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A Chemiluminescence Method for the Determination of Ascorbic Acid based upon Its Reaction with Cerium(IV) in the Presence of $Ru(bipy)_3^{2+}$

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A chemiluminescence (CL) method for the determination of ascorbic acid was proposed, which is based on the enhancement of the CL intensity of $Ru(bipy)_3^{2+}$ - Ce (IV) system with the addition of ascorbic acid. The CL intensity is proportional to the concentration of ascorbic acid in the range of 1×10^{-10} to 1×10^{-5} M with a correlation coefficient of 0.9985. The detection limit is 3×10^{-11} M. The relative standard deviation (R.S.D.) of 10 determinations of 0.1 mM ascorbic acid was 1.75%.The proposed method has been successfully applied to the determination of ascorbic acid in the commercial drinks. The possible mechanism of the CL reaction was studied.

key words: Chemiluminescence, Cerium, Ascorbic acid, Ruthenium

INTRODUCTION

Ascorbic acid (Vitamin C) is a water-soluble vitamin needed for the growth and repair of tissues in all parts of our body. It is necessary to form collagen, an important protein used to make skin, scar tissue, tendons, ligaments, and blood vessels. It is essential for the healing of wounds, and for the repair and maintenance of cartilage, bones, and teeth.

Ascorbic acid is one of many antioxidants. It is a nutrient that blocks some of the damages caused by free radicals, which are by-products that result when our bodies transform food into energy. The build up of these by-products over time can contribute to the development of various health conditions such as cancer, heart disease, and a host of inflammatory conditions like arthritis. It also helps reduce the damage to the body caused by toxic chemicals and pollutants such as cigarette smoke.

Ascorbic acid deficiency can lead to dry and splitting hair; gingivitis (inflammation of the gums) and bleeding gums; rough, dry, scaly skin; decreased wound-healing rate, easy bruising; nosebleeds; weakened enamel of the teeth; swollen and painful joints; anemia; decreased ability to ward off infection; and, possibly, weight gain because of slowed metabolic rate and energy expenditure. A severe form of vitamin C deficiency is known as scurvy, which mainly affects older, malnourished adults.

The body does not manufacture ascorbic acid on its own, nor does it store it. It is therefore important to include plenty of vitamin C-containing foods in one's daily diet. The European Community recommends that the daily intake of ascorbic acid be 60mg. Various methods have been proposed for the determination of ascorbic acid such as titrimetry [1], spectrophotometry [2,3], fluorimetry [4], chromatography [5] and electrochemical detection [6,7] by a manual or flow injection analysis. Each method has its merits and demerits [8] Chemiluminescence method has the advantage of requiring simple instrumentation, having excellent sensitivity over a wide linear dynamic range and of being appropriate for rapid and on-line analysis using flow systems. Low limits of detection can be obtained in comparison with fluorescence detection because no external light source is required and therefore the background signal is very low. Although chemiluminogenic reagents luminol [9-12] or lucigenin [13, 14] are used for the determination of ascorbic acid, the oxidation product dehydroascorbic acid (DHAA) in alkaline media is not stable.

$Ru(bipy)_3^{2+}$ is an extremely versatile chemiluminogenic reagent and growing interest for detection of various compounds. A chemiluminescence (CL) method for the determination of ascorbic acid was proposed, which is based on the enhancement of the CL intensity of $Ru(bipy)_3^{2+}$ - Ce (IV) system with the addition of ascorbic acid in sulfuric acid media.. The CL intensity is proportional to the concentration of ascorbic acid in the range of 1×10^{-10} to 1×10^{-5} M with a correlation coefficient of 0.9985. The detection limit is 3×10^{-11} M. The experimental conditions were optimized; the proposed method was successfully applied to the determination of ascorbic acid in commercially available drinks. Moreover, the mechanism of chemiluminescence reaction was discussed.

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EXPERIMENTAL

Apparatus

The flow system used for the detection and determination of ascorbic acid is shown in Figure 1. An Ismatec 404 peristaltic pump (two channels, variable speed) was used to drive the carrier and reagent streams and one additional pump of the same model was used for waste disposal. Each stream was pumped using tygon tubing (2 mm i.d) at a constant flow rate. The analyte (sample) was injected through a injection valve which allows the complete mixing of the analyte (sample) with the desired amount of Ru $(bipy)_3^{2+}$ solution and combined with acidified Ce (IV) in a 1 cm quartz cell just before the detector. The light was measured by photomultiplier tube (Hamamatsu, Model R928) operated at 950 V. Spex (Edison, NJ, USA) Model FL111 spectrofluorometer was used for CL measurements and spectrum data were collected by Spex DM3000 software. During the measurements, the light source of the spectrofluorimeter was switched off. The slit width of the emission monochromator used was 1.25 mm. Peak height was measured for each signal and expressed as current output of the PMT.

Chemicals

All chemicals were of analytical grade. L-Ascorbic acid, 99 % was purchased from Sigma- Aldrich and stock ascorbic acid (1mM) was prepared daily in deionized water. Working solutions were prepared by dilution of the stock with deionized water and preserved from exposing to light because of the light sensitiveness. Tris(2,2'-bipyridyl) dichloro-ruthenium (II) hexahydrate (Aldrich Chem. Co) was prepared by dissolving appropriate amount of it in 0.05 M sulfuric acid and standard solutions were prepared by proper dilution with the same solvent. Cerium (IV) sulfate was purchased from Aldrich. The Cerium (IV) solution (50 mM) was prepared by dissolving 0.4153 gm of $Ce(SO_4)_2.4H_2O$ in 10 ml of water and 0.281 ml of concentrated H_2SO_4 and diluting with water to 25 ml.

Basic procedure

The manifold described in Fig. 1 was used. Sample (1ml of 0.1 mM ascorbic acid) was injected into a stream of 1mM Ru $(bipy)_3^{2+}$ solution which was then combined with a stream of 50 mM acidified Ce(IV) solution and the resulting peak height was measured. A calibration graph was constructed by plotting the peak height against the acid concentration. For the time-base scan, the instrument was set at emission wave length of ruthenium 610 nm, integration time 1 sec, time increment 1 sec, total time 100 sec and acquisition mode signal (S). Peak height of CL emission was recorded as a function of time.

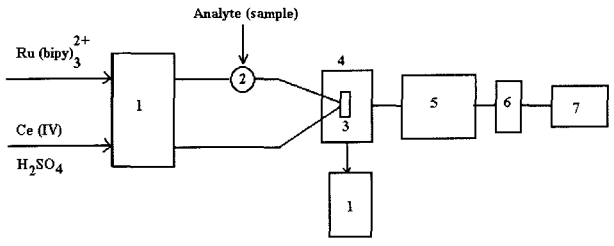


Figure 1. Schematic diagram of the $Ru(bipy)_3^{2+}$ chemiluminescence detection system. Identifications: 1, peristaltic pump; 2, injection valve; 3, quartz cell; 4, light-tight cell compartment; 5, emission mono-chromator; 6, photomultiplier tube; 7, spectroscopy computer.

RESULTS AND DISCUSSION

Effect of the concentration of $Ru(bipy)_3^{2+}$

It is well known that in the CL system containing Ru $(bipy)_3^{2+}$, the CL light emission is due to the reaction of Ru $(bipy)_3^{3+}$ with reductants ([the radical intermediates]*) to give $[Ru(bipy)_3^{2+}]^*$ as follows:

$Ru(bipy)_3^{2+} + Ce(IV) = Ru(bipy)_3^{3+} + Ce^{3+}$

$Ru(bipy)_3^{3+}$ + [the radical intermediates]* = $[Ru(bipy)_3^{2+}]^*$ + products

$[Ru(bipy)_3^{2+}]^* = [Ru(bipy)_3^{2+}] + hv$

So, the chemiluminescent reagent of the system is Ru $(bipy)_3^{2+}$. The concentration of $Ru(bipy)_3^{2+}$ was studied over the range of 0.01 mM (1×10^{-5}M) to 10 mM (1×10^{-2}M). Peak height increased with increasing $Ru(bipy)_3^{2+}$ concentration. Figure 2 shows that 1mM (1×10^{-3}M) provided the greatest intensity, above which the intensity decreased. Therefore, 1×10^{-3}M was used for further experiments.

Effect of the concentration of Ce (IV)

The effect of Ce (IV) concentration on the CL signal was examined over the range 0.1 mM to 100 mM in 0.2 M H_2SO_4 as shown in Figure 3. When the Ce (IV) concentration is below 50 mM, the CL intensity increases with increasing Ce (IV); however, after the Ce (IV) concentration exceeds 50 mM the signal tendency is downward, because of the

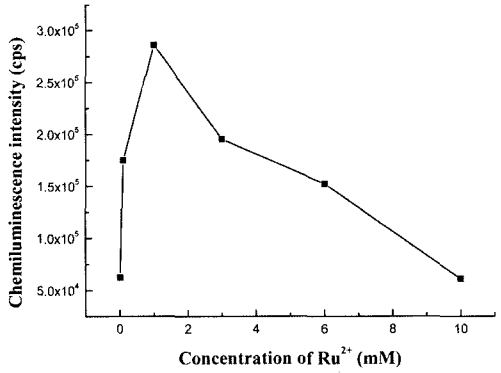


Figure 2. Effect of $Ru(bipy)_3^{2+}$ concentration on the CL intensity. Conditions: [L-AA], 0.1 mM; [Ce(IV)], 50mM; [H_2SO_4], 0.2M; [Flow rate], 3.0 ml/min; [λ_{em}] = 610 nm; [Residence time], 44 sec.

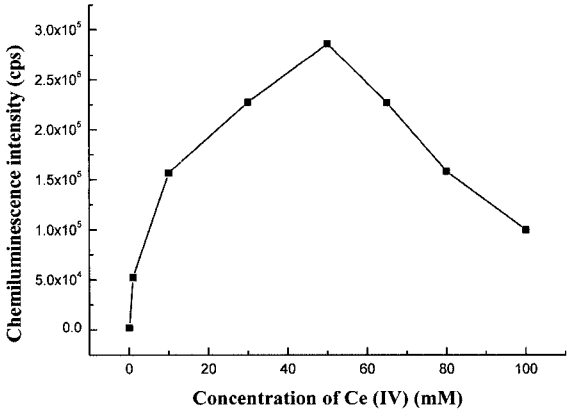


Figure 3. Effect of Ce(IV) concentration on the CL intensity. Conditions: [L-AA], 0.1mM; [$Ru(bipy)_3^{2+}$], 1 mM; [H_2SO_4], 0.2M; [Flow rate], 3.0 ml/min; [λ_{em}] = 610 nm; [Residence time], 44 sec.

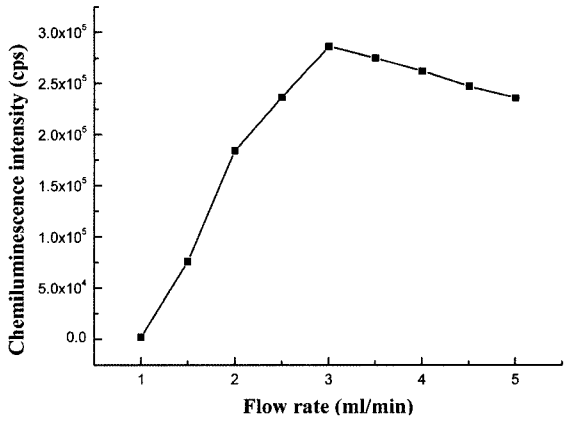


Figure 5. Effect of flow rate on the CL intensity. Conditions:[L-AA], 0.1 mM; [$Ru(bipy)_3^{2+}$], 1 mM; [Ce(IV)], 50 mM; [H_2SO_4], 0.2 M; [λ_{em}] = 610 nm; [Residence time], 44 sec.

absorption of light emission by the colored Ce (IV) solution and the scattering of light emission by the hydrolysis product of Ce (IV) at the experimental acidity [15]. Hence, 50 mM Ce (IV) was selected for the following experiments.

Effect of the concentration of H_2SO_4

Ce (IV) exists as sulfated complexes such as $Ce(SO_4)^{2+}$, $Ce(OH)(SO_4)^{+}$, $Ce(SO_4)_2$, $Ce(SO_4)^{2-}$, $HCe(SO_4)_3^{-}$, $HCe(SO_4)_4^{3-}$ and $Ce(SO_4)_4^{4-}$ in the dilute solution of H_2SO_4 and these species are in a series of equilibria with HSO_4^{-} [16]. It has already been pointed out that the reactive species of the oxidants are Ce (IV), $Ce(SO_4)_2$ and $HCe(SO_4)_3^{-}$ [17]. So, for $Ru(bipy)_3^{2+}$ - ascorbic acid- Ce(IV) system, appropriate concentration of H_2SO_4 is required with a view to producing the optimal amount of reactive oxidants. The experiment was carried out in the range of 0.01mM -900mM H_2SO_4 under the standard conditions mentioned above. The experimental result is shown in the Figure 4. The maximum CL intensity was obtained at 200 mM (0.2M) H_2SO_4, indicating the existence of maximum amount of reactive species in the solution and intensity subsided on either side of this value.

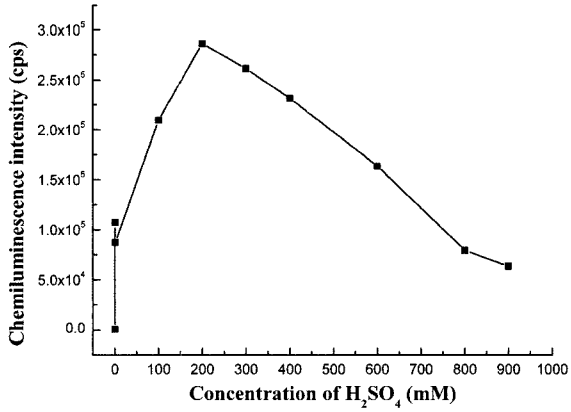


Figure 4. Effect of H_2SO_4 concentration on the CL intensity. Conditions:[L-AA], 0.1 mM; [$Ru(bipy)_3^{2+}$], 1 mM; [Ce(IV)], 50 mM; [Flow rate], 3.0 ml/min; [λ_{em}] = 610 nm; [Residence time], 44 sec.

Effect of flow-rate on intensity

The flow rate is an important parameter in chemiluminescence detection because the time taken to deliver the CL reagents into the cell is critical for maximum collection of the emitted light. Too low or too high flow rates create a decrease or even absence of CL in the cell. The optimum flow rate was found to be 3.0 ml/min as shown in the Figure 5, studying the range of 1 to 5 ml/min with equal flows in each channel. Increasing the flow rate above 3.0 ml/min does not significantly enhance signal and will increase the pressure in the tubes, effects precision and will be uneconomical in the use of the reagents.

Calibration and detection limit

Under the optimum conditions: [$Ru(bipy)_3^{2+}$], 1mM; [Ce (IV)], 50 mM; 0.2M H_2SO_4; [Flow rate], 3.0 ml/min, the calibrations of the responses of chemiluminescence intensity to the concentrations of ascorbic acid 1×10^{-10} M to 1×10^{-3} M were obtained. The linear value of the calibration curve of ascorbic acid was 1×10^{-10} M to 1×10^{-5} M (r= 0.9985). The detection limit of the proposed method is 3×10^{-11} M which was calculated in the ratio of three times the standard deviation of 10 blank measurements to the slope of the calibration curve.

Reproducibility

The reproducibility of the CL signals was obtained for 10 determinations of 0.1 mM ascorbic acid along with other chemiluminogenic reagents under optimum conditions. The relative standard deviation (RSD) was 1.75%.

Evaluation of interferences

The effects of coexisting ions that could be present in real

Table 1. Effect of coexisting foreign ions and compounds on the determination of 0.1 mM ascorbic acid

Foreign ions and compounds	Molar concentrations	Signal change (%) ascorbic acid
None (Only ascorbic acid)	[D] =1× 10^{-5}	Not applicable
Fructose	10 × [D]	-1
	100 × [D]	+1
Vitamin B1	10 × [D]	-1
	100 × [D]	+2
Vitamin B2	10 × [D]	-1
	100 × [D]	+3.8
Vitamin B6	10 × [D]	-1
	100 × [D]	+1
Citric acid	10 × [D]	+1
	100 × [D]	+3
Na^+	10 × [D]	0
	100 × [D]	0
Ca^{2+}	10 × [D]	0
	100 × [D]	0
Fe^{2+} and Fe^{3+}	10 × [D]	0
	100 × [D]	0
Glucose, saccharin, maltose and oxalic acid	10 × [D]	-1
	100 × [D]	+1

sample were carried out on a selected concentration of ascorbic acid (0.01 mM). The concentration of most probable foreign ion was taken 10 and 100 times that of ascorbic acid. The results shown in the Table 1 indicate their effects on the CL response with ascorbic acid. Each organic and inorganic compound was added to the solution of ascorbic acid and concentrations of other reagents were kept constant. A substance was considered not to interfere when the effect on the peak height was <5%. From the table, no interference was observed for metal ions such as [Na^+], [Ca^{2+}] and [Fe^{2+} and Fe^{3+}]. Citric acid and vitamines little bit interfered at high concentration but the effect was reduced by dilution when the ratio [Interfere]/ [Ascorbic acid] was selected at 10. The rest of the organic compounds mentioned in the table showed no interference and permit the use of standard calibration for the determination of ascorbic acid in the real sample of interest.

Application

The proposed method was applied to the determination of ascorbic acid in commercially available 3 types of drinks "Vita 500" (7000 μg/ml), "Fibe-mini" (3000 μg/ml) and "Confidence" (890 μg/ml). Each drink with a nominal value was diluted to 10^4- fold with water to fit the concentration of the analyte within the linear range of the standard curve. The results obtained by the method almost comply with the printed values in the labels (Table 2).

CL mechanism

The mechanism involving the ascorbic acid reduction of cerium(IV) in sulfuric acid media was proposed by Ranjanna et al. [18] that the reaction of Ce (IV) with ascorbic acid was

Table 2. Determination of ascorbic acid in commercial drinks by the proposed method

Drinks	Nominal quantity (μg/ml)	Found[a] (μg/ml)
Vita 500 (100 ml pack)	7000	6643±5.13
Fibe-mini (100 ml pack)	3000	2936.10±1.32
Confidence (230 ml pack)	890	872.20±2.78

[a]Average of three measurements

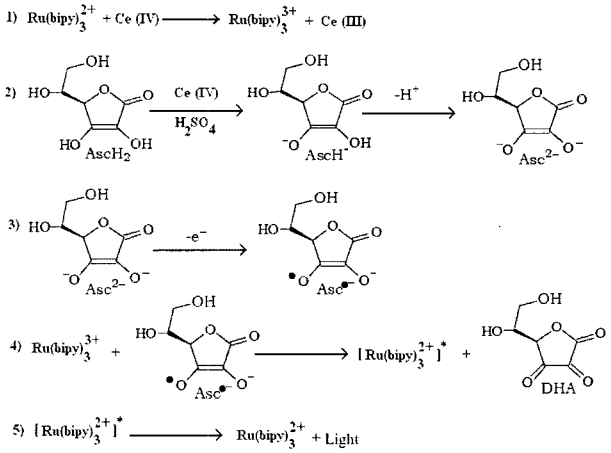
held by rapid complexation of the metal ion by the reductant ($AscH_2$) following by the inner sphere transfer of one electron, so that an ascorbate radical was formed. By analogy, the proposed mechanism involves the oxidation of Ru $(bipy)_3^{2+}$ by Ce (IV) and this product reacts with the radical to form $[Ru(bipy)_3^{2+}]^*$, which turns into $Ru(bipy)_3^{2+}$ with emission at 610 nm. A detailed CL mechanism is shown in scheme 1.

Kinetic curve

The kinetic curves of the systems (a) $Ru(bipy)_3^{2+}$ - Ce (IV) and (b) $Ru(bipy)_3^{2+}$ -Ascorbic acid- Ce (IV) were obtained and shown in the Figure 6. The experimental results indicate that the CL intensity of the system (a) was week but could be enhanced proportionally by ascorbic acid in (system b). The residence times of (a) and (b) are 42 and 44 seconds respectively.

CONCLUSION

The results presented here demonstrated the feasibility of using $Ru(bipy)_3^{2+}$ - Ce (IV) CL system for the rapid, simple, sensitive and universal detection of ascorbic acid with offering good reproducibility. A unique feature of the method was that this CL system gave a low baseline. The method has a linear range of 1×10^{-10} to 1×10^{-5} M and detection limit of 3×10^{-11} which is far lower than most of the chemiluminescence



Scheme 1.

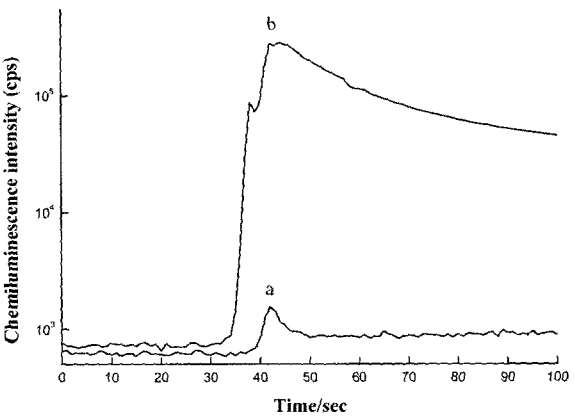


Figure 6. The CL kinetic curves of the proposed system. Conditions: [L-AA], 0.1mM; [$Ru(bipy)_3^{2+}$], 1 mM; [$Ce(^2V)$], 50mM; [H_2SO_4], 0.2M, [Flow rate], 3.0 ml/min; [λ_{em}] = 610 nm. (a), $Ru(bipy)_3^{2+}$ -Ce (IV) system; (b), $Ru(bipy)_3^{2+}$ -Ascorbic acid- Ce(IV) system.

methods. The method can hopefully be applied to the routine analysis of ascorbic acid in quality control laboratory. Furthermore, the system can be utilized to develop a CL sensor for the determination of ascorbic acid because of its low detection limit.

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