A NEW CHEMILUMINESCENCE METHOD FOR DETERMINATION OF CYROMAZINE USING LUMINOL–Ag(III) COMPLEX REACTION IN ALKALINE MEDIUM

Ting Wang, Yajuan Dong, Peiyun Chen, Ming Su, and Hanwen Sun*

College of Chemistry and Environmental Science, Hebei University, Key Laboratory of Analytical Science and Technology of Hebei Province, Baoding 071002, China.

ABSTRACT

A new chemiluminescence (CL) method for the determination of pesticide cyromazine is developed. The mechanism for enhancing effect of cetyl trimethyl ammonium bromide and inhibiting effect of cyromazine to the CL of Ag(III) complex–luminol in alkaline solution was proposed. The inhibition degree of CL emission was proportional to the logarithm of cyromazine concentration from $2 \times 10^{-5}$ to $7 \times 10^{-2}$ g/kg. The effects of the reaction conditions on CL emission and inhibition were examined. Under the optimized conditions, the detection limit ($s/n=3$) was $6 \times 10^{-6}$ g/kg. Recoveries of cyromazine at spiked levels of $5 \times 10^{-4}$, $7 \times 10^{-3}$, and $1.05 \times 10^{-2}$ g/kg ranged from 90.1 to 109%, with relative standard deviation of 1.2–2.7%. The proposed method was used for the determination of cyromazine in spiked milk and milk powder samples. Cyromazine was found in a milk powder sample, which is lower the maximum residue limit (MRL).

KEYWORDS: Chemiluminescence; Ag(III) complex; Enhancing; Inhibition; Cyromazine; Milk.

INTRODUCTION

Bovine milk is one of the most important components of the human diet. There is a significant risk of pesticide in milk. A triazine pesticide cyromazine is used for fly control. In recent years, use of cyromazine has caused the potential environmental and human health problems.\(^1\) China has set maximum residue limit (MRL) of $5 \times 10^{-4}$ g/kg for cyromazine
residue in infant formula.\textsuperscript{[2]} To ensure foods safety, it is necessary to develop an effective and reliable method for determining cyromazine residue in food.

Many analytical methods for cyromazine in foods were reported. A fluorescence gold nanoparticles probe for detecting cyromazine in milk and pet food sample was described.\textsuperscript{[3]} A capillary electrophoresis (CE) method was developed for the detection of cyromazine and melamine.\textsuperscript{[4]} Liquid chromatography (LC) is more attractive for food analysis than gas chromatography(GC) because no preliminary derivatization procedure is required. Several LC-UV and LC–tandem mass spectrometric methods were described for the determination of cyromazine residue in different matrices, such as milk and pork,\textsuperscript{[5]} poultry meats and eggs,\textsuperscript{[6]} milk sample,\textsuperscript{[7]} and chard samples,\textsuperscript{[8]} but the used instrument is not widely available in general laboratories because of its high price, and the methods require large amount of reagents and long analytical time.

Chemiluminescence (CL) is becoming a powerful analytical tool with widespread application in various fields owing to its high sensitivity, wide dynamic range, and simple instrumentation.\textsuperscript{[9,10]} In our previous work, a new CL reaction system with Ag(III) complex in acidic medium without luminol was developed for the determination of fluoroquinolones synthetic antibiotics.\textsuperscript{[11]} A CL reaction of Ag(III) complex with luminol in alkaline medium was used for the determination of cortisol,\textsuperscript{[12]} 10-hydroxycamptothecin,\textsuperscript{[13]} amikacin sulfate,\textsuperscript{[14]} and penicillin antibiotics,\textsuperscript{[15]} but the mechanism of enhancing and inhibiting effects needs to be investigated further. Up to now there is no report for the detection of cyromazine by CL method.

We observed that CL emission of Ag(III) complex–luminol in alkaline medium could be enhanced by cationic surfactant, and then inhibited by cyromazine. The degree of inhibition for CL was linear with the logarithm of cyromazine concentration. The purpose of this work was to develop a new and simple inhibition CL method for the determination of cyromazine in food.

2. MATERIALS AND METHODS

2.1. Instrumentation

The flow-injection system used for CL was an IFFM-E analysis system (Xi’an Remex Electronic Sci-Tech. Co. Ltd., Xi’an, China) consisting of two peristaltic pumps working at a constant flow rate (60 rpm) and a six-way injection valve with a sample loop (120 μL)
automatically operated by a computer-equipped operation system of IFFM-E flow-injection analysis. PTFE tubing (0.8 mm i.d.) was used to connect all components in the flow system. The flow cell was placed close to the window of the photomultiplier tube (PMT, operated at −800 V). An F-7000 Fluorescence spectrophotometer (Hitachi, Japan) and an TU-1901 double beam spectrophotometer (Beijing purkinje general instrument Co., Ltd., China) were used for studying CL mechanism. A TGL-16M centrifuge (Xiangyi Centrifuge Co., Hunan, China) and ultrasonic cleaner (Ultrasonic Instrument Co., Kunshan, China) were used in sample treatment.

2.2. Chemicals and Reagents
Sodium periodate (NaIO₄, 99.5%) was purchased from Tianjin Kermel Chemical Reagent Company (Tianjin, China). Potassium peroxydisulfate (K₂S₂O₈, 99.5%) was purchased from Beijing Chemical Reagent Company (Beijing, China). Silver nitrate (AgNO₃, 99.8%) and potassium hydroxide (KOH, 82%) were purchased from Tianjin Damao Chemical Reagent Company (Tianjin, China). The stock solution of Ag(III) complex {bis(hydrogenperiodato) argentate(III) complex anion, [Ag(HIO₆)₂]⁵⁻} was prepared by oxidizing Ag(I) in the alkaline medium according the known method.¹⁶ The concentration of Ag(III) complex solutions was determined according to the literature.¹⁷

Luminol (purity, 98.0%) was purchased from Aladdin-reagent com. (shanghai, China). Cyromazine (purity, >99.5%) was purchased from Kernel Chemical Reagents Centre (Tianjin, China). Cyromazine stock standard solution, 1 mg/mL, was prepared by dissolving the compound in water, and stored at 4 ºC in amber glass bottles. A fresh working standard solution was prepared daily by diluting the stock solution with water.

Cetyl trimethyl ammonium chloride (CTAC), cetyl trimethyl ammonium bromide (CTAB), sodium dodecyl sulfate (SDS), sodium-dodecylbenzene sulfonate (SDBS), Tweenum-80, and TritonX-100 were purchased from Kernel Chemical Reagents Centre (Tianjin, China). All chemicals were of analytical reagent grade.

2.3. Sample Treatment
3 g of milk sample or milk powder was extracted with 30 mL trichloroacetic acid. The extract was centrifugated at 6500 rpm for 10 min, and the supernatant was applied to a cation exchange column (4 cm×1.2 cm) for clean up. The analytes in the cartridges were eluted with 3 mL of a 25% ammonia solution–methanol (1: 20, v/v). The eluate was evaporated to
dryness under a stream of nitrogen at 40 °C. The obtained residue was re-dissolved with 3.0 mL of water for CL analysis.

2.4. Procedures

The flow-injection system is shown in Fig. 1.

![Fig.1 Schematic diagram of flow injection chemiluminescence analysis system](image)

P—peristaltic pump; V—sampling inlet valve; F—flowing cell: a flat spiral-coiled colorless glass tube (i.d. 1.0 mm, total diameter of the flow cell 3 cm, without gas between loops); PMT—photomultiplier tube; AMP—amplifier; PC—recorder; W—waste; a—[Ag(HIO₆)₂]₅⁻ solution; b—cyromazine solution; c—luminol alkaline solution; d—CTAB solution. The flow lines c and d were inserted into luminol alkaline solution and CTAB solution, respectively. When the injection valve was switched to the position of injection, [Ag(HIO₆)₂]₅⁻ solution and cyromazine solution were injected through the flow lines (a) and (b), respectively, producing CL emission. The peak height of signal from the CL reaction was recorded by the IFFM-E analysis system. The concentration of cyromazine was quantified by the decrement of peak height of the CL signals.

3. RESULTS AND DISCUSSION

3.1. Possible Enhancing and Inhibiting Mechanism

The investigation of CL intensity–time profiles was performed with the static CL analysis. The CL kinetic characteristics of the reactions system were investigated in detail. The kinetic curves are shown in Fig. 2.
Figure 2 shows that the reaction rate in solution was very fast, from reagent mixing to peak maximum only 2 s was needed for $[\text{Ag(HIO}_6\text{)}_2]^{5-}$–luminol–KOH system. The CL could be enhanced by CTAB and the enhanced CL could be inhibited by cyromazine obviously.

In order to obtain more information about the enhancing mechanism of $[\text{Ag(HIO}_6\text{)}_2]^{5-}$ and CTAB CL spectra were recorded by an F-7000 fluorescence spectrophotometer (taken off lamphouse), as shown in Fig. 3. Luminol in alkaline medium only could produce a weak CL emission. Active center of Ag(III) complex, $[\text{Ag(HIO}_6\text{)(OH)(H}_2\text{O})]^{2-}$ (Ag(III)*), could oxidize luminol to diazonaphthoquinone, resulting in conjugation effect to be increased due to the formation of –NQN– in diazonaphthoquinone. Therefore, like H$_2$O$_2$, Ag(III)* could oxidize luminol to produce luminol free radicals, which could be excited by Ag(III)* further $^{[18]}$. Otherwise, a reaction of Ag(III)* with dissolved oxygen in the solution had taken place, which could produce superoxide radical ($\text{O}_2^*$), and $\text{O}_2^*$ forms singlet oxygen ($^1\text{O}_2^*$), which could oxidize the luminol free radicals.$^{[18, 19]}$

Surfactant can produce some effects on CL. In this work, the effects of cation surfactant (CTAB and CTAC), anion surfactant (SDS and SDBS) and nonionic surfactant (Tweenum-80 and TritonX-100) on the CL were compared. The result showed that the presence of the six surfactants did not change the centre wavelength. The presence of cation surfactants (CTAB and CTAC) enhanced intensity of the CL system, because $[\text{Ag(HIO}_6\text{(OH)(H}_2\text{O})]^{2-}$ and
superoxide radical $O_2^\bullet^-$ could be congregated on CTAB, increasing the CL reaction opportunity.

Fig. 3. CL of $\text{[Ag(HIO}_6\text{)_2]}^{\cdot}$–luminol–KOH (a) without and (b) with cyromazine

Luminol, 4.1×10$^{-7}$ g/mL; $\text{[Ag(HIO}_6\text{)_2]}^{\cdot}$, 6.2×10$^{-5}$ M; cyromazine, 1.03×10$^{-5}$ g/ mL; KOH, 0.01 M

Fig. 2(c) and Fig. 3 (b) show that in the presence of cyromazine the CL intensity was inhibited. The UV absorption peak of cyromazine in the presence of $\text{[Ag(HIO}_6\text{)_2]}^{\cdot}$ moved from 226 nm to 217 nm (Fig. 4). This suggested that Ag(III) and cyromazine formed a complex, reducing the luminescence quantum number and resulting in inhibition effect to CL emission. Otherwise, the cyromazine could be oxidized, there might be a competitive oxidation reaction to $\text{[Ag(HIO}_6\text{)_2]}^{\cdot}$, singlet oxygen ($^1O_2^\bullet$) and superoxide (O$_2$)$_2^*$ between cyromazine and luminol.$^{[18, 20]}$ The reaction might consume $\text{[Ag(HIO}_6\text{)_2]}^{\cdot}$, $^1O_2^\bullet$ and (O$_2$)$_2^*$, leading to a decrease in CL intensity.

Fig. 4 UV absorption spectra of (a) $\text{[Ag(HIO}_6\text{)_2]}^{\cdot}$, (b) cyromazine, and (c) $\text{[Ag(HIO}_6\text{)_2]}^{\cdot}$ + cyromazine,
3.2. Effect of Alkaline Medium

Alkaline medium used in the reaction had a very significant influence on the CL emission intensity. Therefore, Na$_2$CO$_3$, NaHCO$_3$, Na$_2$CO$_3$–NaHCO$_3$, KOH or NaOH with the same concentration was added to the luminol solution to test the effect of alkaline medium on the CL signal. The highest and most stable CL intensity was observed in KOH medium. The effect of KOH concentration on CL intensity was investigated in the presence of 2.1×10$^{-4}$ M, [Ag(HIO$_6$)$_2$]$^{5-}$, 1.0×10$^{-5}$ g/mL cyromazine, 4.06×10$^{-7}$ g/mL luminol and 1.89×10$^{-5}$ g/mL CTAB. KOH concentration of 0.01 M was used as the optimum concentration for further test.

3.3. Effect of Luminol and [Ag(HIO$_6$)$_2$]$^{5-}$ Concentrations

The dependence of luminol concentration in the range of 1.46×10$^{-7}$–7.01×10$^{-7}$ g/mL on CL intensity in 0.01 M KOH medium was investigated. The result showed that the CL intensity increased obviously with increasing luminol concentration from 1.46×10$^{-7}$ to 4.06×10$^{-7}$ g/mL, but standed when over 4.06×10$^{-7}$ g/mL. After the analysis of S/N ratio and the sensitivity of the system, the best concentration of luminol was selected to be 4.06×10$^{-7}$ g/mL for next CL test.

In the CL system, [Ag(HIO$_6$)$_2$]$^{5-}$ was used as an oxidant. Its concentration not only influenced the sensitivity, but also influenced the linear range for the assay. The influence of [Ag(HIO$_6$)$_2$]$^{5-}$ concentration from 7.0×10$^{-5}$ to 4.2×10$^{-4}$ M on CL intensity was tested. The result showed that the intensity increased with increasing [Ag(HIO$_6$)$_2$]$^{5-}$ concentration from 7.0×10$^{-5}$ to 1.8×10$^{-4}$ M, and decreased when over 2.1×10$^{-4}$ M, so which was selected as the optimum concentration.

3.4. Effect of Surfactant on CL Intensity

The effect of cationic surfactant (CTAC and CTAB), anionic surfactant (SDS and SDBS), and nonionic surfactant (Tweenum-80 and TritonX-100) on CL intensity was investigated. The result showed that CTAB, CTAC, and Tweenum-80 could enhance the intensity of CL system. Among them, CTAB was better enhancing reagent. The effect of CTAB concentration on CL intensity was investigated further (Fig. 5). Using 3.78×10$^{-4}$ g/mL CTAB highest CL intensity was achieved.
3.5. Interference Effect

The interfering effects from foreign species were investigated for CL determination. The tolerance content was defined as the amount of coexisting species that produced an error not exceeding ±5% in the determination of cyromazine. The tolerated ratios of foreign substances to 3.50×10^{-3} g/kg cyromazine were: 100-fold for melamine, sucrose, starch, Zn^{2+}, and Ba^{2+}; 50-fold for citric acid, Mg^{2+}, Br^{-}; 25-fold for dextrin, lactose, Ca^{2+}, and Fe^{3+}; 10-fold for glucose, galactose, I, and Ni^{2+}; and 5-fold for Cu^{2+}. Since samples were pretreated by cation exchange column, so there was no interference in the analysis of real samples.

3.6. Analytical Performance

Under the optimized experimental conditions, the calibration graphs of the relative CL intensity (ΔI) versus cyromazine concentration or the logarithm of cyromazine concentration were measured. The regression equations of the calibration curves were as follows: in the range of 2×10^{-5}–7.0×10^{-2} g/kg, ΔI=11665.59+1822.12×\log C, with correlation coefficient (r) of 0.997, where ΔI was inhibition degree of CL intensity, and C was concentration of analyte. The limit of detection (LOD) was determined as the sample concentration that produces a peak with a height three times of the level of baseline noise. The limit of quantification (LOQ) was determined as the sample concentration that produces a peak with a height ten times of the level of baseline noise. The LOD was 6×10^{-6} g/kg, and LOQ was 2.0×10^{-5} g/kg, which is lower than the MRL value. Sensitivity of the method is higher than equal or higher than
HPLC-UV/MS method (LOD: 2×10⁻⁵–5×10⁻⁵ g/kg ). The relative standard deviation (RSD) was 2.1% for 11 determinations of the analyte at 3.0×10⁻³ g/kg. The proposed system has satisfactory linearity, sensitivity and precision.

3.7. Application
A total of 20 milk and milk powder samples were treated using the procedure described above. Cyromazine of 4.5× 10⁻⁵ g/kg in a milk powder sample was detected, which was less than the MRL. For other milk powder and milk samples, it was not detected. The recovery at three spiked levels (1×MRL, 14×MRL, and 21×MRL) was in the range of 90.1–104% with RSDs of 1.2–2.3% for spiked milk sample, and 92.0–109% with RSDs of 1.5–2.7% for spiked milk powder samples (Table 1).

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4. CONCLUSION
Based on both enhancing of cetyl trimethyl ammonium bromide and inhibiting effect of cyromazine on CL reaction of luminol–[Ag(HIO₆)₂]⁵⁻ in KOH medium, a new flow-injection CL method was proposed for the determination of cyromazine. The new method offers several advantages over other methods, such as being faster, using simple instrumentation, and the reagents being stable and inexpensive. The proposed method can meet the requirement for analysis of cyromazine in milk and milk products.

Conflict of Interest Statement
We declare that we have no conflict of interest.
ACKNOWLEDGEMENT

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