Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography B, 831 (2006) 17–23
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Determination of difenidol hydrochloride by capillary electrophoresis with electrochemiluminescence detection

Wen Pan [a], Yingju Liu [b], Yan Huang [a], Shouzhuo Yao [a,*]

[a] *State Key Laboratory of Chemo/Biosensing & Chemometrics, College of Chemistry & Chemical Engineering, Hunan University, Changsha, Hunan Province, China*
[b] *College of Science, South China Agriculture University, Guangzhou, Guangdong Province, China*

Received 10 August 2005; accepted 11 November 2005
Available online 20 December 2005

Abstract

A novel and sensitive method for the determination of difenidol hydrochloride has been established using capillary electrophoresis coupled with end-column electrogenerated chemiluminescence (ECL) detection, based on the ECL reaction of tris(2,2′-bypyridine)ruthenium(II) ($Ru(bpy)_3^{2+}$) with the tertiary amino groups of the difenidol analyte. Parameters that affect separation and detection were optimized. Calibration curve was linear over the range from 1×10^{-6} M to 6×10^{-5} M with a detection limit of 1×10^{-7} M (S/N = 3). Separation of difenidol hydrochloride from clomifene citrate and lidocaine was achieved using the proposed method. This method was successfully utilized to the assay of the active ingredients of the "difenidol hydrochloride" tablets and to the investigation on the interaction of difenidol hydrochloride with hemoglobin. The number of binding sites and the binding constant were estimated as (11.2 and 2.5) $\times 10^3$ M^{-1}, respectively.

Keywords: Capillary electrophoresis; Electrochemiluminescence; Difenidol hydrochloride; Hemoglobin

1. Introduction

During the last two decades, capillary electrophoresis (CE), based on the separation of charged molecules through a small capillary under the influence of an electric field, has emerged as a powerful and popular analytical separation technique for the determination of numerous analytes from organic molecules to macromolecules such as DNA and proteins [1,2] with attractive features, such as high resolving power and small sample volume [3]. Nowadays, it is widely utilized for the daily assays as an excellent complement to high-performance liquid chromatography. Some kinds of conventional detection methods coupled with capillary electrophoresis, including UV–vis absorbance [4], mass spectrometry [5], electrochemistry [6], and fluorescence, have been developed. However, the sensitivity of UV–vis absorbance detection limited by the short optical path length cannot support the application of capillary electrophoresis in trace-level analysis. Laser-induced-fluorescence (LIF) detection can be very sensitive but costly in the same time, considered not to be ideal detection method interfacing with capillary electrophoresis [7].

It was reported for chemiluminescence (CL) detection to be suited to the small volume and low detection limit requirements characteristics of capillary electrophoresis separation. Various CL reagents, such as luminol [8,9] and peroxyoxalate [10], are available for capillary electrophoresis. Based on the extremely low background noise, CL detection offers satisfactory detection limit. Hence, reports on CL detection combined with CE have increased remarkably in recent years [11,12].

Electrogenerated chemiluminescence (ECL) detection is a special chemiluminescence where chemiluminescence emission correlates directly or indirectly with oxidation or reduction at an electrode surface, and has become a sensitive and valuable detection tool. Tri(2,2′-bipyridyl)ruthenium(II), namely $Ru(bpy)_3^{2+}$, may be the most efficient, thoroughly examined, and widely used ECL reagent due to high luminescence efficiency and stability in aqueous media. The light signal is produced in the time when $Ru(bpy)_3^{2+}$ is oxidized to $Ru(bpy)_3^{3+}$ and then react with tertiary, secondary, or primary alkyl amines on the electrode surface. $Ru(bpy)_3^{2+}$ ECL has been extensively utilized as

* Corresponding author. Fax: +86 731 8821848.
E-mail address: szyao@hnu.net.cn (S. Yao).

1570-0232/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2005.11.020

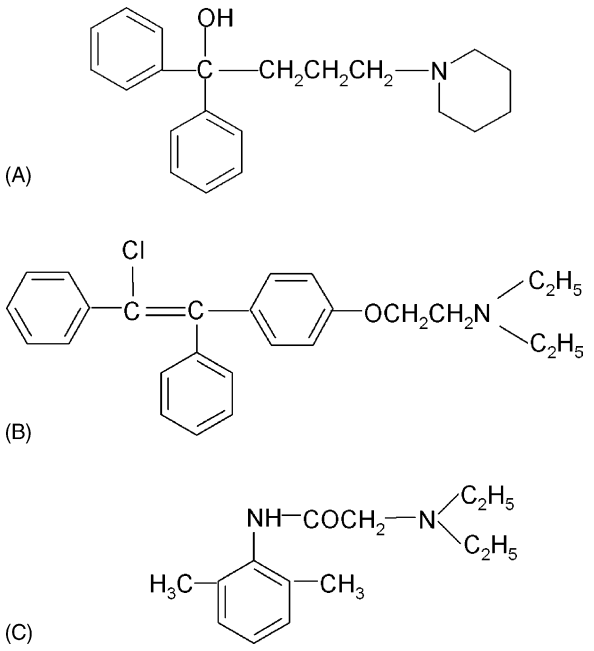

Fig. 1. Molecular structures of difenidol (A), clomifene (B), and lidocaine (C).

a detection means for a lot of amine-containing analytes in flowing streams, such as HPLC [13–15] and flow injection analysis [16–18]. More recently, Wang combined an ECL detection system with capillary electrophoresis and then applied it for the determination of various drugs [19–23].

In this paper, a sensitive method combining the capillary electrophoresis and the end-column $Ru(bpy)_3^{2+}$ ECL detector to determine difenidol hydrochloride is presented. Difenidol hydrochloride is used to relieve or prevent nausea, vomiting, and dizziness caused by certain medical problems. As far as we know, there was scarcely a sensitive analytical method reported for its determination. Since difenidol contains a tertiary amine group in the molecular structure (Fig. 1A), it is expected to be detected through ECL with high sensitivity [16]. Dealing with the ECL detection of difenidol hydrochloride, a detailed process of the optimization to the parameters affecting separation and detection is described. The method was utilized to the assay of the active ingredients of the "difenidol hydrochloride" tablets and to the investigation on the interaction of hemoglobin with difenidol hydrochloride.

2. Experimental

2.1. Reagents

All chemicals and reagents used in this experiment were of analytical grade. Difenidol hydrochloride was extracted from Tablets "difenidol hydrochloride 25 mg" purchased from Hunan Qianjin Pharmaceutical Co. Ltd. (Hunan, China) and was purified to be of analytical grade. Hemoglobin (bovine red cells) was purchased from Worthington Biochemical Corporation (USA). Tris(2,2′-bipyridyl)ruthenium(II) chloride hexahydrate was from J&K Chemical Company. Na_2HPO_4 and KH_2PO_4 were used to prepare buffer solutions with a series of pH values as migration electrolyte. The water employed throughout this experiment to prepare solutions was double-distilled and the solutions were filtered through 0.22 μm membrane prior to use.

2.2. Apparatus

MPI-A CE-ECL was purchased from Xi'an Remax Electronic Science-Tech Co. (China). An uncoated fused-silica capillary (25 μm I.D., 375 μm O.D.) with the length of 50 cm bought from Yongnian Optical Conductive Fiber Plant (Hebei, China) was set between two buffer reservoirs and electrophoresis was driven by the high potential apparatus. Electrochemical analyzer was adopted to carry out constant potential control and its three-electrode system was composed of a 300 μm Pt disk working electrode, an Ag/AgCl reference electrode (KCl saturated), and a Pt wire counter electrode. Multi-channel data acquirement and analysis apparatus was employed to collect the intensity of the electrogenerated chemiluminescence.

CHI 660A electrochemical analyzer was bought from Shanghai Chenhua Apparatus Company (China). Pt wire with the diameter of 1 mm was employed as working electrode, with an Ag/AgCl electrode as reference electrode and a platinum electrode as counter electrode, respectively.

2.3. Preparation of standard solutions

The stock solution of 0.01 M difenidol hydrochloride was prepared by dissolving 34.6 mg difenidol hydrochloride in 10 mL water and stored at 4 °C in a refrigerator. Before use, the stock solution is diluted with water to prepare standard solutions with a series of concentrations for the construction of calibration curve, the study of reproducibility, and the study of recovery.

3. Results and discussion

3.1. Cyclic voltammograms of $Ru(bpy)_3^{2+}$ and difenidol hydrochloride

The cyclic voltammogram of bare Pt electrode in a phosphate buffer solution containing 5 mM $Ru(bpy)_3^{2+}$ displayed reversible electrochemical properties of $Ru(bpy)_3^{2+}$, distinctly different with the cyclic voltammogram in a blank phosphate buffer solution (Fig. 2a and b). Curve c shows the cyclic voltammogram of the mixture composed of 2 mM difenidol hydrochloride and 5 mM $Ru(bpy)_3^{2+}$ in phosphate buffer solution. As shown in curve c, the oxidation peak became stronger while the reduction peak became weaker. According to the earlier reports [24,25], the mechanism can be expressed as following. Firstly, $Ru(bpy)_3^{2+}$ was oxidized to $Ru(bpy)_3^{3+}$ on the working electrode under the applied potential. Secondly, $Ru(bpy)_3^{3+}$ reacts with difenidol hydrochloride and reversed to the excited state $Ru(bpy)_3^{2+*}$, which can release light by returning to the ground state $Ru(bpy)_3^{2+}$. Therefore, $Ru(bpy)_3^{2+}$ concentration greatly increased, leading to the remarkable increase of the oxidation current with a decrease in the reduction current.

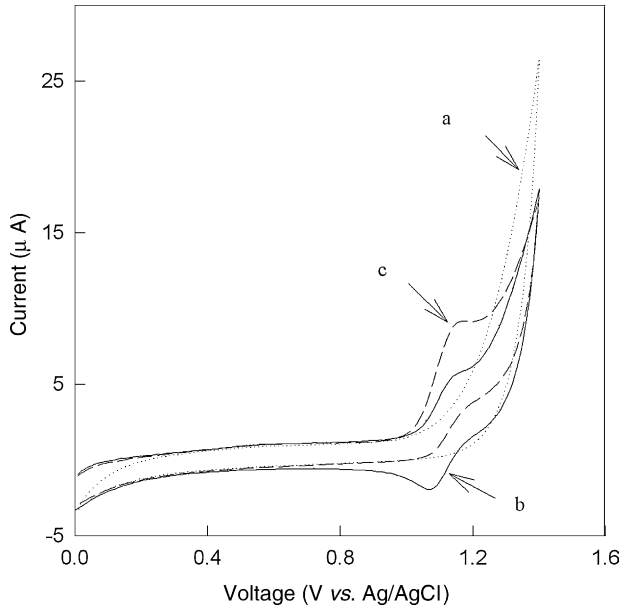

Fig. 2. Cyclic voltammograms for $Ru(bpy)_3^{2+}$ and difenidol hydrochloride in phosphate buffer solution (pH 7.4) at bare Pt electrode. Scan rate, 50 mV/s. (a) Blank phosphate buffer solution only; (b) containing 5 mM $Ru(bpy)_3^{2+}$; and (c) containing 5 mM $Ru(bpy)_3^{2+}$ and 2 mM difenidol hydrochloride.

3.2. Optimization of experimental conditions

Based on the phenomenon observed from the above-mentioned cyclic voltammograms of $Ru(bpy)_3^{2+}$ and difenidol hydrochloride, light signal can be obtained when the solution containing difenidol hydrochloride is injected into the capillary combined with the end-column $Ru(bpy)_3^{2+}$ ECL detector. To obtain a higher S/N ratio, several influencing factors that may affect the separation and the ECL intensity of difenidol hydrochloride were studied in detail, including potential applied at the working electrode, injection time, injection voltage, and the buffer pH value.

3.2.1. Effect of the applied potential

The intensity of the emitted light has significant correlation with the rate of the oxidation of the ECL reagent, namely $Ru(bpy)_3^{2+}$ here, and this oxidation rate is related to the potential applied to the working electrode [26]. Therefore, the influence of the applied potential on the ECL intensity was studied. The relationship of ECL intensity with the applied potential over the range from 1.05 to 1.25 V is plotted in Fig. 3. As shown, while the applied potential increased, the ECL intensity increased to a maximum value at 1.20 V, close to the peak of the oxidation of $Ru(bpy)_3^{2+}$ produced at about 1.15 V in the cyclic voltammogram (Fig. 2). When the applied potential is above 1.20 V, the ECL intensity decreased markedly, possibly resulted from the negative effect of the oxidation of water on the ECL intensity. Hence, in the following experiment, the applied potential was fixed at 1.2 V.

3.2.2. Effect of injection voltage and injection time

Investigation on the influence of the injection voltage and injection time on the ECL intensity and the number of theoretical plates was carried out in detail. The number of theoretical plates (N) was calculated according to the equation:

$$N = 5.54\left(\frac{t_m}{W_{1/2}}\right)^2 \quad (1)$$

where t_m is the migration time and $W_{1/2}$ is the width at half height of the electrophoretic peak. As shown in Fig. 4, when injection voltage increased from 6 to 16 kV and the injection time was fixed at 10 s, the ECL intensity increased but N decreased. The ECL intensity is dependent on the concentration of analyte in the diffusion layer on the working electrode, where the chemiluminescence reaction occurs [27]. At higher injection voltage, more analyte can enter into the diffusion layer, so higher ECL

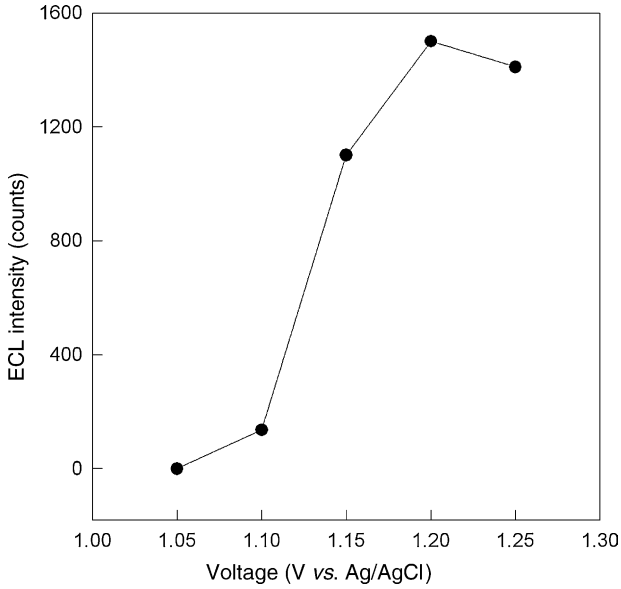

Fig. 3. Effect of applied potential on the ECL intensity. Conditions: $Ru(bpy)_3^{2+}$, 5 mM; difenidol hydrochloride, 1.0×10^{-5} M; injection voltage, 10 kV; injection time, 10 s; phosphate buffer solution, pH 8.0.

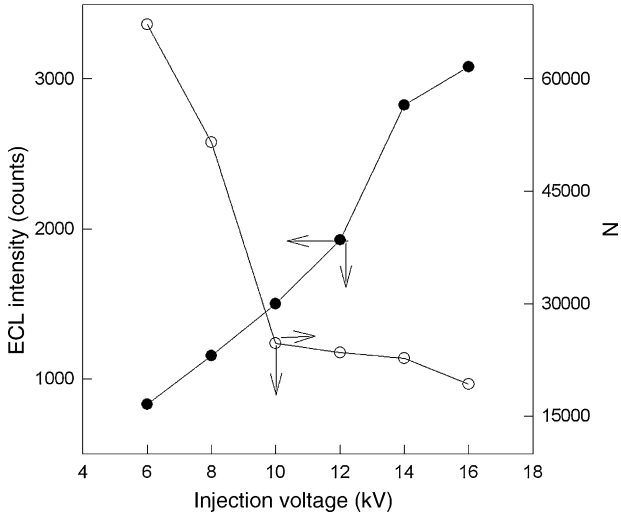

Fig. 4. Effect of injection voltage on the ECL intensity (●) and the number of theoretical plate (○). Conditions: $Ru(bpy)_3^{2+}$, 5 mM; difenidol hydrochloride, 1.0×10^{-5} M; injection time, 10 s; phosphate buffer solution, pH 8.0.

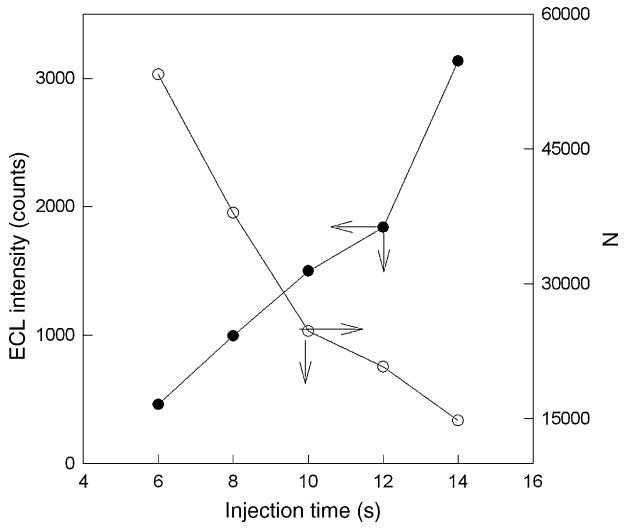

Fig. 5. Effect of injection time on the ECL intensity (●) and the number of theoretical plate (◯). Conditions: $Ru(bpy)_3^{2+}$, 5 mM; difenidol hydrochloride, 1.0×10^{-5} M; injection voltage, 10 kV; phosphate buffer solution, pH 8.0.

signal may be produced. However, dispersion of the analyte may also cause the peak to broaden and the number N to decrease. As illustrated in Fig. 5, the ECL intensity increased but N decreased when injection time increased. This change is attributed to the same reason as that for the effect of injection voltage on the ECL intensity and N. Hence, the injection voltage and the injection time were fixed at 10 kV and 10 s, respectively, to gain a higher ECL signal and a larger N in the following experiments.

3.2.3. Effect of pH

Since $Ru(bpy)_3^{2+}$ ECL reaction with alkylamine depends on the buffer pH value to a great extent [28], the ECL intensity as a function of the buffer pH value over the pH range from 4.5 to 8.0 was investigated. At lower pH value, lower ECL response for difenidol hydrochloride is observed, attributed to the protonation of difenidol. However, when the pH increased up to 7.4, the ECL intensity increased with the protonation on difenidol weakened. OH^- ion can compete with tertiary amine group to react with $Ru(bpy)_3^{3+}$ and lead to considerable consumption of $Ru(bpy)_3^{3+}$ at higher pH [29]. Hence, the phenomenon that the ECL intensity decreased above pH 7.4 can be explained with the reduction of $Ru(bpy)_3^{3+}$ which can react with difenidol hydrochloride. This pH-dependence might be also relative to the phenomenon that maximum ECL activity of tertiary amine occurs at a pH value lower than the pK_a of the amine [28].

3.3. Standard concentration curves of difenidol hydrochloride

Under the optimized experimental conditions: 1.2 V applied potential, pH 7.4, 10 kV injection voltage, 10 s injection time and 15 kV separation voltage, ECL response for a series of concentrations of difenidol hydrochloride were detected. When the concentration of difenidol hydrochloride increased from 1.0×10^{-6} M to 6.0×10^{-5} M, the ECL intensity increased linearly. The linear regression equation is presented as follows:

$$Y = 827 + 151X \quad (r = 0.987) \tag{2}$$

where Y is the ECL intensity (counts), and X is the concentration of difenidol hydrochloride (μM). The limit of detection (LOD) is 1.0×10^{-7} M with 3 as the signal-to-noise ratio, while the relative standard deviation (R.S.D.) of the ECL intensity of 1.0×10^{-5} M difenidol hydrochloride is 3.7% ($n = 6$). The recovery of difenidol hydrochloride at 3.0×10^{-6} M, 5.0×10^{-6} M, 7.0×10^{-6} M, 9.0×10^{-6} M, 1.0×10^{-5} M and 2.0×10^{-5} M were 106, 94, 98, 102, 98 and 104%, respectively. Therefore, CE-ECL method is a sensitive and reliable analytical technique for the determination of difenidol hydrochloride.

3.4. Separation of difenidol hydrochloride from clomifene citrate and lidocaine

It is well known that the most important advantage for the capillary electrophoresis is the powerful ability to separate complex mixture, so the separation of difenidol hydrochloride from other similar drugs by the capillary electrophoresis was studied in this work. Clomifene citrate and lidocaine (Fig. 1B and C) both contain a tertiary amine group similar with difenidol hydrochloride. Hence, we selected them as interferents to be mixed with difenidol hydrochloride and injected the mixture into the capillary to examine whether the mixture can be separated or not under the experimental conditions optimized for the determination of difenidol hydrochloride. The individual ECL responses were obtained at migration time, 307, 391 and 413 s for clomifene citrate, difenidol hydrochloride and lidocaine, respectively. As shown in Fig. 6, under the optimized conditions, clomifene citrate, difenidol hydrochloride and lidocaine, represented with the peaks a–c, respectively, can be separated. But

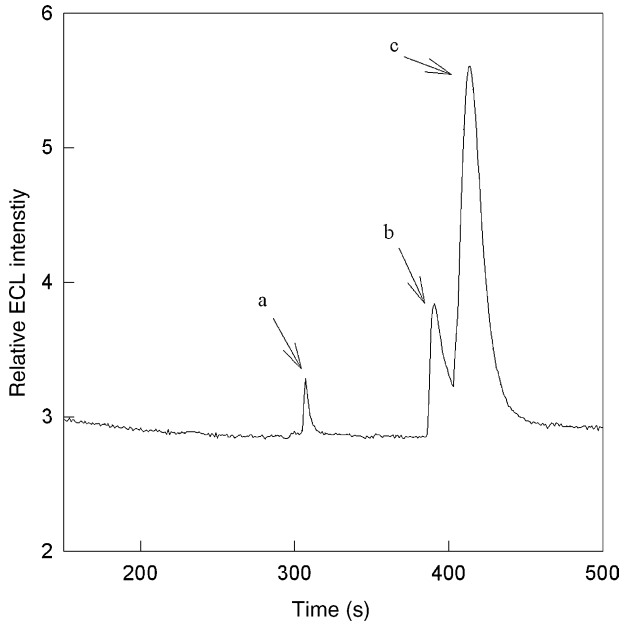

Fig. 6. The separation electrophoregram of three pharmaceuticals: (a) 100 μM clomifene citrate, (b) 1 μM difenidol hydrochloride, and (c) 7 μM lidocaine.

the separation between difenidol hydrochloride and lidocaine is not ideal and the resolution for the two peaks is calculated to be 0.8.

3.5. Interference of other substances

Various representative substances were selected to study the ability of this method to resist interference on the determination of difenidol hydrochloride. These substances included eight inorganic salts, eleven organic compounds, three sulfa drugs and a tertiary amine drug. Firstly, 1.0×10^{-5} M difenidol hydrochloride was tested under the optimized conditions and then a peak was found at the migration time of about 391 s. Secondly, a series of 1.0×10^{-4} M selected substances was detected under the same condition as that of difenidol hydrochloride, respectively. Results are presented in Table 1. The separation factor α is defined as,

$$\alpha = \frac{\Delta t}{t} \quad (3)$$

and the relative ECL intensity factor β is defined as,

$$\beta = \frac{I_1}{I_2} \quad (4)$$

where Δt is the difference of migration time between difenidol hydrochloride and the interferent, t the migration time of difenidol hydrochloride, I_1 represents the ECL intensity resulted from the interferent and I_2 represents the ECL intensity resulted from difenidol hydrochloride. As can be seen from Table 1, no interference was observed for some substances such as potassium chloride, urea, sulfathiazole sodium, and so on. For ferric nitrate, citric acid, formaldehyde, Vitamin B_6, Vitamin C, aminoacetic acid and clomifene citrate, the α values are 0.078, 0.019, 0.328, 0.033, 0.031, 0.012 and 0.174, respectively, while the β values are 0.051, 0.088, 0.024, 0.026, 0.192, 0.102 and 0.097, respectively. It is clear that the smaller the α value is and the bigger the β is the more serious the interference is. However, as shown in Table 1, these substances do not cause any significant interference on the determination of difenidol hydrochloride.

Table 1
Study on interference of other substances on the determination of difenidol hydrochloride

Interfering substance	$\alpha(t)$	$\beta(I)$
Potassium chloride Sodium chloride Zinc chloride Mercury chloride Calcium nitrate Nickel nitrate Ammonium acetate Acetonitrile Ethanol Acetic acid Urea Tartaric acid *o*-Phthalic acid Sulfathiazole sodium Sulfadiazine sodium Sulfacetamide	No peak observed	No peak observed
Ferric nitrate	0.078	0.051
Citric acid	0.019	0.088
Formaldehyde	0.328	0.024
Vitamin B_6	0.033	0.026
Vitamin C	0.031	0.192
Aminoacetic acid	0.012	0.102
Clomifene citrate	0.174	0.097

Table 2
Determination of difenidol hydrochloride in tablets

Number	Labeled (mg/tablet)	Found (mg/tablet)[a]	R.S.D. (%)
040711	25	24.40	3.7
041109	25	24.87	3.2
050108	25	25.34	3.5

[a] Average value of five measurements.

3.6. The analysis of drug tablets

Three batches of drug tablets of "difenidol hydrochloride 25 mg" were purchased from Hunan Qianjin Pharmaceutical Co. Ltd. (Hunan, China) and were analyzed with the proposed CE-ECL method. A number of tablets were ground to exiguous powder in a mortar, dissolved in water, and sonicated for 10 min. The so obtained solution was filtered through a 0.22 μm membrane and diluted 100-fold before injection. The content of difenidol hydrochloride was detected with standard calibration curve. It can be seen from Table 2 that the results obtained with the CE-ECL method are close to the labeled value in every batch. ECL intensity was reported to be easily influenced by the matrix material [30], but the matrix here in this experiment shows that, because of the satisfactory separation of difenidol hydrochloride from the matrix by CE, no significant interference was caused by the matrix material of the tested tablets.

3.7. Study on binding of hemoglobin with difenidol hydrochloride

The distribution and the effect of the drug in the human body are undoubtedly related to the content of unbound drug in total drug and hence it is of interest to study the interaction of protein with drugs. In this work, 50 μL of 100 μM hemoglobin and 200 μL of 0.01 M difenidol hydrochloride were injected into a dialysis bag (21 mm diameter, molecular weight cut-off 8000–14400) and then the dialysis bag was incubated in 2 mL phosphate buffer solution at 37 °C until the equilibrium between the inside and the outside of the dialysis bag was established, which is estimated by the change of the ECL intensity with time for the solution outside the dialysis bag. After the equilibrium, 100 μL of the solution inside the dialysis bag was injected in 2 mL phosphate buffer solution to examine whether hemoglobin bound with difenidol hydrochloride or not, by the UV–vis spectrophotometry. For comparison, 100 μL of 0.01 M difenidol hydrochloride and 40 μL of 100 μM hemoglobin were also injected in 2 mL phosphate buffer solutions, separately, and 100 μL of 0.01 M difenidol hydrochloride was mixed with 40 μL

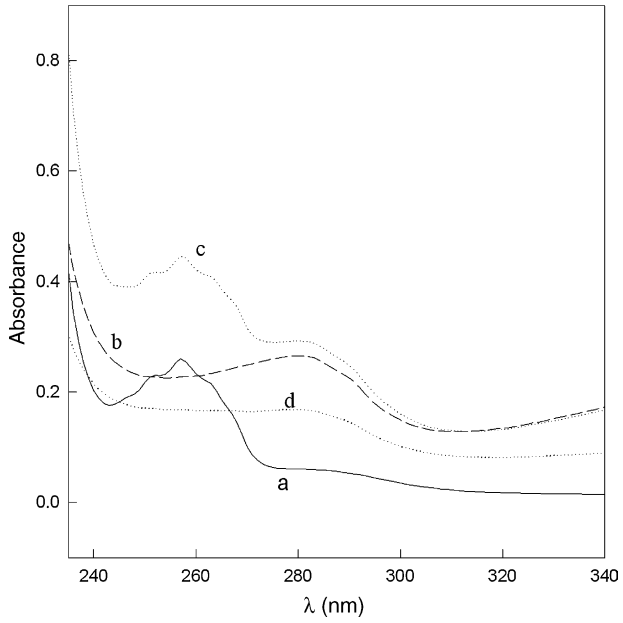

Fig. 7. The UV–vis spectra of 100 μL of 0.01 M difenidol hydrochloride (a), 40 μL of 100 μM hemoglobin (b), 100 μL of 0.01 M difenidol hydrochloride +40 μL of 100 μM hemoglobin (c), and 100 μL of the solution inside the dialysis bag (d), in 2 mL phosphate buffer solution, pH 7.4.

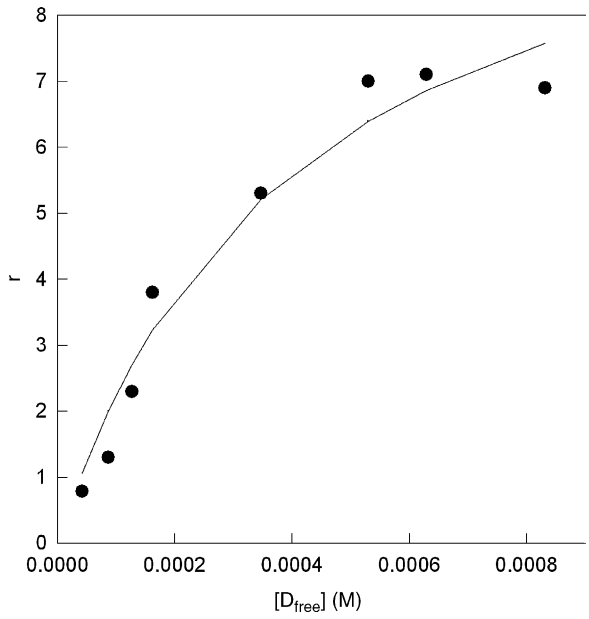

Fig. 8. Binding curve of difenidol hydrochloride with hemoglobin in phosphate buffer solution (pH 7.4) at 37 °C; [D_{free}] is the concentration of free difenidol hydrochloride.

of 100 μM hemoglobin and injected into 2 mL phosphate buffer solution as an example without incubation. The UV–vis spectra corresponding to above solutions were recorded and are shown in Fig. 7. The peak of curve d differs greatly with the peak of curve b, and the peak of curve c, which both attested to the binding of hemoglobin with difenidol hydrochloride under the experimental conditions.

Subsequently, the binding curve was fitted to estimate the binding constant and the number of sites involved in binding. A series of different volumes of difenidol hydrochloride solution was mixed with 100 μL of 200 μM hemoglobin in the dialysis bags, and the dialysis bags were incubated in 2 mL phosphate buffer solution at 37 °C, respectively. After the equilibrium, the ECL intensity of the outside solution was detected. Fig. 8 shows the relationship of the fraction of bound drug molecules per protein molecule versus the concentration of free difenidol hydrochloride. The fraction of bound difenidol hydrochloride per hemoglobin can be expressed by [31]:

$$r = \frac{[D_{bound}]}{[P_{total}]} = n\frac{K[D_{free}]}{1 + K[D_{free}]} \quad (5)$$

where [D_{free}], [D_{bound}], and [P_{total}] stand for the concentrations of free difenidol hydrochloride, bound difenidol hydrochloride, and total hemoglobin, respectively; n denotes the number of sites involved in binding; and K is the binding constant. [D_{free}] can be obtained according to the ECL intensity of the solution outside dialysis bag. The concentration of total difenidol hydrochloride minus [D_{free}] just equals [D_{bound}]. In Fig. 8, using the above equation, the fraction of bound difenidol hydrochloride per hemoglobin can be expressed as:

$$r = 11.2 \times \frac{2500[D_{free}]}{1 + 2500[D_{free}]} \quad (6)$$

Hence, the number of binding sites is 11.2, and the binding constant is $2.5 \times 10^3\ M^{-1}$.

4. Conclusion

The proposed CE-ECL method for the detection of difenidol hydrochloride is simple, reproducible, selective and sensitive. Factors that influence on the ECL intensity and the column efficiency were optimized. Under the optimized conditions, satisfactory separation of difenidol hydrochloride from clomifene citrate and lidocaine was achieved. A linear ECL response for difenidol hydrochloride over the range from 1.0×10^{-6} M to 6.0×10^{-5} M was established with a detection limit of 1.0×10^{-7} M. This method can be utilized for the routine quality control analysis. The suggested method was also successfully utilized to investigate the interaction of hemoglobin with difenidol hydrochloride. The number of binding sites and the binding constant were calculated as (11.2 and 2.5) $\times 10^3\ M^{-1}$, respectively.

Acknowledgments

This work was supported by the Research Fund for the Doctoral Program of Higher Education (20030523017) and the National Natural Science Foundation of China (20335020).

References

[1] J.Z. Xing, J. Lee, S.A. Leadon, M. Weinfeld, X.C. Le, Methods 22 (2000) 157.
[2] M. Ye, S. Hu, W.W.C. Quigley, N.J. Dovichi, J. Chromatogr. A 1022 (2004) 201.
[3] J.W. Jorgenson, K.D. Lukacs, Anal. Chem. 53 (1981) 1298.

[4] C. Vogt, J. Vogt, A. Becker, E. Rohde, J. Chromatogr. A 781 (1997) 391.
[5] Q. Yang, L.M. Benson, K.L. Johnson, S. Naylor, J. Biochem. Biophys. Methods 38 (1999) 103.
[6] X. Sun, W. Cao, X. Bai, X. Yang, E. Wang, Anal. Chim. Acta 442 (2001) 121.
[7] K. Swinney, D.J. Bornhop, Electrophoresis 21 (2000) 1239.
[8] B. Huang, J. Li, L. Zhang, J. Cheng, Anal. Chem. 68 (1996) 2366.
[9] M. Hashimoto, K. Tsukagoshi, R. Nakajima, K. Kondo, J. Chromatogr. A 832 (1999) 191.
[10] K. Tsukagoshi, Y. Okumura, R. Nakajima, J. Chromatogr. A 813 (1998) 402.
[11] C. Kuyper, R. Milofsky, Trends Anal. Chem. 20 (2001) 232.
[12] X. Huang, Z. Fang, Anal. Chim. Acta 414 (2000) 1.
[13] J.B. Noffsinger, N.D. Danielson, J. Chromatogr. A 387 (1987) 520.
[14] W.Y. Lee, T.A. Nieman, J. Chromatogr. A 659 (1994) 111.
[15] K. Uchikura, M. Kirisawa, A. Sugii, Anal. Sci. 9 (1993) 121.
[16] J.B. Noffsinger, N.D. Danielson, Anal. Chem. 59 (1987) 865.
[17] L. He, K.A. Cox, N.D. Danielson, Anal. Lett. 23 (1990) 195.
[18] A.W. Knight, G.M. Greenway, Analyst 120 (1995) 2543.
[19] X. Sun, J. Liu, W. Cao, X. Yang, E. Wang, Y.S. Fung, Anal. Chim. Acta 470 (2002) 137.
[20] J. Yan, J. Liu, W. Cao, X. Sun, X. Yang, E. Wang, Microchem. J. 76 (2004) 11.
[21] J. Liu, W. Cao, X. Yang, E. Wang, Talanta 59 (2003) 453.
[22] W. Cao, X. Yang, E. Wang, Electroanalysis 16 (2004) 169.
[23] J. Liu, W. Cao, H. Qiu, X. Sun, X. Yang, E. Wang, Clin. Chem. 48 (2002) 1049.
[24] H. Qiu, J. Yan, X. Sun, J. Liu, W. Cao, X. Yang, E. Wang, Anal. Chem. 75 (2003) 5435.
[25] R.D. Gerardi, N.W. Barnett, S.W. Lewis, Anal. Chim. Acta 378 (1999) 1.
[26] K.E. Haapakka, J.J. Kankare, Anal. Chim. Acta 138 (1982) 263.
[27] W.Y. Lee, Mikrochim. Acta 127 (1997) 19.
[28] S.N. Brune, D.R. Bobbitt, Talanta 38 (1991) 419.
[29] M. Zorzi, P. Pastore, F. Magno, Anal. Chem. 72 (2000) 4934.
[30] Q. Song, G.M. Greenway, T. McCreedy, Analyst 126 (2001) 37.
[31] A.V. Rudnev, S.S. Aleksenko, O. Semenova, C.G. Hartinger, A.R. Timerbaev, B.K. Keppler, J. Sep. Sci. 28 (2005) 121.