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A novel flow-injection chemiluminescence determination of uric acid based on diperiodatoargentate(III) oxidation

Chunyan Yang, Zhujun Zhang*

College of Chemistry and Materials Science, Shaanxi Normal University, 199 South Chang'an Road, Xi'an 710062, PR China

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ABSTRACT

A novel and high selectivity flow-injection chemiluminescence (FI-CL) system with diperiodatoargentate(III) (DPA) is developed for the determination of uric acid for the first time. It is based on the reaction of diperiodatoargentate(III) (DPA) with uric acid in alkaline medium to emit CL. With the peak height as a quantitative parameter applying optimum working conditions, the relative CL intensity was linear with the uric acid concentration in the range of 4.0×10^{-7}–2.0×10^{-4} mol L^{-1} with a detection limit of 1.2×10^{-7} mol L^{-1} (3σ). The relative standard deviation (RSD) was 2.1% for 5.0×10^{-5} mol L^{-1} uric acid ($n = 7$). The proposed method held higher selectivity than other CL methods and was applied to determination of uric acid in human serum. The possible CL reaction mechanism was also discussed briefly.

1. Introduction

Uric acid, the final product of the purine metabolism, is mainly excreted by the kidneys. Most of uric acid produced from the catabolism is reabsorbed into the blood circulation system after primary filtration and partial secretion by the kidney. Uric acid levels in physiological fluids serve as valuable indicators for certain clinical conditions. Abnormal level of uric acid in blood serum leads to several diseases such as gout, renal failure, hypertension, insulin resistance, and metabolic syndrome [1–4]. So determination of uric acid in serum plays an important role in laboratory medicine and thus is routinely determined in the clinical laboratory.

Common methods for serum uric acid assay include phosphotungstate assay [5], HPLC-UV or MS [6–8] and enzymatic method [9–12]. CL [13–18] and electrochemical [19–21] methods were used to determine the concentration of uric acid. The phosphotungstate assay is unreliable for accurate determination of the concentration of uric acid due to turbidity or the presence of ascorbic acid, aspirin, glutathione and various antibiotics [22]. Although enzymatic assays are promising due to their high level of selectivity, but still suffer from drawbacks, including the effect of temperature, unstable and expensive reagents, and large volumes of samples. HPCL methods have received considerable attention due to good efficiency of separation, but they often suffer from a variety of disadvantages, such as expensive equipment, time-consuming. Electrochemical methods for the determination of uric acid are more selective, less expensive and less time-consuming than the other methods, whose major problem is also interference (e.g. ascorbic acid and uric acid oxidation occurs at the same potential) [23,24].

Chemiluminescence (CL) method is known to be a powerful analytical technique, which owns high sensitivity, fast response time, wide dynamic range, and simple instrumentation which has been extensively applied in the different fields of analytical chemistry. CL methods have been used to determine the concentration of uric acid in human serum and urine combined with capillary electrophoresis or uricase [13,14], because the CL methods hold low selectivity. The goal of the present work was to develop a direct CL method coupled with flow-injection for the determination of uric acid with high sensitivity and selectivity.

The existence of some transition metals in highest oxidation state has been known. Silver in trivalent state can be stabilized by chelating with suitable polydentate ligands for their unstable character in an aqueous solution. Silver chelates such as $[Ag(H_3IO_6)_2]^-$ (DPA) and $[Ag(H_2TeO_6)_2]^{5-}$ are good oxidants in a medium with an appropriate PH value. The polydentate chelates of trivalent silver takes on character of strong oxidation with a redox potential of 1.74 V in alkaline medium, because of their strong versatile nature of the two electron oxidants [25,26]. To our knowledge, there is no report using DPA for direct CL analysis in alkaline medium. It was found that DPA could directly oxidize uric acid to emit strong CL. The relative CL intensity was proportional to the concentration of uric acid. The result of interference studies showed that

* Corresponding author. Tel.: +86 29 85308748.
E-mail address: zhangzhujun18@yahoo.com.cn (Z. Zhang).

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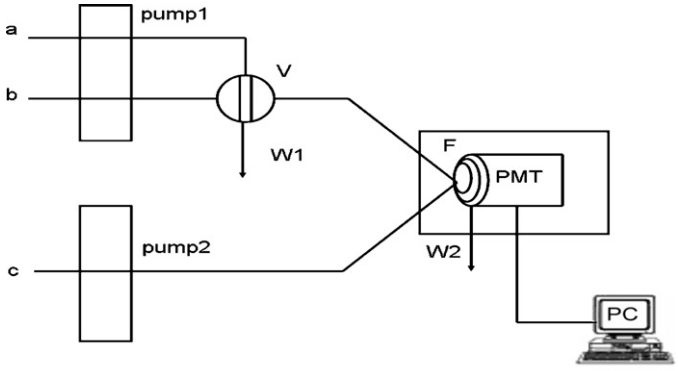

Fig. 1. Schematic diagram of the FI-CL system; (a) uric acid standard solution or samples; (b) distilled water; (c) DPA solution; (V) injection valve; (F) spiral glass flow cell; PMT: photomultiplier; pump1, pump2: peristaltic pumps; W1, W2: waste.

the proposed method possesses high sensitivity and selectivity. The interference of various compounds usually present in serum could be ignored when the serum was ultrafiltered and diluted twice fold. Therefore, a novel flow-injection CL method was developed for the determination of uric acid in serum.

2. Experimental

2.1. Reagents

Potassium hydroxide, sodium nitrate, sodium periodate, silver nitrate, uric acid, and sodium tungstate were obtained from Shanghai Chemical Reagent Company. Phosphoric acid and lithium sulfate were obtained from Xi'an Chemical Reagent Company. All the reagents were of analytical grade and deionized and double-distilled water was used throughout.

The uric acid standard stock solution was prepared in 1.0×10^{-3} mol L^{-1} KOH solution to give a final concentration of 1.0×10^{-3} mol L^{-1}. Working solutions were freshly prepared by diluting standard stock solution with water. All solutions were stored in a refrigerator at 4 °C.

2.2. Apparatus

The FI-CL system used in this work is shown in Fig. 1. Two peristaltic pumps (HL-2, Shanghai Huxi, China) were used to deliver all the chemicals. Polytetrafluoroethylene (PTFE) flow tubes (0.8 mm i.d.) were employed to connect all the components of the flow system. Injection was accomplished using an eight-way injection valve equipped a sample loop (90 μL). The flow cell was made by coiling 20 cm of colorless glass tube (2 mm i.d.) into a spiral disk shape and was located directly facing the window of the photomultiplier tube (PMT). The CL signal was monitored with an IFFM-A multifunction chemiluminescence analyzer (Remex Analytical Instrument Co. Ltd., Xian, China). The UV absorbance was detected with the TU1901 UV–vis spectrophotometer (Beijing Purkinje General Instrument Co. Ltd., China). The chemiluminescence spectrum was monitored with an F-4600 fluorescence spectrophotometer (Hitachi, Japan).

2.3. Procedures

2.3.1. Synthesis of diperiodatoargentate(III)

Diperiodatoargentate was synthesized by the suggested method [27]. In briefly, $AgNO_3$ (1.36 g), $NaIO_4$ (3.42 g), $K_2S_2O_8$ (3 g), and KOH (8 g) were taken in a 500 mL round bottomed flask. 100 mL of demineralised water were added to this mixture. The mixture was heated to boiling while stirring. After 15 min of boiling an orangish-yellow froth was obtained and the mixture was heated for another 15 min. The mixture was left to cool to room temperature and filtered through a Gooch crucible (the complex is instantaneously reduced on a filter paper). The solution was cooled in an iced bath to eliminate as much of potassium sulfate as possible and the solution filtered again while cold. The resulting orangish-red clear filtrate was left to attain room temperature. In order to isolate the complex, 40 mL of $NaNO_3$ solution (50%, in excess) were added to the solution and the mixture left to crystallise. Almost immediately crystals started appearing and crystallisations is complete when the supernatant liquid is colorless. The crystals were filtered and washed several times with demineralised water until the complex itself starts dissolving, which is indicated by the orange-red drops being formed under the crucible. In the way one can be sure of eliminating sodium and potassium hydroxide since this complex is insoluble in concentrated hydroxide solution The DPA solutions were freshly prepared by dissolving amount of complex in 1.0×10^{-2} mol L^{-1} KOH solution before use. The complex was characterized by the UV–visible spectrum, which showed two absorption maxima at 362 and 253 nm (Fig. 2). The concentration of DPA solution was determined by the absorbency at 362 nm (molar absorptivity $\varepsilon = 1.26 \times 10^4$ L mol^{-1} cm^{-1}).

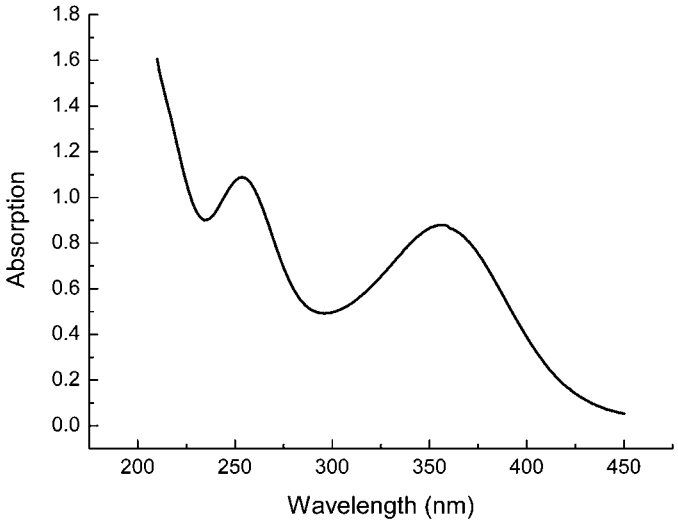

Fig. 2. The UV–vis absorption spectra of DPA.

2.3.2. Sample preparation

The serum sample was supplied by the Hospital of Shaanxi Normal University. 0.5 mL serum sample was collected and transferred into an ultra-filtration tube and centrifuged at 10,000 rpm for 10 min. 0.1 mL filtrate was diluted with double-distilled water to 10 mL and mixed thoroughly for CL analysis.

2.3.3. FI-CL method

As shown in Fig. 1, the distilled water that was propelled by pump delivered the uric acid or the sample solution in the sample loop to merge directly with DPA solution in the flow cell to produce CL. The CL signal was detected with IFFS-A multifunction chemiluminescence analyzer. The determination was based on the proportional relationship between relative CL intensity and corresponding concentration of uric acid.

3. Result and discussion

3.1. Kinetics curve of the CL reaction

Before the flow-injection method was carried out, the kinetic characteristics of the proposed CL reaction were studied by using

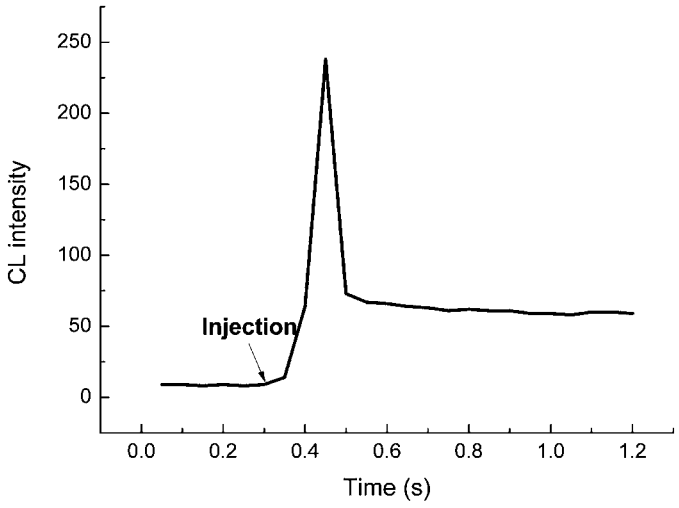

Fig. 3. Kinetics curves of uric acid–DPA; DPA: 1.0×10^{-4} mol L^{-1}; KOH: 0.1 mol L^{-1}; uric acid: 1.0×10^{-6} mol L^{-1}.

the batch method. In the batch mode, the experimental parameters were kept constant. The typical response curve of uric acid that reacted with DPA was recorded to study the kinetic characteristic of the CL reaction. Fig. 3 demonstrates that the CL reaction was very quick. The CL intensity peak appeared within 0.3 s since the uric acid solution was injected. The CL signal would also decrease instantly to baseline at within 0.5 s. Therefore, the length of the mixing tube of DPA and uric acid should be as short as possible in the flow-injection method. The tube delivering uric acid was inserted into the flow cell in order to obtain strong CL intensity.

3.2. Optimization of the reaction conditions

A series of experiments were performed to optimize analytical conditions with 1.0×10^{-6} mol L^{-1} uric acid.

The flow rate was an important factor which influences the analytical sensitivity. The effect of the flow rate of two pumps on CL intensity was examined in the range of 0.5–3.5 mL min^{-1}. The results showed that the CL signal increased with flow rate increased, because the CL reaction is rapid. So the flow rate of 3.5 mL min^{-1}, the maximal flow rate under present conditions, was selected as optimum.

The CL reaction was performed in alkaline condition and the alkalinity of reaction medium was adjusted by varying the concentration of KOH in DPA solution. The effect of KOH concentration on the CL reaction was examined in the range of 0.01–0.5 mol L^{-1}. As can be seen from Fig. 4a, the suitable concentration of KOH was 0.2 mol L^{-1} because a maximal CL intensity could be obtained under this alkalinity.

DPA was the oxidant in the CL reaction which effect on CL intensity was investigated. The range of the concentration of DPA was 5.0×10^{-4}–1.0×10^{-3} mol L^{-1}. The experiments showed the maximal CL signal could be obtained when the concentration of DPA was 4.0×10^{-4} mol L^{-1} (Fig. 4b).

Table 1
Figures of merit of comparable CL methods 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	0.4	[13]
CL biosensor	6–600	0.6	[14]
Luminol-$K_3[Fe(CN)_6]$	4.8–179	3	[15]
$KMnO_4$-OP	0.6–3600	0.3	[16]
Luminol-H_2O_2-HRP	0.05–250	0.05	[17]
Luminol-H_2O_2-Co^{2+}	1.0×10^{-4}–7.0	1.1×10^{-5}	[18]
Luminol-DPA	0.4–200	0.12	This work

3.3. Analytical performance

When the optimized experimental conditions showed above were employed, the relative CL intensity (ΔI) was linearly proportional to the uric acid concentration (C, μmol L^{-1}) in the range of 0.4–200 μmol L^{-1} with the regression equation $\Delta I = 16.2C + 94.2$ ($r^2 = 0.9903$). The determination limit was 1.2×10^{-7} mol L^{-1} (3σ). The relative standard deviation was (RSD) was 2.1% for 5.0×10^{-5} mol L^{-1} uric acid ($n = 7$). Eight standard uric acid solutions (4.0×10^{-7} mol L^{-1}, 6.0×10^{-7} mol L^{-1}, 4.0×10^{-6} mol L^{-1}, 1.0×10^{-5} mol L^{-1}, 4.0×10^{-5} mol L^{-1}, 6.0×10^{-5} mol L^{-1}, 1.0×10^{-4} mol L^{-1}, 2.0×10^{-4} mol L^{-1}) were measured continuously (three replicates for one solution) with FI-CL system, the result of a series of measurements are shown in Fig. 5. Figures of merit of comparable CL methods for determination of uric acid were shown in Table 1.

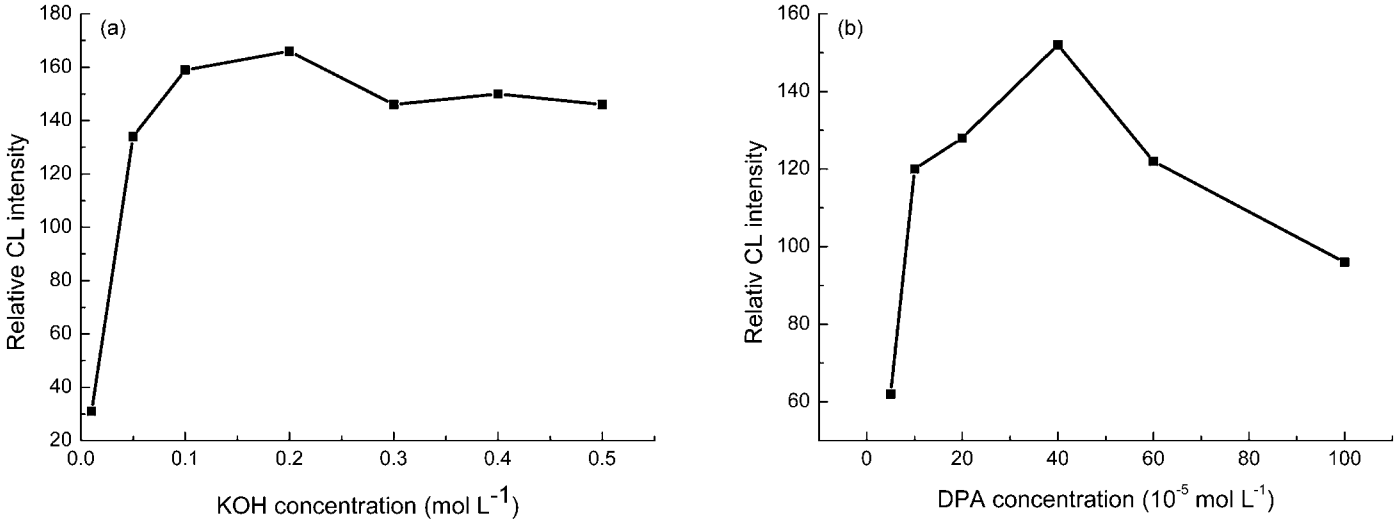

Fig. 4. Effects of reaction conditions on the CL intensity (a) KOH concentration, condition: 1.0×10^{-4} mol L^{-1} DPA; (b) DPA concentration, condition: 0.2 mol L^{-1} KOH.

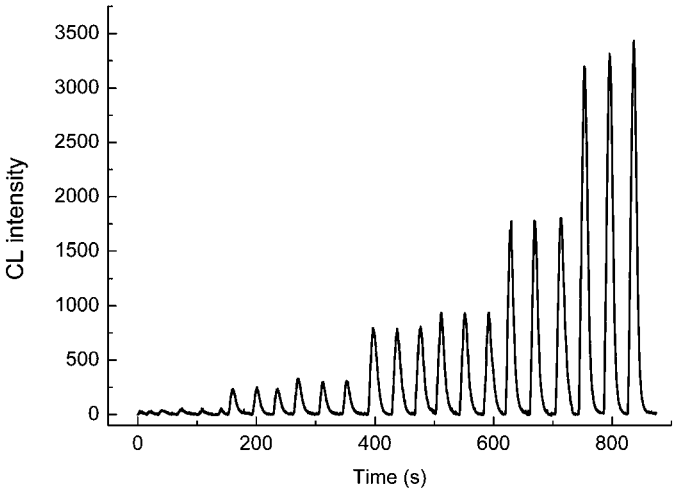

Fig. 5. Results of a series of measurements made with the flow CL system.

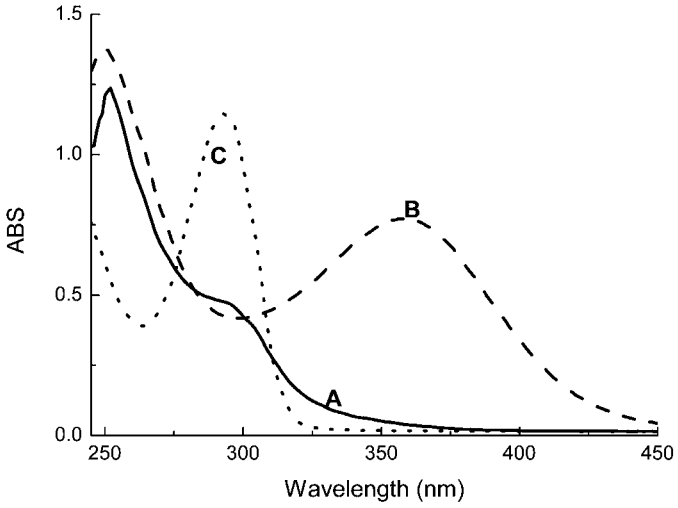

Fig. 6. UV–vis absorption spectra; (a) DPA (3.0×10^{-5} mol L^{-1}) + uric acid (3.0×10^{-4} mol L^{-1}); (b) DPA (3.0×10^{-5} mol L^{-1}); (c) uric acid (3.0×10^{-4} mol L^{-1}).

3.4. Interference studies

To evaluate the selectivity of the method developed the effect of various compounds usually present in serum was studied. The inference of foreign substances were tested by analyzing standard solution of 5.0×10^{-6} mol L^{-1} uric acid into which increasing an amount of interfering analytes were added. The tolerable concentration of foreign species was taken as a relative error <5% and the results demonstrated that 1000 folded Na^+, Ca^{2+}, Al^{3+}, Cu^{2+}, Mg^{2+}, Fe^{3+}, Zn^{2+}, NH_4^+, Cl^-, SO_4^{2-}, $H_2PO_4^-$, carbamide, oxalate, lysine, glutamic acid, leucine, aspirin, serine, lactose, glucose, aspartic acid, hydroxyproline, creatinine; 100 folded lactic acid, glutathione; 10 folded ascorbic acid. The interference of protein in human serum could be ignored when human serum is ultrafiltered. So it is concluded that the present method could be directly applied to the determination of uric acid in human serum.

3.5. Application

In order to evaluate the applicability and reliability of the proposed method, the concentration of uric acid in human serum was determined. The results of the proposed method agree well with those obtained by the phosphotungstate method (phosphotungstate method is a colorimetric assay by reaction of uric acid and phosphotungstate in alkaline medium to produce tungsten blue, which was determined at 700 nm). Recovery studies were also carried out on real samples to which known amounts of uric acid were added. The results are shown in Table 2.

3.6. Possible CL mechanism of the CL reaction

The UV–vis absorption spectra of DPA, uric acid and DPA–uric acid were measured (Fig. 6). The absorption of DPA and uric acid almost disappear as they mixed. When DPA and uric acid were mixed in alkaline medium, the color of DPA faded. However, when strong oxidant ($K_2S_2O_8$ or HNO_3) was added into this near colorless solution, the primary color of DPA recovered. Considering of above experiments, it is obvious that a redox reaction takes place between uric acid and DPA lead to emit CL.

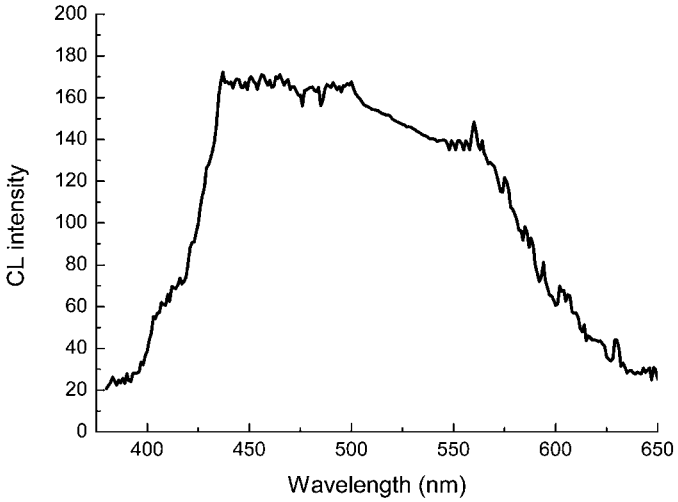

Fig. 7. CL spectrum DPA: 5.0×10^{-4} mol L^{-1} in 0.2 mol L^{-1} KOH; uric acid: 5.0×10^{-4} mol L^{-1}.

In order to get an idea about the CL reaction, the CL spectrum of the reaction of DPA and uric acid was examined by a modified F-4600 fluorescence spectrophotometer (Shimadzu, Japan) com-

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

Sample	Found (μmol L^{-1})			Added (μmol L^{-1})	Total found (μmol L^{-1})	Recovery (%)	RSD (%)
	Proposed method	Reference[a] [5] ± RSD%	Relative error (%)				
No. 1	232	231 ± 1.2	0.4	100	328	96.0	1.3
				200	439	103.5	0.5
				400	630	99.5	2.5
No. 2	297	284 ± 0.8	4.5	100	390	93.0	2.0
				300	605	102.7	2.2
				900	1195	99.8	0.8

[a] Average of three measurements.

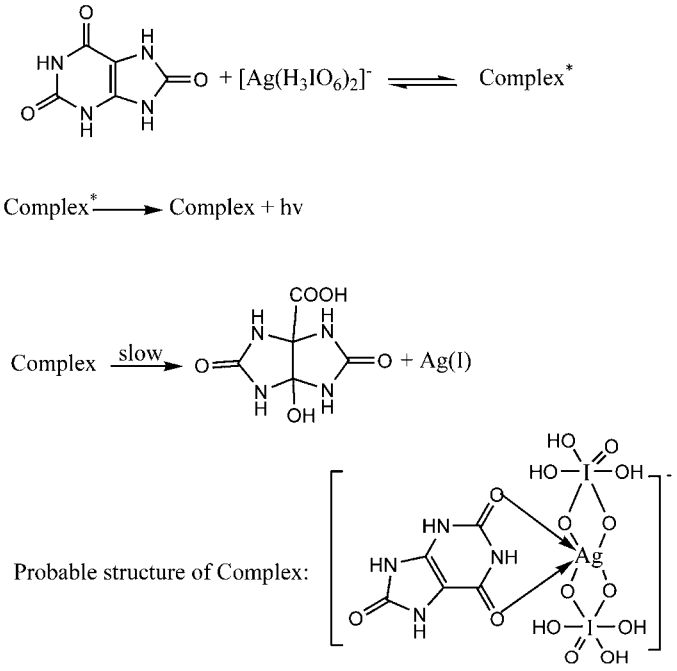

Fig. 8. The most possible reaction mechanism.

bined with flow-injection system, with the light source taken off. The obtained CL spectrum is shown in Fig. 7. It was found that there was one broad spectrum without obvious biggest wavelength located. The fluorescence spectra of the mixture of DPA and uric acid were measured. It was found that no fluorescence emission of DPA and uric acid solution alone or the mixture together.

Based on the experiments mentioned above, the CL emitter should be exited intermediate product. It was reported that DPA could take place complex reaction [25,26,28], so the CL emitter maybe the exited intermediate complex. According to the mechanism of oxidation of some organic compounds by DPA [25,26,28,29] and the study of uric acid oxidation [30], the mechanism of the DPA and uric acid reaction in alkaline medium can be explained as shown in Fig. 8, although more evidences are not available.

4. Conclusion

The use of DPA for direct CL analysis in alkaline medium was developed for the first time. The novel method has been proposed to determine the concentration of uric acid based on CL reaction of DPA and uric acid. The new method is rapid and reproducible with high sensitivity and selectivity in determination of uric acid in human serum. Moreover, DPA can be readily prepared and stable in alkaline media. Thus, the method may find wide application in determination of other biologically important substances with high sensitivity and selectivity. Otherwise, the CL system has a potential application in HPLC and capillary electrophoresis detection.

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