Research article

Drug Testing and Analysis

Received: 15 November 2010 Revised: 21 April 2011 Accepted: 21 April 2011 Published online in Wiley Online Library: 14 June 2011

(www.drugtestinganalysis.com) DOI 10.1002/dta.299

A sensitive flow injection chemiluminescence method for the determination of progesterone

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A sensitive flow injection (FI) chemiluminescence (CL) method was developed for the determination of trace amounts of progesterone. This method was based on the luminescent properties of the tris(1,10-phenanthroline) ruthenium(II) - potassium permanganate ($KMnO_4$) - progesterone in acidic medium sensitized by Na_2SO_3. With the peak height as a quantitative parameter applying optimum conditions, the relative CL intensity was linear with progesterone concentration in the range of $1.0 \times 10^{-10} \sim 6.0 \times 10^{-9}$ g·ml^{-1} and $6.0 \times 10^{-9} \sim 4.0 \times 10^{-8}$ g·ml^{-1} with a detection limit of 7.1×10^{-11} g·ml^{-1}. The relative standard deviation (RSD) was 2.79% for 1.0×10^{-8} g·ml^{-1} progesterone ($n = 11$). The proposed method held low detection limit and was successfully applied to determination of progesterone in pharmaceutical preparations. The possible CL reaction mechanism was also discussed.

Keywords: flow injection; chemiluminescence; progesterone; potassium permanganate; tris(1,10-phenanthroline)ruthenium(II)

Introduction

Progesterone (chemical structure in Figure 1) is a steroid hormone that is involved in regulating female reproductive processes. Its concentration in blood is measured to determine ovarian function. Progesterone participates in the regulation of the menstrual cycle and is especially important in preparing the uterus for the implantation of the blastocyst and in the maintenance of pregnancy. In non-pregnant women, progesterone is secreted mainly by the corpus luteum in the ovary formed by an ovarian follicle following the discharge of its ovum. During pregnancy, the placenta becomes the major source of this hormone.[1] Progesterone is a progestin commonly used in clinical drug analysis; the determination of progesterone is very important.

A variety of techniques is reported for the determination of progesterone in raw medicines, such as fluorescence,[2] high performance liquid chromatography (HPLC),[3] polarography,[4] chemiluminescence (CL) methods,[5,6] and immunoassay[7,8]. Ren *et al.*[5] have developed chemiluminescence enzyme immunoassay for the determination of progesterone using luminal-hydrogen as chemiluminescence system catalyzed by horseradish peroxidase. The linear range for the determination of progesterone is from 0.5 ~ 60 μg·l^{-1} with a detection limit of 0.08 μg·l^{-1}. Deftereos and Calokerinos[6] have developed cerium (IV)-sulfite chemiluminescence system for the determination of steroids. The linear range for the determination of progesterone is from 1.0 ~ 20 μg·ml^{-1} with a detection limit of 0.05 μg·ml^{-1}.

Some methods for the determination of progesterone are shown in Table 1.

CL has received much attention in various fields, especially combination with flow injection (FI). The method of CL relying on the effects related to the chemical reaction only, i.e. without the need of external energy supply, has been found to be more advantageous than other luminescence methods. It is characterized by high sensitivity, wide dynamic range of concentrations of the substances determined, minimum background, no disturbances and light scattering, reproducibility and the possibility of simple and quick analysis.[11,12]

Experimental

Reagents and solutions

The stock standard solution (1.0×10^{-4} g ·ml^{-1}) of progesterone (National institute for the control of pharmaceutical and biological products, Beijing, China) was prepared by dissolving 0.0100 g progesterone with ethanol and diluting into 100-ml volumetric flask with water.

Stock solution (1.0×10^{-2} g ·ml^{-1}) of tris(1,10-phenanthroline) ruthenium(II) ($Ru(phen)_3^{2+}$, which was prepared in our laboratory) was prepared by dissolving 1.0000g $Ru(phen)_3^{2+}$ in a 100-ml volumetric flask with water.

Tris(1,10-phenanthroline) ruthenium(II) ($Ru(phen)_3^{2+}$) was prepared according to the method outlined by Meyer *et al.*:[13] $Ru(phen)_3^{2+}$ a mixture of 0.2g $RuCl_3 \cdot 3H_2O$ (Sigma, St. Louis, USA) and 4.55g 1,10-phenanthroline was heated at reflux for 10 h in 10 ml N,N-dimethylformamide (DMF). At the end of 10 h, the DMF was slowly distilled off, until the solution was about 3 ml. The DMF solution which remained was added dropwise to a saturated solution of Tetrabutyl ammonium bromide in reagent grade acetone, which precipitated the bromide salts, $Ru(phen)_2Br_2$. The product was recrystallized in acetone and dried to use for following experiments.

Stock solution (2×10^{-2} mol ·l^{-1}) of $KMnO_4$ was prepared by dissolving 0.7902 g $KMnO_4$ in a 250-ml volumetric flask with water.

The 2.5×10^{-3} mol·l^{-1} of Na_2SO_3 and 0.4 mol·l^{-1} HNO_3 were also prepared.

All reagents were of analytical grade and double-distilled water was used throughout the experiment.

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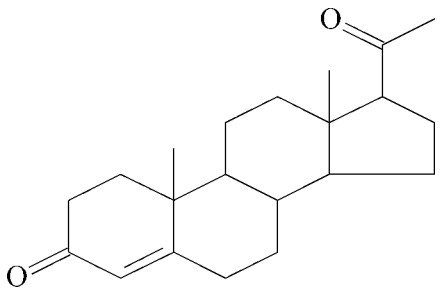

Figure 1. Chemical structure of progesterone.

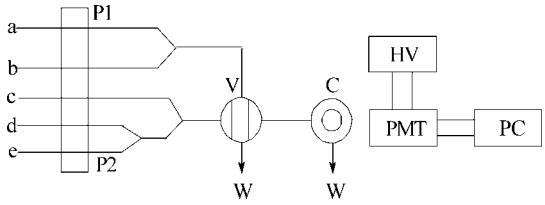

Figure 2. Schematic diagram of the FI system with CL detection for determination of progesterone **P1**, P2: peristaltic pump; V: injection valve; C: flow cell; W: waste; HV: negative high voltage; PMT: photomultiplier tube; PC: computer; a: progesterone; b: Na_2SO_3; c: $Ru(phen)_3^{2+}$; d: $KMnO_4$; e: HNO_3.

Apparatus

All CL detection was done with the IFFM-E mode FI-CL analysis system (Xi'an Remex Analysis Instrument Co., Ltd, Xi'an, China). It had two peristaltic pumps and injection system synchronized by microprocessors. All the reactor coils were made of Teflon tubing (i.d. 0.8 mm). The flow cell was a glass tube (i.d. 0.5 mm) connected with a selected high sensitivity, low noise, photomultiplier tube (PMT). The CL emission was converted by PMT to amplify the signal and the signal was fed to luminescence analyzer, recorded with a computer via special software. The FI manifold was shown schematically in Figure 2. The peak height emission was measured. A CL spectrum was obtained by LS 55 Fluorescence Spectrometer (Perkin Elmer Inc., Massachusetts, USA).

Procedure

As shown in Figure 2, Flow tubes a–e were injected with progesterone standard or sample solution, Na_2SO_3 solution, $Ru(phen)_3^{2+}$ solution, $KMnO_4$ solution and HNO_3 solution respectively. The sample solution was injected through the sample injection valve, which allowed the mixing of the sample with Na_2SO_3 solution, then combination with $Ru(phen)_3^{2+}$, $KMnO_4$ and HNO_3 just before the detector. The CL signal was monitored by the detector and displayed on the computer screen simultaneously.

Take five mixed injection, and then take 2 ml (equivalent to 20 mg of progesterone) dissolved with ethanol, with a sub-page to the lower liquid funnel, transfer the upper layer of liquid to 250 mL flask, and finally set the volume with water.

Results and discussion

Kinetic curve

Before the method was carried out, the kinetic characteristics of the proposed CL reaction were studied by using the batch method. In the batch mode, the experimental parameters were kept constant. The kinetic curve of CL reaction in the presence of progesterone

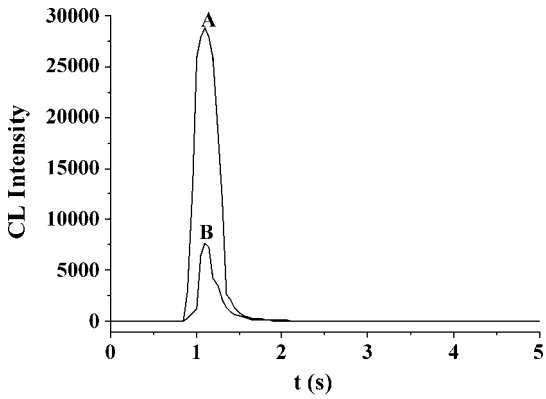

Figure 3. Kinetic curve: (A) in the presence of progesterone, (B) in the absence of progesterone. Conditions: $KMnO_4$: 1.3×10^{-3} mol·l^{-1}; HNO_3: 0.4 mol·l^{-1}; $Ru(phen)_3^{2+}$: 1.0×10^{-4} g·ml^{-1}; Na_2SO_3: 2.5×10^{-3} mol·l^{-1}; progesterone: 1.0×10^{-8} g·ml^{-1}.

was investigated with a static method (Figure 3A). This system showed a fast-type luminescence and the CL intensity reached a maximum value within 2 s after mixing. Comparing with that without progesterone (Figure 3B), the emission intensity increased significantly. This showed the characteristic of this system was suitable for FI-CL determination.

Effect of $KMnO_4$ concentration

The effect of $KMnO_4$ concentration on the CL intensity was examined over the range $2.0 \times 10^{-4} \sim 2.0 \times 10^{-3}$ mol·l^{-1}. Maximum CL intensity was observed at 1.3×10^{-3} mol·l^{-1}, as shown in Figure 4. Therefore, 1.3×10^{-3} mol·l^{-1} was used for all the following work.

Effect of acid type and concentration

The reaction could be carried out in acidic medium. The CL reaction of progesterone was examined in the system by using

Table 1. Results of determination of progesterone using different methods

Analyte	Method	Linear range	Limits of detection	Reference
Human serum	Liquid Chromatography/Tandem Mass Spectrometry	0.151-24.42 ng/g	1.8 pg	[1]
serum	HPLC	$0 - 1 \times 10^{-6}$ g·mL^{-1}	5.5×10^{-8} mol·L^{-1}	[3]
progesterone	sweep polarography	$8 \times 10^{-6} - 4 \times 10^{-8}$ mol·L^{-1}	2×10^{-8} mol·L^{-1}	[4]
progesterone	Chemiluminescence immunoassay	5.0–50 nmol·L^{-1}	0.64 nmol·L^{-1}	[9]
serum	Chemiluminescence immunoassay	0.98–127 nmol·L^{-1}	0.54 nmol·L^{-1}	[10]

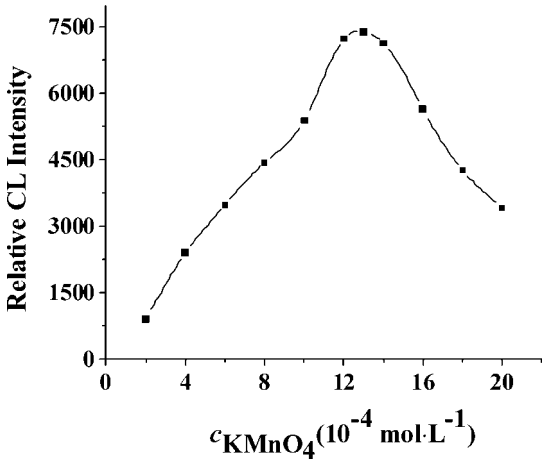

Figure 4. Effect of $KMnO_4$ concentration on the intensity
Conditions: HNO_3: 0.4 mol·l^{-1}; $Ru(phen)_3^{2+}$: 1.0×10^{-4} g·ml^{-1}; Na_2SO_3: 2.5×10^{-3} mol·l^{-1}; progesterone: 1.0×10^{-8} g·ml^{-1}.

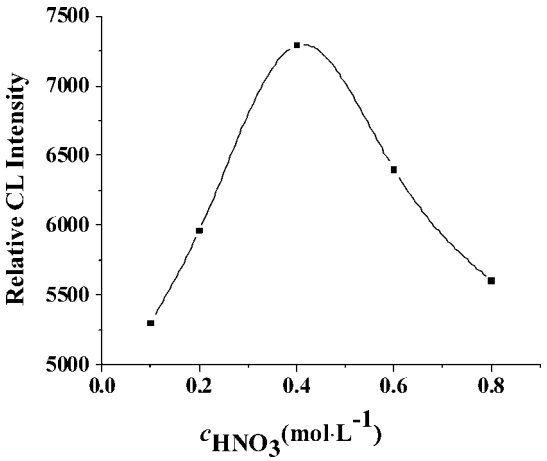

Figure 5. Effect of HNO_3 concentration on the intensity
Conditions: $KMnO_4$: 1.3×10^{-3} mol·l^{-1}; $Ru(phen)_3^{2+}$: 1.0×10^{-4} g·ml^{-1}; Na_2SO_3: 2.5×10^{-3} mol·l^{-1}; progesterone: 1.0×10^{-8} g·ml^{-1}.

H_3PO_4, H_2SO_4, HCl, HNO_3, and $HClO_4$ as medium. The CL intensity reached maximum and kept stable, when HNO_3 was used as the medium in the system.

The effect of HNO_3 concentration over the range 0.1 ~ 0.8 mol·l^{-1} on the CL intensity was studied in Figure 5. The result showed that the optimal concentration of HNO_3 solution was 0.4 mol·l^{-1}, the relative CL intensity decreased on either side of this value. For obtaining the highest sensitivity and accuracy, the concentration 0.4 mol·l^{-1} HNO_3 was selected as optimum.

Effect of $Ru(phen)_3^{2+}$ concentration

The effect of the $Ru(phen)_3^{2+}$ solution concentration was compared in the range of 2.0×10^{-5} g·ml^{-1} to 1.6×10^{-4} g·ml^{-1} in Figure 6. The result showed that the optimal concentration of $Ru(phen)_3^{2+}$ solution was 1.0×10^{-4} g·ml^{-1}. Low concentrations of $Ru(phen)_3^{2+}$ gave decrased CL emission, the reaction produced stable CL signal, when the concentration of $Ru(phen)_3^{2+}$ is larger than 1.0×10^{-4} g·ml^{-1}. Therefore, 1.0×10^{-4} g·ml^{-1} of $Ru(phen)_3^{2+}$ was adopted for further experiment.

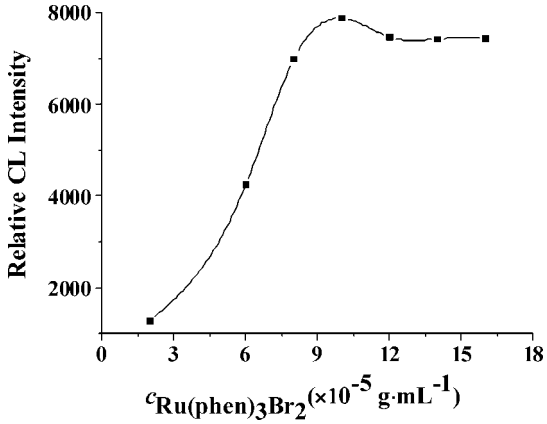

Figure 6. Effect of concentration of $Ru(phen)_3^{2+}$ on the intensity
Conditions: $KMnO_4$: 1.3×10^{-3} mol·l^{-1}; HNO_3: 0.4 mol·l^{-1}; Na_2SO_3: 2.5×10^{-3} mol·l^{-1}; progesterone: 1.0×10^{-8} g·ml^{-1}.

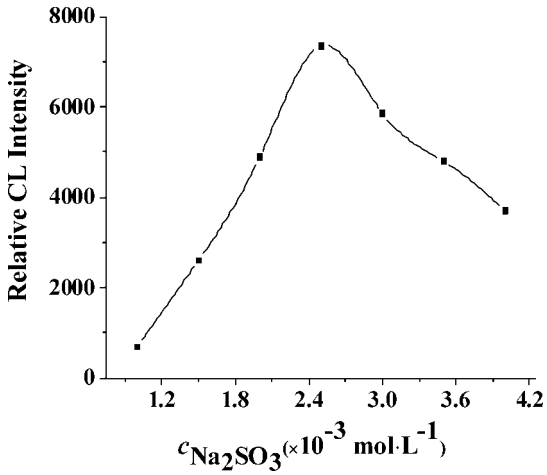

Figure 7. Effect of concentration of Na_2SO_3 on the intensity
Conditions: $KMnO_4$: 1.3×10^{-3} mol·l^{-1}; HNO_3: 0.4 mol·l^{-1}; $Ru(phen)_3^{2+}$: 1.0×10^{-4} g·ml^{-1}; progesterone: 1.0×10^{-8} g·ml^{-1}.

Effect of Na_2SO_3 concentration

The primary investigation showed that Na_2SO_3 could enhance the CL emission intensity in acidic medium. Therefore, the concentration of Na_2SO_3 was investigated in the range of 1.0×10^{-3} ~ 4.0×10^{-3} mol·l^{-1} in Figure 7. When the concentration of Na_2SO_3 was below 2.5×10^{-3} mol·l^{-1}, the CL intensity increased with increasing Na_2SO_3 concentration. When Na_2SO_3 concentration exceeded 2.5×10^{-3} mol·l^{-1}, the intensity decreased. Hence, 2.5×10^{-3} mol·l^{-1} Na_2SO_3 was chosen for the analytical procedure.

Selection of flow rate

The flow rate was an important parameter which influences the analytical sensitivity. Unsuitable flow rates resulted in a decrease or even disappearing of CL signal in the flow cell. The flow rates of progesterone and Na_2SO_3 were investigated in the range of 1.1 ~ 4.2 ml·min^{-1}, When the flow rates of progesterone and Na_2SO_3 were 2.5 ml·min^{-1}, the CL intensity reached a maximum and then remained constant, so the optimum flow rates of progesterone and Na_2SO_3 were 2.5 ml·min^{-1}. In the same way,

Table 2. Interference species

Interferons	Tolerance ratios
Cu^{2+}, I^-	1
Br^-	2
Estrogen, Zn^{2+}, Ni^{2+}	20
Ca^{2+}	50
L-tryptophan	60
Mg^{2+}, PO_4^{3-}	100
β-cyclodextrin	150
Sodium benzoate, Al^{3+}	300
Citric acid	800
Bovine Serum Albumin, Starch	1000
Glucose	1500
CO_3^{2-}, L-leucine, L-aspartic acid	2000
Na^+, K^+	5000

the flow rates of $Ru(phen)_3^{2+}$, $KMnO_4$ and HNO_3 were set to 2.5 ml·min^{-1}.

Interference studies

To evaluate the selectivity of this system, the influences of some possibly co-existing inorganic and organic with progesterone were investigated. The tolerable limit of foreign species was taken as a relative error not greater than ±5% in the recovery at a concentration of 1×10^{-8} g·ml^{-1} standard solution. The results indicated that the CL intensity of progesterone was not affected by the foreign species which were shown in Table 2.

Calibration

Under the selected experimental conditions, the CL intensity increased linearly with increasing progesterone concentration. Under optimum experimental conditions, the linear range of progesterone was from $1.0 \times 10^{-10} \sim 6.0 \times 10^{-9}$ g·ml^{-1} to $6.0 \times 10^{-9} \sim 4.0 \times 10^{-8}$ g·ml^{-1} ($r = 0.9993$) with a detection limit was 7.1×10^{-11} g·ml^{-1}. The relative standard deviation (RSD) for 1×10^{-8} g·ml^{-1} progesterone measurement was 2.79% ($n = 11$).

Application

In order to evaluate the applicability and reliability of the proposed method, the concentration of progesterone in the injection was determined. The recoveries of standard addition were also carried out. It could be seen that the accuracy and precision of the proposed method were satisfactory. Following the procedure described above, the results were obtained and summarized in Table 3.

Possible CL mechanism of the CL reaction

It had been reported that the CL method containing $Ru(phen)_3^{2+}$ was a particularly sensitive detection system. Zhike He *et al*. had detected barbituric acid based on the system of Ce(IV)-$Ru(phen)_3^{2+}$.[14] They proposed a possible reaction mechanism in the study and speculated that the light of the system was excited $Ru(phen)_3^{2+}$. The dynamic CL spectrum generated from the reaction was examined by using LS 55 Fluorescence Spectrometer

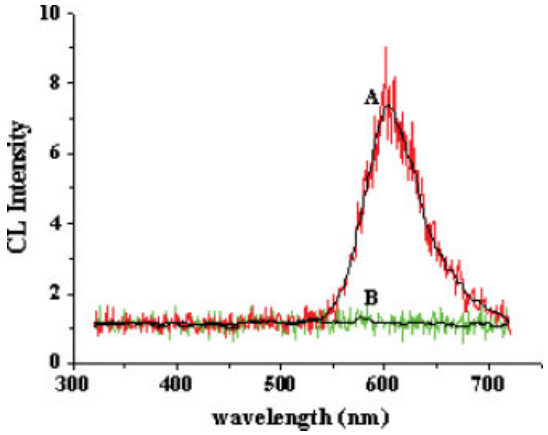

Figure 8. CL spectrum of the reaction system
A: CL spectra of KMnO4 was added into $Ru(phen)_3^{2+} + HNO_3 + Na_2SO_3 +$ progesterone; B: CL spectra of KMnO4 was added into $Ru(phen)_3^{2+} + HNO_3 + Na_2SO_3$.

and was shown in Figure 8. It could be seen that the peak of CL spectrum was at 600 nm, which was very similar to that of $Ru(phen)_3^{2+}$ fluorescence emission spectrum,[15] the addition of progesterone didn't change the luminophor of the system, but enhanced the CL intensity, therefore, it could be concluded that the above assumption. We speculated that the luminophor was the excited state of ruthenium based on previous studies.[14,16–19] And the possible reaction mechanism was suggested as following:

$$Ru(phen)_3^{2+} + KMnO_4 \rightarrow Ru(phen)_3^{3+} + Mn^{2+} \quad (1)$$

$$\text{Progesterone} + KMnO_4 \rightarrow [\text{the radical intermediates}]^* + Mn^{2+} \quad (2)$$

$$Ru(phen)_3^{3+} + [\text{the radical intermediates}]^* \rightarrow [Ru(phen)_3^{2+}]^* \quad (3)$$

$$[Ru(phen)_3^{2+}]^* \rightarrow Ru(phen)_3^{2+} + h\nu(\lambda em \approx 600\ nm) \quad (4)$$

Koukli and Calokerinos reported the determination of quinine based on its enhancing effect on the emission from the reaction between sulphite and cerium (IV)[20] and in a related study. Qu *et al*. used permanganate and quinine for the determination of sulfite.[21] Adcock *et al*.[22] have collected chemiluminescence spectra for the oxidation of sodium sulfite with cerium (IV) sulfate or potassium permanganate, enhanced by quinine. Both spectra matched the characteristic fluorescence of quinine. It is clear that, as suggested by Koukli *et al*.,[23] the mechanism of enhancement in this system involves energy transfer from an excited intermediate to the fluorescent enhancer. In the study, we have collected chemiluminescence spectra for the oxidation of Na_2SO_3 with $KMnO_4$, enhanced by $Ru(phen)_3^{2+}$, the spectra matched the characteristic fluorescence of $Ru(phen)_3^{2+}$. The mechanism of enhancement in this system involves energy transfer from an excited intermediate to the fluorescent enhancer.

Conclusion

In this paper, a simple, rapid and sensitive FI-CL method is described to determine trace progesterone based on strong chemiluminescence generated by progesterone mixed with $KMnO_4$-$Ru(phen)_3^{2+}$ and Na_2SO_3 in acidic medium. The proposed method

Table 3. The results of determination of progesterone in the injection

Batch number	Specifications ml : mg	Found mg	RSD(%, $n = 7$)	Added ($\times 10^{-9}$ g·ml^{-1})	Found ($\times 10^{-9}$ g·ml^{-1})	Recovery (%)	RSD (%, $n = 7$)
100320	2 : 20	20.8	0.81	2	2.07	103.5	1.44
				4	4.98	99.3	1.55
				6	6.04	101.0	2.37
100116	1 : 20	20.1	3.21	2	1.97	98.5	1.72
				4	3.85	96.3	0.89
				6	6.25	104.2	2.10

does not require special reagents and equipment, and has a wide calibration range and a relative low detection limit. It has been applied to determine progesterone in pharmaceutical preparations with satisfactory results. The mechanism of the chemiluminescence reaction is explained. The method is better in terms of sensitivity and sample throughput than the spectrophotometric methods.

Acknowledgements

This work was supported by the Natural Science Foundation of China (No. 20575035) and the University of Jinan (No.XBS0908).

References

[1] S. C. T. Susan, B. Xu, J. W. Michael. Development and evaluation of a candidate reference measurement procedure for the determination of progesterone in human serum using isotope-dilution liquid chromatography/tandem mass spectrometry. *Anal. Chem.* **2006**, *78*, 6628.

[2] R. Short, I. Levett. The fluorimetric determination of progesterone in human plasma during pregnancy and the menstrual cycle. *J. Endocrin.* **1962**, *25*(2), 239.

[3] M. Katayama, R. Nakane, Y. Matsuda, S. Kaneko, I. Hara, H. Sato. Determination of progesterone and 17-hydroxyprogesterone by high performance liquid chromatography after pre-column derivatization with 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-propionohydrazide. *Analyst* **1998**, *123*(11), 2339.

[4] S. Hu, Y. Yan, Z. Zhao. Determination of progesterone based on the enhancement effect of surfactants in linear sweep polarography. *Anal. Chim. Acta* **1991**, *248*(1), 103.

[5] S. Q. REN, W. Xu, B. J. Tang, G. M. Hu, Z. J. Li, G. N. Chen, J. M. Lin. Micro-plate chemiluminescence enzyme immunoassay for clinical determination of progesterone in human serum. *Chin. J. Anal. Chem.* **2008**, *36*(6), 729.

[6] N. T. Deftereos, A. C. Calokerinos. Flow-injection chemiluminometric determination of steroids. *Anal. Chim. Acta* **1994**, *290*(1–2), 190.

[7] F. Amant, K. Schurmans, E. Steenkiste, L. Verbist, V. M. Abeler, G. Tulunay, E. de Jonge, L. Massuger, P. Moerman, I. Vergote. Immunohistochemical determination of estrogen and progesterone receptor positivity in uterine adenosarcoma. *Gynecol. Oncol.* **2004**, *93*(3), 680.

[8] C. Munro, G. Stabenfeldt. Development of a microtitre plate enzyme immunoassay for the determination of progesterone. *J. Endocrinol.* **1984**, *101*(1), 41.

[9] A. P. Richardson, J. B. Klm, G. J. Bamard, W. P. Collins, F. McCapra. Chemiluminescence immunoassay of plasma progesterone, with progesterone-acridinium ester used as the labeled antigen. *Clin. Chem.* **1985**, *31*(10), 1664.

[10] J. D. Boever, F. Kohen, D. Vandekerchkhove, G. V. Maele. Solid-phase chemiluminescence immunoassay for progesterone in unextracted serum. *Clin. Chem.* **1984**, *30*(10), 1637.

[11] A. M. Powe, S. Das, M. Lowry, B. El-Zahab, S. O. Fakayode, M. Geng, G. A. Baker, W. Lin, M. E. McCarroll, G. Patonay, L. Min, M. Aljarrah, S. Neal, I. M. Warner. Molecular fluorescence, phosphorescence, and chemiluminescence spectrometry. *Anal. Chem.* **2010**, *82*(12), 4865.

[12] B. J. Hindson, N. W. Barnett. Analytical applications of acidic potassium permanganate as a chemiluminescence reagent. *Anal. Chim. Acta* **2001**, *445*(1), 1.

[13] J. N. Braddock, T. J. Meyer. Kinetics of the oxidation of hexaaquoiron (2+) by polypyridine complexes of ruthenium (III). Negative enthalpies of activation. *J. Am. Chem. Soc.* **1973**, *95*(10), 3158.

[14] Z. J. Xi, Z. J. Zhang, Y. H. Sun, Z. L. Shi, W. Tian. Determination of indole-3-acetic acid and indole-3-butyric acid in mung bean sprouts using high performance liquid chromatography with immobilized $Ru(bpy)_3^{2+}$–$KMnO_4$ chemiluminescence detection. *Talanta* **2009**, *79*(2), 216.

[15] T. Huang, R. W. Murray. Quenching of $[Ru(bpy)_3]^{2+}$ fluorescence by binding to Au nanoparticles. *Langmuir* **2002**, *18*, 7077.

[16] Z. J. Lin, X. M. Chen, Z. M. Cai, X. Chen, X. R. Wang. Chemiluminescence of tryptophan and histidine in $Ru(bpy)_3^{2+}$-$KMnO_4$ aqueous solution. *Talanta* **2008**, *75*, 544.

[17] C. Thongpoon, B. Liawruangrath, S. Liawruangrath, R. A. Wheatley, A. Townshend. Flow injection chemiluminescence determination of cephalosporins in pharmaceutical preparations using tris (2,2′-bipyridyl) ruthenium (II)-potassium permanganate system. *Anal. Chim. Acta* **2005**, *553*, 123.

[18] M. Karim, S. Lee, H. Lee, Z. U. Bae, K. H. Choi. A batch chemiluminescence determination of enoxacin using a Tris-(1, 10-phenanthroline) ruthenium (II)–cerium (IV) system. *J. Fluoresc.* **2006**, *16*(4), 535.

[19] J. Xi, B. A. Shi, X. Ai, Z He. Chemiluminescence detection of isoniazid using $Ru(phen)_3^{2+}$ – isoniazid–Ce(IV)system. *J. Pharmaceut. Biomed.* **2004**, *36*(1), 237.

[20] I. I. Koukli, A. C. Calokerinos. Determination of quinine and quinidine by continuous-flow chemiluminescence. *Anal. Chim. Acta* **1990**, *236*, 463.

[21] P. Qu, B. X. Li, Z. J. Zhang. A flow-through chemiluminescence sensor for sulfite determination. *Chinese J. Anal. Lab.* **2004**, *23*(4), 19.

[22] J. L. Adcock, P. S. Francis, N. W. Barnett. Chemiluminescence spectra for the oxidation of sulphite in the presence of fluorescent and non-fluorescent enhancers. *Anal. Chim. Acta* **2009**, *652*, 303.

[23] I. I. Koukli, E. G. Sarantonis, A. C. Calokerinos. Effect of sensitisers on the chemiluminescent reduction of cerium (IV) by sulphite. *Anal. Lett.* **1990**, *23*(7), 1167.