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The influence of temperature on the ECL behavior of the benzhexol hydrochloride -$Ru(bpy)_3^{2+}$ system on graphite electrode

Cheng Hao[1,2], Jiang Shaotong[1,*], Yanqing Li[2]

[1]College of Biotechnology and Food Engineering, Hefei University of Technology, Hefei, 230009, (CHINA)

[2]Department of Biological and Chemical Engineering, Guangxi University of Science and Technology, Liuzhou, 545006, (CHINA)

E-mail: chhfut@126.com

ABSTRACT

The influence of temperature on the electrochemiluminescence (ECL) behavior of the benzhexol hydrochloride -$Ru(bpy)_3^{2+}$ system was studied by means of electrochemistry, chemiluminescence and fluorescence on graphite electrode. The results showed that the temperature has a significant impact on the ECL behavior of the benzhexol hydrochloride - $Ru(bpy)_3^{2+}$ system from 0℃ to 70℃, the current intensity was enhanced with the increase of temperature, and the chemiluminescence intensity of the benzhexol hydrochloride-$Ru(bpy)_3^{2+}$ system increased from 0℃ to 30℃ and then decreased. Under the optimum conditions, the linear range for benzhexol hydrochloride from 1×10^{-8}-1×10^{-4}mol•L^{-1} and the detection limit (S/N=3) of 6.0×10^{-9} mol•L^{-1} were obtained, and the satisfactory reproducibility, relative errors and recoveries were gained. These results indicated that the testing temperature had an significant influence on the ECL behavior of the $Ru(bpy)_3^{2+}$ system.

KEYWORDS

Electrochemiluminescence; Graphite electrode; Temperature; Fluorescence.

INTRODUCTION

The electrochemical detection technology, which has the advantages of high sensitivity, low detection limit, wide linear range, simple apparatuses and operations, has received considerable attention in the analytical field during the past decades[1-3], moreover, $Ru(bpy)_3^{2+}$ (bpy=2,2′-bipyridine) has remarkable features of water solubility, chemical stability and electrochemical reversibility. With the development of electrochemical technology, the $Ru(bpy)_3^{2+}$ system is widely used in the determination of inorganic substances, drug testing and life sciences[4-7], and electrode modification technologies were also continuously improved. To enhance ECL performances, it was also combined with other instruments [8,9], such as flow injection analysis-electrochemiluminescence (FIA-ECL), high performance liquid chromatography-electrochemiluminescence (HPLC-ECL), capillary electrophoresis-electrochemiluminescence (CE-ECL) and microchip capillary electrophoresis-electrochemiluminescence (MCE-ECL). However, it was a common problem that the stability and repeatability were bad in the detection process.

To improve the detection performance, thermoelectric technology was applied in ECL, and the literatures had proved that the temperature exactly had a significant influence on the detection when the temperature on electrode surface was changed. Wu Shaohua[10] used electrically heated graphite cylindrical electrodes (HGCEs) to perform electrochemical behavior studies and adsorptive stripping square wave voltammetric (SWV) measurements of flavin adenine dinucleotide, the SWV stripping peak current was significantly enhanced with the electrode temperature during preconcentration step, the detection limit measured at 72℃ was about one magnitude less than that at 27℃, and the sensitivity was obviously increased by the thermal convection. Chen Yiting combined ECL with CE to detect triethylamine and tri-n-propylamine with an electrically heated carbon paste electrode(CPE), compared with the conventional electrode at the room temperature, using heated CPE could improve peak shape and gain good reproducibility with lower detection limits and wider linearity ranges[11], then, a heated glassy carbon electrode was used to make cephalexin rapidly hydrolyze on the electrode surface and achieved the effective determination of cephalexin[12]. Wu Aihong[13] fabricated a platinum coated heated pencil graphite electrode (Pt/HPGE) to detect riboflavin based on the quenching effect of riboflavin on $Ru(bpy)_3^{2+}/C_2O_4^{2-}$ electrochemiluminescence system. It was demonstrated that Pt/HPGE had unique properties such as good reproducibility and sensitive temperature response, and the detection limit at an electrode temperature of 58℃ was clearly lower than that at room temperature. Ye Ruihong[14] designed an electrically heated microelectrode chip (HMEC), which was applied to the $Ru(bpy)_3^{2+}$/carbofuran electrochemiluminescence system,to characterize the performance of the HMEC, it showed that the ECL intensities increased at elevated electrode temperature, and the detection limit at 60℃ (electrode surface temperature) was about 10 times lower than that at 30℃.

In the study of improving the detection performance by enhancing the temperature, the main means was to change surface temperature of electrode, so the different types of hot electrodes were used for the determination of samples in electrochemistry (EC) and ECL. However, the influence law of temperature on detection performance was not revealed, and the effect of temperature on stability and reproducibility of instruments was not investigated, too. In view of the importance of temperature, to study the influence of temperature on the ECL behavior will be of great importance and necessity with the in-depth study on ECL. In order to investigate effect of temperature of the detection system on ECL, we selected the $Ru(bpy)_3^{2+}$ system as the objective[15,16], the gold electrode was applied as working electrode, and the effects of temperature on pH, current intensity, luminous intensity, electrical conductivity, luminious intensity and the stability of instruments were analysed. Meanwhile, with the help of fluorescence technique[17,18], the ECL intensity was compared with the level of fluorescence intensity to obtain the effect law of temperature on the ECL behavior.

EXPERIMENTAL

Apparatus and regents

The ECL behavior was measured with an MPI-E electrogenerated chemiluminescence analyzer (Xi'an ruimai Analytical Instruments Co., Ltd., Xi'an, China), fluorescence spectrometer LS45 (Perkin Elmer), DDS-11A conductivity meter (Shanghai Rex Xinjing Instrument Co., Ltd., Shanghai, China), DL-60D ultrasonic cleaner (Shanghai zhi sun Instrument Co. Ltd., Shanghai, China), micropipettor (Eppendorf, Germany). The three-electrode system including gold electrode as working electrode, Pt electrode as counter electrode and Ag/AgCl (in a saturated KCl solution) as the reference electrode.

1×10^{-4}mol/L benzhexol hydrochloride standard stock solution: accurately weighed 0.0034g azithromycin (Mational Institutes for Food and Drug Control, Beijing, China), dissolved in water and constant volume in 100mL brown volumetric flask, and then stored it in the refrigerator (4℃) standby after degassing by ultrasonic.

1×10^{-3}mol/L $Ru(bpy)_3^{2+}$ stock solution: accurately weighed 0.0187g $Ru(bpy)_3Cl_2\cdot6H_2O$ (St. Louis, MO,USA), dissolved in water and constant volume in 100mL brown volumetric flask, stored it in the refrigerator (4℃) standby after degassing by ultrasonic.

All other chemicals were obtained from Xilong Chemical CO.,LTD. (Shantou, China) and were of analytical reagent grade, and the water were two quartz sub-boiling water.

Methods

The MPI-E workstation was used as a working platform, and the three-electrode system (Figure 2) was chosen to research the ECL behavior after the gas of the solution removed by the ultrasound for 5 min. By changing the temperature of the system, the influence of temperature on the ECL behavior of the benzhexol hydrochloride-$Ru(bpy)_3^{2+}$ system was studied in a certain negative pressure condition. At the same time, the fluorescent technique was used to analyse the law of effects of temperature on ECL, and the recovery of standard addition could be applied to demonstrate the instrument has a good stability at a constant temperature.

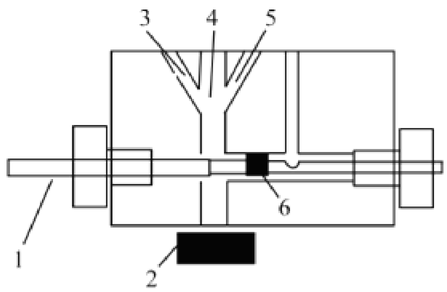


Figure 2 : The schematic diagram of the ECL detection cell

1. working electrode, 2. photomultiplier tube, 3. auxiliary electrode, 4. Import reagents and samples, 5. reference electrode, 6. sealing film

RESULTS AND DISCUSSION

The sensitizing effect of benzhexol hydrochloride on $Ru(bpy)_3^{2+}$

To study the ECL behavior of $Ru(bpy)_3^{2+}$ system, the blank experiments were assayed in mixed solution of 100μL $Ru(bpy)_3^{2+}$ (1×10^{-4}mol/L), 100μL borate buffer solution (BBS, pH=7.5) and 100μL water, and the sensitization test was also detected when water was replaced to benzhexol hydrochloride (1×10^{-4}mol/L). The sensitizing effect of benzhexol hydrochloride on $Ru(bpy)_3^{2+}$ was notable (Figure 2), which provides a new method for the detection of benzhexol hydrochloride.

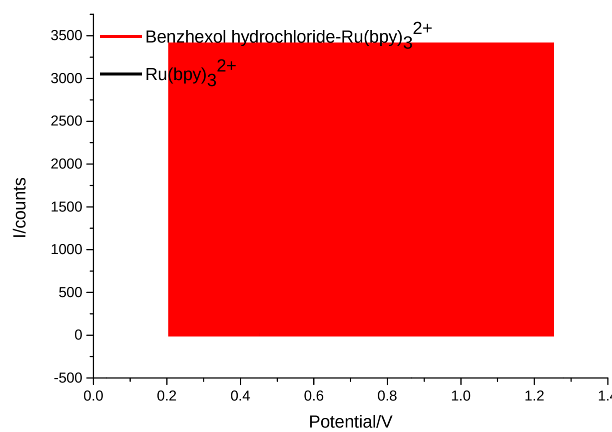


Figure 2 : ECL curves of $Ru(bpy)_3^{2+}$ and Benzhexol hydrochloride-$Ru(bpy)_3^{2+}$

In the blank experiment, the electrochemical luminescence intensity is weak because of the annihilation reaction of $Ru(bpy)_3^{2+}$, after adding benzhexol hydrochloride, the reaction system became a redox reagent system, and benzhexol hydrochloride had a significant sensitizing effect on $Ru(bpy)_3^{2+}$ by contrast with the blank experiment.

Optimization of factors affecting ECL

Effect of MPI-E parameters on the detection system

Selection of negative high voltage

In the detection process, the negative high voltage of photomultiplier tube have an important role, it can directly impact on the sensitivity and the linear range. In the condition of respectively adding 100μL $Ru(bpy)_3^{2+}$ (1×10^{-4}mol/L), 100μL BBS (pH=7.5) and 100μL benzhexol hydrochloride (1×10^{-4}mol/L), the negative high voltage from -700V to -900V were investigated so as to make signal-to-noise ratio (SNR) best. The results shown that the SNR of the benzhexol hydrochloride-$Ru(bpy)_3^{2+}$ system first increases and then decreases between -700V and -900V, and the optimum SNR was generated at -850V.

Selection of scanning rate

Under the condition of 1×10^{-4}mol/L Ru(bpy)$_3^{2+}$, PH 7.5 BBS, 1×10^{-4}mol/L benzhexol hydrochloride and -850V, we separately selected 0.06V/s, 0.08V/s, 0.10V/s and 0.12V/s to determine the ECL intensity, the results showed that the luminous intensity of benzhexol hydrochloride-Ru(bpy)$_3^{2+}$ system was enhanced with the increase of scanning rate, and the best effect was achieved at 0.1V/s, so the scanning rate was 0.1V/s.

Effects of the concentration of Ru(bpy)$_3^{2+}$ on ECL

Under the condition of PH 8.0 BBS, 1×10^{-4} mol/L benzhexol hydrochloride and -850V, the concentrations of Ru(bpy)$_3^{2+}$, which were 2×10^{-5}mol/L, 6×10^{-5}mol/L, 1×10^{-4}mol/L, 1.2×10^{-4}mol/L, 1.4×10^{-4}mol/L and 1.8×10^{-4}mol/L, were taken as the object to research the effect of the concentration on the luminous intensity. It was clear that the effect is obvious and the luminous intensity also raised with concentration increasing. To ensure the best SNR, the optimum concentration of Ru(bpy)$_3^{2+}$ was 1×10^{-4}mol/L.

Selections of the buffer solution and pH

In the detection system of benzhexol hydrochloride-Ru(bpy)$_3^{2+}$, the buffer solution as the main ingredient not only provided acid-base environment but also impacted on both electrical conductivity and luminous efficiency[1,19], the selection of the buffer solution had the vital significance to the effects of determination, as shown in Figure 3.

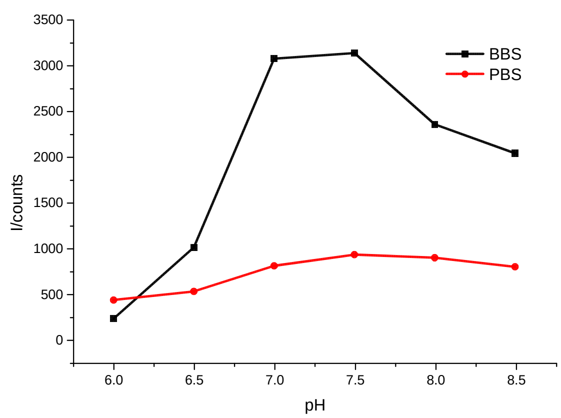


Figure 3 : Effects of pH on ECL intensity

The condition of 1×10^{-4} mol/L Ru (bpy)$_3^{2+}$,1×10^{-4} mol/L benzhexol hydrochloride and -850V was fixed, through the study of the borate buffer solution (BBS) system and the phosphate buffer solution (PBS) system, the ECL intensity of benzhexol hydrochloride-Ru(bpy)$_3^{2+}$ system increased in the range of pH 6.0-7.5, and it decreased from 7.5 to 9.0, moreover, the BBS system was better than the PBS system. The reason was that the amine free radical with high energy formed easily in the weak alkaline condition[20], therefore, the pH 7.5 BBS system was used as the optimal mediums for the following studies.

The influence of temperature on the supporting electrolyte

Effect of temperature on pH

For ascertaining the effect of temperature on the ECL intensity, the regularity of pH variation with temperature was first inspected in BBS, the Fib. 4 showed that the temperature had a little effect on pH.

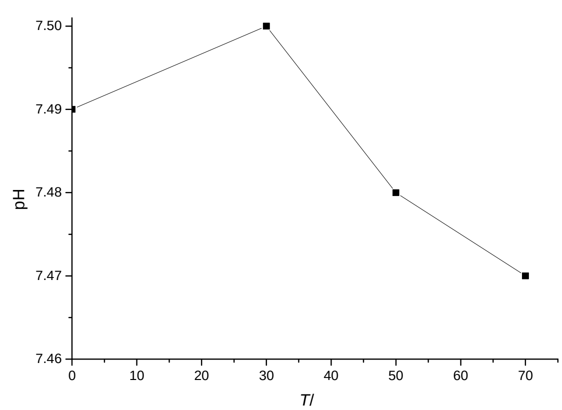


Figure 4 : Effect of temperature on pH

Effect of temperature on conductivity

According to the parameters of the MPI-E and the ECL mechanism, the voltage range of the detector cell is from 0.1V to 1.25V, so the conductivity would have a influence on the current intensity and the luminous intensity, and it also played an important role to study the effect of temperature on the ECL behavior, the relationship between temperature and conductivity in pH 7.5 BBS was showed in Figure 5.

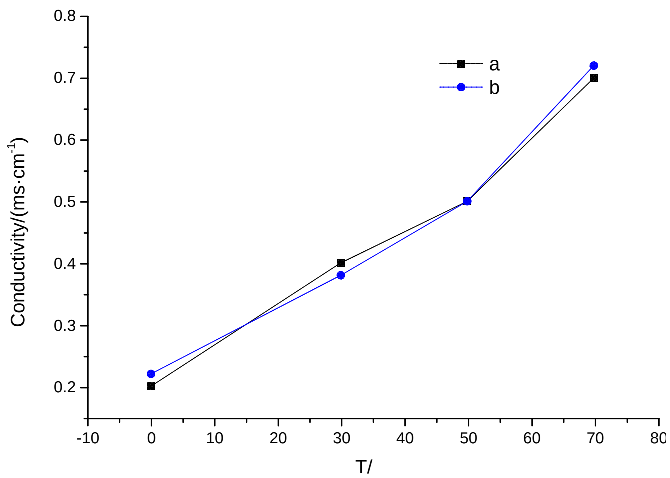


Figure 5 : Effects of temperature on electroconductibility

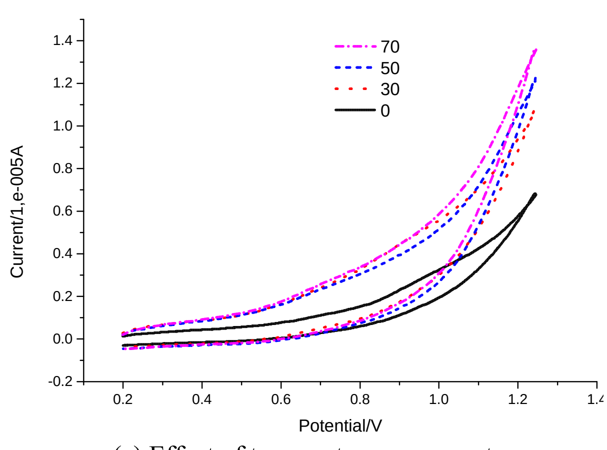
(a. $Ru(bpy)_3^{2+}$-water-BBS, b. benzhexol hydrochloride-$Ru(bpy)_3^{2+}$-BBS)

Under the condition of 1×10^{-4} mol/L Ru (bpy) $_3^{2+}$, pH 7.5 BBS and 1×10^{-4} mol/L benzhexol hydrochloride, electrical conductivity is greatly influenced by temperature in the $Ru(bpy)_3^{2+}$-water-BBS (1：1：1, V/V) system and the $Ru(bpy)_3^{2+}$-benzhexol hydrochloride-BBS (1：1：1, V/V) system, electrical conductivity increased with temperature increasing, and the conductivity at 30℃ approximately 2 times than that in 0℃. By contrast with the $Ru(bpy)_3^{2+}$-water-BBS system, benzhexol hydrochloride has little effect on electrical conductivity.

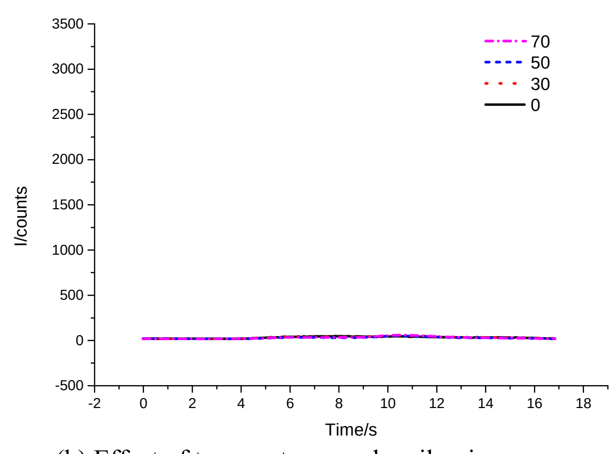
Effect of temperature on the ECL behavior and fluorescence emission spectra

Effect of temperature on the ECL behavior of the benzhexol hydrochloride system and its fluorescence emission spectra

To discuss the influence factors on the ECL behavior of the $Ru(bpy)_3^{2+}$-benzhexol hydrochloride-BBS system, under the condition of PH 7.5 BBS, 1×10^{-4} mol/L benzhexol hydrochloride and -850V, the benzhexol hydrochloride ECL behavior was investigated in the benzhexol hydrochloride-water-BBS system, and fluorescence emission spectra of benzhexol hydrochloride was obtained when it was excited by UV at 250 nm at 30℃.



(a) Effect of temperature on current



(b) Effect of temperature on chemiluminescence

Figure 6 : Effects of temperature on current and chemiluminescence of the benzhexol Hydrochloride ECL system

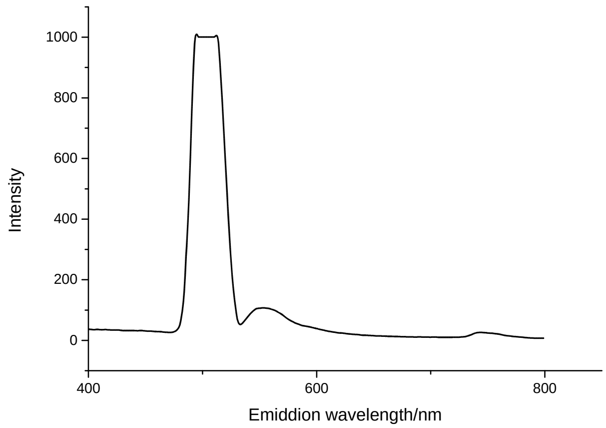
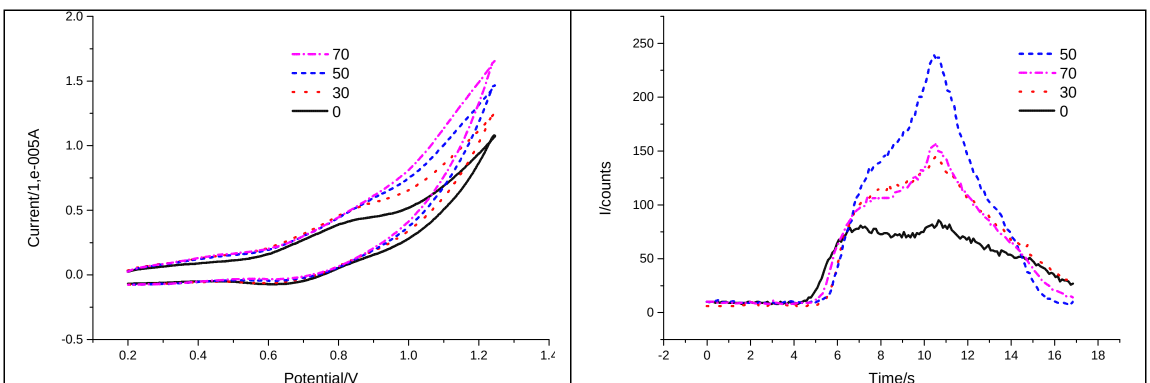


Figure 7 : Fluorescence emission spectrum of benzhexol hydrochloride

Figure 6(a) shows that the current rose with temperature increasing, which corresponds to the temperature variation of conductivity, and this phenomenon is due to the increased conductivity, besides, there was no electrochemical luminescence signal in Figure 6(b) and no fluorescence emission spectrum in Figure 7 (it was the scattered light in the range of about 500nm). The experiments showed that benzhexol hydrochloride just possessed the sensitizing effect (Figure 1) and have no luminescence properties (Figure 7).

Effect of temperature on the ECL behavior of the Ru(bpy)$_3^{2+}$ system and its fluorescence emission spectra

According to the literatures[21,22], the maximum chemiluminescence wavelength of Ru(bpy)$_3^{2+}$ was 610nm, which was consistent with fluorescence spectrum, it indicated that the electronic transition energy between the electrochemical redox reaction and fluorescence excitation was the same[18,19]. To make the analysis more intuitive and avoid instrument error, fluorescence spectrum could be used to analyse the effect of temperature on the Ru(bpy)$_3^{2+}$ ECL behavior in the Ru(bpy)$_3^{2+}$-water-BBS system (1∶1∶1, V/V).



(a) Effect of temperature on current | **(b) Effect of temperature on chemiluminescence**

Figure 8 Effects of temperature on current and chemiluminescence of Ru(bpy)$_3^{2+}$

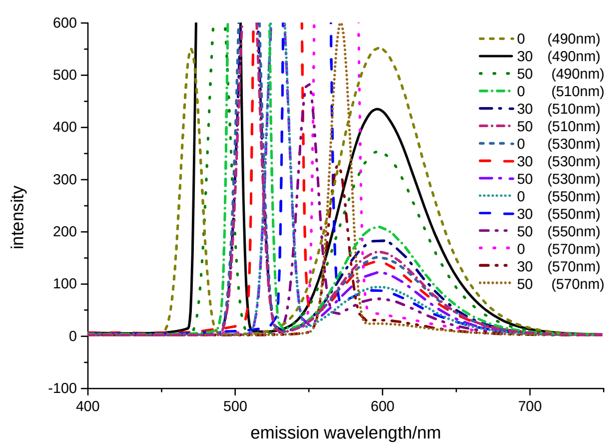


Figure 9 : Effects of excitation wavelength on Fluorescence spectrum of $Ru(bpy)_3^{2+}$

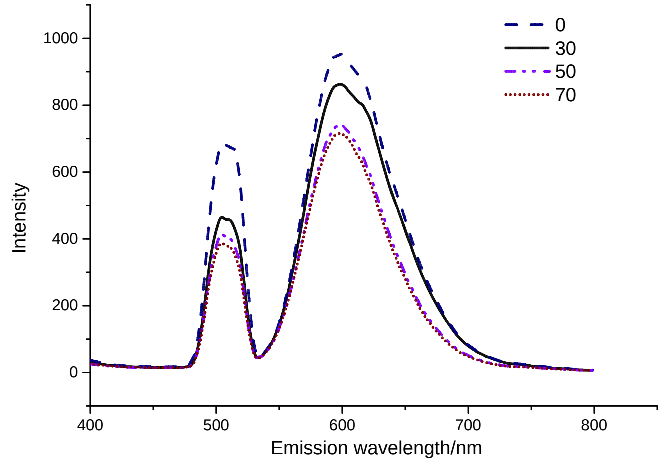


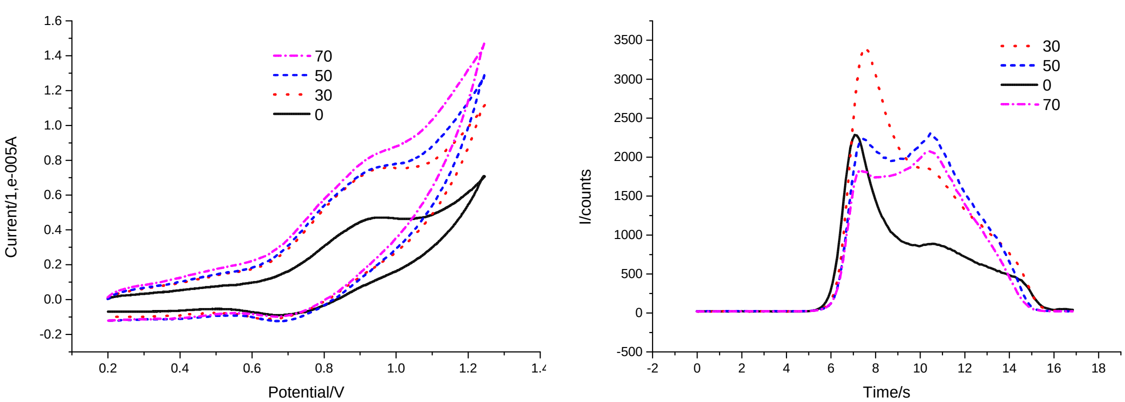
Figure 10 : Effects of different temperatures on Fluorescence spectrum of $Ru(bpy)_3^{2+}$ at 250nm

In the condition of 1×10^{-4} mol/L Ru (bpy) $_3^{2+}$, PH 7.5 BBS and -850V, Figure 8(a) showed that the current trend was consistent with the tendency of the conductivity change, the current increased with temperature increasing, however, Figure 8(b) showed chemiluminescence intensity first increased and then decreased, the peak value appeared in 50℃, the reasons for this phenomenon could be summarized as follows.

The annihilation reaction of $Ru(bpy)_3^{2+}$ would happen in the $Ru(bpy)_3^{2+}$-water-BBS system when the electrode surfaces were applied step voltage. In the process of ECL, the adequate energy was provided to produce free radical oxidation state and the reduced state of free radicals, then the luminescent material was excited after annihilation reaction, the light then emitted after relaxation[23]. Figure 9 showed the fluorescence intensity increased with the excitation energy increasing and declined from 0℃ to 50℃ at different excitation wavelengthes, so the energy had a great impact on the fluorescence intensity, as the excitation energy was lower, such as 510nm, 530nm, 550nm, 570nm, the fluorescence intensity was also lower, and there was little difference between 0℃ and 50℃, it means that fluorescence quantum yield of $Ru(bpy)_3^{2+}$ was less affected by temperature when the energy is lower. As shown in Figure 8(a), the current was lower at 0℃ than that at 50℃, meanwhile, the mass transfer speed, which would speed up oxidation rate and strengthen the luminous intensity, was heightened on the electrode surface with the increase of temperature[10], so the chemiluminescence intensity climbed up from 0℃ to 50℃. When it was at high temperature, the energy was enough to produce free radical oxidation state and the reduced state of free radicals at 50℃, and oxidation rate also became constant, in this case, fluorescence quantum yield of $Ru(bpy)_3^{2+}$ affected by temperature played a main role in Figure 10, so the chemiluminescence intensity declined from 50℃ to 70℃[15,24-25].

Effect of temperature on the ECL behavior of the benzhexol hydrochloride-$Ru(bpy)_3^{2+}$ system and its fluorescence emission spectra

On the basis of the above study, under the condition of 1×10^{-4} mol/L Ru (bpy) $_3^{2+}$, PH 7.5 BBS, 1×10^{-4} mol/L benzhexol hydrochloride and -850V, we decided to further investigate the effect of temperature on benzhexol hydrochloride - $Ru(bpy)_3^{2+}$ ECL system, as shown in Figure 11 and Figure 12.



(a) Effect of temperature on current (b) Effect of temperature on chemiluminescence

Figure 11 : Effects of temperature on current and chemiluminescence of benzhexol hydrochloride-Ru(bpy)$_3^{2+}$

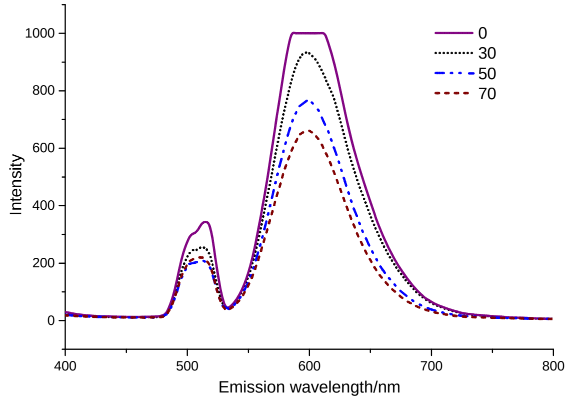


Figure 12 : Effect of different temperatures on Fluorescence spectrum of benzhexol hydrochloride-Ru(bpy)$_3^{2+}$ at 250nm

Comparing Figure 11 and Figure 8, Figure 11(a) had the same change trend with Figure 8(a), however, the influence of temperature on the chemiluminescence intensity is not consistent. The optimum temperature was 50℃ in Figure 8 (b), but it was 30℃ Figure 11(b). By comparison, due to the existence of benzhexol hydrochloride, it not only can be used as electron donor to transfer electrons to the electrode surface, but also can produce free radical intermediates which has strong reducibity, ECL reagent reacted with free radical intermediates and formed excited state, and the light was emitted after the relaxation, so amine oxide played an important role in ECL[8]. Meanwhile, we took into account the effect of temperature on mass transfer rate and Ru(bpy)$_3^{2+}$ oxidation efficiency, compared with annihilation reaction, co-reaction reagent system may be more prone to being excited[26], and fluorescence quantum yield of Ru(bpy)$_3^{2+}$ affected by temperature decreased from 30°C to 70°C, these factors result in shifting optimum temperature forward.

Method evaluation

Under the condition of 1×10^{-4}mol/L Ru(bpy)$_3^{2+}$, PH 7.5 BBS and -850V, the luminous intensity curves with different concentrations of benzhexol hydrochloride was recorded by the workstation at 30℃, as shown in Figure 13, there was a good linear relationship between peak height and concentrations of benzhexol hydrochloride in the range of 1×10^{-8}-1×10^{-4}mol•L^{-1}, the equation of linear regression for benzhexol hydrochloride determination is I (counts) $=27.469\times 10^6$ C+436.88, the detection limit is 6.0×10^{-9} mol•L^{-1} (S/N=3). Meanwhile, the RSD for 6 times determination of 1.0×10^{-4}mol/L benzhexol hydrochloride was 3.01%.

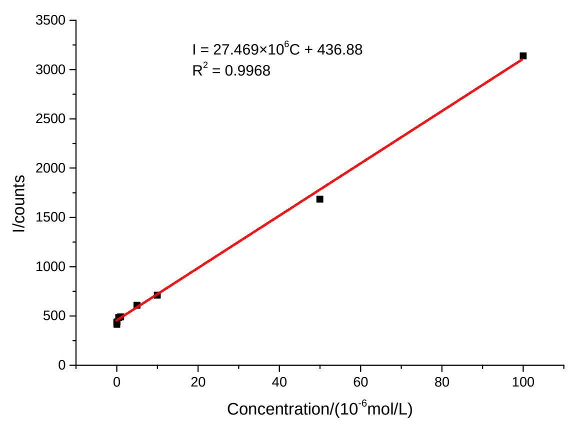


Figure 13 : Linear regression equation of benzhexol hydrochloride-Ru(bpy)$_3^{2+}$

CONCLUSIONS

In the paper, we have proved the temperature had a marked effect on the ECL behavior of the Ru(bpy)$_3^{2+}$ system, and the influence laws of temperature on the Ru(bpy)$_3^{2+}$ system and the benzhexol hydrochloride-Ru(bpy)$_3^{2+}$ co-reagent system were different due to the existence of benzhexol hydrochloride. The main advantage of this study lies in following aspects. First, the ECL intensity first increased and then decreased with temperature increasing from 0°C to 70°C, which provided a new concept in contrast with the view of the ECL intensity increasing with the temperature raising, and the chemiluminescence intensity climbed up from 0°C to 50°C and declined from 50°C to 70°C in the Ru(bpy)$_3^{2+}$ system and the optimum temperature was 30°C in the benzhexol hydrochloride-$Ru(bpy)_3^{2+}$ co-reagent system. Secondly, the fluorescence analysis technique was used to intuitively reveal the effect of temperature on fluorescence quantum yield of $Ru(bpy)_3^{2+}$ and explain the interrelation between the luminous intensity of $Ru(bpy)_3^{2+}$ and temperature. Thirdly, the temperature has a significant influence on the instrument stability, under the same test temperature, the linear range for benzhexol hydrochloride and the detection limit were obtained satisfactorily. So the environment temperature should be kept constant when the electrochemiluminescence technique was applied to determinate samples in the $Ru(bpy)_3^{2+}$ system.

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