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Short communication

Hydrogen peroxide–sodium hydrosulfite chemiluminescence system combined with high-performance liquid chromatography for determination of 1-hydroxypyrene in airborne particulates

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ABSTRACT

In this research, a highly sensitive chemiluminescence method based on a sodium hydrosulfite ($NaHSO_3$)–hydrogen peroxide (H_2O_2) reaction for the determination of 1-hydroxypyrene (1-OHP) was developed. The response of this system was linear in the range from 0.5 to 50 pmol ($R^2 = 0.9983$). The limit of detection for 1-OHP was 100 fmol ($S/N = 3$). 1-OHP in airborne particulates was well separated from interfering compounds using an ODS column with 75% methanol as the mobile phase in isocratic mode. The proposed method was successfully applied to determine the 1-OHP in airborne particulates collected in Kanazawa, Japan. The average concentration of 1-OHP in the atmosphere was 2.0 pg/m^3 (9.2 fmol/m^3).

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants [1,2] that are generated from incomplete combustion of organic matters such as fuel oil and coal [3,4]. In the atmosphere, PAHs react with the co-pollutants, such as ozone and nitrogen oxides (NO_x) to produce substituted PAHs [5,6]. One type of substituted PAHs is hydroxylated PAHs (OH-PAHs). They may be generated from photochemical reactions of PAHs or combustion of coal [7–9]. OH-PAHs have endocrine disrupting activities such as estrogenic activities or antiestrogenic activities [10,11]. Atmospheric concentrations of OH-PAHs were reported to be in the pg/m^3 range, which are 1–3 orders of magnitude lower than that of PAHs [8,9]. Therefore, it is necessary to establish a highly sensitive and reliable method for determination of OH-PAHs in atmosphere [12].

Gas chromatography/mass spectrometry (GC–MS) is one of the most commonly used methods in detecting OH-PAHs in the atmosphere [7–9,13,14]. A disadvantage of GC–MS is that it requires a derivatization step before analysis, which is time-consuming, and increases the risk of contamination or loss of OH-PAHs [15]. Another method for determining OH-PAHs is high-performance liquid chromatography (HPLC) with electrochemical detection [16], but the sensitivity was too low to detect OH-PAHs in the atmosphere. Kishikawa et al. determined six kinds of OH-PAHs by HPLC with a fluorescence detector using the internal standard method [15]. However, interference peaks were found in the chromatogram, which may affect the accuracy of the method.

In this study, we developed a highly sensitive and simple chemiluminescence (CL) method to determine 1-OHP in the airborne particulates for the first time. Airborne particulate sample might contain many interfering compounds. Therefore, an ODS separation column was connected to the system to separate 1-hydroxypyrene from interfering compounds. The method avoids the time-consuming derivatization step required by GC–MS method [14]. Moreover, the method was simple and convenient. The possible mechanism of this CL reaction was proposed. We used the method to determine 1-OHP in airborne particulates in Kanazawa city in Japan.

2. Experimental

2.1. Reagents and chemicals

Sodium hydrosulfite ($NaHSO_3$) and hydrogen peroxide (H_2O_2) were purchased from Kanto Chemical Co. Inc. (Tokyo, Japan).

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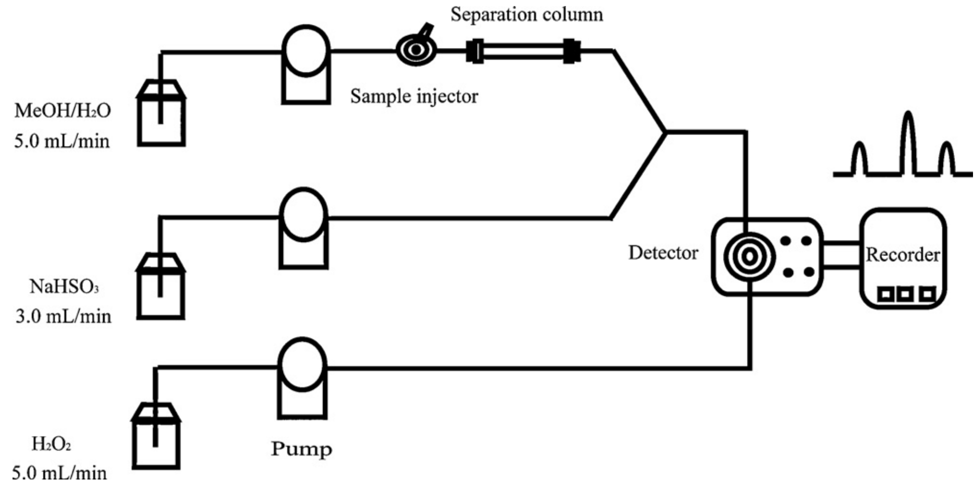

Fig. 1. Schematic diagram of the $NaHSO_3$–H_2O_2 CL system to detect 1-OHP.

$NaHSO_3$ solution was freshly prepared by dissolving appropriate amounts of $NaHSO_3$ powder in ultrapure water. H_2O_2 solution was freshly prepared by volumetric dilution of commercial 30% H_2O_2 solution. 1-OHP was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Solution of 1-OHP was prepared by dissolving powder with methanol. Standard solution of 1-OHP was diluted with 75% methanol. All the organic solvents (methanol, hexane, dichloromethane, diethyl ether, benzene, and ethanol) were HPLC grade and from Wako Pure Chemical Industries, Ltd. Ultrapure water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. *Sample collection*

Airborne particulates were collected on the roof of a seven-storey building of Kanazawa University (Kakuma-machi) in Kanazawa, Japan. Sampling was conducted in August, 2010 using a high-volume air sampler (Model 120, Kimoto Electrics, Osaka, Japan) with a 2500 QAT-UP quartz fiber filter (8″ × 10″, Pallflex Products, Putnam, CT, USA) for 24 h at a flow rate of 1000 L/min (total volume 1434.9 m^3). The filters with airborne particulates were stored in a freezer at −20 °C until analysis.

2.3. *Sample preparation*

The whole sample filter was cut into small pieces and extracted ultrasonically with 100 mL benzene/ethanol mixture solvent (1:1, v/v) for 15 min twice. Both extracts were combined and filtered with a cellulose acetate filter (No. 5C, Advantec MFS, Dublin, CA, USA) to remove solid residue. Then the solution was evaporated to near dryness at 30 °C in a rotary evaporator under reduced pressure. The residues were reconstituted in 10 mL hexane (under ultrasonication for 2 min), and filtered with a 0.45 μm membrane filter.

The residues were loaded onto a Sep-Pak®Plus Silica cartridge (Waters, Milford, MA, USA) which was preconditioned with 10 mL hexane. The target compound was eluted by 10 mL hexane/ethyl acetate (7/1, v/v). The eluate was gently evaporated to near dryness under the gentle nitrogen gas stream, and then re-dissolved in 1.0 mL methanol/water mixture solvent (75/25, v/v). An aliquot of 100 μL of the sample solution was injected into the HPLC system.

2.4. *HPLC-CL system*

The HPLC system with a CL detector is shown in Fig. 1. This HPLC-CL system consists of three HPLC pumps (LC-10A, LC-10A, and LC-6000, Shimadzu, Tokyo, Japan), a sample injector with a 100 μL loop, a separation column (Cosmosil 5C18-AR-II; 10.0 mm i.d. 250 mm, Nacalai Tesque), and a column oven (Sugai, U-620). The temperature of the column oven was set at 30 °C. The CL detector is S-3400 (Soma Optics, Tokyo, Japan). An aliquot of 100 μL of the standard mixture or the airborne particulate sample was injected into the HPLC system, and detected directly by the CL detector.

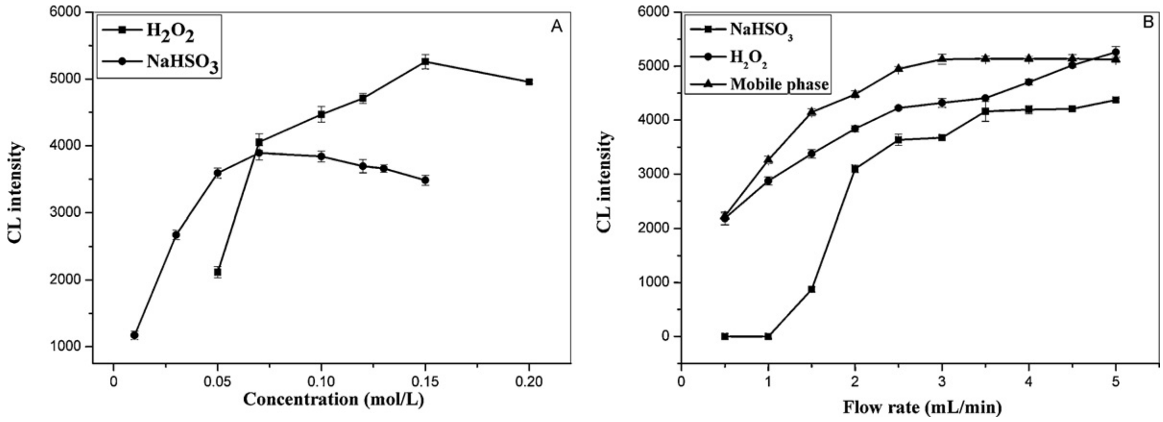

Fig. 2. Effects of concentrations and flow rates on CL intensity: (A) concentrations of $NaHSO_3$ and H_2O_2. Flow rates of $NaHSO_3$, H_2O_2 and mobile phase were 5.0, 3.0, and 5.0 mL/min, respectively; (B) flow rates of $NaHSO_3$, H_2O_2 and mobile phase. Concentrations of $NaHSO_3$ and H_2O_2 were 0.1 mol/L and 0.15 mol/L.

Table 1
Precision of the method for determining 1-OHP in airborne particulates and recovery of 1-OHP in airborne particulates.

	Intra-day ($n=3$)			Inter-day ($n=3$)		
Added (nmol/L)	0	50	100	0	50	100
Found (nmol/L)	7.0	53	110	7.0	53	110
Recovery (%)[a]	–	92%	103%	–	92%	103%
RSD (%)	9.5%	8.2%	2.4%	15.5%	11.5%	4.7%

[a] Expressed as [(found concentration – non-spiked concentration)/added concentration] × 100.

Mobile phase was methanol/water mixture (75/25, v/v) in isocratic mode at a flow rate of 5.0 mL/min.

3. Results and discussion

3.1. Optimization of HPLC-CL system

In order to obtain a high sensitivity for determination of 1-OHP, factors influencing CL intensity, such as concentration and flow rate of CL reagents were investigated independently.

We investigated the concentration of H_2O_2 in the range from 0.05 to 0.2 mol/L. CL intensity increased with H_2O_2 concentration up to 0.15 mol/L and then leveled off (Fig. 2A). In addition, too high concentration of H_2O_2 caused gas bubble to form in the tube, which would affect the stability of the CL system. Therefore, a H_2O_2 concentration of 0.15 mol/L was used for the following experiments.

The effect of $NaHSO_3$ concentration on CL intensity was studied in the range 0.01–0.15 mol/L. The CL intensity increased linearly with the increasing of $NaHSO_3$ concentration, but at concentrations of $NaHSO_3$ higher than 0.10 mol/L, the CL intensity began to decrease (Fig. 2A). Therefore, the concentration of $NaHSO_3$ solution was selected to be 0.1 mol/L for further study.

The flow rate of the CL reagent solution strongly affected sensitivity of the CL method. We investigated the flow rates of $NaHSO_3$, H_2O_2, and mobile phase from 0.5 to 5.0 mL/min. The optimized flow rates of $NaHSO_3$ and H_2O_2 were 5.0 mL/min and 3.0 mL/min (Fig. 2B). When the flow rate of the mobile phase was higher than 3.0 mL/min, the CL intensity remained constant (Fig. 2B). But the separation efficiency of the column was the highest at a flow rate of 5.0 mL/min. Therefore, the flow rate of the mobile phase was set at 5.0 mL/min.

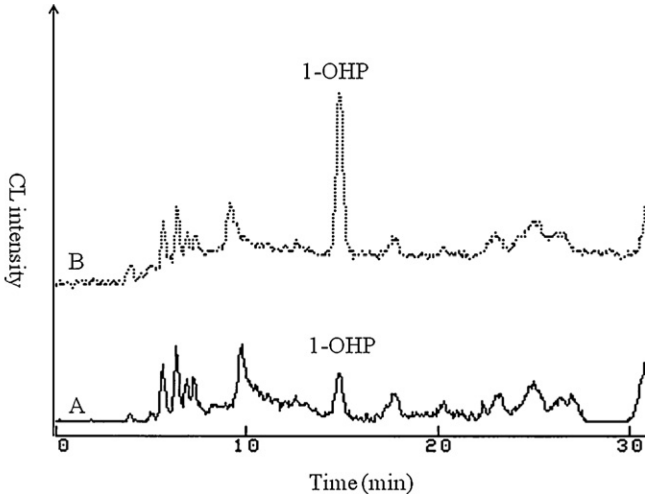

Fig. 3. Chromatograms of 1-OHP (A) in airborne particulates and (B) airborne particulates spiked with 1.0 pmol 1-OHP. Mobile phase was 75% methanol at 5.0 mL/min; concentrations of $NaHSO_3$ and H_2O_2 were 0.10 mol/L and 0.15 mol/L; their flow rates were 3.0 mL/min and 5.0 mL/min respectively.

Methanol and acetonitrile are organic solvents that are often used as the mobile phase in HPLC analyses. The effect of methanol and acetonitrile on the CL intensity was compared. Methanol gave higher sensitivity for 1-OHP and better separation. Therefore, methanol was selected for further study.

3.2. Possible mechanism of CL system

In the $NaHSO_3$–H_2O_2 CL reaction, HSO_3^- was oxidized by H_2O_2 to produce sulfite radical ($^{\bullet}SO_3^-$) [17], which dimerized to dithionate ion ($S_2O_6^{2-}$) [18,19].

$$HSO_3^- + H_2O_2 \rightarrow {}^{\bullet}OH + SO_3^{\bullet -} + H_2O \tag{1}$$

The OH radical, a strong oxidant, rapidly reacted with HSO_3^- to give $^{\bullet}SO_3^-$ radical [20,21].

$$^{\bullet}OH + HSO_3^- \rightarrow SO_3^{\bullet -} + H_2O \tag{2}$$

$$k = 1.5 \times 10^9\ M^{-1}\ s^{-1}$$

$$^{\bullet}SO_3^- + {}^{\bullet}SO_3^- \rightarrow S_2O_6^{2-} \tag{3}$$

Excited sulfur dioxide (SO_2^*) was generated by the decomposition of $S_2O_6^{2-}$ [18,19]. The emission wavelength of SO_2^* is 260–480 nm [22,23], which overlaps the excitation wavelength of 1-OHP [15]. Therefore, when 1-OHP is present in the CL system, SO_2^* will transfer its energy to 1-OHP. This greatly increased the CL emission. CL intensity was highly correlated with 1-OHP concentration. Based on this mechanism, 1-OHP could be detected sensitively through the following reactions.

$$S_2O_6^{2-} \rightarrow SO_4^{2-} + SO_2* \tag{4}$$

$$SO_2* \rightarrow SO_2 + h\nu \tag{5}$$

$$SO_2* + \text{1-OHP} \rightarrow SO_2 + \text{1-OHP}* \tag{6}$$

$$\text{1-OHP}* \rightarrow \text{1-OHP} + h\nu \tag{7}$$

3.3. Calibration curve, detection limit and reproducibility

Under the optimized conditions, standard 1-OHP was successfully determined by the $NaHSO_3$–H_2O_2 CL system without a separation column. The calibration curve was obtained with a good linear relationship between the concentrations of 1-OHP and peak heights. The linear range was from 0.005 to 10 pmol/injection (seven calibration points; $R^2 = 0.9994$). The slope and intercept of the regression equation of calibration curve (mean ± error) were $5.77 \times 10^{11} \pm 5.63 \times 10^9$ and 635 ± 220. The detection limit ($S/N = 3$) was 1.0 fmol/injection. This CL detection method for detecting 1-OHP showed higher sensitivity than the previous methods reported [15,24]. However, 1-OHP cannot be detected exactly only with the proposed CL method due to the interference compounds in airborne particulates. An ODS column was incorporated to separate 1-OHP and the interference compounds in airborne particulates. A good linear relationship was observed between the peak height and concentrations of 1-OHP in the range from 0.5 to 50 pmol/injection (ten calibration points) with 100 μL per injection

($R^2 = 0.9983$). The slope and intercept of the regression equation (mean ± error) were $2.92 \times 10^{11} \pm 8.94 \times 10^9$ and 674 ± 65.7. The detection limit ($S/N = 3$) for 1-OHP detected by the proposed method was 100 fmol/injection.

The precision of the determination of 1-OHP and the recovery of 1-OHP were investigated by spiking standard 1-OHP solutions into the airborne particulates. The results are shown in Table 1. The relative standard deviation (RSD) of intra-day and inter-day ranged from 2.4% to 11.5%. The recoveries for the spiked airborne particulates of intra-day and inter-day were in the range 93–103%.

3.4. Determination of 1-OHP in airborne particulates

Pyrene is one of major PAHs in the atmosphere and exists in both gas and particulate phase. The vapor pressure of 1-OHP is much lower than that of pyrene because of the hydroxy group; 1-OHP may mainly exist in particulate phase. Therefore, the proposed method was applied to determine 1-OHP in airborne particulates collected in Kanazawa, Japan in August 2010. The chromatogram of real airborne particulates showed the peak at the retention time of 1-OHP and the peak height increased by the addition of 1-OHP standard solution (Fig. 3(A) and (B)). From the chromatogram, the average concentration ($n = 3$) of 1-OHP in airborne particulates in Kanazawa city was 2.0 ± 0.2 pg/m^3 (9.2 ± 0.9 fmol/m^3). Although only a few reports have examined the concentrations of 1-OHP in airborne particulates, our results were in the range of previous values. For example, the concentration of 1-OHP in Nagasaki, Japan was 5.97–63.25 pg/m^3 [15].

4. Conclusions

In this research, a highly sensitive HPLC-CL method based on $NaHSO_3$–H_2O_2 coupled with an ODS separation column to detect 1-OHP in airborne particulates was proposed. This is the first time that the $NaHSO_3$–H_2O_2 CL system reaction was combined with an HPLC system to detect air pollutants. The proposed method was successfully applied to detect trace levels of atmospheric 1-OHP in Kanazawa city in summer.

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