Determination of L-Ascorbic Acid by a Chemiluminescence Method using a Metal-MWCNT Immobilized Microfluidic Chip

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Abstract
The determination of L-ascorbic acid (AA) by a high throughput chemiluminescence (CL) method using a metal ion attached multiwalled carbon nanotubes (MWCNTs) immobilized microfluidic chip has been developed. The method is based on enhancing CL intensity of the luminol-hydrogen peroxide system with the immobilized metal-MWCNTs in the microfluidic chip and on the quenching of CL intensity with addition of AA. The effects of pH, concentrations of luminol, hydrogen peroxide and metal ions on the CL intensity were investigated and optimized. The calibration curve for AA was linear over the range of 1.6×10⁻⁸ M to 6.4×10⁻⁷ M, the correction coefficient was 0.98913 and the detection limit was 1.11×10⁻⁹ M.

Key words: Metal-MWCNTs, Microfluidic chip, L-ascorbic acid, chemiluminescence

Introduction
L-ascorbic acid (AA, (5R)-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxyfuran-2(5H)-one) act as an antioxidant in various biochemical pathways [1]. Various determination methods including spectrophotometric and electrochemical methods were reported to determine AA in food or pharmaceutical samples [2,3]. Recently, microfluidic chip based analytical methods have high interest as a high throughput analytical platform to determine various chemical or biological samples in water based solutions [4-9]. Especially, microfluidic chip based analytical methods provide rapid determination using very small amount of sample and reagent solutions such as nano-liter level [4-9].

In this study, metal ions as a catalyst were attached to the multiwalled carbon nanotubes (MWCNTs) and a metal-MWCNTs immobilized microfluidic chip was fabricated for determination of AA. Then the AA was determined using the microfluidic chip on the optimized conditions.

Experimental
Oxidized MWCNTs were prepared using 60 % nitric acid for 12 hrs at 120 °C. Then various metal ions such as Co²⁺, Cu²⁺, Fe³⁺ were attached on the oxidized MWCNTs respectively. The prepared metal-MWCNTs were immobilized on the microchamber of a polydimethylsiloxane (PDMS) microfluidic chip (Fig. 1a and 1b). The PDMS microfluidic chip was fabricated by soft-lithographic procedure using a microfluidic chip mold that prepared by a photolithographic procedure. The microfluidic chip have three inlets to introduce AA, luminol, and hydrogel peroxide solutions and a microchamber having the metal-MWCNTs. The experimental conditions including concentration of luminol, hydrogen peroxide, and metal ions, pH, flow rate of each solution were investigated and optimized. Then the CL intensity was measured at the 425 nm. A spectrofluorimeter (Model F-4500, Hitachi, Japan) equipped with a photo multiplier tube was used to detect CL intensities from the reaction at the microchamber. While the CL intensity was obtaining, the light source of the...
A pH meter (Model Orion, 520A, USA) was used. On the optimized conditions, the determination of AA was performed using the metal-MWCNTs immobilized microfluidic chip.

**Results and discussion**

Metal ions such as Co\(^{2+}\), Cu\(^{2+}\), Fe\(^{3+}\) have catalytic activity at luminol-hydrogen peroxide CL reaction that enhance CL intensity. Furthermore the immobilized metal-MWCNTs in the microfluidic chip was highly enhanced the CL intensity of the luminol-hydrogen peroxide system as shown in Fig. 2. Thus, the metal-MWCNTs immobilized microfluidic chip was fabricated to determine an antioxidant such as AA. By adding an antioxidant, the CL intensity of luminol system could be reduced. Therefore, a highly enhanced CL signal is necessary to determine a very low concentrated antioxidant sample. Among the metal ions, Co\(^{2+}\), Cu\(^{2+}\), Fe\(^{3+}\), attached MWCNTs, Cu\(^{2+}\)-MWCNTs produced highest CL intensity. Thus, the Cu\(^{2+}\)-MWCNTs immobilized microfluidic chip was used to determine AA in a sample solution. By adding AA into the microfluidic channel of the chip, the CL intensity was reduced according to the concentration of AA. Based on the results, the AA was determined using the microfluidic chip as shown in Fig. 3. The linear range of the calibration curve for AA was obtained from 1.6 x 10\(^{-8}\) M to 6.4 x 10\(^{-7}\) M, the correction coefficient was 0.98913 and the detection limit was 1.11 x 10\(^{-9}\) M.

**Conclusion**

A microfluidic chip based high throughput determination of L-ascorbic acid has been presented. The immobilized metal-MWCNTs on the microchamber of the microfluidic chip highly enhanced CL intensity of luminol. Thus, very low concentrated AA could be determined by using the enhanced CL signal of the metal-MWCNTs immobilized microfluidic chip. The metal-MWCNTs immobilized microfluidic chip could be applicable to determination of an antioxidant in a water based solution.

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