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Determination of Catechin in Aqueous Solution by Chemiluminescence Method

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A method to determine catechin in aqueous solution by measuring chemiluminescence intensities using a stopped flow system has been studied. The lucigenin-hydrogen peroxide chemiluminescence reaction was chosen for the determination of catechin. Fe(II) ion was added to the chemiluminescence system to increase the sensitivity. The chemiluminescence intensity from the lucigenin system was increased by the addition of catechin. Effects of flow rates of reagent and sample and concentrations of lucigenin, hydrogen peroxide, Fe(II) ion and KOH were investigated. The calibration curve for catechin was linear over the range from 1.0×10^{-6} to 1.0×10^{-3} M and the detection limit was 3.0×10^{-7} M under the optimal experimental conditions.

KEY WORDS: Catechin; chemiluminescence.

INTRODUCTION

Catechins are a group of polyphonic compounds abundantly contained in green tea. The main polyphonic components in green tea are (−)-epicatechin (EC), (−)-epicgallocatechin (EGC), (−)-epicatechin gallate (ECG), and (−)-epigallocatechin gallate (EGCG). Catechins are considered to exert protective effects against cancer and inflammatory and cardiovascular diseases [1–3]. This suggests that polyphenolic compounds like catechins may play an important role in scavenging free radicals such as hydroxyl radicals, peroxyl radicals, superoxide anion radicals, and nitric oxide in living systems [4–12]. Several conventional detection techniques for catechin exist: HPLC-UV detection [13–16], electrochemical detection [2,18] and chemiluminescence method [2,3,18]. Among these approaches, the chemiluminescence method is considered the most sensitive due to the fact that it does not require an excitation light sources as do fluorometry and spectrophotometry analyses.

The purpose of this paper is to evaluate the antioxidant activity of catechin using chemiluminescence in order to determine the catechin concentration. The optimum analytical conditions such as concentrations of KOH, H_2O_2, lucigenin, and Fe (II) ion and flow rates were studied.

EXPERIMENTAL

Materials

Lucigenin (*bis-N*-methylacridinum nitrate) and catechin hydrate (98%) were obtained from Aldrich (Milwakukee, WI). Hydrogen peroxide (30%) and KOH (min 85%) were purchased from Junsei chemical Co. Ltd. and Duksan (Duksan Pure chemical Co. Ltd) respectively. Ferrous Ammonium sulfite was obtained fromWako Pure chemical industries. Ltd. Deionized water was obtained by means of a Millipore (Bedford, MA) Milli-Q water system and used through out the whole experiment. Catechin stock solution was prepared by dissolving an appropriate amount in deionizied water, and then diluted with deionized water to give a concentration of 1.0×10^{-2} M.

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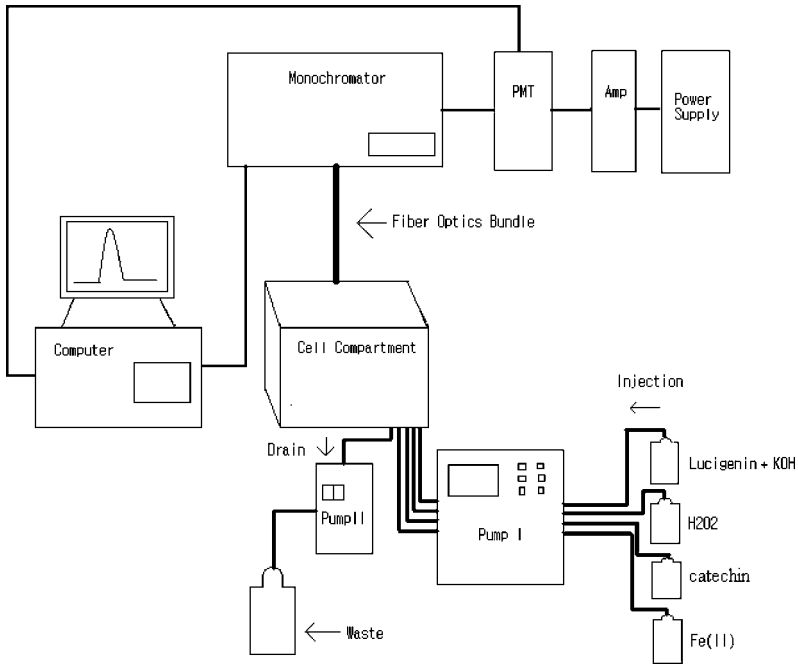

Fig. 1. The diagram of a flow system for chemiluminescence: Pump I; injection pump, Pump II; drain pump.

Apparatus

The diagram of an automated flow injection analyzer used in the chemiluminescence measurement is shown in Fig. 1. The flow system employed in this work consisted of two peristaltic pumps (Ismatec Model MS-4 Reglo/6-100, Glattbrugg-Zich, Switzerland). One (Pump I) delivered a chemiluminogenic reagent solutions, H_2O_2, KOH, and Fe (II). The other (Pump II) drained all sample solution. The sample solution was mixed with flow cell. PTFE tubing (i.d. 0.040″) was used to connect all the components of this system. A bifurcated optical fiber bundle (Model 77533, Oriel, Straford, CT) was screwed to the flow cell for the position of the sensing tip of the optical fiber to be the same for each measurement. The flow cell was housed in a laboratory made light tight chamber to remove all the unnecessary stray light. One end of the fiber bundle was fixed at 10 mm before the emission port and the other end at 10 mm before the excitation port of the cell component of spectrofluorometer (Model FL 111, Spex, Edison, NJ). To record emission and excitation spectra, a 450 W Xe lamp was used. To measure chemiluminescence intensity, the Xe lamp was shut off and the luminescence emitted from the cell was fed to a photomultiplier tube (Model R928, Hamamatsu, USA). The voltage used for the photomultiplier tube was 950 V. The acquisition mode used was signal/reference (S/R) for the excitation and emission spectra and signal (s) for the chemiluminescence measurements. The chemiluminescence intensity at 473 nm was monitored for the determination of catechin. For the chemiluminescence measurements, the integration time and slit width were 1 s and 0.50 mm, respectively.

Procedure

A chemiluminogenic reagent solution containing 1.0×10^{-3} M lucigenin was used for calibration. The chemiluminogenic reagent solution was not stable under ambient conditions, and a fresh solution was made daily. The catechin standard solutions were freshly prepared by appropriate dilution of the 1.0×10^{-2} M stock solution with the deionized water. The flow rate flowing through the flow cell was programmed to be 3.5 mL/min. Calibration plot of chemiluminescence intensities measured at 473 nm versus concentrations of catechin standard solutions were carried out. For each standard solution, three successive measurements were conducted.

RESULTS AND DISCUSSION

Spectral Characteristics

The excitation spectrum of a 1.0×10^{-3} M lucigenin solution recorded at 500 nm emission by continuously pumping only a lucigenin stream of the flow injection analyzer (Fig. 1) showed four bands. The maximum

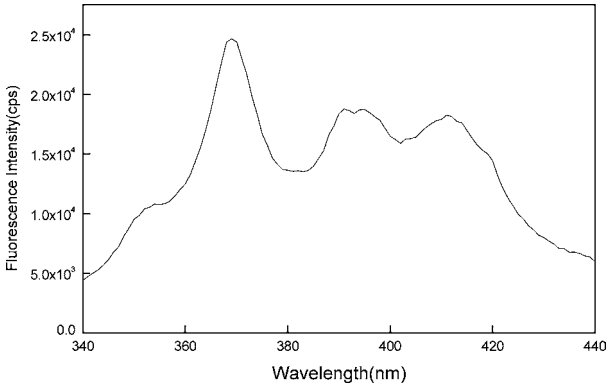

Fig. 2. Excitation spectrum of lucigenin in aqueous solution: [lucigenin], 1.0×10^{-3} M; λ_{em} 500 nm.

wavelength appeared at 369 nm (Fig. 2). The corrected emission spectrum obtained at 369 nm excitation for the same solution gave one broad band with the emission maximum at 501 nm (Fig. 3). The chemiluminescence spectrum recorded by pumping 1.0×10^{-3} M lucigenin and 1.0 M H_2O_2 solution is shown in Fig. 4. The peak maximum of chemiluminescence spectrum observed at 473 nm is in good agreement with previous reports [19, 20]. Therefore, the optimum emission wavelength was 473 nm; this value was chosen for further work.

Effect of KOH Concentration

The effect of KOH concentration on the chemiluminescence intensity is shown in Fig. 5. As the concentration of KOH increases, the chemiluminescence intensity increases up to 1.0 M, beyond which the chemiluminescence intensity starts decreasing. Therefore, the optimum concentration of KOH was 1.0 M; this value was chosen for subsequent work.

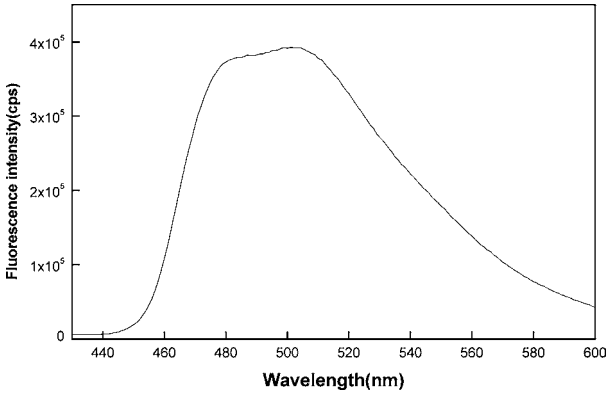

Fig. 3. Emission spectrum of lucigenin in aqueous solution: [lucigenin], 1.0×10^{-3} M; λ_{ex} 369 nm.

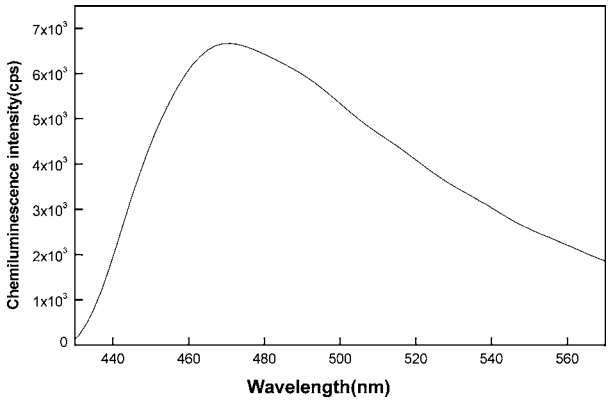

Fig. 4. Chemiluminescence spectrum of lucigenin solution containing hydrogen peroxide in aqueous solution: [lucigenin], 1.0×10^{-3} M; [H_2O_2], 1.0 M; flow rate, 3.5 mL/min.

Effect of H_2O_2 Concentration

The effect H_2O_2 concentration on the chemiluminescence intensity is shown in Fig. 6. As the concentration of H_2O_2 increases, the chemiluminescence intensity increases up to 1.5 M, beyond which the chemiluminescence intensity decreases. Therefore, the optimum concentration of H_2O_2 was 1.5 M; this value was selected for the present system.

Effect of Lucigenin Concentration

The effect of lucigenin concentration on the chemiluminescence intensity is shown in Fig. 7. As the concentration of lucigenin increases, the chemiluminescence intensity increases up to 1.0×10^{-3} M, beyond which the chemiluminescence intensity started to be constant.

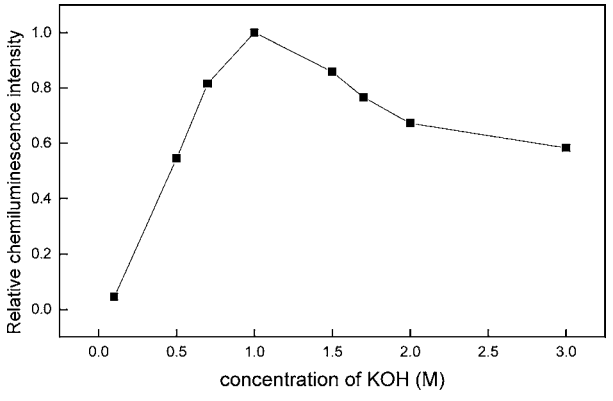

Fig. 5. Effect of KOH concentration on chemiluminescence intensity: [lucigenin], 1.0×10^{-5} M; [H_2O_2], 1.5 M; flow rate, 3.5 mL/min; λ_{em} 473 nm.

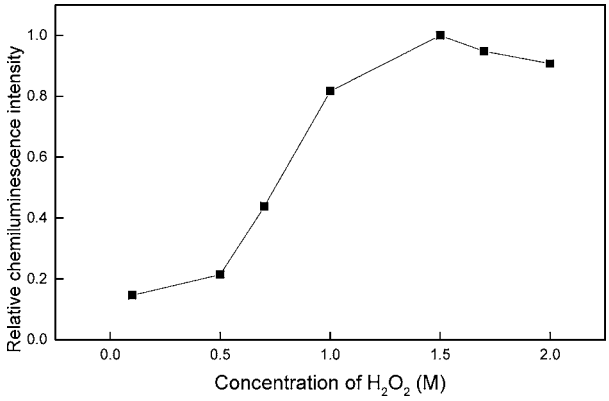

Fig. 6. Effect of H_2O_2 concentration on chemiluminescence intensity: [lucigenin], 1.0×10^{-3} M; [KOH], 1.0 M; flow rate, 3.5 mL/min; λ_{em} 473 nm.

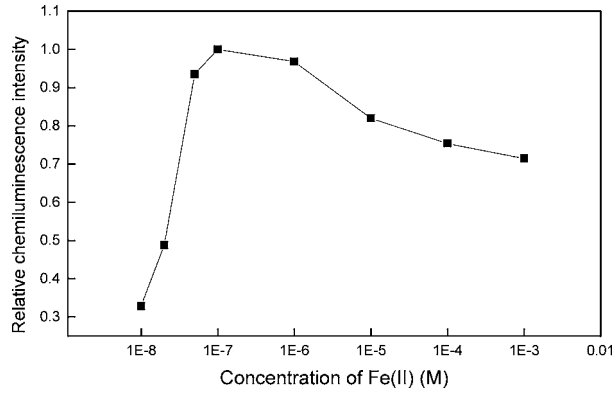

Fig. 8. Effect of Fe(II) ion concentration on chemiluminescence intensity: [lucigenin], 1.0×10^{-3} M; [H_2O_2], 1.5 M; [KOH], 1.0 M; flow rate, 3.5 mL/min; λ_{em} 473 nm.

Therefore, the optimum concentration of lucigenin was 1.0×10^{-3} M; this value was chosen for subsequent studies.

Effect of Fe(II) Ion Concentration

The effect of Fe(II) ion concentration on the chemiluminescence intensity is shown in Fig. 8. As the concentration of sensitizer increases, the chemiluminescence intensity increases up to 1.0×10^{-7} M, beyond which the chemiluminescence intensity started decreasing. Therefore, the optimum concentration of Fe(II) was 1.0×10^{-7} M; this value was chosen for further work.

Effect of Flow Rates

The flow rate of reagents delivered to a flow cell is an essential factor for chemiluminescence measurements using a flow injection system because it determines the contact time between reactants and a sensing tip. It also controls, to some extent, the diffusion of reactants from the flowing solution to a sensing tip. Therefore, the influence of the flow rate of the chemiluminogenic reagent solution on the chemiluminescence response was investigated in the 2.0–5.5 mL/min range. For this work, the volumes taken on the basis of the results of initial crude optimization were 3.5, 3.5, 3.5 and 3.5 mL/min for lucigenin, H_2O_2, KOH, and Fe(II) solution, respectively. The result is shown in Fig. 9. The lower flow rates resulted in higher contact time for the sensing tip of optical fiber but they were found to be unfavorable for the sensitivity because the chemiluminescence reaction is a very fast process. A flow rate of 3.5 mL/min was chosen in this work for optimum value to have a fast response as well as a high chemiluminescence intensity.

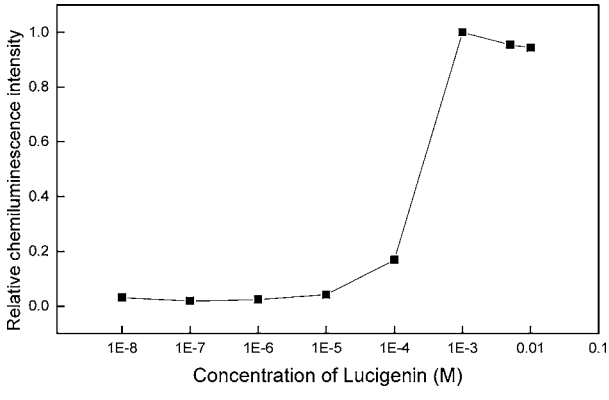

Fig. 7. Effect of lucigenin concentration on chemiluminescence intensity: [H_2O_2], 1.5 M; [KOH], 1.0 M; flow rate, 3.5 mL/min; λ_{em} 473 nm.

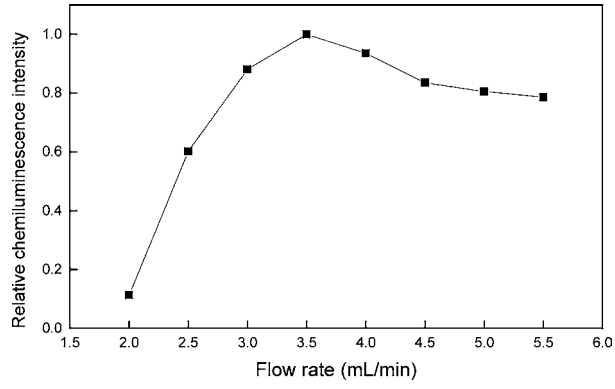

Fig. 9. Effect of flow rates on chemiluminescence intensity: [lucigenin], 1.0×10^{-3} M; [H_2O_2], 1.5 M; [KOH], 1.0 M; Fe(II), 1.0×10^{-7} M; λ_{em} 473 nm.

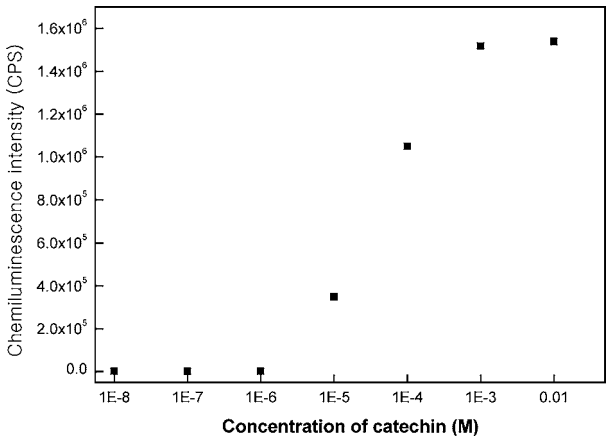

Fig. 10. Calibration curve for catechin standard solution: [lucigenin], 1.0×10^{-3} M; [H_2O_2], 1.5 M; [KOH], 1.0 M; [Fe(II)], 1.0×10^{-7} M; flow rate, 3.5 mL/min; λ_{em} 473 nm.

Calibration Curve for the Determination of Catechin

The average of peak heights of three successive chemiluminescence signals obtained under the optimum experimental conditions for each catechin standard solution was used for calibration. Fig. 10 shows a typical calibration curve for different catechin concentrations. A linear response to catechin concentration was established over the range of 1.0×10^{-6} to 1.0×10^{-3} M. The correlation coefficient in this range was 0.9942. The detection limit (3σ) was found to be 3.0×10^{-7} M.

CONCLUSION

A novel CL method was developed for catechin based on the increased CL intensity in the presence of catechin into a solution of lucigenin and hydrogen peroxide. The proposed CL method is simple and precise, it allows determination of catechin over the range of 1.0×10^{-6} M to 1.0×10^{-3} M, the coefficient of correlation was 0.9942 and the detection limit was 3.0×10^{-7} M under the optimal experimental conditions.

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