

Determination of Ketamine in Urine Using Flow Injection Chemiluminescence

Zhang P, Sun Y, Li J and Li Y*

Department of Reproductive Genetic Family, Hebei General Hospital, Shijiazhuang 050000, China

*Corresponding author: Yali Li Department of Reproductive Genetic Family, Hebei General Hospital, Shijiazhuang 050000, China, Tel: 86-85988701; E-Mail: li_y_li@sina.com

Received: June 30, 2018; Accepted: July 11, 2018; Published: July 18, 2018

Abstract

A simple, rapid and sensitive flow-injection chemiluminescence (FL-CI) method has been developed for the determination of ketamine (KT) in urine; strong chemiluminescence (CL) signal was produced when KT was injected into the mixed stream of KMnO_4 with Na_2SO_3 in acidic medium. Under the optimized conditions, the proposed method allowed the measurement of KT over the range of 5.0-600 ng mL^{-1} with a correlation coefficient of 0.9987, and the detection limit was 1.3 ng mL^{-1} . The relative standard deviation for 50.0 ng mL^{-1} KT (n=11) was 3.2%. The optimal conditions for the detection of KT were evaluated and the interferences were studied. The utility of this method was demonstrated by determining KT in urine sample.

Keywords: Chemiluminescence; Ketamine; Potassium permanganate; Sodium sulfite; Urine

Introduction

Ketamine [2-(2-chlorophenyl)-2-methylamino-cyclohexan-1-one] (KT), a general anaesthetic, was first synthesized in 1962 as an alternative to its analogue, phencyclidine [1]. Recently, mounting studies have shown that a sub-anesthetic dose of ketamine exerts rapid, robust, and lasting antidepressant effects in depressed animals and patients [2]. Furthermore, it was initially abused by medical personnel due to its hallucinogenic properties, and gradually became popular among young user population at dance and rave parties, being one of the recreational drugs known as “club drugs” [3,4]. Therefore, ketamine has been included in the screening list of illicit drugs and quantified with many other abused drugs [5,6].

Different analytical techniques including gas chromatography (GC) [3,7,8], electrochemical method [9], electrokinetic chromatography (EC) [10], and high-performance liquid chromatography (HPLC) [11,12] have been employed for KT testing. These techniques are suitable for assay samples and exhibit good sensitivity for KT. However, GC often required chemical derivatization. EC and HPLC have the advantage of separating and quantifying different forms of folic acid and its derivations and minimum interference from enzymes but involve set up cost, a complex extraction, and purification

Citation: Li Y*, Zhang P, Sun Y and Li J. Determination of Ketamine in Urine Using Flow Injection Chemiluminescence.

Anal Chem Ind J. 2018;18(2):135

© 2018 Trade Science Inc.

procedure. The useful life period of the modified electrode is shorter. Chemiluminescence (CL) is a phenomenon in which chemically-generated molecules emit light in excited states. It has attracted considerable attention in a number of fields such as clinical research, biotechnology, pharmacology, environmental chemistry and food analysis [13-15]. The attractiveness of CL techniques lies in their simplicity, rapidity, high sensitivity, low cost of instrumentation and maintenance, and requiring no back ground light.

In this paper, it was found that KT could enhance CL signal of the KMnO_4 with Na_2SO_3 and the CL intensity was dependent on KT concentration. Based on this phenomenon, a new, simple, and sensitive method was proposed for the determination of KT. In addition, a series of parameters affecting the CL were optimized, validation of the methodology and interferences were investigated systematically. The proposed method has been successfully used to detect KY urine samples with satisfactory results.

Experimental Section

Reagents and solutions

KT was purchased from National Institutes for Food and Drug Control (Beijing, China), standard stock solution (0.1 mg mL^{-1}) was prepared in water and stored in refrigerator (4°C). A stock solution of Na_2SO_3 ($0.1000 \text{ mol L}^{-1}$, Shanghai Chemical Reagent Company, China) was freshly prepared daily and diluted as required. A stock solution of KMnO_4 (0.2 mol L^{-1} , Beijing Chemical Reagent Company, China) was prepared daily in $1.0 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ (Beijing Chemical Reagent Company, China) solution. NaOH, ethylacetate, HCl, and HNO_3 were obtained either from Beijing Chemical Reagent Company (Beijing) or from Tianjin Chemical Reagent Company (Tianjin, China). All reagents were analytically pure unless otherwise stated and prepared in doubly distilled water.

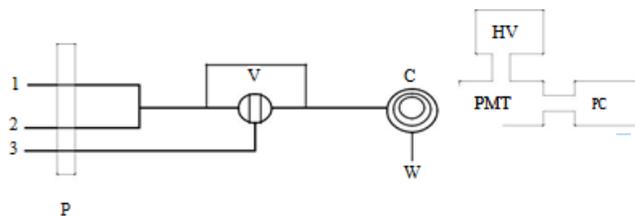


FIG. 1. Schematic diagram of the flow injection CL system for the determination of KT. 1. Mixture of KMnO_4 and H_2SO_4 ; 2. Na_2SO_3 solution; 3. KT solution P. Peristaltic pump; C. Flow cell; V. Injection valve; W. Waste solution

Apparatus and procedure

Flow injection chemiluminescences were performed with IFFL-D flow injection chemiluminescence analysis system (Xi'an Ruike Electronic equipment Corporate, Xi'an, China). The schematic diagram of the FI-CL analyzer is shown in **FIG. 1**. Reagent solutions were delivered by peristaltic pumps (P, working at a constant flow rate: 30 rpm) through three flow-lines. The mixture of KMnO_4 and H_2SO_4 (1) and the Na_2SO_3 solution (2) were mixed through a three-way piece, and then reacted in a flow cell (C) with the sample solution which was injected into the carrier stream using an eight-way injection valve equipped with a 100 μL sample loop. The enhanced CL signals were produced immediately and were recorded. The flow cell was a 10-cm long spiral glass tubing (2.0 mm i.d.) and the distance between injection valve and flow cell was about 20 cm. The fluorescence and absorption spectra were monitored using an F-7000 fluorescence spectrometer (Hitachi, Tokyo, Japan). The spectrofluorimeter was also adapted for the measurement of CL spectra.

Pretreatment of sample

Three urine samples were collected from the Department of Anesthesiology, The Third Hospital of Hebei Medical University. 2.0 mL of each sample were adjusted to pH 10 to 11 with 2 mol L^{-1} NaOH. Centrifuged at 4000 rpm for 10 min to remove precipitates, and then extracted with 2 ml of ethylacetate. The organic layer was evaporated to dryness under a stream of nitrogen gas. The dried extracts were dissolved in 2 ml of water for FL-CL analysis.

Results and Discussion

Kinetic characteristic of the chemiluminescence reaction

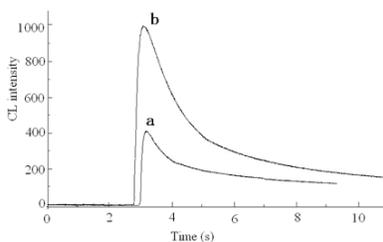
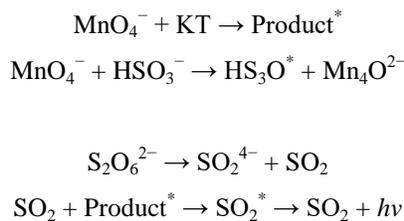


FIG. 2. The characteristics of FL-CL reaction: (a) $1.6 \times 10^{-3} \text{ KMnO}_4$ (in $1.0 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$) + $7.0 \times 10^{-3} \text{ mol L}^{-1} \text{ Na}_2\text{SO}_3$, (b) a + $100 \text{ ng L}^{-1} \text{ KT}$.

Before the FL-CL analysis was carried out, the CL kinetic characteristic of the proposed CL reaction of $\text{KMnO}_4\text{-Na}_2\text{SO}_3\text{-KT}$ was studied by using a static system. FIG. 2 showed the CL intensity-time profile. It was found that the rate of the present reaction was very fast, only 0.5s being needed from reagent mixing to the maximum emerging. It took only 3-4s from reaching the peak maximum to declining to the baseline level. It also showed that the CL intensity increased sharply in the presence of $100 \text{ ng L}^{-1} \text{ KT}$ as FIG. 2 (b) shown.

In order to investigate the reaction mechanism of chemiluminescence enhancement, the fluorescence spectrum of (a) $\text{KMnO}_4\text{-Na}_2\text{SO}_3$; (b) $\text{KMnO}_4\text{-Na}_2\text{SO}_3\text{-KT}$ were detected using fluorescence spectrum, respectively (showed in FIG. 3). The CL intensity of the $\text{KMnO}_4\text{-Na}_2\text{SO}_3\text{-KT}$ system has a significant increase, but the two spectrums showed a similar range and profile. It suggested that these chemiluminescence reactions shared a common emitting species, which was SO_2^* species [16,17]. There is no new emitter produced in the reaction, the role of KT was only as an enhancement reagent. Therefore, it was indicated that KT did not change the mechanism of the CL reaction.

As a result, the chemiluminescence reaction process is assumed as following:



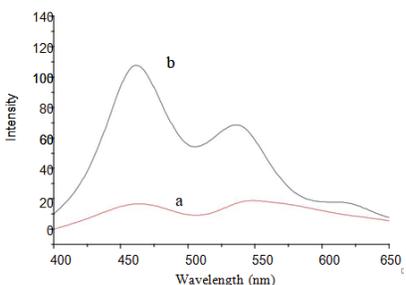


FIG 3: The comparison of fluorescence spectrum: (a) $1.6 \times 10^{-3} \text{ KMnO}_4$ (in $1.0 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$) + $7.0 \times 10^{-3} \text{ mol L}^{-1} \text{ Na}_2\text{SO}_3$, (b) a + $100 \text{ ng L}^{-1} \text{ KT}$. Ex: 300 nm .

Optimization of CL conditions

Effect of flow rate on CL intensity: The flow rate is an important factor which influences not only the analytical efficiency but also the sensitivity of the system. In order to achieve satisfactory emission intensity, the effect of flow rate on the CL reaction was examined in the range of 10-50 rpm. It was found that the biggest CL signal was obtained at a flow rate of 30 rpm. Too low or too high flow rates result in a decrease or even absence of CL in the flow cell and thus was employed in our measurements.

Selection of acid medium: The kinds and concentration of acid in the reaction system influence the chemiluminescence emission intensity. Therefore, four different acids including HCl, HNO₃, and H₂SO₄ of different concentration in the range of 0.2-1.5 mol L⁻¹ were tested, respectively. The results showed the best signal was obtained in H₂SO₄ medium, and the highest chemiluminescence intensity was produced when the concentration of H₂SO₄ was 1.0 mol L⁻¹. Therefore, 1.0 mol L⁻¹ H₂SO₄ was selected for further research.

Effect of concentration of Na₂SO₃ on CL intensities: The effect of varying the concentration of Na₂SO₃ solution was tested on the analytical response corresponding to a KT solution concentration of 50.0 ng L⁻¹ over the range 2.0×10^{-3} - 1.0×10^{-2} mol L⁻¹. It showed that the relative CL intensity was increased as Na₂SO₃ concentration increased from 2.0×10^{-3} to 7.0×10^{-3} mol L⁻¹. When the concentration of Na₂SO₃ was $>7.0 \times 10^{-3}$ mol L⁻¹, the relative CL intensity was decreased. Considering the sensitivity of system, 7.0×10^{-3} mol L⁻¹ Na₂SO₃ was chosen as optimum for the consequent experiments.

Effect of KMnO_4 concentration on CL intensities: KMnO_4 was the important oxidant for determination of KT in this CL system. Therefore, influence of varying concentration of KMnO_4 between 1.0×10^{-3} and $2.0 \times 10^{-3} \text{ mol L}^{-1}$ was examined. The result showed that the ratio of signal and noise (S/N) increased with the increasing of KMnO_4 concentration. When the concentration of KMnO_4 was more than $1.6 \times 10^{-3} \text{ mol L}^{-1}$, the S/N decreased gradually. Thus, $1.6 \times 10^{-3} \text{ mol L}^{-1}$ was selected as optimum concentration of KMnO_4 for the further research.

Calibration and Detection

In order to obtain a calibration curve for KT, a series of standard solutions ($n=8$) of KT were added to the $\text{KMnO}_4\text{-Na}_2\text{SO}_3$ system under the optimized experimental conditions: $[\text{KMnO}_4]=1.6 \times 10^{-3}$, $[\text{Na}_2\text{SO}_3]=7.0 \times 10^{-3} \text{ mol L}^{-1}$, $[\text{H}_2\text{SO}_4]=1.0 \text{ mol L}^{-1}$. The recording outputs of the proposed CL system for measurements of KT were recorded, and the experimental results showed that under the optimum conditions noted above, the responses of CL intensity are linear to the concentrations of KT in the range of 5.0-600 ng mL^{-1} . The linear regression equation is $\text{Intensity}=12.8+16.3c$ ($c: \text{ng mL}^{-1}$, $r=0.9987$). The detection limit (3σ) for the regression equation was 1.3 ng mL^{-1} and the relative standard deviation (R.S.D., $n=11$) was 3.2% for $50.0 \text{ ng mL}^{-1}\text{KT}$.

Study of interferences

TABLE 1. The Response for a variety of potential interferences.

Interference	Concentration(ng mL^{-1})	FA (%)
KT	50.0	100 (Int=867)
K^+	1000	0.54
Ca^{2+}	1000	0.52
Mg^{2+}	1000	-0.36
Ni^{2+}	1000	2.2
Cu^{2+}	500	-3.5
NH_4^+	500	-3.1
NO_3^-	200	-2.0
Cl^-	200	2.1

Zn ²⁺	50	2.5
Fe ²⁺	50	1.3
Fe ³⁺	50	3.2
Tartaric acid	1000	-0.35
Uric acid	1000	-1.5
Sucrose	1000	0.66
Lactose	1000	0.21
Glucose	1000	-0.45
Dextrin	500	-0.88
Maltose	500	-2.2
Galactose	500	-3.5
VB1	500	-2.8
Ethanol	100	2.4
VB2	100	4.2

In order to assess the possible analytical application of the proposed CL system to samples, the influences of some common inorganic ions and a couple of relevant organic compounds on CL intensities were investigated for measuring 50.0 ng mL⁻¹KT. Results in **TABLE 1** show a fairly satisfactory selectivity of the method.

Application

Three real urine samples obtained from the KT injected volunteers were collected after injected 4 h and the amount of KT were determined by the FL-CL method described above. Five replicate analyses were performed for each time points. To evaluate the validity of the proposed method, the recovery experiment of adding standard had been done at the same time. The results for the assay of KT were given in **TABLE 2**. It can be seen that the recoveries were found 96.6, 98.2 and 95.4% and the RSD were 3.4, 4.2, and 3.5, respectively.

TABLE 2. Determination results of KT in urine.

Sample	Added (ng mL ⁻¹)	Found (ng mL ⁻¹)	Recovery (%)	RSD (n=5, %)
1	0	-	-	3.4
	50	48.3	96.6	
2	0	62.5	-	4.2
	50	111.6	98.2	
3	0	12.8	-	3.5
	50	60.5	95.4	

Conclusions

In this paper, a FL-CL detection method was developed for the determination of KT. The presented method had a good sensitivity, precision and wider linear range, which allowed application in determination of KT in urine and biological sample analysis.

Acknowledgments

We acknowledge financial support from the Department of Health of Hebei province (20180117) and Foundation of Hebei science and technology Committee (1727728D).

REFERENCES

1. Hirota K and Lambert DG. Ketamine: its mechanism of action and unusual clinical use. *Br J Anaesth.* 1996;77:441-4.
2. Zhou W, Wang N, Yang C, et al. Ketamine-induced antidepressant effects are associated with AMPA receptors-mediated upregulation of mTOR and BDNF in rat hippocampus and prefrontal cortex. *Eur Psychiatry.* 2014;29:419-23.
3. Lian K, Zhang P, Niu L, et al. A novel derivatization approach for determination of ketamine in urine and plasma by gas chromatography-mass spectrometry. *J of Chromatogr.* 2012;16:104-9.
4. Bairros AV, Lanaro R, Almeida RM, et al. Determination of ketamine, norketamine and dehydronorketamine in urine by hollow-fiber liquid-phase microextraction using an essential oil as supported liquid membrane. *Forensic Sci Int.* 2014;243:47-54.

5. Sporkert F and Pragst F. Use of headspace solid-phase microextraction (HS-SPME) in hair analysis for organic compounds. *Forensic Sci Int.* 2000;107:129-48.
6. Kohrs R, Durieux ME. Ketamine: Teaching an old drug new tricks. *Anesth Analg.* 1998;87:1186-1193.
7. Xiong J, Chen J, He M, et al. Simultaneous quantification of amphetamines, caffeine and ketamine in urine by hollow fiber liquid phase microextraction combined with gas chromatography-flame ionization detector. *Talanta.* 2010;82:969-75.
8. Moore KA, Kilbane EM, Jones R, et al. Tissue Distribution of Ketamine in a Mixed Drug Fatality. *J of Forensic Sci.* 1997;42:1183-5.
9. Chen Y, Yang Y, Tu Y. An electrochemical impedimetric immunosensor for ultrasensitive determination of ketamine hydrochloride. *Sens Actuators B Chem.* 2013;183:150-6.
10. Jen HP, Tsai YC, Su HL, et al. On-line preconcentration and determination of ketamine and norketamine by micellar electrokinetic chromatography: Complementary method to gas chromatography/mass spectrometry. *J of Chromatogr A.* 2006;1111:159-65.
11. Niedorf F, Bohr HH, Kietzmann M. Simultaneous determination of ketamine and xylazine in canine plasma by liquid chromatography with ultraviolet absorbance detection. *J Chromatogr B.* 2003;791:421-6.
12. Rosas MER, Patel S, Wainer IW. Determination of the enantiomers of ketamine and norketamine in human plasma by enantioselective liquid chromatography–mass spectrometry. *J Chromatogr B.* 2003;794:99-108.
13. Liu B, He Y, Duan C, et al. Platinum nanoparticle-catalyzed lucigenin-hydrazine chemiluminescence. *J Photochem Photobiol A: Chem.* 2011;217:62-7.
14. Sakharov I. Microplate Chemiluminescent Assay for DNA Detection Using Apoperoxidase-Oligonucleotide as Capture Conjugate and HRP-Streptavidin Signaling System. *Sensors.* 2018;18:1289.
15. Amjadi M, Manzoori JL, Hallaj T. Chemiluminescence of graphene quantum dots and its application to the determination of uric acid. *J Luminescence.* 2014;153:73-8.
16. Li Y, Lu J. Direct chemiluminescence determination of ibuprofen by the enhancement of the KMnO_4^- sulphite reaction. *Luminescence.* 2007;22:326-330.
17. Yi L, Zhao H, Chen S, et al. Flow-injection analysis of two fluoquinolones by the sensitizing effect of terbium (III) on chemiluminescence of the potassium permanganate-sodium sulfite system. *Talanta.* 2003;61:403-9.