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Article

Investigation of a Novel Ag(III) Chemiluminescence System and its Mechanism for Determination of Uric Acid in Human Urine

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Um método simples e sensível baseado na análise de quimiluminescência por injeção em fluxo acoplado ao complexo luminol-Ag(III) foi desenvolvido para se determinar ácido úrico (UA) em urina humana. O método se baseia na reação de quimiluminescência (CL) do luminol com Ag(III) em solução alcalina, sendo que o UA da urina aumenta dramaticamente as intensidades CL. Em condições ótimas, a intensidade CL relativa aumentava linearmente com a concentração de UA, na faixa de 1.0×10^{-8} a 5.0×10^{-6} mol L^{-1} com um limite de detecção de 2.0×10^{-9} mol L^{-1} (3σ). O desvio padrão relativo (n = 11) era de 0.71% para 1.0×10^{-7} mol L^{-1} UA. Os mecanismos de reação possíveis do sistema CL foram sugeridos com base nos espectros de absorção UV, espectros CL e no experimento de *trapping* de radicais livres feitos neste trabalho.

A simple and sensitive method based on flow-injection chemiluminescence analysis coupled with luminol-Ag(III) complex was developed for the determination of uric acid (UA) in human urine. The method was based on the chemiluminescence (CL) reaction of luminol with Ag(III) in alkaline solution. UA in the urine can dramatically enhance CL intensities. In optimum conditions, the relative CL intensity had a linear relationship with UA concentration in the range of 1.0×10^{-8} to 5.0×10^{-6} mol L^{-1} with a detection limit of 2.0×10^{-9} mol L^{-1} (3σ). The relative standard deviation (n = 11) was 0.71% for 1.0×10^{-7} mol L^{-1} UA. Possible reaction mechanisms for the CL system were suggested based on the UV absorption spectra, CL spectra, and the free radical trapping experiment performed in this work.

Keywords: Ag(III) complex, UA, CL, determination, mechanism

Introduction

Uric acid (2,6,8-trihydroxypurine, UA) is a relatively water-insoluble end product of purine metabolism in humans and is excreted via urine.[1] The normal UA level in urine for healthy adults is about 250 to 750 mg L^{-1}.[2] Excessive production of UA may lead to hyperuricemia, gout, and kidney disorders.[3]

One suggested function of UA in human body fluids is to act as an antioxidant. A recent study suggested that low serum/cerebrospinal fluid levels of UA are one of the biomarkers of Parkinson's disease and that high UA levels could slow the progression of this disease.[4] Consequently, rapid, reliable, and sensitive analytical methods for UA determination in human fluids are needed for the diagnosis and treatment of various disorders.

Several methods for UA determination include the conventional uricase enzyme method,[5,6] spectrophotometry,[7] chromatography including capillary electrophoresis,[8-11] electrochemical analysis,[12,13] and chemiluminescence (CL).[14,15] Enzymatic method is the conventional method for the determination of UA levels; however, this method requires expensive reagents and suffers from interferences. High-performance liquid chromatography has also been employed for UA quantification in blood and urine samples; however, the use of this technique for routine analysis in clinical laboratories is hindered by low sample throughput and high acquisition and operational costs, as well as toxic waste generation.[7]

In the last few years, we have been focusing on the interactions between bis(hydrogenperiodato)argentate(III) complex anion, $[Ag(HIO_6)_2]^{5-}$, and bioactive and medically important molecules.[16,17] Our investigations on Ag(III) complex consist of two aspects: (a) the kinetic investigation of the Ag(III) oxidation reactions that help to gain some insight on the mechanisms of these oxidations; (b) the appplication in the determination of some biological samples[18-20] and some drugs[21,22] based on the characteristic

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that Ag(III) complex can oxidize some reducible substances to produce chemiluminescence.

Compared with other methods, CL has better sensitivity, lower detection limits, wide linear ranges, and lower cost. It is also simple and convenient. Thus far, limited reports on the determination of UA by CL have been published, such as with luminol-$K_3[Fe(CN)_6]$,[23] and $KMnO_4$-octylphenyl polyglycol ether ($KMnO_4$-OP).[15] In the current study, we found UA can markedly enhance CL intensity of luminol-Ag(III) complex system. So we present a simple, sensitive, and accurate analytical method by using the luminol-Ag(III) complex system for the determination of UA in human urine. The results of the interference experiments show that the main substances excreted in human urine do not interfere with UA determination. Based on the UV absorption spectra, chemiluminescent spectra and the results of the free radical trapping experiment performed in this study, possible reaction mechanisms for the CL system are suggested.

Experimental

Reagents and solutions

All reagents were of analytical reagent grade. Double-distilled water was used throughout the experiment. Bis(hydrogen periodato)argentate(III) complex anion (Ag(III)) was synthesized according to the procedure described previously.[24] The concentration of Ag(III) stock solution was determined spectrophotometrically at 362 nm by use of the molar absorptivity of $\varepsilon = 1.26 \times 10^4$ L mol^{-1} cm^{-1}. Luminol and UA were purchased from Sigma-Aldrich. The luminol stock solution (0.02 mol L^{-1}) was prepared by dissolving 0.886 g of luminol in 7.0 mL of 1 mol L^{-1} NaOH**.** The solution was then transferred into a 250 mL volumetric flask and diluted with water. All the solutions were freshly prepared using ultra-pure water prior to use.

Instruments

The flow-injection analysis (FIA) CL system (Xi'an Remax Electronic Science-Tech Co. Ltd., shown in Figure 1) consisted of two peristaltic pumps, a six-way injection valve and photomultiplier tube detector. $[Ag(HIO_6)_2]^{5-}$ and luminol solution were delivered by pump P_1 and mixed in manifold M_1. The sample solution was injected into the carrier stream (water) via the six-way injection valve and met with mixed $[Ag(HIO_6)_2]^{5-}$/luminol solution in manifold M_2. The chemiluminescence was detected by photomultiplier tube detector D.

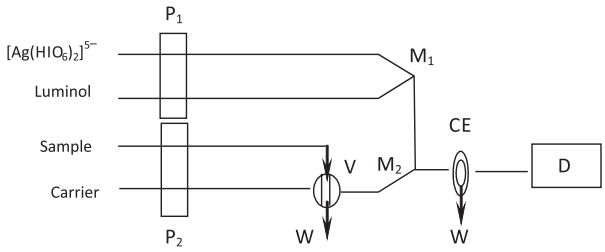


Figure 1. Schematic diagram of the FIA-CL system for the determination of UA: P, peristaltic pumps; V, six-way injection valve; M, manifold; CE, CL reaction cells; W, Waste; D, photomultiplier tube detector.

The UV absorbance was detected using a TU-1901 UV-Vis spectrophotometer (Beijing Purkinje General Instrument Co. Ltd.). The CL spectra were obtained using an F-7000 fluorescence spectrophotometer (Hitachi, Japan).

Results and Discussion

Effect of luminol and Ag(III) concentration

The CL intensity and noise increased with increasing luminol concentration. The optimum signal to noise (S/N) ratio was observed at a luminol concentration of 4.0×10^{-7} mol L^{-1}. Hence, 4.0×10^{-7} mol L^{-1} was the concentration used in our measurements.

Preliminary experiments showed that the concentration of Ag(III) in KOH medium greatly influences the CL emission intensities. The effect of Ag(III) concentration within a range of 1.0×10^{-5} to 5.0×10^{-4} mol L^{-1} on the intensities was investigated. An Ag(III) concentration of 1.0×10^{-4} mol L^{-1} was used for further studies.

Effect of [OH$^-$] in the Ag(III) solution

The hydroxide ion concentration influenced the oxidative capability of Ag(III). With the increasing of [OH$^-$], CL intensity remarkably increased. The results (Figure 2) showed that the S/N ratio gradually increased with increasing KOH concentrations up to 0.20 mol L^{-1}, where a maximum CL signal was reached. Increasing the KOH concentration further resulted in a decrease in the S/N ratio signal. Based on these results, a KOH concentration of 0.20 mol L^{-1} was chosen for further studies.

Effect of flow rate

The speed of the two pumps is an important parameter that influences the analytical sensitivity of the system. The effect of solution flow rate on CL intensity was observed in the range of 0.5 to 3.5 mL min^{-1}. The results showed that the CL signal increased with increasing flow rate. The

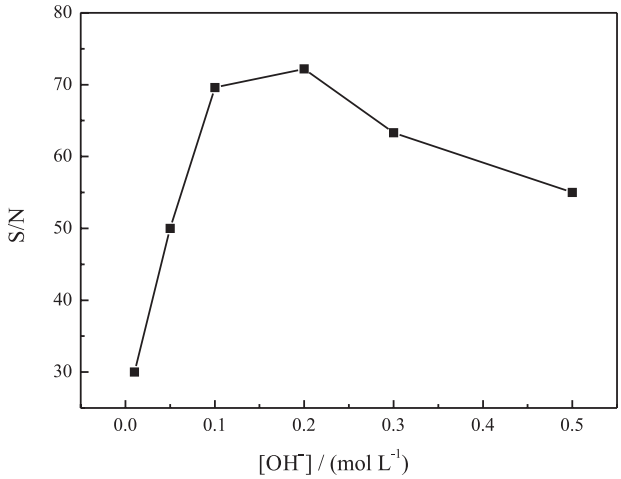


Figure 2. Effect of $[OH^-]$ in Ag(III) solutions on CL intensity. Reaction conditions: [UA] = 2.0×10^{-7} mol L^{-1}; Ag(III) = 3.0×10^{-5} mol L^{-1}; [luminol] = 4.0×10^{-7} mol L^{-1}.

optimal flow rates were all kept at 2.5 mL min^{-1} because the consumption of the reaction reagent was considered.

Interference study

The influence of interferences, which were probably present in the human urine samples during the determination of UA, was investigated. A foreign substance is considered to not interfere if the substance caused a relative error of ≤ ± 5%. Results showed that 1000-fold Na^+, Ca^{2+}, K^+, Fe^{3+}, Zn^{2+}, SO_4^{2-}, creatinine, sarcosine; 200-fold Mg^{2+}, NH_4^+, Cl^-, urea, creatine, PO_4^{3-}; and 50-fold $H_2PO_4^-$, and Cu^{2+} did not interfere with the CL system.

Analytical characteristics

In optimum conditions, the relative CL intensity (ΔI) exhibited a linear relationship with UA concentration (C) in the range of 1.0×10^{-8} to 5.0×10^{-6} mol L^{-1} ($\Delta I = -68.33 + 1.22 \times 10^{10}$ C ($r^2 = 0.999$)). The detection limit was 2.0×10^{-9} mol L^{-1}, and the relative standard deviation was 0.71% for 11 repeated measurements of 1.0×10^{-7} mol L^{-1} UA. This new CL system has a lower detection limit compared with those reported in other studies (shown in Table 1).

Sample analysis

The validity of the proposed method was evaluated by determining UA concentrations in several human urine samples and performing a recovery study. No further pretreatment was performed on the urine samples when the urine samples were diluted 10^3-fold by water. The results of the proposed method agree well with those obtained by the HPLC method.[10]

The results are summarized in Table 2.

Kinetic characteristics of the CL reaction

The kinetic characteristics of the CL reaction is shown in Figure 3. CL signals were quickly enhanced upon the addition of UA to the luminol-Ag(III) system. This result demonstrates that the speed of the CL reaction is so rapid and the CL system is sensitive enough for the determination of UA.

Table 1. Comparison of various CL systems for the determination of uric acid

Method	Linear range / (μmol L^{-1})	Detection limit / (μmol L^{-1})	Reference
CE-luminol-$K_3[Fe(CN)_6]$	0.6-30	0.35	10
Luminol-$K_3[Fe(CN)_6]$	4.8-179	3.0	23
$KMnO_4$-OP[a]	0.6-3600	0.3	15
$[Ag(H_3IO_6)_2]^-$	0.4-200	0.12	25
Luminol-H_2O_2-Co^{2+}	1.0×10^{-4}-7.0	1.1×10^{-5}	26
Luminol-Ag(III)[b]	0.01-5	0.002	This work

[a]OP: octylphenyl polyglycol ether; [b]Ag(III): $[Ag(HIO_6)_2]^{5-}$.

Table 2. The results of determination of uric acid in human urine

Sample	Found / (10^{-7} mol L^{-1})		Added / (10^{-7} mol L^{-1})	Total found / (10^{-7} mol L^{-1})	Recovery / %	RSD / % (n = 3)
	Proposed method	HPLC				
No. 1	5.24	5.41	1	6.20	95.8	1.12
			5	10.02	95.6	0.89
No. 2	3.28	3.36	1	4.33	105.1	1.23
			5	8.38	102.0	0.91
No. 3	8.51	8.65	1	9.47	96.3	0.75
			5	13.59	101.6	0.78

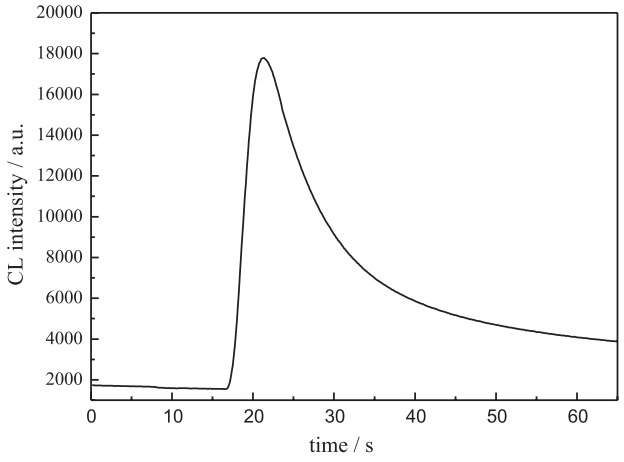


Figure 3. CL kinetics curve for the luminol-Ag(III) system (1.0×10^{-6} mol L^{-1} UA dropped in luminol-Ag(III) solution).

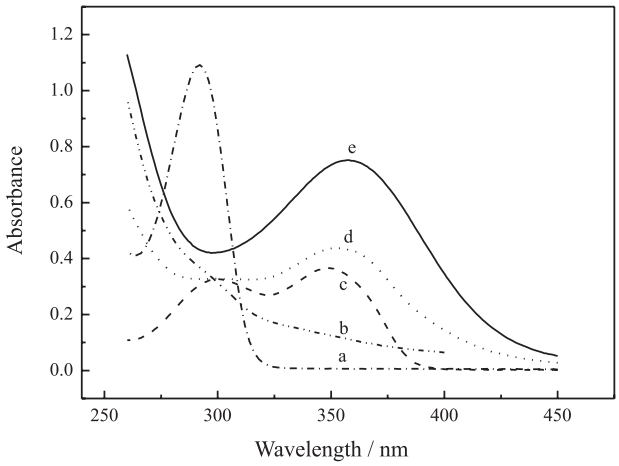


Figure 4. UV spectra of UA (a), UA-Ag(III) (b), Ag(III)-luminol (c), luminol (d), and Ag(III) (e). The concentrations of UA, luminol and $[Ag(HIO_6)_2]^{5-}$ in mixed solution were all 5.0×10^{-5} mol L^{-1}.

UV absorption spectra

The UV absorption spectra of luminol, UA, Ag(III), UA-Ag(III) and luminol-Ag(III) were recorded (Figure 4). Luminol has two absorption bands at 300 and 350 nm (curve c). Ag(III) possesses a characteristic absorption band at 362 nm (curve e), and UA has an absorption band at 280 nm (curve a). The addition of luminol or UA to the Ag(III) solution caused the color of the Ag(III) solution to change from yellow to colorless, which resulted in a decrease of the absorbance at 362 nm. This result indicates that luminol and UA can be oxidized by Ag(III). It can be concluded that UA was more easily oxidized by Ag(III) by comparing the speed of absorbance decrease at 362 nm of the Ag(III) solution from the time-scan spectra.

CL spectra

The mechanism of the CL reaction was investigated using the CL spectra (shown in Figure 5) obtained from an F-7000 fluorescence spectrophotometer. The CL spectra of the three reaction systems indicated four points, as follows: (A) Ag(III) in 0.2 mol L^{-1} KOH oxidized UA without luminescence; (B) luminol-Ag(III) system produced CL; (C) UA enhanced CL emission of the luminol-Ag(III) system; and (D) the two peaks at 425 nm suggest that the luminophor of luminol-Ag(III)-UA or luminol-Ag(III) is 3-aminophthalate.[27]

Free radical trapping experiment

The participation of the free radical in the CL reaction was confirmed via a free radical trapping experiment. In the same optimal conditions used for the CL measurements, 50 mL of 5×10^{-4} mol L^{-1} Ag(III) alkaline solution was

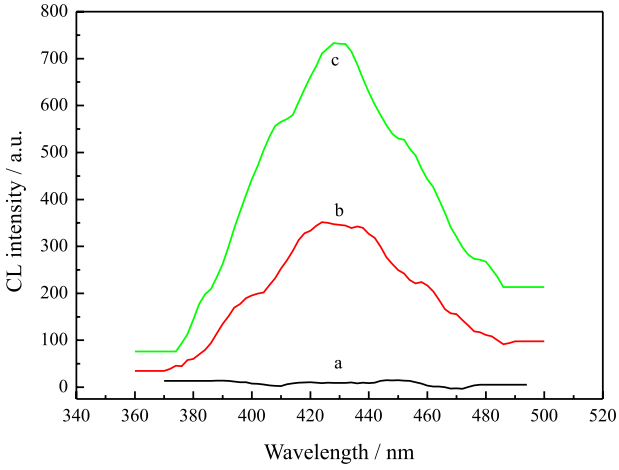
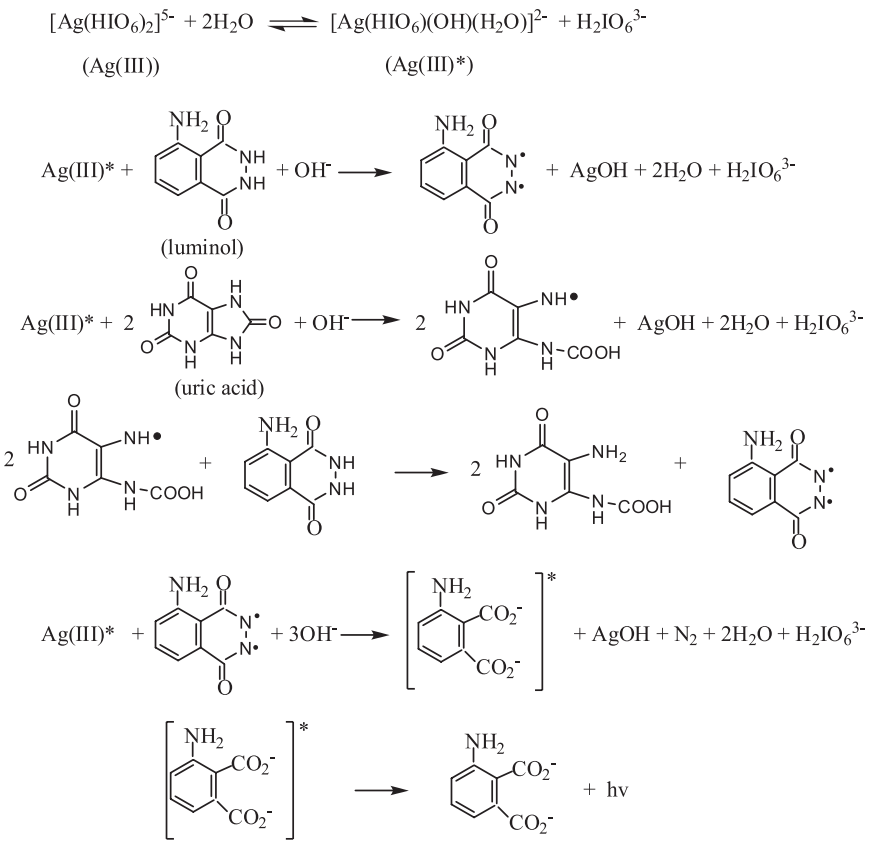


Figure 5. CL spectra for the luminol Ag(III) CL system: (a) UA-Ag(III); (b) luminol-Ag(III); and (c) UA-luminol-Ag(III). [Luminol]: 1.0×10^{-5} mol L^{-1}; [Ag(III)]: 1.0×10^{-4} mol L^{-1}; [OH^-]: 0.10 mol L^{-1}; [UA]: 1.0×10^{-7} mol L^{-1}.

added dropwise to 50 mL solution of luminol and UA, both of which had a concentration of 1.0×10^{-5} mol L^{-1} and contained 1 mol L^{-1} acrylonitrile. The solutions of luminol and UA were flushed for 30 min with nitrogen gas before the Ag(III) solution was added. The reaction mixture was then stirred at 40 °C for about 3 h under nitrogen gas. Both the luminol and UA solutions produced polyacrylonitrile precipitates, which indicates that the free radicals were probably involved in the reactions of luminol and UA with Ag(III).

Possible CL reaction mechanism

The involvement of the hydroxyl radical (·OH) and the superoxide radical ($\cdot O_2^-$) in the CL reaction was investigated in our CL experiment because a previous study suggested



Scheme 1. Possible mechanism for the UA-luminol- Ag(III) CL system.

that ·OH and $\cdot O_2^-$ were involved in the CL reaction of luminol.[28] Ascorbic acid (10^{-4} mol L^{-1}) and methanol (10%, v/v) were used separately as CL reaction scavengers in the UA-luminol-Ag(III) system. Instead of inhibiting the CL reaction, the CL signals were all markedly enhanced, which indicates the presence of free radicals that were neither ·OH or $\cdot O_2^-$ during the reactions of luminol and UA with Ag(III).

The results above show that free radicals are likely present during the reactions of luminol and UA with Ag(III). UA is very easily oxidized and the reaction rate of UA with Ag(III) is faster compared with that of luminol with Ag(III). We presume that the free radicals generated from the oxidation of Ag(III) and UA were transferred to luminol, which resulted in enhanced CL intensity. Based on the experiments mentioned above, a CL mechanism is suggested as described in Scheme 1.

Conclusions

UA enhanced the CL signal of luminol reacting with Ag(III) in an alkaline solution. Thus, a flow injection CL method for the determination of UA in human urine was developed. The method is simple, rapid, sensitive, and cost-efficient. In addition, the proposed method does not need sample pretreatment and expensive instrumentation. The method was successfully applied to the analysis of UA in human urine samples. Based on the UV absorption spectra, the CL spectra, and the free radical trapping experiment performed in this study, possible reaction mechanisms for this reaction system were suggested.

Acknowledgments

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