Full Paper

Highly Sensitive Electrogenerated Chemiluminescence Detecting Ranitidine Based On Chemically Modifying Microenvironment of the Chemiluminescence Reaction

Xingwang Zheng, Yingjuan Qu, Zhujun Zhang, Chunmei Zhang*

College of Chemistry and Materials Science, Shaanxi Normal University, Xi 'an 710062, P. R. China
*e-mail: zhengxw@snnu.edu.cn

Received: August 15, 2004
Accepted: October 18, 2004

Abstract

Using a graphite electrode modified with vaseline and NiO, ranitidine showed a strongly ECL enhancing effect for the weak ECL signal of electrooxidation of luminol. Based on this finding, a more sensitive ECL method for ranitidine was firstly proposed. Under the optimum experimental conditions, the ranitidine hydrochloride concentration in the range of $3.0 \times 10^{-8} - 9.0 \times 10^{-6}$ mol/L was proportional to the enhancing ECL signal and offered a 9×10^{-9} mol/L detection limit for ranitidine hydrochloride. At the same time, based on the investigation on this ECL reaction mechanism, a new concept, to improve the suitable ECL reaction micro-environment with chemically modified electrode technique for the better analytical performances of ECL analysis was also firstly proposed.

Keywords: Ranitidine hydrochloride determination, Chemically modified graphite electrode, Electrogenerated chemiluminescence, Luminol, Nickel oxide

1. Introduction

Ranitidine hydrochloride acts as histamine H_2-receptor antagonist and is used medicinally in the treatment of gastric hyperacidity and peptic ulceration [1]. Many methods such as polarography [2] differential pulse polarography [3], capillary electrophoresis (CE) [4, 5], high-performance liquid chromatography (HPLC) [6–9], fluorimetry [10], and chemiluminescence (CL) detection method [11] have been developed for ranitidine. Among these methods, the CL method for ranitidine has been given much attention due to its excellent sensitivity, wide dynamic concentration range and the simple instrumental setup. For example, based on the stronger enhancing CL effect of the ranitidine to the weak CL reaction of MnO_4^- with $Ru(bpy)_3^{2+}$, Barnett and co-workers developed a sensitive CL method for ranitidine and a 6.0×10^{-7} mol/L detection limit for ranitidine was achieved [11]. However, due to the higher oxidation potential of MnO_4^- ($E^0 = 1.50$ V vs NHE) and the stronger background CL effect, this CL method suffered from either the poor selectivity or the sensitivity. Thus, a more sensitive method for ranitidine is still desirable.

In recent years, electrogenerated chemiluminescence (ECL) has emerged as a useful analytical technique by which a chemiluminescence (CL) reaction is produced in the vicinity of the electrode surface when suitable potential is applied to the electrode. Compared with CL, the electro-initiation of CL reactions introduces a large number of additional advantages [12, 13]. Firstly, because the reagents needed for the reactions are electrochemically generated in situ, it not only eliminates the problems associated with the solution addition, but also makes the ECL method suitable for the reactions in which the reagents are unstable. Secondly, because the ECL reactions can be controlled and manipulated by alterations to the applied potential, it can be used for the selective determination of some substances without the use of some tedious separating technique. Thirdly, because the ECL emission is concentrated close to the electrode surface, it can be shaped and accurately positioned in relation to the optical measurement system for maximum sensitivity. Lastly, since the ECL-based emitter, which is formed by the related chemical reaction, was sensitive to the chemical micro-environment of the surface of the electrode, thus, compared to CL, the analytical performances of the ECL emitter can be easily improved with chemically modified electrode technique. Based on these advantages, ECL has been widely used for different analytical purposes, such as the fabrications of the ECL-based sensors[14, 15], the design of the useful detectors in many high performance separating techniques [16, 17], the development of many powerful analytical methods for bioactive species [18, 19] and the design of the nano-scale probes in the ECL microscopy [20] etc., respectively.

Of these useful ECL systems reported, two of them, the $Ru(bpy)_3^{2+}$-based ECL system and the luminol-based ECL system, were given much attention by analysts [21, 22] because these ECL systems offered super analytical advantages, such as the high ECL emission quantum yields, wide analytical application fields and lower experimental cost. However, on reviewing the investigating results on these two ECL reaction mechanisms, it was found that, in the most cases, these ECL reaction procedures concern the gener-

  DOI: 10.1002/elan.200403210

ation of the highly active but unstable intermediators from the electrooxidation analytes and its subsequent CL reaction. For example, in the luminol ECL system, the active-oxygen-based species, such as superoxygen anion, hydroxide free radical, were often concerned [23]. Similarly, in the $Ru(bpy)_3^{2+}$-based ECL system, the N-based free radical intermediator was often the typical example of this kind of intermediators [24]. However, due to the naturally instability properties of these intermediators and their high chemical reacting activity, most of these highly active intermediators at the same time do no other CL reaction besides doing the CL reaction of interest. Thus, in most case, the sensitivity of this kind of ECL method was limited.

On the other hand, as a powerful analytical technique, the chemically modified electrodes (CME) has played an important role in electroanalytical chemistry and other analytical fields since this technique could offer the conventional electrodes the novel and super analytical performances [25–27]. Based on the consideration of ECL analysis using chemically modified electrode technique, many CME-based ECL works were developed and the analytical performances of many conventional ECL-based methods at bare electrodes were improved by using different CME-based ideas. For example, after the first introduction of Nafion modifying graphite electrode for the immobilization of ECL reagent ($Ru(bpy)_3^{2+}$) by Bard and co-workers [28], many novel ECL-based sensors have been fabricated with different modifiers (such as sol-gel film [29], self-assemble film [30], L–B film [31], electro-polymer [32], nanoparticle materials [33] etc,) along this line. The immobilization of ECL reagent with CME technique not only made the ECL analytical procedure simple, but also offered the lower experimental cost due to the regeneration of the ECL reagents on the surface of the CME. In addition, for improving the sensitivity of the conventional ECL method, Dong and co-workers [34] applied the chemically modified carbon paste electrode to the field of ECL analysis. It was found that, while lauric acid was modified onto the surface of the CPE, this CME could not only preconcentrate selectively the chlorpromazine into the CME, but also achieve the sensitive detection of the analyte with ECL method. In this case, compared to that of the conventional ECL method, the selectivity or the sensitivity of the new CME-based ECL method was greatly improved. More recently, based on either the paraffin or the nanoparticle Au modifying conventional electrodes, Lin and co-workers [35] developed the multichannel ECL reaction mechanisms of luminol and the ECL properties of the luminol were further broaden. At the same time, these findings also indicated strongly that, with this CME, the multianalytes might be simultaneous ECL detected by the similar way to that of sweep-potential electroanalysis technique.

However, on reviewing the key factors of the ECL reaction, it was found that, besides the effect of the electrochemical reaction, the micro-environment or the structure of the electrode surface often strongly affected the properties of the subsequent CL reaction, which was generated from the chemical reaction of electrogenerated species with themselves or other species existing in solution. The main reason was that either the electrochemical reaction or subsequent CL reaction of the electrogenerating species were all made in the same spatial zone of the electrode surface. But up to now, to the best our knowledge, although some useful CME-based ECL ideas have been developed for ECL reaction, especially for the electrochemical reaction alone, little attention was paid to modify the microenvironment of the electrode for creating the better ECL performances.

In this paper, it was found that: while the NiO modified graphite electrode was initially modified with vaseline, the blank ECL signal of electrooxidation luminol itself did not present any obviously changes, but the enhancing ECL effect of the ranitidine for the weak ECL signal of electrooxidation luminol was greatly improved in the aspect of either the sensitivity or the stability. Based on this finding, a more sensitive ECL method for ranitidine was firstly proposed. At the same time, based on the investigation on this ECL reaction mechanism, a new concept with chemically modified electrode technique to improve the analytical performances of ECL analysis was also firstly proposed.

2. Experimental

2.1. Reagents

All solutions were prepared from analytical-reagent grade materials with deionised water. Stock solution of ranitidine (1.0×10^{-3} mol/L) was prepared by accurately weighing the ranitidine (National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China) into a 100 mL calibrated flask and diluting to volume with water. 1.0×10^{-3} mol/L luminol solution was prepared by dissolving 0.1772 g of luminol (Sigma, America) in 0.1 mol/L NaOH solution and diluted to 1 L with water. When not in use, the stock solution was kept at about 4 °C in a dark bottle. Other reagents used were all analytical-reagent grade materials or higher.

2.2. Apparatus

The applied potential for single-step potential electrolysis was achieved by a MPI-A electrochemiluninescence analytical system (Xi'an remax Electronic Science Tech. Co. Ltd, Xi'an, China). The ECL intensity was transformed into an electrical signal by an R456 photomultiplier (PMT) (Hamamatsu), which was operated at – 800 V, and the ECL cell was placed in front of the PMT.

The electrolytic cell utilizes a conventional three-electrode setup and was arranged as shown in Figure 1. The cell was made of a microbeaker (high 3.5 cm, i.d. 2.5 cm). The working electrode was the vaseline and nickel oxide modified graphite electrode (Shanghai, 28.3-mm^2 surface area), a Pt flake (7 mm × 7 mm) and Ag wire were used as the counter electrode and psuedo-reference electrode, respectively.

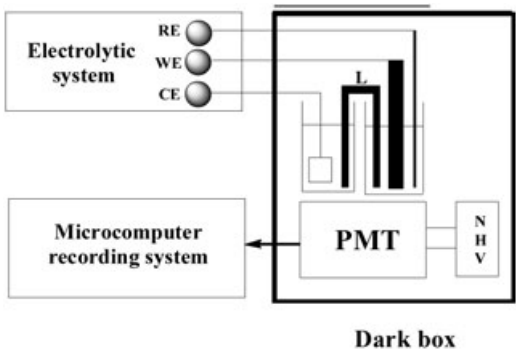

Fig. 1. The block diagram of ECL detection system. W: working electrode; R: Ag reference electrode; C: counter electrode; L: KNO_3 salt bridge; PMT: photomultiplier; NHV: negative high voltage supply.

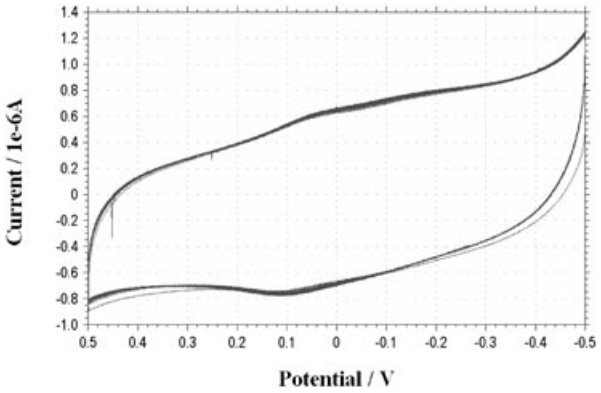

Fig. 2. The cyclic voltammograms of chemically modifying graphite electrode with nickel oxide. The CV was performed in 1.0×10^{-3} mol/L $Ni(NO_3)_2$ solution on the vaseline-impregnated-based graphite electrode with a 100 mV/s scan rate, the number of scans was 50.

In addition, all the potentials appearing in this paper were relative to the silver – metal psuedo-reference electrode.

2.3. The Fabrication of the Chemically Modified Electrode

Prior to modification, the graphite working electrode was firstly impregnated with vaseline until it was saturated in boiling vaseline. Then the hot vaseline-impregnated-based graphite electrode was cooled at room temperature, rinsed with water, dried with filter paper, and polished with polishing paper.

Thereafter, the vaseline-impregnated-based graphite electrode was further modified with nickel oxide by cyclic potential sweep in the range of -0.5 to 0.5 V for 100 cycles (as shown in Fig. 2) in the aqueous solution of 1.0×10^{-3} mol/L $Ni(NO_3)_2$.

2.4. Procedure

The 5.0 mL blank solution which contained 3.0×10^{-7} mol/L luminol and 0.1 mol/L borax was added to the ECL cell and a stable blank signal was recorded when the electrolytic potential was applied to the working electrode; The sample or standard ranitidine solutions which contained not only 3.0×10^{-7} mol/L luminol and 0.1 mol/L borax but also an appropriate concentration of ranitidine was added to the ECL cell, and the ECL signal was recorded. The concentration of ranitidine was quantified *via* the peak height of the relative ECL emission intensity which was obtained by subtracting the blank ECL emission intensity from that of the sample or standard ranitidine solution.

3. Results and Discussion

3.1. The Possible ECL Reaction Mechanism

In our initial studies, it was found that the ranitidine did not show any enhancing ECL effect for electrooxidation of the luminol at the surface of the bare graphite electrode. However, it was found that, when the bare graphite electrode with potential sweeping in the 1.0×10^{-3} mol/L $Ni(NO_3)_2$ solution and the nickel oxide was modified on the surface of the graphite electrode, the ranitidine offered a greatly ECL enhancing effect for the weak ECL signal of the electrooxidation luminol.

For explaining this novel function of the nickel oxide modified electrode, the CV responses of the ranitidine at the bare graphite electrode as well as the CME were investigated respectively. As can be seen, for the bare graphite electrode, only a very weak irreversible oxidation peak at 0.70 V appeared at the bare graphite electrode (as shown in Fig. 3a) for the ranitidine when the potential sweep range was 0.20 V to 1.0 V.

However, when NiO was electrodeposited to the surface of the graphite electrode, a couple of irreversible redox peaks at about 0.78 and 0.50 V, corresponding to the redox reaction between NiO and NiO_2, were occurred in the borax buffer medium, and the oxidation peak at 0.78 V was greatly enhanced and the reduction peak at 0.50 V was obviously decreased when the ranitidine was present in this borax buffer solution. These results suggested that the NiO modified electrode offered the obviously electrocatalytic effect for electrooxidation of ranitidine at 0.78 V.

At the same time, the investigated results on the effect of the electrolytic potential for the enhanced ECL signal of ranitidine showed that (as shown in the Fig. 4), only at 0.80 V electrolytic potential, the ranitidine gave the strongest ECL enhancing effect for electrooxidation of luminol. These results suggested that the electrooxidation product of the ranitidine at 0.80 V electrolytic potential was the key species for presenting its enhancing ECL effect. This key species might be an unstable intermediator (more possible a N-based free radical), these unstable intermediator species may be produced based on the chemical reaction of NiO_2

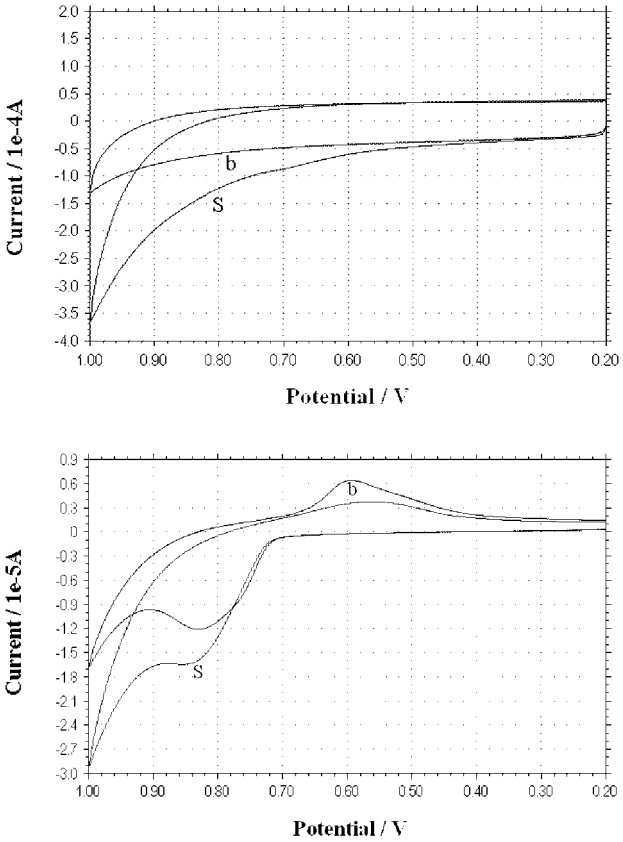

Fig. 3. a) The cyclic voltammograms of the bare graphite electrode in 0.1 mol/L borax buffer solution without (b) and with ranitidine (S, 1.0×10^{-4} mol/L ranitidine) at 100 mV/s sweep rate. b) The cyclic voltammograms of the working electrode in 0.1 mol/L borax buffer solution without (b) and with ranitidine (S, 1.0×10^{-4} mol/L ranitidine) at 100 mV/s sweep rate.

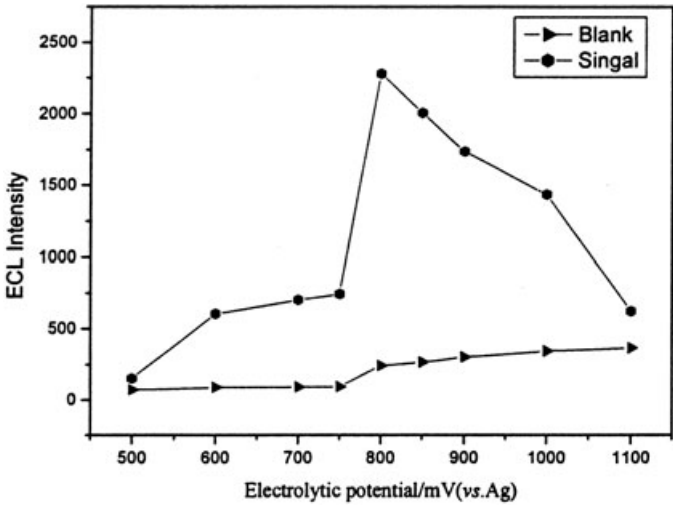

Fig. 4. The effect of the final potential on the ECL enhancement of ranitidine ranitidine concentration: 3.0×10^{-6} mol/L: luminol concentration: 3.0×10^{-7} mol/L; medium: 0.1 mol/L borax buffer solution.

electrogenerated with ranitidine by the similar way to that of chemical reaction between the MnO_4^- and ranitidine [11] because the NiO_2 electrogenerated on the surface of the electrode has the near oxidation potential to that of the MnO_4^- [36]. On the other hand, the previously research works on the $Ru(bpy)_3^{2+}$-based ECL or CL reaction strongly suggested that only the stronger reducing ability species could reacted with $Ru(bpy)_3^{3+}$ to produce the CL signal [24]. Thus, the CL chemical reaction phenomenon of the $Ru(bpy)_3^{3+}$ with an unstable substance, generated from the chemical reaction of the ranitidine with MnO_4^-, indicated that this unstable substance (which generated from the chemical reaction of ranitidine with MnO_4^-) should have the strong reducing ability.

At the same time, it was also found that reproducibility of the enhancing ECL signal of ranitidine at the nickel oxide modifying graphite electrode was too poor to develop a useful ECL method for ranitidine. ON overcoming this limit, it was found that, after the bare graphite electrode was initially impregnated with vaseline and next was further modified with nickel oxide by the electrochemical method mentioned above, the resulting chemically modified electrode could offer either the stronger enhancing ECL effect or the better reproducibility of the ranitidine (as shown in Fig. 3b). The main reason was that due to the typical hydrophobic properties of the vaseline, the vaseline-coating-based graphite electrode offered a suitable hydrophobic environment to make the relating ECL reaction.

On the other hand, many previously investigating results on the luminol-based ECL reaction showed that either the bare graphite electrode or the bare platinum electrode without the coating of the vaseline could offer the better ECL reproducibility [37, 38]. Thus, the key function of the vaseline at the surface of nickel oxide modified electrode may create a hydrophobic micro-environment to improve the stability of the intermediator, which was generated from electrooxidation ranitidine, and make it such as to be converted efficiently into the active oxygen-based species. In this case, the sensitivity and reproducibility of the enhancing ECL signal of the ranitidine was improved at the vaseline and nickel oxide modifying electrode.

In addition to these investigated results, the ECL emission spectrum of the proposed ECL reaction system was made with an Rf-540 fluorenscence spectrophotometer. The result showed that the maximum emission wavelength of the ECL emitter in this proposed ECL system was about 425 nm. This indicated that the possible ECL emitter was the excited state 3-aminophthalate [39].

Moreover, it was further found that, when all the solutions in this ECL system were deoxygenated with pure nitrogen gas, the enhancing function of ranitidine or the weak ECL signals of electrooxidation luminol itself nearly disappeared. This result suggested that the dissolved oxygen existed in the solutions was another key species for the presence of the enhancing ECL effect of ranitidine.

For exploring the relationship of these two key species (dissolved oxygen and the electrochemically oxidation product of ranitidine at 0.8 V) in the proposed ECL system, some active oxygen cleaning reagents such as ascorbic acid, benzophenone etc were added to the solutions of ranitidine

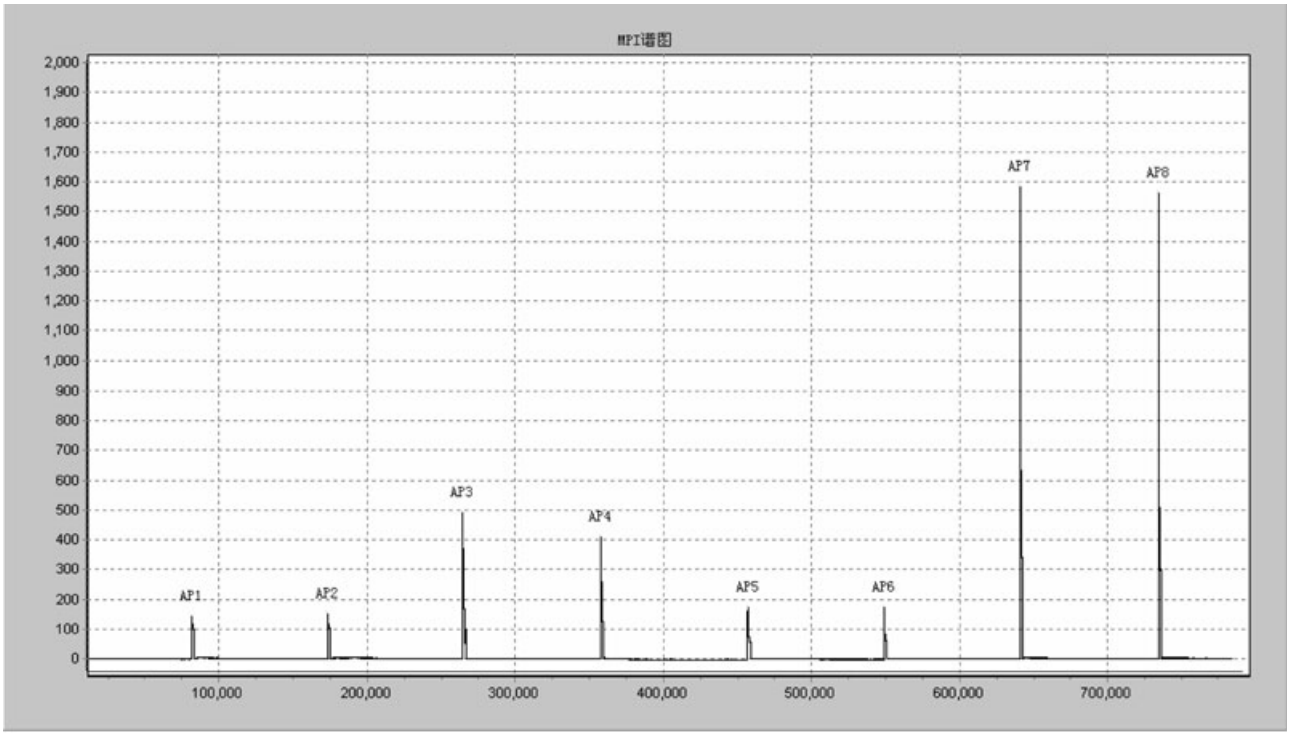

Fig. 5. Comparing of signals from electrodes with and without modifying vaseline. AP1 and AP2 are blank signals of the electrode without vaseline; AP3 and AP4 are sample (1.0×10^{-6} mol/L ranitidine) signals of the electrode without vaseline; AP5 and AP6 are blank signals of the electrode with vaseline; AP7 and AP8 are sample (1.0×10^{-6} mol/L ranitidine) signals of the electrode with vaseline.

or luminol. It was found that the enhancied ECL signal or the weak ECL signal of luminol itself nearly disappeared. These results indicated that the active oxygen, more possibly the superoxygen anion free radical, may be produced in the proposed ECL reaction. For further confirmation of this hypothesis, it was found that, when superoxide dismutase (SOD) was added to the solutions of ranitidine or luminol, the enhancing ECL signal of ranitidine or the background ECL signal of luminol nearly disappeared, respectively. In contrast, when sodium benzoate (one of the typical hydroxyl free radical cleaning reagents) was added to the solutions of ranitidine or luminol, the background or the enhancing ECL signal did not present any obvious changes. Based on these results mentioned above, this active oxygen-based species should be the superoxygen anion free radical but the hydroxyl free radical.

Based on these investigating results and the chemical properties of ranitidine, we presumed the possible ECL reaction mechanism of this system is as follows:

Initially, both the luminol and ranitidine are oxidized at 0.8 V at the surface of the vaseline and nickel oxide modifying electrode, producing the luminol radical and the stronger reducing intermediate from electrocatalytic oxidation ranitidine in the diffusion layer of the working electrode. Then, due to the stronger reducing ability of this intermediate and the better hydrophobic micro-environment offered by vaseline in the surface of the CME, this intermediator could efficiently reduce dissolved oxygen to generate the superoxygen anion radical. Finally, the chemical reaction of the superoxygen anion radical with luminol-based free radical in the solution emitted the stronger light.

3.2. The Choice of Modifier for Creating Suitable ECL Reaction Micro-Environment

Compared to that of the conventional electrochemical reaction in amperometric analysis, the analytical performances of the ECL reaction was affected not only by the relating electrochemical reaction, but also strongly by the micro-environment of the near field of the electrode surface since the same CL reactions offered the different analytical performances in the different reacting micro-environment systems [40]. Based on this consideration, many hydrophobic species (such as vaseline, paraffin, glycerin), which have the ability to improve the stability of the unstable intermediator from electrooxidation ranitidine, were initial modified to the surface of the graphite electrode for this purpose by a hot dipping modified technique. Of these species tested, although the vaseline and paraffin achieved to initial consideration, the vaseline presented the best performances (as shown in Fig. 5) and further used for the fabrication target CME for the ECL analysis.

3.3. Selection of Electrochemical Parameters

The electrochemical parameters are important parameters which strongly affected the properties of the relating ECL

Table 1. The selected electrochemical parameters for ECL analysis.

Waveform	Step-up time	Rest time	Initial potential	Final potential
Single-step	1 s	90 s	0 mV (vs. Ag)	800 mV (vs. Ag)

reactions. For obtaining the higher ECL ratio of the ECL sensing ranitidine, the effect of the electrolytic potential waveform, such as linear sweep, square-wave, triangular wave and pulse wave, were examined, respectively. The results showed that the enhanced ECL emission occurred while any of them was applied, but the strongest ratio of the ECL signal to the noise was given by the use of pulse wave. Therefore, pulse potential was chosen in this work.

The effect of the electrolytic parameters on the ECL performances of ranitidine was also investigated in details. The electrolytic parameters as shown in Table 1 presented the best analytical performances and selected for the subsequent works.

3.4. Effect of ECL Reaction Medium

It is well known that the medium plays an important role in ECL reaction. The enhancing ECL signal of ranitidine in different mediums, such as borax buffer medium, Na_2CO_3, $NaHCO_3$, CH_3COONa, NaOH, were investigated in a suitable concentration range, respectively. The results showed that, although the enhanced ECL signals of ranitidine could occur in borax buffer or Na_2CO_3 media, the maximal ratio of ECL signal to the noise for detecting ranitidine and the better ECL reproducibility was obtained only in borax buffer solution. The effect of the borax buffer concentration on the enhanced ECL of ranitidine was also studied in a suitable concentration range, the result showed that 0.1 mol/L borax buffer solution offered the best results and was used for the determination of ranitidine in this ECL system.

3.5. Effect of Luminol Concentration

The effect of luminol concentration on the relative ECL intensity was investigated from 1.0×10^{-7} mol/L to 1.0×10^{-6} mol/L and the results are shown in Figure 6. When the concentration of luminol was lower or higher than 3.0×10^{-7} mol/L, the enhancing ECL intensity of the ranitidine decreased. Hence, 3.0×10^{-7} mol/L luminol was selected as optimum for the following experiments.

3.6. Analytical Performances

Under the above optimum conditions, the relative ECL intensity had a linear relationship with the concentration of ranitidine in the range from $3.0 \times 10^{-8} - 9.0 \times 10^{-6}$ mol/L. The regression equation was $\Delta I = 37.5\,C + 340.4$ (C: $\times$

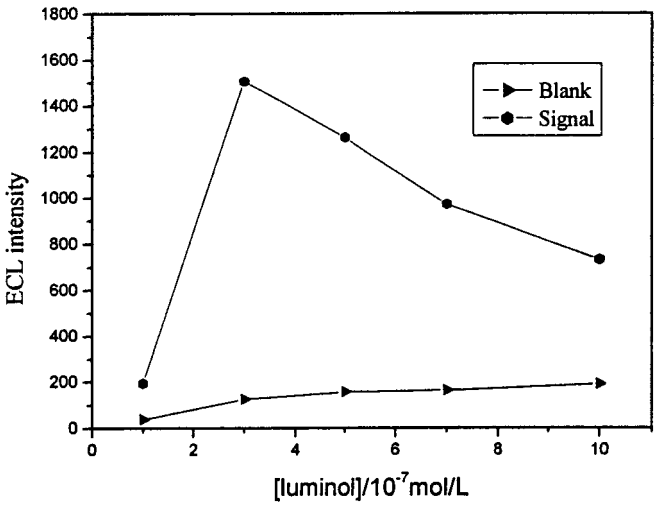

Fig. 6. Effect of luminol concentration on ECL intensity. Ranitidine concentration: 3.0×10^{-6} mol/L; medium: 0.1 mol/L borax buffer solution; electrolytic potential: 800 mV (vs. Ag)

10^{-8} mol/L) and the correlation coefficient of 0.9990. The relative standard deviation for 1.0×10^{-7} mol/L ranitidine was 4.8% ($n = 11$). The limit of detection (defined as the concentration that could be detected at the signal-to-noise ratio of 3) was 9×10^{-9} mol/L.

3.7. Interferences Study

In order to apply the method to the analysis of ranitidine in the dosage form, the commonly used concomitant species and additives was investigated for the determination of 1.0×10^{-7} mol/L ranitidine solution as the relative error not larger than 5%. No interferences could be observed when including up to 1000-fold weight of concentration of oxalate, citrate, carbamide, Tween 20, β-CD, SDS, Ca^{2+}, Mg^{2+}, 100-fold weight of concentration of uric acid, glucose, 50-fold weight of concentration of starch, 10-fold weight of concentration of CTMAB, 1-fold weight of concentration of ascorbic acid, BSA and thiourea.

3.8. The Analytical Applications

The proposed method was applied to the determination of ranitidine in pharmaceutical formulations. The results are shown in Table 2 and agree well with those obtained by an official method [41]. These suggested that the proposed ECL method is accurate.

Table 2. The analysis of the sample with the proposed ECL method.

Sample NO	Official method (mg per capsule) [a]	Proposed method (mg per capsule) [b]
1	149.4	148.6 (±3.4)
2	150.2	150.8 (±4.4)
3	149.6	148.9 (±3.8)

[a] Average value of five determinations; [b] mean of three replicates (± RSD, %).

4. Conclusions

In this paper, based on the modification of the vaseline and nickel oxide on the surface of the graphite electrode, a new and sensitive ECL method for determination of ranitidine was developed. At the same time, compared to reported ECL ideas, a new idea, which used chemically modified electrode technique to improve the ECL reaction micro-environment for better analytical performances, was firstly proposed. Our results also showed that the combination of the ECL method with the chemically modified electrode technique will offer the greatest potential to improve the analytical performance of ECL analysis.

5. Acknowledgement

This study was supported by the national Natural Science Foundation of China (No. 20175039)

6. References

[1] J. Thomas, *Australian Products Prescription Guide* **1996**, Vol. 2, pp. 2494, 2415
[2] A. Z. Abu-Zuhri, S. I. Hannoun, A. H. Hasna, *Anal. Lett*. **1988**, *21*, 1845.
[3] P. S. Sankar, S. J. Reddy, *J. Pharm. Sci*. **1989**, *51*, 263.
[4] V. M. Morris, C. Hargreaves, K. Overall, P. J. Marriott, J. G. Hughes, *J Chromatogr. A* **1997**, *766*, 245.
[5] T. Pérez-Ruiz, C. Martýnez-Lozano, V. Toma's, E. Bravo, R. Galera. *J. Pharm. Biomed. Anal.* **2002**, *30*, 1055.
[6] C. Lüpez-Calull, L. García-Capdevila, C. Arroyo, J. Bonal, *J. Chromatography B* **1997**, *693*, 228.
[7] C. F. Wong, K. K. Peh, K. H. Yuen, *J. Chromatography B* **1998**, *718*, 205.
[8] A. Ahmadiani, H. Amini, *J. Chromatography B* **2001**, *751*, 291.
[9] D. Zendelovska, T. Stafilov, *J. Pharm. Biomed. Anal.* **2003**, *33*, 165.
[10] C. Lopez-Erroz, P. Vinas, N. Campillo, M. Hernandez-Cardoba, *Analyst* **1996**, *121*, 1043.
[11] N. W. Barnett, B. J. Hindson, S. W. Lewis, *Anal. Chim. Acta* **1999**, *384*, 151.
[12] A. W. Knight, *Trends in Anal. Chem.* **1999**, *18*, 47.
[13] A. W. Knight, G. M. Greenway, *Analyst* **1994**, *119*, 879.
[14] W. Zhan, J. Alvarez, R. M. Crooks, *Anal. Chem.* **2003**; *75*, 313.
[15] H. N. Choi, S.-H. Cho, W.-Y. Lee, *Anal. Chem.* **2003**; *75*, 4250.
[16] G. Chen, Y. Chi, X. Wu, J. Duan, N. Li, *Anal. Chem.* **2003**; 75, 6602.
[17] H. Morita, M. Konishi, *Anal. Chem.* **2002**, *74*, 1584.
[18] W. Miao, A. J. Bard, *Anal. Chem.* **2003**, *75*, 5825.
[19] M. Zhou, J. Roovers, G. P. Robertson, C. P. Grover, *Anal. Chem.* **2003**, 75, 6708.
[20] J. P. Preston, T. A. Nieman, *Anal. Chem.* **1996**, *68*, 966.
[21] R. D. Gerardi, N. W. Barnett, S. W. Lewis, *Anal. Chim. Acta* **1999**, *378*, 1.
[22] K. A. Fahnrich, M. Pravda, G. G. Guilbault, *Talanta* **2001**, *54*, 531.
[23] G. P. Jirka, A. F. Martin, T. A. Nieman, *Anal. Chim. Acta* **1993**, *284*, 345.
[24] A. W. Knight, G. M. Greenway, *Analyst* **1996**, 121, 101R.
[25] R. W. Murray, *Anal. Chem.* **1980**, *13*, 135.
[26] M. I. Prodromidis, P. G. Veltsistas, M. I. Karayannis, *Anal. Chem.* **2000**, *72*, 3995.
[27] X. M. Liu, X. G. Zhang, *Electrochim. Acta* **2004**, *49*, 229.
[28] I. Rubinstein, A. J. Bard, *J. Am. Chem. Soc.* **1980**, *102*, 6641.
[29] M. Sykora, T. J. Meyer, Chem. Mater. 1999; *11*, 1186.
[30] Y. Sato, K. Uosaki, *J. Electroanal. Chem.* **1995**, *384*, 57.
[31] C. J. Miller, P. McCord, A. J. Bard, *Langmuir* **1991**, *7*, 2781.
[32] K. M. Maness, R. H. Terrill, T. J. Meyer, R. W. Murray, R. M. Wightman, *J. Am. Chem. Soc.* **1996**, *118*, 10609.
[33] Y. Bae, N. Myung, A. J. Bard, *Nano Lett.* **2004**, *4*, 1153.
[34] G. B. Xu, Sh. Dong, J. *Anal Chem*. **2000**, *72*, 5308.
[35] H. Cui, G.Zh. Zou, X. Q. Lin, *Anal Chem.* **2003**, *75*, 324.
[36] *Handbook of Analytical Chemistry*, 2nd ed., Chinese Science Press **1998.**
[37] L. D. Zhu, Y. X. Li, F. M. Tian, B. Xu, G. Y. Zhu, *Sens. Actuators B* **2002**, *84*, 265.
[38] X. W. Zheng, M. Yang, Zh. J. Zhang, *Anal. Chim. Acta* **2001**, *440*, 143.
[39] E. H. White, D. F. Roswell, *Chemi- and Bioluminescence* (Ed: J. G. Burr), Marcel Dekker, New York **1985**, p. 215.
[40] J. M. Lin, M. Yamada, *Trends in Anal. Chem.* **2003**, *22*, 99.
[41] *Chinese Drug Analysis Handbook*, Chinese Chemical Press **2000**, p. 690.

