Chinese Chemical Letters Vol. 14, No. 12, pp 1275 – 1277, 2003 1275
http://www.imm.ac.cn/journal/ccl.html

Studies on the Interaction of Beryllon Ⅲ with Proteins by Voltammetric Technique and its Application

Wei SUN[1,2], Kui JIAO[2,*], Xue Liang WANG[2], Lu De LU[1]

[1]College of Chemical Engineering, Nanjing University of Science and Technology, Nanjing 210094
[2]College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042

Abstract: A new quantitative determination method of proteins using beryllon Ⅲ by voltammetric technique was developed in this paper. In pH 3.5 Britton-Robinson (B-R) buffer solution, beryllon Ⅲ can bind with human serum albumin (HSA) to form an electro-inactive supermolecular complex. Beryllon Ⅲ has a well-defined voltammetric reduction peak at –0.38 V (*vs*. SCE) and the addition of protein in this solution can cause the decrease of the reductive peak current. Based on the decrease of the reduction peak current, a new electrochemical method for the determination of HSA was established with linear range of 1.0~40.0 mg/L and the detection limit of 1.0 mg/L. This method is further applied to the determination of real sample of healthy human serum.

Keywords: Beryllon Ⅲ, serum albumin, voltammetry, binding reaction.

The determination of protein is a very important requisite in biochemistry and clinical diagnosis. Many methods have been developed for the determination of serum proteins such as spectrophotometry[1], fluorometry[2] and Rayleigth light scattering technique[3]. But there are seldom electrochemical methods for the detection of protein concentration in human serum sample. Electrochemical assay is a simply, reliable, practical method with lower detection limit and wider dynamic range. Because the electrochemical reaction occurs at the electrode/liquid surface, it is especially suitable for a little amount of sample and avoids the interferences from color or turbid biological substances in the sample that plague the spectroscopic technique. In this paper, a new electrochemical method for the determination of proteins was developed and further applied to the real sample determination with satisfactory result.

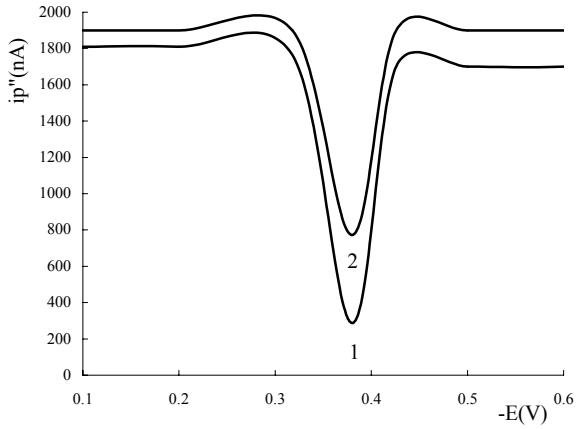
The binding reaction of organic dyes with protein is based on the weak interactions such as ionic, Van der Waals, hydrogen bonding and hydrophobic effect. In an acidic solution, the lysine, arginine and other amino acid residues on the serum albumin molecular chains are positively charged, while beryllon Ⅲ species is negatively charged, so it would be easy for them to bind together by electrostatic attraction to form a super-molecular complex. Some experiments such as electro-capillary curve, diffusion coefficients determination, electron transferred number *et al* were carried out in the

* E-mail:Kjiao@qust.edu.cn

absence and presence of human serum albumin (HSA), respectively, to examine the changes of electrochemical parameters of the reaction system. On the addition of HSA, the reductive current of beryllon Ⅲ decreased apparently, due to the formation of electro-inactive complex. The concentration of beryllon Ⅲ in solution is decreased and it is difficult for beryllon Ⅲ to take place the reductive reaction in this complex on the surface of a mercury electrode. So the reduction current of beryllon Ⅲ is decreased. The decrease of peak current is proportional to the concentration of protein in the range of 1.0~40.0 mg/L, so this new method can be applied to the determination of the healthy human serum albumin, the content of which is in the range of 50.0~100.0 mg/mL.

Figure 1 shows the second order derivative linear sweep voltammograms of this binding reaction system. It can be seen that before the addition of the protein to beryllon Ⅲ solution, beryllon Ⅲ has a well-defined reductive peak at –0.38 V (*vs*.SCE). The addition of HSA to this solution leads to the decrease of the peak current with little movement of the reductive peak potentials. So the difference of peak current ($\Delta ip''=ip_0''- ip''$) was used to determine the concentration of protein.

Figure 1 The typical second order derivative linear sweep voltammetric curves of beryllon Ⅲ (curve 1) and mixture of beryllon Ⅲ with HSA (curve 2)



The concentration of beryllon and HSA are 1.5×10^{-5} mol/L and 10 mg/L, respectively, at pH 3.5 Britton-Robinson (B-R) buffer solution.

The conditions for the binding reaction were optimized. The pH of the buffer solution greatly influences the interaction and at pH 3.5 of Britton-Robinson (B-R) buffer solution the difference of peak current reaches its maximum. So pH 3.5 was chosen as the suitable pH. Under pH 3.5, the reaction occurs rapidly at room temperature (<10 min) and the peak current remains stable for at least 2 hours, which is enough for routine determination.

The influences of coexisting substances such as metal ions, amino acids and glucose were studied. The results were listed in **Table 1** and it can be seen that few of them influenced the assay results and good selectivity can be achieved. But the addition of some surfactants such as cetyl trimethyl ammonium bromide, Tween-20 and

sodium dodecyl sulfate greatly influenced the value of $\Delta ip''$.

Table 1 The influences of coexisting substances, C_{HSA}=10 mg/L

Substances	Cd^{2+}	Cu^{2+}	Co^{2+}	Ba^{2+}	Gln	Lys	Gys	Val	Ser	Glucose	C.A.*
	μg/mL				μg/mL						
Concentration	20	20	20	20	5	5	5	5	5	80	10
Error (%)	-4.2	1.3	-4.6	-4.4	0.49	0.04	0.78	2.7	1.8	-3.3	-4.5

*Citric acid

Under the optimal conditions, a calibration curve for HSA was obtained and good linearity was got in the range of 1.0~40.0 mg/L with the linear regression equation as $\Delta ip''$ (nA) =179.14 + 42.115 C (μg/mL) (n=11, γ=0.9987). The relative standard deviation for 30.0 mg/L HSA was 2.21% (11 replication determinations) and the detection limit was 1.0 mg/L.

The healthy human serum albumin sample is measured with this method and compared with the traditional Coomassie brilliant blue G-250 (CBB G-250) method, the results are in great accordance with each other.

Table 2 Assay results of protein in human serum sample (n≥3)

Method	Content (mg/mL)	Recovery (%)
This method	93.16	99.66
CBB G-250	92.17	

Acknowledgment

The work was supported by the National Natural Science Foundation of China (Grant No. 20075013) and the Younger Foundation of Qingdao University of Science and Technology (Grant No. 22000219).

References

1. Y.J. Wei, K. A. Li, S. Y. Tong, *Talanta.*, **1997**, *44*, 923.
2. N. Li, S. Y. Tong, *Anal. Biochen.*, **1996**, *233*, 151.
3. Q.C. Ma, K. A. Li, S. Y. Tong, *Chin. Chem. Lett.*, **1997**, *8*, 57.
4. M. Bradford, *Anal. Biochem.*, **1976**, *72*, 248.

Received 15 November, 2002