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Time measurement-visual analysis of L-cysteine using the autocatalytic sodium sulfite/hydrogen peroxide reaction system and its application to length detection–flow analysis

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ABSTRACT

Trace amounts of L-cysteine can function as a trigger, i.e., reaction initiator, in the autocatalytic sodium sulfite/hydrogen peroxide reaction system. Rapidly changing of pH after induction time is visually confirmed by color changing of bromothymol blue in this autocatalytic reaction. Based on this finding, $\mu g\,L^{-1}$ levels of L-cysteine were measured over time using the autocatalytic reaction system. The determination range using the above method was 5.0×10^{-8}–2.5×10^{-6} M, the detection limit (3σ) was 1.8×10^{-8} M ($1.94\,\mu g\,L^{-1}$), and the relative standard deviation was 2.41% at an L-cysteine concentration of 5×10^{-7} M ($n = 5$). This method was also applied to length detection–flow injection analysis. The determination range for the flow injection analysis was 2.0×10^{-7}–1.0×10^{-5} M. The detection limit (3σ) was 1.4×10^{-7} M ($17.0\,\mu g\,L^{-1}$), and the relative standard deviation was 0.91% at an initial L-cysteine concentration of 10^{-6} M ($n = 5$).

1. Introduction

In autocatalytic reactions, there is a chemical amplification reaction that generates catalysts exponentially from an indicator [1]. The substance that acts as the reaction initiator is called the trigger. Generally speaking, when indicators decompose or are oxidized during catalysis, the signal intensity (e.g., absorbance) changes smoothly in the catalytic reaction system, which provides a means to determine the concentration of the catalysts [2–5]. However, during the course of an autocatalytic reaction, catalysts are produced as the indicator decomposes from the action of the trigger. Therefore, the amount of the catalyst increases exponentially as the reaction progresses and the signal intensity is damped for a stretch followed by a sudden increase in the catalyst concentration after an arbitrary induction time. Because the induction time of the reaction is determined by the initial concentration of the trigger, spectrophotometric measurement of the trigger can be performed over time up to the reaction end point [6–9].

The following systems have been introduced in previous reports as measurement methods for autocatalytic reactions. In the autocatalytic reaction with the cobalt(III)-bis[(2-(5-bromo-2-pyridylazo)-5-(N-propyl-N-sulfopropyl-amino)phenolate] complex [Co(III)-5-Br-PAPS]/peroxomonosulfate salt system [6], the cobalt complex indicator is decomposed by oxidation due to the cobalt(II) ion which acts as the trigger. In the sodium sulfite/hydrogen peroxide system, which utilizes the change in pH over time [7], horse radish peroxidase (HRP), an antibody label in an enzyme immunoassay, acts as the trigger. In addition, in the copper(II)-phthalocyanine tetrasulfonic sodium salt (Cu-PTS)/potassium bromate system [8], Cu-PTS is decomposed by the oxidation with ruthenium(III) acting as the trigger. Each reaction system has a characteristic that makes it possible to determine trace amounts of the trigger.

These measurement methods for autocatalytic reaction systems can detect extremely small (trace) amounts of the trigger. The trigger's detection limit is not influenced by the signal intensity, because these methods have a constant sensitivity. The autocatalytic reaction can theoretically be detected at a limit of 10^{-30} M trigger in 20 min using computer simulations with kinetic analysis [9]. Currently, 10^{-14} M of ruthenium(III) was determined by adding citrate to the autocatalytic reaction with the Cu-PTS/potassium bromate system [8].

Recently, the length detection–flow analytical system as an automatic analytical system using an autocatalytic reaction was proposed [10]. Determination of the concentration of the trigger by the conventional autocatalytic reaction system using time measurement could be substituted with that using a length measurement. Briefly, the reaction solution is pumped into a trans-

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parent tubing at a constant flow rate, and the length of the color band is measured by a ruler when the color of the solution changes. An autocatalytic reaction with the sodium sulfite/hydrogen peroxide system was utilized as a model reaction in the foregoing report [10]. Furthermore, this system was recently applied to microfluidic device [11,12]

In this study, it was found that L-cysteine acts as a trigger, and it was further found that the autocatalytic reaction system can be selectively and simply used to determine L-cysteine. Furthermore, this simple determination of L-cysteine was applied to the length detection–flow analytical system.

L-Cysteine is an amino acid having a thiol basis (SH basis), and exists widely in nature as a component of proteins. Recently, it has been used in various kinds of cosmetics, medical supplies and food additives [13]. The Ellman method [14] is well known for the determination of L-cysteine, but a lot of methods except it are reported, too. Although kinetic analyses such as the catalysis method [13,15] and the ligand exchange reaction method [16,17] do not need special analytical instrumentation, determination of L-cysteine at the sub-mg L^{-1} level is not realized. Therefore, the search for a new measurement system for the determination of L-cysteine is essential. The kinetic analytical method has infinite possibilities for measurements of trace chemical substances. Furthermore, application to autoanalysis systems such as flow injection analysis is easy.

In this paper, a simple determination of $\mu g\,L^{-1}$ levels of L-cysteine by the autocatalytic reaction with the sodium sulfite/hydrogen peroxide system and its application to the length detection–flow analytical system will be described in detail.

2. Experimental

2.1. Reagents

Bromothymol blue (BTB), sodium sulfite and 30% hydrogen peroxide solution were purchased from Wako (Osaka, Japan). The L-cysteine and indigo carmine were provided by Kanto Chemicals (Tokyo, Japan). All other reagents were of analytical grade.

2.2. Apparatus

An F-8AT (Horiba Co., Kyoto, Japan) was used as the pH meter. Two TCI-NOX1000ω pumps (Tokyo Kasei Kogyo Co., Tokyo, Japan) were used for the double plunger-type pump. A PFA (tetrafluoroethylene-perfluoro alkylvinyl ether copolymer) tube (length: 10 m, internal diameter: 1.0 mm; GL Sciences Co., Tokyo, Japan) was used for flow tubing and mixing coil. The size of the glass stick was 7 mm in diameter and 300 mm in length. A CTE-42A (Yamato Co., Tokyo, Japan) thermostat was used. The ultraviolet–visible absorption spectra were measured using a V-570 (JASCO Co., Tokyo, Japan).

2.3. Preparation of each solution in the batch system

2.3.1. Preparation of sodium sulfite/BTB aqueous solution

Both 0.32 g sodium sulfite and 15 mg BTB were dissolved in small amounts of distilled water. The mixture was placed in a 50-mL volumetric flask, and distilled water was added to the mark.

2.3.2. Preparation of hydrogen peroxide solution

A solution of 30% hydrogen peroxide (3 mL) was placed in a 100-mL volumetric flask, and was diluted to the mark with distilled water.

2.4. Preparation of each solution in the flow system

2.4.1. Preparation of sodium sulfite/BTB aqueous solution

Both 0.64 g sodium sulfite and 15 mg BTB were dissolved in small amounts of distilled water. The mixture was placed in a 50-mL volumetric flask, and diluted to the mark with distilled water. The solution was further diluted 5 times.

2.4.2. Preparation of hydrogen peroxide solution

A solution of 30% hydrogen peroxide (750 μL) was placed in a 100-mL volumetric flask, and was diluted to the mark with distilled water.

2.5. Preparation of L-cysteine solution as a model sample

A 0.1212-g sample of L-cysteine was weighed, dissolved and diluted to 100 mL with distilled water. This L-cysteine solution was regarded as the mother liquor. The mother liquor was further diluted with distilled water before use.

The encapsulated formulation sample solution was prepared as follows. An encapsulated formulation (L-cysteine content, 500 mg) was added to 80 mL of dilute sodium hydroxide solution, and it was stirred for 10 min. The pH was adjusted to the neighborhood of 10 with 0.1 M sodium hydroxide solution, and the solution was stirred for an additional 10 min. The residual capsule contents were removed by a Teflon® membrane filter with an aperture diameter of 1 μm. The filtrate was placed in a 100-mL volumetric flask, and diluted to the mark with distilled water. Three solutions A, B, and C were prepared. Sample solution A: 5.00×10^{-7} M L-cysteine sample solution. Sample solution B: 98 mL of the L-cysteine sample solution diluted 100,000-fold was diluted to the mark of a 100-mL volumetric flask. Sample solution C: 98 mL of L-cysteine sample solution diluted 100,000-fold and 1 mL of 5.00×10^{-5} M L-cysteine solution were diluted to the mark of a 100-mL volumetric flask. If all the L-cysteine (500 mg) in the encapsulated formulations dissolved at first, the theoretical L-cysteine concentration of each sample solution becomes A: 5.00×10^{-7} M; B: 4.03×10^{-7} M; C: 9.03×10^{-7} M finally.

2.6. Determination procedure in the time detection–batch system

The sulfite/BTB aqueous solution (2 mL) was mixed with 16 mL of sample solution containing L-cysteine and added to a 50-mL beaker, and then a pH electrode was placed in the solution. Hydrogen peroxide solution (2 mL) was then added to start the reaction. The determination of the L-cysteine concentration was carried out by measuring the time from the start of the reaction to the point where the pH decreased sharply.

2.7. Determination procedure in the length detection–flow system

The sulfite/BTB aqueous solution and hydrogen peroxide solution were each pumped into the tubing at 0.2 mL min^{-1}. The L-cysteine sample solution was pumped into another passage at 0.4 mL min^{-1}. Determination of the L-cysteine concentration was carried out by measuring the length of the blue band with a ruler.

3. Results and discussion

3.1. Autocatalytic reaction with the sodium sulfite/hydrogen peroxide system

The following reaction (Eq. (1)) occurs in the sodium sulfite/hydrogen peroxide autocatalytic reaction system [7]. In this reaction, a hydrogen sulfite ion is oxidized by hydrogen peroxide, generating a proton and a sulfuric ion. This oxidative reaction is