Study on Chemiluminescence Reactions of Lucigenin and Reductants by Flow Injection Analysis

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Abstract: The chemiluminescence reactions between lucigenin and reductants such as Mo(III),V(II), U(III), W(III), Cr(II), Ti(III) and Fe(II), which were produced on-line by passing Mo(VI),V(V),U(VI), W(VI), Cr(VI,III),Ti(IV) and Fe(III) through a micro Jones column, are studied in detail. Results show that the reactions can be used directly for the determination of these substances. The mechanism of the reactions is also investigated.

Keywords: Reductant, lucigenin, chemiluminescence, flow injection analysis.

Preceding studies involved two kinds of chemiluminescence (CL) reactions of Lucigenin (Lu),one was the reaction of Lu with H_2O_2 catalyzed by metal ions, the other was that of Lu with organic reductants such as glucose, uric acid and so on.

We found that Mo (III),V (II),U (III),W (III),Cr (II),Ti (III) and Fe (II), which were produced on-line by passing Mo (VI),V (V),U (VI), W (VI), Cr (VI,III),Ti (IV) and Fe (III) through a micro Jones column[1], can react with Lu to generate strong CL light. Conditions of these reactions and the probability of their applications to analysis were examined in detail. The mechanism of the reactions was also investigated thoroughly.

A stock solution of Lu $(1.0 \times 10\text{-}3 mol/L)$ was stored in the refrigerator at the temperature below 5℃. Working solution was prepared from the stock solution by appropriate dilution. Standard solutions $(1.0 \times 10^{-4} g/mL)$ of Mo (VI),V (V),U (VI), W (VI), Cr (VI), Cr (III), Ti (IV) and Fe (III) were prepared from $(NH_4)_6Mo_7 \bullet 4H_2O$, NH_4VO_3, $UO_2(NO_3)_2 \bullet 6H_2O$, Na_2WO_4, $K_2Cr_2O_7$, $CrCl_3 \bullet 6H_2O$, TiO_2 and $NH_4Fe(SO_4)_2 \bullet 12H_2O$ respectively.

All the reagents were of analytical grade. Distilled-deionized water was used throughout.

A flow injection on-line reduction chemiluminescence analyzer with a micro Jones column made in this laboratory, is shown in **Figure. 1**. A peristaltic pump was used to deliver all flow streams at a constant rate of 3mL/min on each flow line.

A known amount of standard solution was pipetted into a 25 mL volumetric flask, an appropriate amount of 0.01 mol/L H_2SO_4 was used to adjust the acidity, and the flask was filled with water as sample solution and its CL intensity was measured with the analyzer.

Figure.1 Schematic diagram of the flow injection system
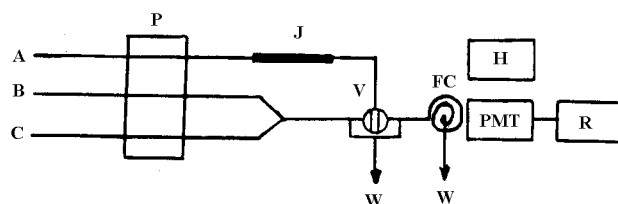
A---Samp4le;   B---Lu;   C---NaOH;   P---Peristaltic   pump;   J---Jones   column;   V---Valve; PMT---Photomultiplier tube; FC---Flow cell; H---High voltage power;   R---Recorder; W---Waste.

The reaction conditions including the pH value of sample solution, Lu concentration, NaOH concentration, value-cell distance and so on, were optimized in detail, the experimental results are listed in **Table 1**.

Table 1   The optimized reaction conditions

Compounds	pH of sample solution	C_{Lu}/mol/L	C_{NaOH}/mol/L	Valve-cell distance/cm
Mo(VI)	3.5	5×10^{-4}	0.4	5
V(V)	3.0	5×10^{-4}	0.1	10
U(VI)	3.0	5×10^{-4}	0.5	5
W(VI)	2.8	5×10^{-4}	0.8	5
Cr(VI)	3.0	5×10^{-4}	0.2	5
Cr(III)	4.0	5×10^{-4}	0.2	5
Ti(IV)	1.0	5×10^{-4}	0.4	5
Fe(III)	3.0	5×10^{-4}	0.5	5

The linear relationship between CL intensity and the concentrations of eight species mentioned above, was investigated under optimized conditions. The parameters for CL determination are shown in **Table 2.** The results show that the CL reactions between Lu and reductants can be used for the determinations of reductants, which are active to luminescence in Lu system and for substances which can be converted into active reductants.

By comparison of the CL spectra of the Lu — reductants system and Cr (III) — H_2O_2 —Lu system. It was found that the two systems have identical maximum emission wavelength and halfpeak width. It is obvious that the two systems have the same emission species, *i. e*, N—methylacridone (NMA[*])[2].

By flushing nitrogen to remove the oxygen dissolved in all solutions, the CL intensity sharply decreased. this demonstrated that oxygen participats the reactions, moreover by addition of sodium benzoate to clean out superoxide radical $O_2^{\cdot-}$, the CL signal almost vanished.
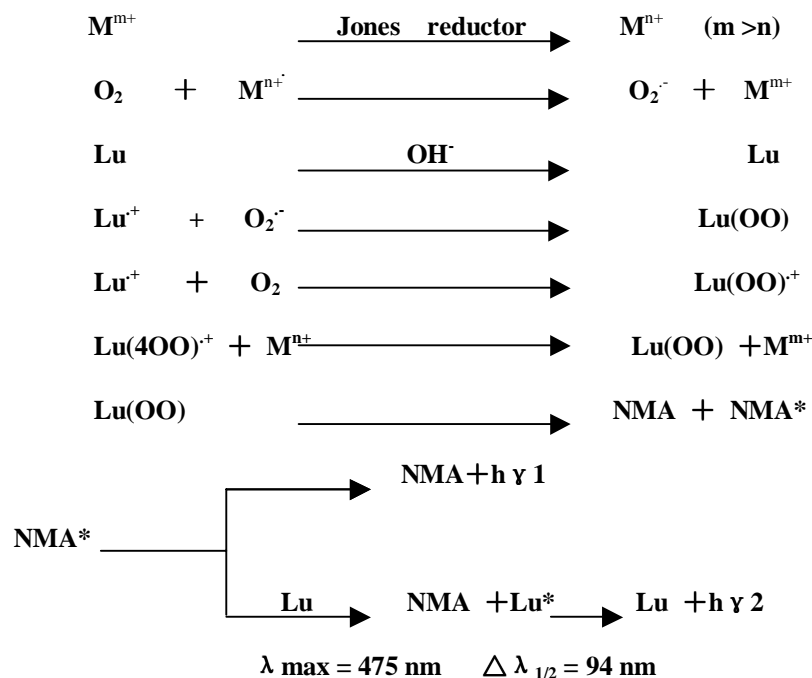
Table 2 The parameters for CL determination[**]

Species	Species state	Linear range(g/mL)	Detection limit(g/mL)
Mo(VI)	Mo(III)	$1\times10^{-10} \sim 1\times10^{-6}$	4×10^{-11}
V(V)	V(II)	$1\times10^{-9} \sim 8\times10^{-5}$	3×10^{-10}
U(VI)	U(III)	$1\times10^{-9} \sim 1\times10^{-5}$	3×10^{-10}
W(VI)	W(III)	$1\times10^{-9} \sim 1\times10^{-5}$	4×10^{-10}
Cr(VI)	Cr(II)	$1\times10^{-7} \sim 1\times10^{-5}$	3×10^{-8}
Cr(III)	Cr(II)	$1\times10^{-7} \sim 1\times10^{-5}$	4×10^{-8}
Ti(IV)	Ti(III)	$1\times10^{-9} \sim 6\times10^{-6}$	3×10^{-10}
Fe(III)	Fe(II)	$1\times10^{-11} \sim 1\times10^{-5}$	3×10^{-12}

** Jones reductor was prepared by zine with 2-5% of amalgam for W and 0.1-1.0% of amalgam for other species；Jones reductor must be kept under weak acidic condition while not used.

According Ref. 3 and 4, the possible mechanism is that reductant reduce O_2 to O_2^-, Lu radical (Lu^{+}) existing in alkaline solution can react with O_2^- to form a dioxetane intermediate Lu (OO), with O_2 to form Lu $(OO)^{+}$, Lu $(OO)^+$ can be reduced continuously by inorganic reductant to Lu (OO), then Lu (OO) decomposes immediately to produce NMA*, the excited state of NMA[3].

One part of NMA* directly emits energy resulting CL (h γ $_1$), the other part of NMA* transfers energy to produce the excited state of Lu (Lu*). The unstable Lu* then releases energy to generate C L (h γ $_2$). The observed CL is the sum of the two emissions. λ max of the emission is 475 nm and the $\triangle \lambda_{1/2}$ is 94 nm.

In a word, the mechanism can be expressed as follows:

$$M^{m+} \xrightarrow{\text{Jones reductor}} M^{n+} \quad (m > n)$$

$$O_2 + M^{n+} \longrightarrow O_2^- + M^{m+}$$

$$Lu \xrightarrow{OH^-} Lu$$

$$Lu^+ + O_2^- \longrightarrow Lu(OO)$$

$$Lu^+ + O_2 \longrightarrow Lu(OO)^{+}$$

$$Lu(4OO)^{+} + M^{n+} \longrightarrow Lu(OO) + M^{m+}$$

$$Lu(OO) \longrightarrow NMA + NMA*$$

$$NMA* \longrightarrow NMA + h γ 1$$

$$NMA* \xrightarrow{Lu} NMA + Lu* \longrightarrow Lu + h γ 2$$

λ max = 475 nm $\triangle \lambda_{1/2}$ = 94 nm

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