

Short communication

Chemiluminescence detection of isoniazid using $\text{Ru}(\text{phen})_3^{2+}$ –isoniazid–Ce(IV) system

Juan Xi^a, Bo'an Shi^{a,b}, Xinping Ai^a, Zhike He^{a,*}

^a College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, PR China

^b Department of Chemistry and Chemical Engineering, Hubei Institute for Nationalities, Enshi 445000, PR China

Received 29 November 2003; received in revised form 24 May 2004; accepted 29 May 2004

Available online 28 July 2004

Abstract

In our experiment, it was observed that isoniazid could enhance the chemiluminescence (CL) emission of tris-(1,10-phenanthroline) ruthenium(II) ($\text{Ru}(\text{phen})_3^{2+}$)–cerium(IV) (Ce(IV)) system and this enhancement effect was dependent on the concentration of isoniazid, based on which, a novel CL system was established for the detection of isoniazid. Under the optimum experimental conditions, the dynamic range and detection limit are 7.0×10^{-2} to $6.5 \mu\text{g ml}^{-1}$ and $2.5 \times 10^{-2} \mu\text{g ml}^{-1}$, respectively. The R.S.D. is 3.4% ($n = 11$). The proposed method has been applied to detect the content of isoniazid in the injection solution with satisfactory results. The possible mechanism of the CL reaction was studied.

© 2004 Elsevier B.V. All rights reserved.

Keywords: Chemiluminescence; Isoniazid; Tris-(1,10-phenanthroline)ruthenium(II)

1. Introduction

Isoniazid, also called isonicotinic acid hydrazide or Rimifon and chemically named pyridine-4-carboxylic acid hydrazide, is an antitubercular drug and with many advantages, such as highly effective, orally administered, well-tolerated and minimal toxicity, now widely used for the chemotherapy of tuberculosis [1,2]. Quantitative detection of isoniazid is, therefore, very important and significant.

Many analytical methods have been applied to detect isoniazid, including titration [3], spectrophotometry [4,5], fluorimetry [6], electrogenerated chemiluminescence (ECL) [7] and chemiluminescence (CL) [1,2,8–12]. From the above cases reported, it was indicated that, for the detection of isoniazid, the most commonly used approach is the CL method which has been an attractive detection means for trace analysis due to its low detection limits, wide dynamic linear ranges and relatively simple instrumentation. In the above-mentioned literatures on the CL and ECL detection

of isoniazid, the CL determination was based on either luminol system [1,2,7–10] or direct oxidation of isoniazid [11,12] and to our knowledge, there is no report involving in application of tris-(1,10-phenanthroline)ruthenium(II) ($\text{Ru}(\text{phen})_3^{2+}$).

$\text{Ru}(\text{phen})_3^{2+}$ is a sensitive CL reagent and has been used to determine many chemicals, such as organic acids [13], sulfite [14], nucleic acids [15], barbituric acid [16], and so on. In the experiments of this paper, it was observed that isoniazid could enhance the CL emission of $\text{Ru}(\text{phen})_3^{2+}$ –cerium(IV) (Ce(IV)) system and the enhancement degree was proportional to the content of isoniazid. Hence, a novel CL system was established to detect isoniazid. Under the optimum experimental conditions, the CL intensity is linear to the concentration of isoniazid in the range of 7.0×10^{-2} to $6.5 \mu\text{g ml}^{-1}$ and the detection limit is $2.5 \times 10^{-2} \mu\text{g ml}^{-1}$. The proposed method exhibits higher precision and has been successfully applied to detect isoniazid injection solution. The possible mechanism of corresponding CL reaction was also studied. By the work in this paper, a novel method for the detection of isoniazid was developed and furthermore, the analytical application of $\text{Ru}(\text{phen})_3^{2+}$ was expanded.

* Corresponding author. Tel.: +86 2787162672; fax: +86 2787647617.
E-mail address: zhkhe@whu.edu.cn (Z. He).

2. Experimental

2.1. Apparatus

LKB-1251 luminometer based upon collection of all light emitted (no wavelength discrimination), with dispenser SVD and dispenser controller DC (Pharmacia LKB Biotechnology, Uppsala, Sweden) and YEW-3066 pen recorder (Chongqing the fourth equipment factory, China) were used.

2.2. Reagents

All the reagents were of analytical-reagent grade and were directly used for following experiments without purification and all water used was doubly distilled in fused-silica apparatus.

A 2 mg ml^{-1} stock standard solution of $\text{Ru(phen)}_3\text{Br}_2$ was prepared by dissolving 200 mg of $\text{Ru(phen)}_3\text{Br}_2$ prepared in our laboratory in 100 ml of water. A 0.5 mol l^{-1} solution of H_2SO_4 was prepared by dissolving 6.90 ml of concentrated sulfuric acid (Shanghai the first reagent factory, China) in 250 ml of water. A 0.1 mol l^{-1} stock standard solution of Ce(IV) ($0.5 \text{ mol l}^{-1} \text{ H}_2\text{SO}_4$) was prepared by dissolving 4.04 g of $\text{Ce(SO}_4)_2 \cdot 4\text{H}_2\text{O}$ (China medicine (group) Shanghai chemical reagent corporation, China) in 40 ml of water and 2.80 ml of concentrated sulfuric acid and diluting with water to 100 ml. A 1 mg ml^{-1} stock standard solution of isoniazid was prepared by dissolving 100 mg of isoniazid (Shanghai the first reagent factory, China) in 100 ml of water. The working solutions used in experiments were prepared by diluting the stock standard solutions with water.

$\text{Ru(phen)}_3\text{Br}_2$ was prepared in our laboratory according to reference [17]: a mixture of 0.2 g of $\text{RuCl}_3 \cdot 3\text{H}_2\text{O}$ (Sigma, USA) and 4.55 g of 1,10-phenanthroline (Shanghai the third reagent factory, China) was heated at reflux for 3 h in 10 ml of *N,N*-dimethylformamide (DMF, Shanghai the first reagent factory, China). At the end of 3 h, the DMF was slowly distilled off, until the solution volume was about 2 ml. The DMF solution that remained was added dropwise to a saturated solution of tetra-*n*-butylammonium bromide (China medicine (group) Shanghai chemical reagent corporation, China) in acetone (Shanghai the first reagent factory, China), which is precipitated in bromide salt, $\text{Ru(phen)}_3\text{Br}_2$. The product was recrystallized in water for two times and dried to use for following experiments.

2.3. Procedures

A 0.40 ml of $15 \text{ } \mu\text{g ml}^{-1}$ $\text{Ru(phen)}_3\text{Br}_2$ and 0.40 ml of $5 \text{ } \mu\text{g ml}^{-1}$ isoniazid were mixed in sample cuvettes and then the cuvettes were transferred into the measuring chamber of the luminometer. After the start button of the dispenser controller had been pushed, 0.20 ml of 2 mmol l^{-1} Ce(IV) ($0.12 \text{ mol l}^{-1} \text{ H}_2\text{SO}_4$) was injected into the sample cuvettes automatically by the dispenser and the signal of CL produced

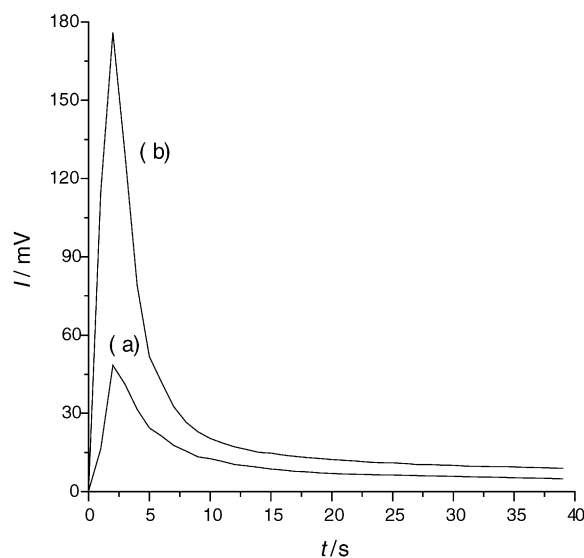


Fig. 1. The CL kinetic curves of the systems. Conditions: $2.0 \text{ } \mu\text{g ml}^{-1}$ isoniazid, $6.0 \text{ } \mu\text{g ml}^{-1}$ $\text{Ru(phen)}_3\text{Br}_2$, 0.4 mmol l^{-1} Ce(IV) and 24 mmol l^{-1} H_2SO_4 . (a) Ru(phen)_3^{2+} - Ce(IV) system, (b) Ru(phen)_3^{2+} -isoniazid- Ce(IV) system.

was measured immediately. The maxima of the CL kinetic profiles were recorded for quantitative analysis.

3. Results and discussion

3.1. Establishment of Ru(phen)_3^{2+} -isoniazid- Ce(IV) CL system

The CL intensity of Ru(phen)_3^{2+} - Ce(IV) system in the absence of and in the presence of isoniazid were recorded continuously with the recorder, respectively, and the obtained CL kinetic curves were shown in Fig. 1. The experimental results indicated that the CL emission of the investigated system was weak but could be enhanced proportionably by isoniazid. Hence, a novel CL system, Ru(phen)_3^{2+} -isoniazid- Ce(IV) , was established and employed to detect isoniazid.

3.2. Selection of the optimum experimental conditions

3.2.1. Selection of the concentration of Ru(phen)_3^{2+}

It is well known that for the CL system involved in Ru(phen)_3^{2+} , the light emission is generated from the excited state species $[\text{Ru(phen)}_3^{2+}]^*$ which is the reaction product of Ru(phen)_3^{3+} with the appropriate reductants, commonly the analytes, so Ru(phen)_3^{2+} is the luminophor of the system [13]. With the solutions containing a variable amount of $\text{Ru(phen)}_3\text{Br}_2$ from 2.0 to $16 \text{ } \mu\text{g ml}^{-1}$, $2.0 \text{ } \mu\text{g ml}^{-1}$ isoniazid, and 0.4 mmol l^{-1} Ce(IV) ($24 \text{ mmol l}^{-1} \text{ H}_2\text{SO}_4$), the effect of the concentration of $\text{Ru(phen)}_3\text{Br}_2$ on the systems was investigated by determining the CL intensity of Ru(phen)_3^{2+} - Ce(IV)

Table 1
Effect of the concentration of $\text{Ru}(\text{phen})_3^{2+}$

Concentration of $\text{Ru}(\text{phen})_3\text{Br}_2$ ($\mu\text{g ml}^{-1}$)	I_0 (mV)	I (mV)	I/I_0
2.0	8	31	3.9
4.0	15	65	4.3
6.0	19	113	5.9
8.0	29	167	5.8
10	36	196	5.4
12	46	225	4.9
14	58	273	4.7
16	72	308	4.3

Conditions: $2.0 \mu\text{g ml}^{-1}$ isoniazid, 0.4 mmol l^{-1} $\text{Ce}(\text{IV})$ and 24 mmol l^{-1} H_2SO_4 .

system (I_0) and $\text{Ru}(\text{phen})_3^{2+}$ -isoniazid- $\text{Ce}(\text{IV})$ system (I), respectively, and calculating the ratio of I to I_0 (I/I_0) and the results were listed in Table 1. From Table 1, it was shown that with the concentration of $\text{Ru}(\text{phen})_3\text{Br}_2$ varying from 2.0 to $16 \mu\text{g ml}^{-1}$, both I_0 and I increasingly increase, which proved the conclusion of the literature, that is, $\text{Ru}(\text{phen})_3^{2+}$ is the luminophor of the systems [13]. Here, I/I_0 value was used to select the concentration of $\text{Ru}(\text{phen})_3\text{Br}_2$. The experimental results showed that with the amount of $\text{Ru}(\text{phen})_3\text{Br}_2$ increasing, I/I_0 increased firstly and then decreased and the maximum value was obtained at $6.0 \mu\text{g ml}^{-1}$. To maximize the relative increase of the CL signal in the presence of isoniazid, $6.0 \mu\text{g ml}^{-1}$ of $\text{Ru}(\text{phen})_3\text{Br}_2$ was selected for the following experiments.

3.2.2. Selection of the concentration of $\text{Ce}(\text{IV})$

As the oxidant, the concentration of $\text{Ce}(\text{IV})$ could affect the CL intensity of the systems and the corresponding experiments were carried out under the fixed amount of $\text{Ru}(\text{phen})_3\text{Br}_2$ and H_2SO_4 and the variational concentration of $\text{Ce}(\text{IV})$ in the range of 0.1 – 0.6 mmol l^{-1} . From the experimental results shown in Fig. 2, it was seen that when the concentration of $\text{Ce}(\text{IV})$ increased from 0.1 to 0.4 mmol l^{-1} ,

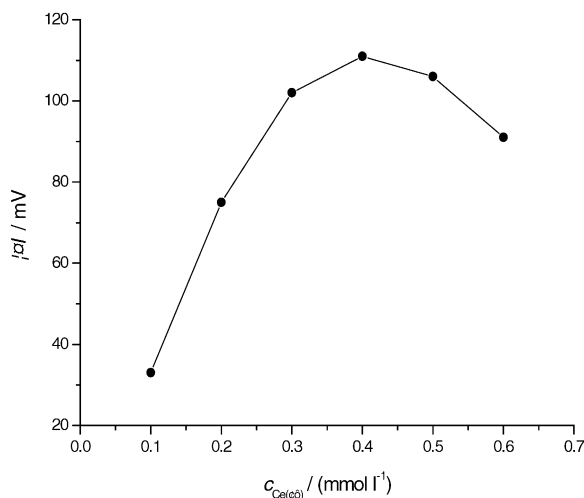


Fig. 2. Effect of the concentration of $\text{Ce}(\text{IV})$. Conditions: $2.0 \mu\text{g ml}^{-1}$ isoniazid, $6.0 \mu\text{g ml}^{-1}$ $\text{Ru}(\text{phen})_3\text{Br}_2$ and 24 mmol l^{-1} H_2SO_4 .

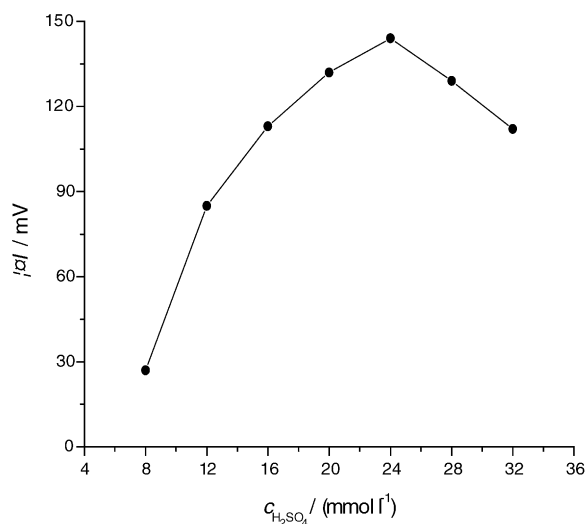


Fig. 3. Effect of the concentration of H_2SO_4 . Conditions: $2.0 \mu\text{g ml}^{-1}$ isoniazid, $6.0 \mu\text{g ml}^{-1}$ $\text{Ru}(\text{phen})_3\text{Br}_2$ and 0.4 mmol l^{-1} $\text{Ce}(\text{IV})$.

ΔI value ($\Delta I = I - I_0$) which was used to evaluate the enhancement degree of isoniazid to the CL emission of $\text{Ru}(\text{phen})_3^{2+}$ - $\text{Ce}(\text{IV})$ system increasingly increased and after the concentration of $\text{Ce}(\text{IV})$ exceeded 0.4 mmol l^{-1} , ΔI value decreased, which maybe was due to the absorption of light emission by the colored $\text{Ce}(\text{IV})$ solution and the scattering of light emission by the unsolvable hydrolysis product of $\text{Ce}(\text{IV})$ at the experimental acidity. In order to obtain the maximum ΔI value, 0.4 mmol l^{-1} of $\text{Ce}(\text{IV})$ was selected for the further experiments.

3.2.3. Selection of the concentration of H_2SO_4

In the medium of dilute H_2SO_4 , $\text{Ce}(\text{IV})$ exists in the form of multifarious sulfated complexes, such as $\text{Ce}(\text{SO}_4)_2^{2+}$, $\text{Ce}(\text{OH})(\text{SO}_4)^+$, $\text{Ce}(\text{SO}_4)_2$, $\text{Ce}(\text{SO}_4)_3^{2-}$, $\text{HCe}(\text{SO}_4)_3^-$, $\text{HCe}(\text{SO}_4)_4^{3-}$ and $\text{Ce}(\text{SO}_4)_4^{4-}$, and these species are in a series of equilibria with HSO_4^- [18,19]. It has already been pointed out that the reactive species of the oxidants are $\text{Ce}(\text{IV})$, $\text{Ce}(\text{SO}_4)_2$ and $\text{HCe}(\text{SO}_4)_3^-$ [20]. So, for $\text{Ru}(\text{phen})_3^{2+}$ -isoniazid- $\text{Ce}(\text{IV})$ CL system, in order to produce the maximum amount of reactive oxidants, appropriate concentration of H_2SO_4 is required. As shown in Fig. 3, under the experimental conditions noted above, the concentration of H_2SO_4 was selected in the range of 8 – 32 mmol l^{-1} and when the concentration of H_2SO_4 was 24 mmol l^{-1} , the maximum ΔI value was obtained, that is, at this concentration of H_2SO_4 , there were the maximum amount of reactive oxidants. Hence, 24 mmol l^{-1} H_2SO_4 was selected for the detection of isoniazid.

3.3. Calibration, detection limit and precision of the method proposed

Under the optimum experimental conditions selected above, the calibrations of the responses of CL intensity (ΔI)

Table 2
Analysis of injector solution of isoniazid ($n = 5$)

Claimed value (mg ml^{-1})	Determined value (mg ml^{-1})	Contained ($\mu\text{g ml}^{-1}$)	Added ($\mu\text{g ml}^{-1}$)	Found ($\mu\text{g ml}^{-1}$)	Recovery (%)	R.S.D. (%)
50.0	49.7 ± 1.6	1.59×10^{-1}	4.00×10^{-1}	5.80×10^{-1}	105.2	3.7
		1.59	2.00	3.55	98.0	3.3

to the concentrations of isoniazid (c) are shown as follows: $\Delta I = 17.8 + 9.8 \times 10c$ ($\mu\text{g ml}^{-1}$) ($r = 0.9984$) in the range of 7.0×10^{-2} to $1.0 \mu\text{g ml}^{-1}$ and $\Delta I = 94.7 + 2.6 \times 10c$ ($\mu\text{g ml}^{-1}$) ($r = 0.9949$) in the range of 1.0 – $6.5 \mu\text{g ml}^{-1}$. The detection limit (DL) of the proposed method is $2.5 \times 10^{-2} \mu\text{g ml}^{-1}$, which was calculated in the ratio of three times the standard deviation of I_0 ($n = 11$) to the slope of the calibration graph. The precision of the proposed method is evaluated by determining samples containing 4.0×10^{-1} and $4.0 \mu\text{g ml}^{-1}$ isoniazid, and the R.S.D. is both 3.4% for low concentrations ($n = 11$).

3.4. Effect of common substances

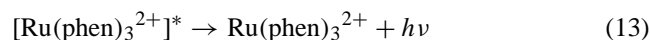
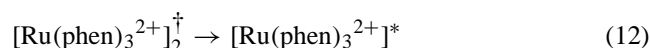
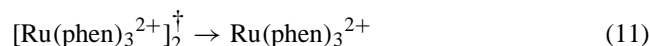
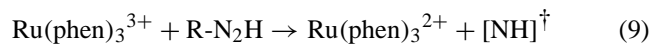
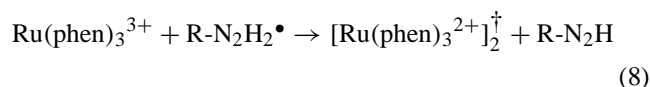
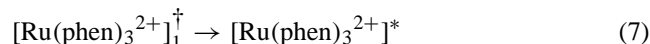
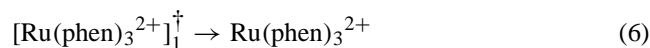
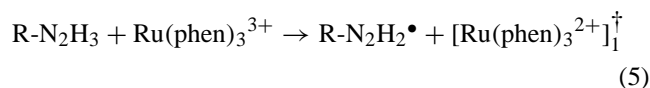
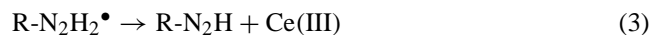
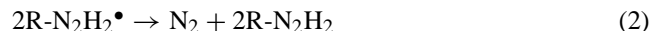
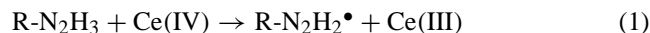
The effect of common substances in the laboratory on detection of isoniazid was investigated with the solution containing $1.0 \mu\text{g ml}^{-1}$ isoniazid. The experimental results showed that a 1000-fold excess of K^+ , Na^+ , Cl^- , Br^- or NO_3^- , a 500-fold excess of Mg^{2+} or NH_4^+ , a 100-fold excess of Ca^{2+} , Co^{2+} , CO_3^{2-} , starch, sucrose or *o*-benzene dicarboxylic acid, and a 50-fold excess of Mn^{2+} , Cu^{2+} or PO_4^{3-} could not interfere the detection of isoniazid (the relative deviation $\leq 5\%$).

3.5. Detection of isoniazid injection solution

The isoniazid injection solution (Guangzhou Mingxing Pharmacy Factory, China) was analyzed by the proposed CL method in this paper and the determined value was consistent with the claimed one. The recovery experiments were carried out by adding standard isoniazid solutions to injection samples and the satisfactory results were obtained. The above experimental data were listed in Table 2.

3.6. Possible mechanism of corresponding CL reaction

Concerning the reaction of hydrazine with Ce(IV) or ruthenium(III) chelates, the in-depth investigation has been carried out and the more detailed mechanism has been discussed [21,22]. As its derivative, the reaction of isoniazid with those two substances should be analogous to hydrazine. Then collecting the conclusions of the literatures [13,21,22], the possible mechanism of $\text{Ru}(\text{phen})_3^{2+}$ –isoniazid–Ce(IV) CL system was proposed as shown by Eqs. (1)–(13) where $\text{R-N}_2\text{H}_3$, $[\text{Ru}(\text{phen})_3^{2+}]^\dagger$ and $[\text{NH}]^\ddagger$ were used to represent isoniazid, some activated reductive products of $\text{Ru}(\text{phen})_3^{3+}$ and certain intermediate in the oxidation reaction of N_2H_2 , respectively.



Acknowledgements

The authors acknowledge the supports of Alexander von Humboldt Foundation, National Natural Science Foundation of China (20275028), National Key Basic Research and Development Program (973 Program) (2002CB2118), Key Technologies R&D Programme of Hubei Province, Educational Ministry of China, and Alumni Association of Wuhan University.

References

- [1] A. Safavi, M.A. Karimi, M.R.H. Nezhad, J. Pharm. Biomed. Anal. 30 (2003) 1499–1506.
- [2] Z.H. Song, J.H. Lü, T.Z. Zhao, Talanta 53 (2001) 1171–1177.
- [3] P. Nagendra, H.S. Yathirajan, K.N. Mohana, K.S. Rangappa, J. Indian Chem. Soc. 79 (2002) 75–78.
- [4] B.G. Gowda, M.B. Melwanki, J. Seetharamappa, K.C. Srinivasa Murthy, Anal. Sci. 18 (2002) 839–841.

- [5] N. Erk, *Spectrosc. Lett.* 34 (2001) 745–761.
- [6] R.A.S. Lapa, J.L.F.C. Lima, J.L.M. Santos, *Anal. Chim. Acta* 419 (2000) 17–23.
- [7] X.W. Zheng, Z.H. Guo, Z.J. Zhang, *Anal. Sci.* 17 (2001) 1095–1099.
- [8] S.C. Zhang, H. Li, *Anal. Chim. Acta* 444 (2001) 287–294.
- [9] Y.M. Huang, Z.J. Zhang, D.J. Zhang, J.G. Lü, *Fresenius J. Anal. Chem.* 368 (2000) 429–431.
- [10] J.G. Lü, Y.M. Huang, Z.J. Zhang, *Anal. Lett.* 34 (2001) 1323–1330.
- [11] B.X. Li, Z.J. Zhang, W. Liu, *Talanta* 54 (2001) 697–702.
- [12] Y.M. Huang, Z.J. Zhang, *Anal. Lett.* 34 (2001) 1703–1710.
- [13] Z.K. He, H. Gao, L.J. Yuan, Q.Y. Luo, Y.E. Zeng, *Analyst* 122 (1997) 1343–1345.
- [14] H. Meng, F.W. Wu, Z.K. He, L.J. Yuan, Q.Y. Luo, Y.E. Zeng, *Int. J. Environ. Anal. Chem.* 75 (1999) 299–307.
- [15] H.Y. Han, Z.K. He, Y.E. Zeng, *Fresenius J. Anal. Chem.* 364 (1999) 782–785.
- [16] J. Xi, X.P. Ai, Z.K. He, *Talanta* 59 (2003) 1045–1051.
- [17] N.B. James, J.M. Thomas, *J. Am. Chem. Soc.* 95 (1973) 3158–3162.
- [18] B.S.S. Muhammad, R.K. Vijayachander, *Bull. Chem. Soc. Jpn.* 36 (1963) 949–953.
- [19] W.A. Waters, I.R. Wilson, *J. Chem. Soc.* (1966) 534–536.
- [20] S. Lakshmi, R. Renganathan, *Int. J. Chem. Kinet.* 28 (1996) 713–720.
- [21] J.I. Morrow, G.W. Sheeres, *Inorg. Chem.* 11 (1972) 2606–2608.
- [22] F.E. Lytle, D.M. Hercules, *Photochem. Photobiol.* 13 (1971) 123–133.