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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

Table 1a
Searching for inorganic chemical substances which influence the reaction.

Chemical substanses	Limited concentration[a] of non-influence
Inductive time was shortened	
Ru^{3+}	1×10^{-11} M
Os^{4+}	1×10^{-11} M
Pt^{2+}	1×10^{-11} M
Ir^{3+}	1×10^{-10} M
Pd^{2+}	1×10^{-10} M
Ni^{2+}	1×10^{-10} M
Cu^{2+}	1×10^{-9} M
Fe^{3+}	1×10^{-8} M
Mn^{2+}	1×10^{-8} M
Co^{2+}	1×10^{-7} M
Fe^{2+}	1×10^{-7} M
Ta^{5+}	1×10^{-7} M
Ti^{4+}	1×10^{-7} M
Nb^{5+}	1×10^{-6} M
Zr^{4+}	1×10^{-6} M
V^{5+}	1×10^{-6} M
Ce^{3+}	1×10^{-6} M
Cr^{6+}	1×10^{-6} M
Mo^{6+}	1×10^{-5} M
CO_3^{2-}	1×10^{-8} M
H_2O_2	1×10^{-7} M
BrO_3^-	1×10^{-7} M
SO_4^{2-}	1×10^{-6} M
ClO_3^-	1×10^{-6} M
ClO_4^-	1×10^{-6} M
NO_3^-	1×10^{-6} M
PO_4^{3-}	1×10^{-6} M
K^+	1×10^{-6} M
Cl^-	1×10^{-5} M
Na^+	1×10^{-5} M
Inductive time was longed	
W^{6+}	1×10^{-5} M

[a] The concentration was evaluated by the following equation: {[Time(blank)−Time(each substances)]/Time(blank)} × 100 < ±5%.

catalyzed by the proton, and becomes an autocatalytic reaction in which the number of protons increases sharply [16].

$$HSO_3^- + H_2O_2 \xrightarrow{H^+} H^+ + SO_4^{2-} + H_2O \quad (1)$$

As a result, the pH of the solution, which is ca. 9.0 at the beginning of the reaction, rapidly decreases to ca. 5.5 after an inductive period. When BTB is added to this solution as an indicator, it is possible to visually confirm the rapid decline in pH due to the quick blue to yellow color change in the solution.

3.2. Searching for the trigger in the autocatalytic reaction with the sodium sulfite/hydrogen peroxide system

According to a previous report [7], it was possible to determine trace amounts of horse radish peroxidase, which acts as a trigger in the measurement method, using this autocatalytic reaction system. In this study, a search was carried out to find other substances that can act as triggers in this reaction. The results of the search of inorganic and organic substances are summarized in Tables 1a and 1b, respectively. The experiment was carried out by the batch system procedure. The induction time of the blank reaction which contains only distilled water was compared with that of a sample reaction which contained each substance, and the concentration at the tolerance limit (the calculation method is described in the footnote of Tables 1a and 1b) was determined by a fluctuation of less than ± 5%. In inorganic substances, the concentration at the tolerance limit was from 10^{-11} to 10^{-10} M for platinum group elements; 10^{-10} M for nickel(II), the lowest among the transition metals; and 10^{-8} M for CO_3^{2-}, the lowest among the inorganic anions and oxidants;

Table 1b
Searching for organic chemical substances which influence the reaction.

Chemical Substanses	Limited concentration[†] of non-influence
Inductive time was shortened	
cysteine	1×10^{-8} M
histidine	1×10^{-7} M
tryptophan	1×10^{-7} M
asparagine	1×10^{-7} M
aspartic acid	1×10^{-7} M
phenylalanine	1×10^{-6} M
tyrosine	1×10^{-6} M
leusine	1×10^{-6} M
alanine	1×10^{-6} M
proline	1×10^{-6} M
arginine	1×10^{-6} M
threonine	1×10^{-5} M
methionine	1×10^{-5} M
glutamine	1×10^{-5} M
glutamic acid	1×10^{-5} M
glycine	1×10^{-5} M
lysine	1×10^{-4} M
valine	1×10^{-4} M
isoleusine	1×10^{-4} M
serine	1×10^{-4} M
ascorbic acid	1×10^{-7} M
malonic acid	1×10^{-7} M
uric acid	1×10^{-7} M
EDTA[a]	1×10^{-7} M
ATP[b]	1×10^{-7} M
glucose	1×10^{-6} M
nicotinic acid	1×10^{-5} M
pantothenic acid	1×10^{-5} M
thiamin	1×10^{-5} M
pyridoxine	1×10^{-5} M
RNA[c]	1×10^{-3} g L^{-1}
SOD[d]	1×10^{-3} g L^{-1}
Inductive time was shortened	
folic acid	1×10^{-5} M
riboflavin	1×10^{-5} M
DDTC[e]	1×10^{-5} M
DNA[f]	1×10^{-3} g L^{-1}

[a] Ethylendiaminetetraacetic acid.
[b] Adenosine triphosphate.
[c] Ribonucleic acid.
[d] Superoxide dismutase.
[e] Diethyldithiocarbamate.
[f] Deoxyribonucleic acid.
[†] The concentration was evaluated by the following equation: {[Time(blank)−Time(each substances)]/Time(blank)} × 100 < ±5%.

only tungsten(VI) increased the induction time. For all other substances, the induction time in the autocatalytic reaction decreased. By the way, it is thought that the effect of the additional proton from tested trigger was canceled in the low concentration than the threshold of the pH buffer effect of the sulfite.

Among organic substances, L-cysteine most strongly influenced the reaction, and the concentration at the tolerance limit was 10^{-8} M. In addition, RNA and SOD (superoxide dismutase) were added at a concentration of 10^{-3} g L^{-1}, but the reaction was not affected. Only folic acid, riboflavin, DDTC (diethyldithiocarbamate), and DNA increased the induction time. They have acted in a high concentration. Therefore, it is thought whether they act as reductant in Eq. (1) same as sulfite or that they work as pH buffer. All other substances decreased the induction time in the autocatalytic reaction. As an attention, most of inorganic substances and some organic substances in Tables 1a and 1b have already been reported previously [18].

3.3. Time measurement-visual analysis of L-cysteine

Based on the results of the search, the determination of L-cysteine was developed. Fig. 1 shows the pH-time curve of the

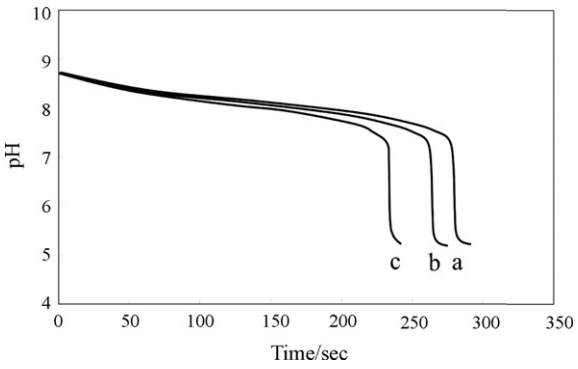

Fig. 1. pH-time curve. $[Na_2SO_3]_T = 5.0 \times 10^{-3}$ M; $[H_2O_2]_T = 2.6 \times 10^{-2}$ M; [L-cysteine]$_T$, a: 0; b: 5×10^{-8}; c: 2.5×10^{-6} M; 25 °C.

autocatalytic reaction with the sodium sulfite/hydrogen peroxide system. Here, L-cysteine acts as a trigger in this system. The induction time of this autocatalytic reaction was shortened with an increase in the initial concentration of L-cysteine.

3.4. Effect of L-cysteine

It is known that L-cysteine [RSH] reacts with dissolved oxygen in a catalyst coexistence condition to accelerate oxidation, producing cystine [RSSR] and hydrogen peroxide (Eq. (2)) [14]. In addition, indigo carmine is oxidized and degraded by hydrogen peroxide. Hence, hydrogen peroxide is detected by degradation of indigo carmine over the passage of time [19]. Therefore, the induction time is thought to decrease, causing the increase in the initial concentration of L-cysteine due to additional hydrogen peroxide generated by the reaction between L-cysteine and dissolved oxygen. The following experiments were performed to test this hypothesis. Indigo carmine was added to the L-cysteine sample solution and hydrogen peroxide was added as a catalyst to the reaction shown in Eq. (2), and the degradation reaction of indigo carmine was started. Next, to show that hydrogen peroxide is produced from the reaction between L-cysteine and dissolved oxygen, the absorbance–time curve at the maximum absorption wavelength of indigo carmine (610 nm) was performed. The result is shown in Fig. 2. The value of Δabsorbance is obtained by absorbance(L-cysteine presence)—absorbance(L-cysteine absence).

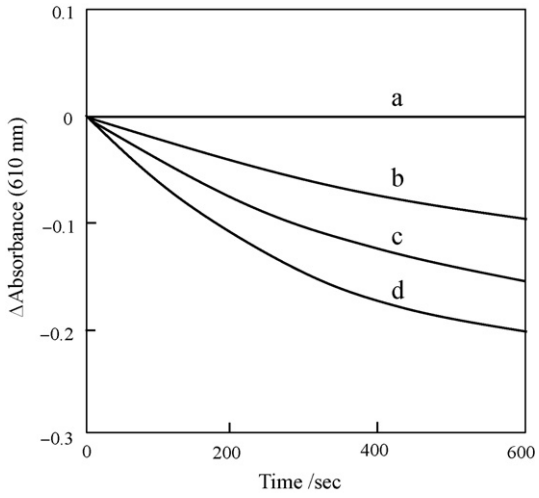

Fig. 2. ΔAbsorbance–time curve at 610 nm: degradation of indigo carmine dependence on L-cysteine concentration. [Indigo carmine]$_T = 1.7 \times 10^{-5}$ M; $[H_2O_2]_T = 13$ mM; [L-cysteine]$_T$, a: 0; b: 1.6; c: 3.3; d: 5.0 mM; [Borax]$_T = 6 \times 10^{-4}$ M; 25 °C.

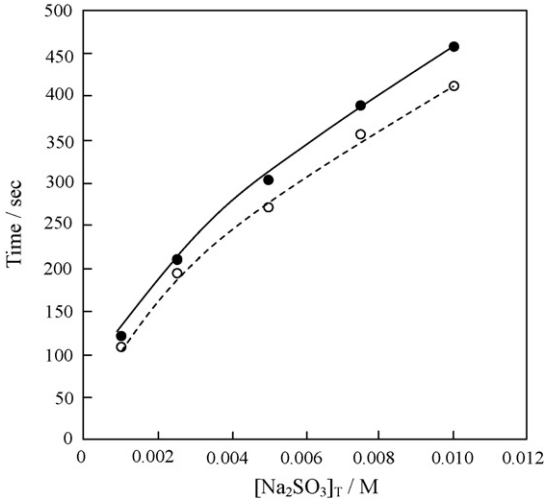

Fig. 3. Effect of sodium sulfite. Solid line curve (—): blank; dashed line curve (—): [L-cysteine]$_T = 10^{-6}$ M. $[H_2O_2]_T = 66$ mM; 25 °C.

When the L-cysteine concentration increased, degradation of indigo carmine (610 nm) was accelerated. This acceleration depends on an increase in the hydrogen peroxide concentration by the reaction between L-cysteine and dissolved oxygen (Eq. (2)). From these experimental results, the induction time decreases, causing the increase in L-cysteine due to increased hydrogen peroxide by the reaction in Eq. (2), and the increased acceleration of the reaction in Eq. (1). This result is consistent with the low tolerance limit of hydrogen peroxide concentration in Table 1a (10^{-7} M).

$$2RSH + O_2 \rightarrow RSSR + H_2O_2 \quad (2)$$

3.5. Effect of sodium sulfite

The effect of the sodium sulfite concentration was examined over the concentration range of 1.9–8.2 mM (Fig. 3). The induction time of the autocatalytic reaction increased with increasing sodium sulfite concentration as the reaction time increased with increasing concentration of sodium sulfite, which acts as a reductant in Eq. (1). The concentration with the largest ΔT% in Eq. (3) (5.0 mM) was chosen as the measurement condition. T(blank) is the induction time in the blank reaction, and T(sample) is induction time in the reaction with 10^{-6} M of total L-cysteine.

$$\Delta T\% = \frac{T(\text{blank}) - T(\text{sample})}{T(\text{blank})} \times 100 \quad (3)$$

3.6. Effect of hydrogen peroxide

The effect of hydrogen peroxide concentration was examined over the concentration range of 17.6–35.2 mM (Fig. 4). The induction time decreased with increasing hydrogen peroxide concentration as the reaction in Eq. (1) is accelerated with increasing concentration of hydrogen peroxide, which acts as an oxidant. The concentration chosen for this measurement condition was 26.4 mM because it had the largest ΔT%.

3.7. Effect of temperature

The effect of temperature was examined over the range of 15–35 °C (Fig. 5). The induction time decreased at higher temperatures as the reaction rate increased. The temperature chosen as the measurement condition was 25 °C because it had the largest ΔT%.

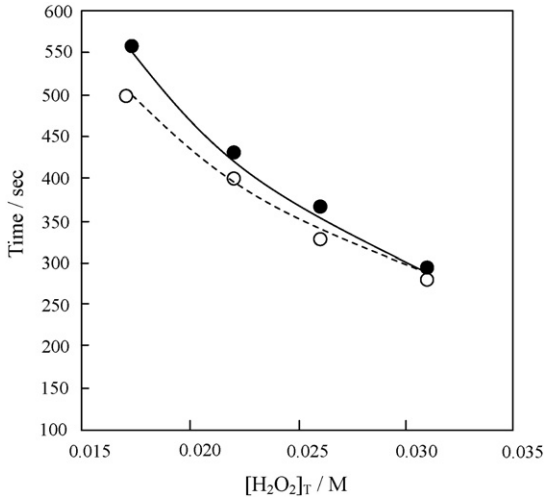

Fig. 4. Effect of hydrogen peroxide. Solid line curve (—): blank; dashed line curve (—): [L-cysteine]$_T$ = 10^{-6} M. [Na_2SO_3]$_T$ = 20 mM; 25 °C.

3.8. The calibration curve for the batch system

The calibration curve for the batch system is shown in Fig. 6. The relationship of the concentration of L-cysteine (x, M) *vs*. the induction time (y, sec) becomes a negative logarithmic proportion. This result agrees well with the relationship of time *vs.* the concentration of the trigger in the theoretical equation for the autocatalytic reaction in a previous report [9]. The equation for the line of best fit is $y = -5.44(+0.30)\ln(x) + 168(+4)$, and the correlation coefficient (r) is $r = 0.9909$. The determination range for L-cysteine was 5.0×10^{-8}–2.5×10^{-6} M, the value of the detection limit (3σ) was 1.8×10^{-8} M (1.94 μg L^{-1}), and the relative standard deviation (RSD) was 2.41% at an L-cysteine concentration of 5×10^{-7} M ($n = 5$). As a result, it was possible to determine visually the concentration of L-cysteine using the autocatalytic reaction with the sodium sulfite/hydrogen peroxide system.

3.9. Interference of coexistent substances

The interference of coexistent materials at the total L-cysteine concentration of 10^{-6} M is shown in Table 2. The tolerance limit of concentration was determined as ΔT% of Eq. (3) becomes within ± 5%. In this case, T(blank) is the induction time for the reaction with a total concentration of 10^{-6} M L-cysteine, and T(sample) is the induction time with the coexistent substance and total concentration of 10^{-6} M L-cysteine. Little interference with the reaction was observed with sodium(I), potassium(I), magnesium(II), calcium(II), chloride, glucose, pantothenate and formate. Moreover, nickel(II), iron(III), nicotinamide, histidine, tryptophan, leucine and lysine were tolerated up to 100-fold. Ascorbate was allowed up to 10-fold. Cystine was allowed up to 5-fold.

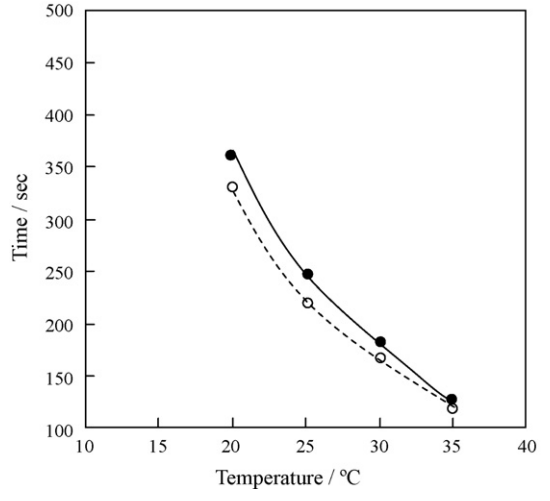

Fig. 5. Effect of temperature. Solid line curve (—): blank; dashed line curve (—): [L-cysteine]$_T$ = 10^{-6} M. [Na_2SO_3]$_T$ = 8 mM; [H_2O_2]$_T$ = 66 mM.

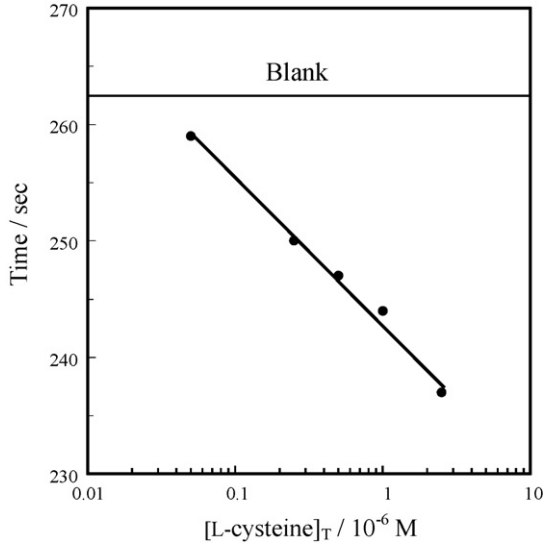

Fig. 6. Calibration curve for batch system. [Na_2SO_3]$_T$ = 5 mM; [H_2O_2]$_T$ = 26 mM; 25 °C.

3.10. Application to the length detection–flow injection analysis

An overview of the flow analytical system used in this study is shown in Fig. 7. The sulfite/BTB solution, hydrogen peroxide solution and L-cysteine sample solution were each pumped through the tubing. The blue solution mixed in the tubing was pumped into the 25 cm mixing coil wound around the glass stick, and the solution turned yellow at a specific length due to the rapid decline in pH. The mode of separation of the blue and yellow bands could be visually determined in the mixing coil on the glass stick. The principle of separation of the blue and yellow solutions in the mixing coil can be explained by Eq. (4).

$$\text{Length(cm)} = \text{Rate(cm/s)} \times \text{Time(s)} \quad (4)$$

The reaction occurred rapidly after a specific time in the autocatalytic reaction system. Therefore, the characteristics of the reaction solution, which flowed at a constant rate, are clearly different before and after the rapid reaction, as time is proportional to length in Eq. (4). In this study, the pH region of BTB for the color change was 6.0–7.6, and the color of the reaction solution changed from blue (pH about 9) to yellow (pH about 5.5) as the reaction progressed.

Table 2
Effect of foreign substances.

Coexistances	Tolerance limit (±5%)
Na^+, K^+, Cl^-, Ca^{2+}, formate Mg^{2+}, glucose, pantothenate	>×1000
Ni^+, Fe^{3+}, nicotinamide, histidine, Tryptophan, leucine, lysine,	×100
Ascorbate	×10
Cystine	×5

[L-cysteine]$_T$: 1×10^{-6} M.

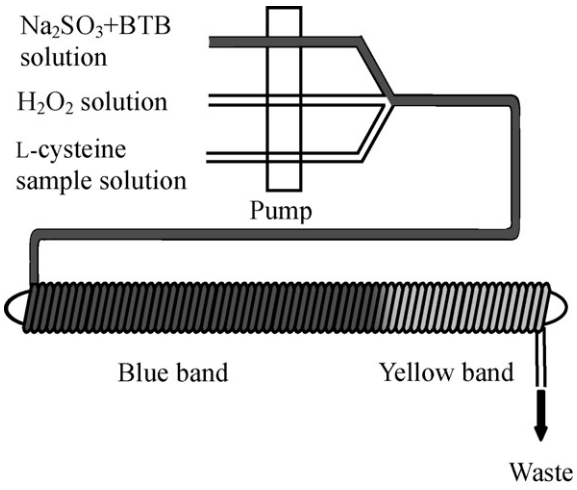

Fig. 7. Color band range-detection analytical flow system.

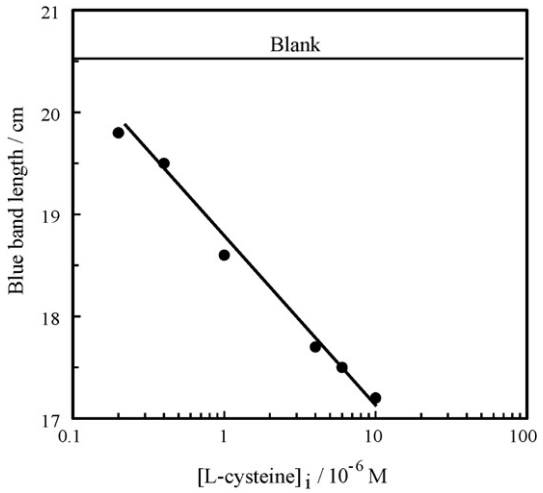

Fig. 9. Calibration curve for color band range-detection analytical flow system. $[Na_2SO_3]_i = 8$ mM; $[H_2O_2]_i = 66$ mM; 25 °C.

The BTB concentration was optimized to 9.61×10^{-5} M as initial concentration that blue band length could identify clearly. The variation in the boundary position between the blue and yellow bands was about ±0.1 cm in 6 h of continuous manipulation. Moreover, as compared with the observation of the color change in conventional batch system which was by human eyes and stopwatch, or time course of absorbance [2–4], the advantage of the proposed flow system is that observation is limited to the moment of the rapid color change of the solution.

3.11. Variation in color band length based on the change in L-cysteine concentration

The change in the blue band length when the L-cysteine concentration progressively changed (0, 10^{-6}, 10^{-5} M) is shown in Fig. 8. The blue band length decreased when the L-cysteine concentration increased and increased when the L-cysteine concentration decreased. This flow system is not only able to simply determine the concentration of a sample but can also be used to follow the change in the concentration of the sample in real-time. Fifteen samples per hour were determined in a 2.5×10^{-6} M L-cysteine sample solution on this occasion. The L-cysteine plays a role as a trigger in the length detection–flow analytical system.

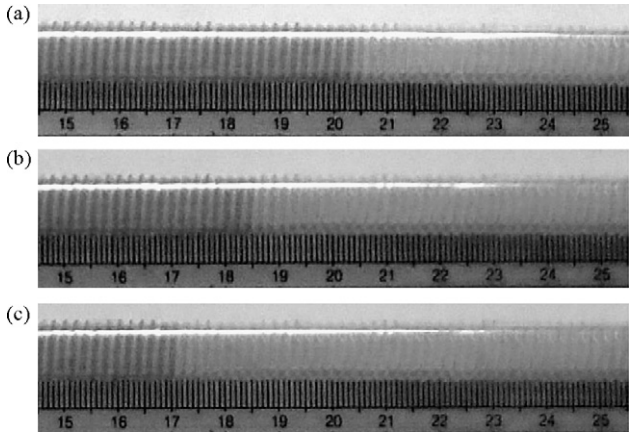

Fig. 8. Relationship between blue band length and L-cysteine concentration. $[Na_2SO_3]_i = 8$ mM; $[BTB]_i = 9.61 \times 10^{-5}$ M; flow rate = 0.1 mL min^{-1}. $[H_2O_2]_i = 66$ mM; flow rate = 0.1 mL min^{-1}. $[L\text{-cysteine}]_i$, a: 0; b: 10^{-6} M; c: 10^{-5} M; flow rate = 0.2 mL min^{-1}; 25 °C. $[]_i$: initial concentration.

3.12. Effect of sodium sulfite

The effect of sodium sulfite was examined over a concentration range of 4.0–20 mM. The blue band length increased with increasing sodium sulfite concentration. The reason is similar to the case of the batch system. The concentration with the largest ΔL% in Eq. (5) (8.0 mM) was chosen as the measurement condition. L(blank) is the blue band length in the blank reaction, and L(sample) is that from 10^{-6} M of initial L-cysteine concentration.

$$\Delta L\% = \frac{L(blank) - L(sample)}{L(blank)} \times 100 \quad (5)$$

3.13. Effect of hydrogen peroxide

The effect of hydrogen peroxide was examined over a concentration range of 0.066–0.132 M. The blue band length decreased with increasing hydrogen peroxide concentration. The reason is similar to the case in the batch system. The concentration chosen as the measurement condition was 66 mM because it had the largest ΔL%.

3.14. Effect of temperature

The effect of temperature was examined over a range of 16 to 31 °C. The blue band length decreased with increasing temperature. The reason is again similar to the case in the batch system. The temperature of the measurement condition was 25 °C because it had the largest ΔL%.

3.15. Calibration curve for the length detection–flow analytical system

The calibration curve for the length detection–flow system is shown in Fig. 9. The relationship of the concentration of L-cysteine (x', M) *vs.* the blue band length (y', cm) is a negative logarithmic proportion. The equation for the line of best fit is $y' = -0.69(+0.03) \ln(x') + 9.2(+0.4)$, and the correlation coefficient is $r = 0.9922$. The determination range for L-cysteine was 2.0×10^{-7}–1.0×10^{-5} M, the value of the detection limit (3σ) was 1.4×10^{-7} M (17.0 μg L^{-1}) and the RSD was 0.91% at an initial L-cysteine concentration of 10^{-6} M ($n = 5$).

Table 3
Comparison with other kinetic analytical methods.

Methods	Determination range (M)	Detection limit (3σ) (M)	Reference
Proposed method (batch method)	5.0×10^{-8}–2.5×10^{-6}	1.8×10^{-8}	–
Proposed method (flow method)	2.0×10^{-7}–1.0×10^{-5}	1.4×10^{-7}	–
Catalytic reaction method	1.0×10^{-6}–6.0×10^{-5}	5.0×10^{-7}	[13]
Catalytic reaction method	2.0×10^{-7}–8.0×10^{-6}	1.2×10^{-7}	[15]
Ligand exchange reaction method	1.0×10^{-7}–5.0×10^{-7}	1.0×10^{-7}	[16]
Ligand exchange reaction method	2.0×10^{-7}–2.0×10^{-6}	2.0×10^{-7}	[17]

3.16. Application to a sample

3.16.1. Determination of L-cysteine in commercial encapsulated formulations

The concentration of the L-cysteine sample solution prepared from a commercial encapsulated formulation was determined. The L-cysteine concentration found in sample solution A was 4.93×10^{-7} M (theoretical concentration, 5.00×10^{-7} M). Recovery was 98.6(+8.9)% and RSD ($n = 5$) was 0.9%. The L-cysteine concentration found in sample solution B was 3.86×10^{-7} M (4.03×10^{-7} M). Recovery was 95.8 (+3.0)% and RSD ($n = 5$) was 4.6%. The L-cysteine concentration found in sample solution C was 8.73×10^{-7} M (9.03×10^{-7} M). Recovery was 96.7 (+0.8)% and RSD ($n = 5$) was 2.5%. The theoretical L-cysteine concentration and detected concentration in each sample solution showed good agreement.

3.17. Comparison of other kinetic analytical methods

The comparison of L-cysteine determination using other kinetic analytical methods is shown in Table 3. The batch method achieved the lowest detection limit in L-cysteine determination compared to other kinetic analytical methods such as the catalytic reaction method [13,15] and ligand exchange reaction method [16,17].

4. Conclusion

In this paper, an autocatalytic reaction was applied to the length detection–flow analytical system. The developed system was accomplished with low cost and high performance as a special detector is not required. In the near future, this flow analytical system may not only be applied to determine L-cysteine concentration in encapsulated formulations but also widely applied to determine L-cysteine concentration in cosmetic and food samples.

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