = 1: 2 because when m = 2, the calculated value  $\Delta I_{\text{max}} = 303$  nA and the equilibrium constant  $\beta_s = 3.30 \times 10^{10}$  approached the experimental values the most closely.

TABLE I. The calculated results for HSA-mCAA

Assumed value/m	$\Delta I_{ m max}/{ m nA}$	$eta_{ m s}$	γ
1	1110	$2.25 \times 10^4$	0.994
2	303	$3.30 \times 10^{10}$	0.981
3	250	$1.33 \times 10^{16}$	0.941

Analytical parameters of different proteins

Under the optimal conditions, this method was applied to determine the concentration of some proteins, such as human serum albumin (HSA), bovine serum albumin (BSA), lipase, *etc*. Obvious linear relationships were found between the decrease of the peak current and the concentration of a protein. The linear regression equations for different proteins are listed in Table II. The results show that the

different proteins had different responses and that the sensitivities were sufficient for routine determination of samples.

TABLE II. The working curves for the determination of different proteins

Protein	Linear range mg l <sup>-1</sup>	Regression equation	Detection limit mg l <sup>-1</sup>	Correlation coefficient
HSA	2.0-70.00	$\Delta I_{\rm p}$ " (nA) = 11.74 c (mg l <sup>-1</sup> ) – 12.33	2.0	0.97
	80.0–350.0	$\Delta I_{p}$ " (nA) = 1.23 c (mg l <sup>-1</sup> ) + 745.33		0.982
BHb	1.0-60.0	$\Delta I_{p}$ " (nA) = 13.14 c (mg l <sup>-1</sup> ) + 43.78	1.0	0.994
	80.0–350.0	$\Delta I_{p}$ " (nA) = 0.48 c (mg l <sup>-1</sup> ) + 774.11		0.991
OVA	0.5–10,0	$\Delta I_{p}$ " (nA) = 9.05 c (mg l <sup>-1</sup> ) + 85.39	0.5	0.997
	10.0–100.0	$\Delta I_{p}$ " (nA) = 9.04 c (mg l <sup>-1</sup> ) + 72.52		0.996
HRP	1.0-10.0	$\Delta I_{\rm p}$ " (nA) = 17.24 c (mg l <sup>-1</sup> ) - 13.78	1.0	0.991
	15.0-50.0	$\Delta I_{p}$ " (nA) = 3.17 c (mg l <sup>-1</sup> ) + 158.12		0.973
BSA	3.0-60.00	$\Delta I_{p}$ " (nA) = 14.51 c (mg l <sup>-1</sup> ) + 12.93	3.0	0.992
	70.0–300.0	$\Delta I_{\text{p}}$ " (nA) = 0.93 c (mg l <sup>-1</sup> ) + 847.65		0.986

<sup>\*</sup>HSA (Human serum albumin), BHb (Bovine hemoglobin), OVA (Oval albumin), HRP (Horseradish peroxidase), BSA (Bovine serum albumin)

# Effect of coexisting substances

The effects of coexisting substances, such as metal ions, amino acids, glucose and surfactants, on the determination of HSA were tested by premixing HSA with the interference substances and the results are shown in Table III. Most of them, such as the metal ions, commonly found in blood or amino acids, could be allowed at higher concentrations and good selectivity could be obtained by this method. The addition of some surfactants, such as sodium dodecylsulfate (SDS), cetryl-trimethylammonium bromide (CTMAB), Tween-20,  $\beta$ -cyclodextrin ( $\beta$ -CD), can greatly influence the value of  $\Delta I_p$ °, which indicated that they could greatly influence the complex formation, or they could be significantly adsorbed on the surface of the mercury electrode.

The effect of NaCl concentration in the range of  $0.1-0.6~\text{mol}~l^{-1}$  on this assay was also examined and the results proved that the NaCl concentration has a signifi-

cant influence on the complex formation. The  $\Delta I_p$ " decreased with increasing salt concentration, which indicates that the interaction of CAA with HSA is mainly caused by electrostatic attraction. The electrostatic shielding effect of the charges on the binding reaction with increasing Na<sup>+</sup> concentration was detrimental to the formation of the HSA–CAA complex.

TABLE III. Tolerance of coexisting substances on the determination of 20.0 mg l<sup>-1</sup> HSA

Coexisting substance	Concentration µg ml <sup>-1</sup>	Relative error	Coexisting substance	Concentration µmol 1 <sup>-1</sup>	Relative error
L-Glutamine	50.0	3.70	$Mn^{2+}$	200.0	0.37
L-Valine	50.0	3.80	$Ca^{2+}$	200.0	0.12
L-Tryptophan	50.0	1.80	$Co^{2+}$	20.0	1.90
L-Leucine	50.0	4.10	$Cu^{2+}$	200.00	1.05
L-Serine	50.0	3.80	Ni <sup>2+</sup>	200.0	0.52
L-Lysine	50.0	5.00	$\mathrm{Ba}^{2+}$	200.0	-0.47
L-Tyrosine	50.0	4.40	$Fe^{3+}$	200.0	1.15
Citric acid	10.0	-0.90	$Pb^{2+}$	200.0	-0.47
Glucose	800.0	-0.21	$\mathrm{Mg}^{2+}$	200.0	0.97
Tween-20	50.0	-46.10	$\beta$ -CD	50.0 μg ml <sup>-1</sup>	- 63.36
SDS	50.0	- 74.67	CTMAB	50.0 μg ml <sup>-1</sup>	- 95.27

# Sample determination

Under the recommended conditions, the proposed method was further applied to determine the albumin content in healthy human serum samples according to the general procedure and the results are listed in Table IV. It can be seen that the results were in good agreement with those obtained by the traditional Coomassie Brilliant Blue G-250 spectrophotometric method. The recovery of HSA was in the range of 93.3–101.0 %. Therefore, the detection of the protein by this new electrochemical method was practicable, reliable and reproducible.

TABLE IV. The results of the determination of HSA in human serum samples

Human serum samples	This method $(n = 5)$			CBB G-250 method ( $n = 5$ )	
samples	Total amount of protein / g l <sup>-1</sup>	RSD/%	Recovery/%	Total amount of protein/g l <sup>-1</sup>	RSD/%
1	112.6	0.30	93.3	106.6	3.22
2	103.8	0.36	98.7	97.1	5.66
3	94.2	5.60	101.0	93.6	4.79

#### **CONCLUSIONS**

The interaction of CAA with HSA was electrochemically investigated in this study. Under the applied experimental conditions, a supramolecular complex of HSA–2CAA was formed, which resulted in a decrease of the reduction peak current of CAA. The decrease of peak current can be further applied for the determination of the concentration of HSA in human serum samples with good selectivity and a low detection limit.

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#### извод

# ВОЛТАМЕТРИЈСКА КВАНТИФИКАЦИЈА АЛБУМИНА ЗАСНОВАНА НА РЕАКЦИЈИ СА КАРМИНСКОМ КИСЕЛИНОМ

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У слабокиселој средини Бритн-Робинсоновог пуфера хумани серумски албумин (HSA) реагује са карминском киселином (КК) уз брзо формирање супрамолекуларног комплекса. Микроелектростатичко поље у молекуларној структури HSA узрокује реакцију електростатичког везивања позитивно наелектрисане КК са негативно наелектрисаним HSA. Применом деривативне линеарне волтаметрије добијен је редукциони пик другог реда, у раствору КК, на потенцијалу - 0.054 V (према SCE) на капљућој живиној електроди. Додатком НЅА у раствор који садржи КК долази до снижења струје пика и померања потенцијала. На основу пада струје пика предложена је осетљива волтаметријска метода за одређивање микро количина протеина. Оптимизовани су услови реакције везивања и волтаметријске детекције. Под оптималним условима смањење струјног пика је сразмерно количини HSA у опсегу 2.0 - 70.0 mg/L и детекционом границом од 2.0 mg/L ( $3\sigma$ ). Метода је примењена за одређивање садржаја албумина у узорцима хуманог серума и добијени резултати су у доброј сагласности са традиционалном Coomassie Brilliant Blue G-250 (СВВ G-250) спектрофотометријском методом. Дискутован је механизам везивања и израчунат однос реактаната при везивању на основу волтаметријских података.

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