Chinese Chemical Letters Vol. 13, No. 11, pp 1093 – 1096, 2002
http://www.imm.ac.cn/journal/ccl.html

Flow Injection Analysis of Histidine with Enhanced Electrogenerated Chemiluminescence of Luminol

Lian De ZHU, Ying Xiu LI, Guo Yi ZHU*

State key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022

Abstract: A simple and sensitive flow injection method is presented for the determination of histidine based on its enhancement of electrogenerated chemiluminescence (ECL) of luminol. After optimization of the experimental parameters, the working range for histidine was in 1.0 x 10^{-6} to 1.0 x 10^{-3} mol/L with a detection limit (S/N = 3) of 0.56 μmol/L. The relative standard deviation was 1.6% for 11 measurements of 5 x 10^{-5} mol/L histidine solution. The proposed method has been successfully applied to the determination of histidine in real pharmaceutical preparation.

Keywords: Electrogenerated chemiluminescence, flow injection analysis, luminol, histidine.

Histidine is one of the necessary basic amino acids in biological bases, which often controls the catalytic activity of enzymes and acts in holding the higher structure of proteins. Furthermore, it is also an important ingredient in pharmaceutical preparations used for treatment of hetpatosis and nephropathy. Therefore, the determination of histidine in biological fluids and pharmaceutical preparations is of great importance. Various methods have been proposed for the detection of histidine such as, amperometry[1], potentiometry[2], liquid chromatography with UV-absorbance[3,4] or fluorometric detection[4,5], electrophoresis with electrochemical detection [6] and chemiluminescence (CL) analysis[7-9]. Some of those methods lack sensitivity or require complex derivatization procedures. We have observed for the first time that histidine could enhance the ECL of luminol. Based on the enhancement effect, a simple, sensitive and rapid flow injection method for the determination of histidine was developed. The results of the method were superior to those obtained by tris (2, 2′ – bipyridyl) ruthenium (II) ECL system[10, 11].

Experimental

Reagents and apparatus

Luminol was purchased from Fluka (>98%). Histidine and other amino acids were

* E-mail: zhuguoyi@ciac.jl.cn

obtained from Shanghai Chemical Reagent Company. All other reagents were of analytical grade or better and used without purification and doubly distilled water was used throughout.

A peristaltic pump was used to deliver carrier (water), luminol and sample solutions through the system. Injection was made using a six-port valve injector equipped with a 120 μL injection loop. A home made ECL flow cell consisted of a polytetrafluoro-ethylene (PTFE) block fitted with a working glassy carbon electrode (6 mm in diameter) and a plexiglass window. A 0.5 mm thick PTFE film (spacer) was inserted between the electrode and the plexiglass. An Ag wire pseudo reference electrode and a stainless steel counter electrode were also assembled in the flow cell. The entire flow cell was placed in a light-tight box. A quartz optical fiber bundle was used to collect and transport the light emission. One end of the optical fiber bundle was directly faced to the working electrode separated by the plexiglass window; the other end was set in front of the photomultiplier tube (PMT) of a luminometer constructed in this laboratory. All ECL measurements (expressed in arbitrary units, a.u.) were recorded on an X-Y-t recorder. A BI-PAD model potentiostat (Tacussel, France) was used for maintaining the potential to induce chemiluminescence.

Procedure

Luminol solution contained in 0.1 mol/L borate buffer and carrier solution were pumped continuously at 2.0 mL/min by the peristaltic pump to a mixing valve, and then the merged stream was introduced into the ECL flow cell while a suitable positive electrode potential was applied by the potentiostat. A continuous blank signal of luminol ECL was observed. Histidine solution was injected by the injector when the blank signal tended to stabilize. The ECL signal was recorded and the concentration of histidine was quantitated by the peak height of the enhanced ECL intensity above the blank signal.

Results and Discussion

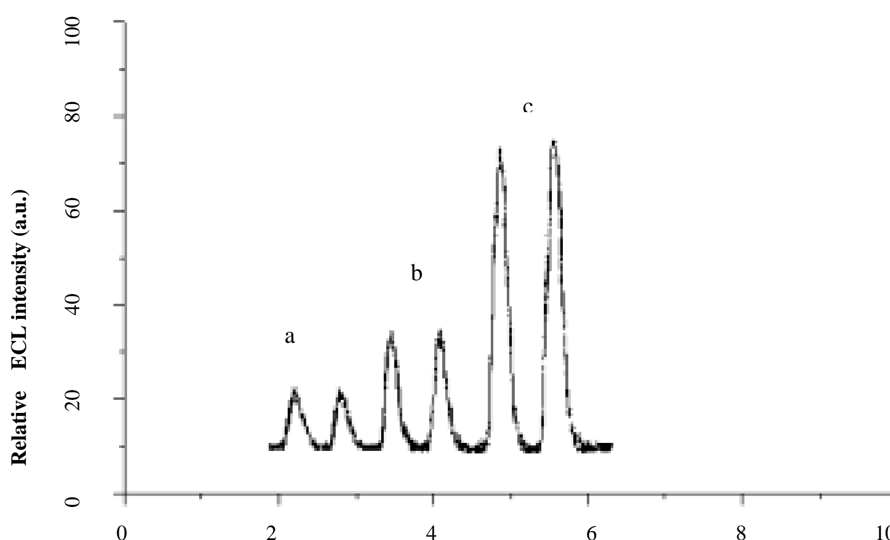
In aqueous alkaline solution containing oxygen, luminol ECL can be triggered by a positive electrode potential in the absence of hydrogen peroxide[12, 13]. In the preliminary study, we found that ECL of luminol could be significantly enhanced in the presence of histidine. A series of positive ECL peaks were obtained with respect to injections of different concentrations of histidine which were shown in **Figure 1**. The ECL peak height was used to quantify the concentration of histidine.

The influence of electrode potential (E) on the enhancement of ECL intensity was studied in the range of 0.2 – 1.0 V *vs.* Ag pseudo reference electrode. It could be seen that the enhanced ECL intensity increased with the increase of electrode potential, however, the enhancement of ECL intensity changed very slightly when the potential was beyond 0.8 V. Therefore, 0.8 V was selected as the optimal electrode potential for the following experimentation.

The effect of pH value of the buffer solution on the ECL intensity was also investigated in the range of 10.0 - 12.5. The enhanced ECL intensity increased with the

increase of pH value until 11.5, and then began to decrease gradually. Therefore, pH value of 11.5 was selected for further study.

Figure 1: Enhancement of luminol ECL in the presence of histidine (a) $5x\ 10^{-6}$ mol/L, (b) 1×10^{-5} mol/L, (c) 5×10^{-5} mol/L; [luminol] = 50 µmol/L, pH = 11.5; electrode potential: 0.8 V *vs* Ag pseudo reference.



The effect of luminol concentration on the ECL intensity was studied in the range of 5×10^{-6} – 5×10^{-4} mol/L. The enhanced ECL intensity increased with the concentration of luminol and changed little at concentrations higher than 5.0×10^{-5} mol/L. It was well known that electrode fouling was much more severe when the luminol concentration was above 0.1 mmol/L [14]. Thus, a relative lower value of 50 µmol/L was chosen in the following studies in order to acquire reproducible and stable ECL signal.

The influence of pump speed was also investigated in detail. With the increase of pump speed, the enhanced ECL intensity increased straightly and thus the consumption of reagents. An intermediate pump speed has to be compromised between those two opposite factors and 2.0 mL/min was selected in this study.

Under the selected conditions described above, the calibration curve for histidine was obtained in the range of 1.0×10^{-6} mol/L to 1.0×10^{-3} mol/L with a correlation coefficient of 0.992. The standard deviation was 1.6% for 11 measurements of 5×10^{-5} mol/L histidine standard solution. The detection limit at a signal to noise ratio of 3 was 5.6×10^{-7} mol/L.

The other nineteen amino acids in human body were tested for their effects on the determination of histidine. The results showed that the tolerable concentration ratio with respect to 1.0×10^{-5} mol/L histidine were 500 for glutamine, glutamic acid and

glycine, 100 for alanine, valine, serine and threonine, 50 for aspartic acid, 10 for asparagine, phenyl alanine, leucine, isoleucine, 5 for methionine, 2 for lysine and proline, 1 for arginine and tryptophan if allowed the signal variations within 10%. Equal amounts of cysteine and tyrosine would interfere with the detection of histidine. Tyrosine caused negative while cysteine caused positive interference, respectively.

A pharmaceutical injection containing nine kinds of amino acid, which was used for treatment of nephropathy (Amino Acid 9R compound Injection, *Shenbian Injection*), was employed to validate the analytical application of the proposed method. The results are listed in **Table 1**. It can be seen that the detected value agreed well with the reference one as well as with satisfactory recovery.

Table 1 Determination of histidine in a pharmaceutical preparation

Detected[a] (mg/mL)	Added (mg/mL)	Recovered (mg/mL)	Recovery (%)	Reference (mg/mL)
4.37 ± 0.08	5.00	9.26	97.8	4.40

[a] Mean of three measurements

References

1. G. N. Chen, X. P. Wu, J. P. Duan, H. Q. Chen, *Talanta*, **1999**, *49*, 319.
2. K. A. Mohamad, S. Said, T. Shahram, *Anal. Chem.*, **1999**, *71*, 2502.
3. S. Wadud, R. Omodera, M. M. Or-rashid, M. R. Amin, *J. AOAC. Int.*, **2000,** *83*, 8.
4. V. Fratti, C. Lionetti, *J. Chromatogr. A*, **1998**, *809*, 241.
5. N. Tateda, K. Matsushisa, K. Hasebe, N. Kitajima, T. Miura, *J. Chromatogr. B*, **1998**, *718*, 235.
6. T. Kapps, P. Schierle, P. C. Hauser, *Anal. Chim. Acta*, **1999**, *393*, 77.
7. S. R. Spurlin, M. M. Cooper, *Anal. Lett.*, **1986**, *19*, 2277.
8. S. Y. Liao, Y. C. Chao, C. W. Whang, *J. High Resol. Chromatogr.*, **1995**, *18*, 667.
9. Y. Zhang, B. Huang, J. Cheng, *Anal. Chim. Acta*, **1998**, *363*, 157.
10. S. N. Brune, D. R. Bobbitt, *Anal. Chem.*, **1992**, *64*, 166.
11. W. Y. Lee, T. A. Nieman, *Anal. Chem.*, **1995**, *67*, 1789.
12. Y. G. Sun, H. Cui, X. Q. Lin, *Acta Chim. Sinica* (Chinese), **2000**, *58*, 567.
13. R. Wilson, H. Akhavan-Tafti, R. DelSilva, A. P. Schaap, *Electroanalysis*, **2001**, *13*, 1083.
14. J. P. Preston, T. A. Nieman, *Anal. Chem.*, **1996**, *68*, 966.

Received 7 February, 2002