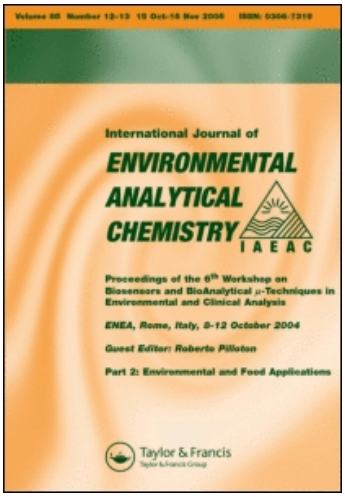

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Determination of picomole amounts of formaldehyde in air and bio-fluids based on its enhanced myoglobin-luminol chemiluminescence reaction

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Determination of picomole amounts of formaldehyde in air and bio-fluids based on its enhanced myoglobin-luminol chemiluminescence reaction

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A rapid and sensitive chemiluminescence determination of formaldehyde with the flow injection technique was presented. It was found that the specific binding of myoglobin with formaldehyde could accelerate the electron transfer between myoglobin and luminol, thus producing quicker and more efficient chemiluminescence at 425 nm than with the myoglobin–luminol reaction. The increased chemiluminescence intensity was linear with the formaldehyde concentration in the range of 1–3000 pmol L^{-1}, and the limit of detection was 0.3 pmol L^{-1} with a relative standard deviation of less than 5.0% (3σ). At a flow rate of 2.0 mL min^{-1}, including sampling and washing, the assay could be accomplished in 0.5 min. It was also found that there was a linear relationship between the vapour pressure of formaldehyde and temperatures of 291.5–315.0 K. The proposed method has been applied successfully for the determination of formaldehyde in air and human serum.

Keywords: Formaldehyde; Myoglobin; Chemiluminescence; Luminol; Serum

1. Introduction

Formaldehyde, a colourless, flammable gas with a strong, pungent odour, is one of the most significant industrial hazards and air pollutants. Formaldehyde is employed as chemical intermediate in the industrial synthesis of a large number of organic compounds [1–3]. It is also formed in the atmosphere by photochemical reactions involving hydrocarbons with a substituent methyl group. Formaldehyde has been reported to irritate the mucous membrane and cause allergic reactions of the respiratory tract, eyes, and skin at levels above 0.1 ppm in air [4, 5]. Due to the widespread use of formaldehyde and reported adverse health effects attributed to this chemical, its monitoring and control of exposure have attracted special attention. There are several methods for the determination of formaldehyde including chromatography [6], spectrofluorimetry [7], electrochemistry [8], biosensing [9], and chemiluminescence (CL) [10], at the lowest detection limit of 2.0×10^{-10} mol L^{-1} of all the above methods.

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It was previously reported that myoglobin (Mb), which contains a single iron protoporphyrin or haem moiety in the ferric state, namely Mb(Fe(III)), could react with luminol yielding CL emission [11]. Also, in this work, it was first found that the specific binding of Mb(Fe(III)) with formaldehyde could accelerate electron transfer between Mb and luminol, producing a stronger CL signal at 425 nm. Based on the linear relationship between the increased CL intensity and formaldehyde concentration, a simple, sensitive, and rapid method was established for the determination of formaldehyde in air samples and human serum, which is promising for the miniature, simple, and automatic procedure for monitoring formaldehyde. Compared with other methods, the novel procedure shows notable advantages, offering a wide linear range from 1.0×10^{-12} to 3.0×10^{-9} mol L^{-1}, a low detection limit down to 0.3×10^{-12} mol L^{-1}, and a high sample efficiency up to 120 times per hour.

2. Experimental

2.1 *Reagents*

All reagents were of analytical pure grade. Water purified in a milli-Q system (Millipore, Bedford, MA) was used throughout. Horse-heart Mb (Sigma) was used as received without further purification. A 2.5×10^{-2} mol L^{-1} solution of luminol (Fluka, Biochemika, Switzerland) was prepared by dissolving 4.4 g of luminol in 1.0 L of 0.2 mol L^{-1} of sodium hydroxide. A 1.0 mmol L^{-1} stock standard solution of formaldehyde was prepared from formaldehyde (37%, Xi'an Chemical Reagent Plant) and was stored at 4°C. A series of testing standards solutions from 1.0 to 3.0 nmol L^{-1} were prepared daily by appropriate dilution of the stock solution of formaldehyde.

2.2 *Apparatus and procedures*

The flow-injection (FI) system used in this work is shown schematically in figure 1. A six-way injection valve and PTFE tubing (2 mm i.d.) were used in the flow system. The carrier (pure water) and the solutions (luminol, Mb, and formaldehyde) were propelled at a flow rate of 2.0 mL min^{-1} on each flow line. Two hundred microlitres

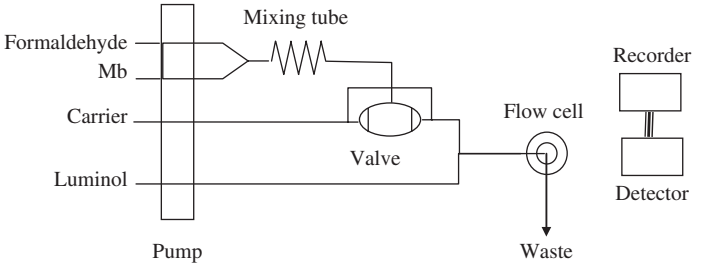

Figure 1. Schematic diagram of the present FI–CL system for formaldehyde. Luminol: 5.0×10^{-5} mol L^{-1} in 0.05 mol L^{-1} sodium hydroxide; Mb: 5.0×10^{-8} mol L^{-1}; flow rate: 2.0 mL min^{-1}; HV: −700 V.

of mixing solution of formaldehyde and Mb was injected into the carrier stream, which was then mixed with the luminol stream. The distance between the CL flow cell and point where luminol was mixed with myoglobin and formaldehyde was 5.0 cm. The mixed solution was delivered to the CL flow cell, a flat spiral consisting of glass tubing (2.0 mm i.d., 15.0 cm length) placed adjacent to the photomultiplier tube (PMT), and the CL signal produced was therefore detected with PMT (Hamamatsu, model IP28) and a luminometer (Xi'an Remax Electronic Instrument Co. Ltd, model GD-1). The concentration of formaldehyde could be quantified by the increment of CL intensity, $\Delta I = I_s - I_o$, where I_s and I_o were the CL signals in the presence and absence of formaldehyde, respectively.

A schematic diagram of the instrument used as absorption system is shown in figure 2. The air stream containing house air was artificially contaminated by purging a small bottle containing formaldehyde. The air stream flowed at a rate of 300 mL min^{-1} for 5–10 min. The concentration of formaldehyde in the air stream can be determined by the temperature of the water bath around the bottle. The absorbent solution was pure H_2O.

The formaldehyde-vapour generation and absorption system is shown in figure 3. The absorbed solution was determined by the present method, and the stream of air was vented by a vacuum pump. The absorption system was full of water to absorb the formaldehyde, the air containing formaldehyde was flowed into the absorption system by a valve, and it can be determined by this FI-CL.

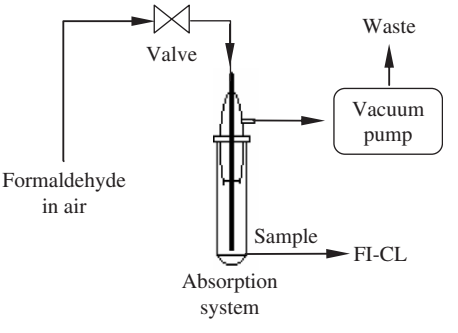

Figure 2. Manifold for formaldehyde absorption apparatus.

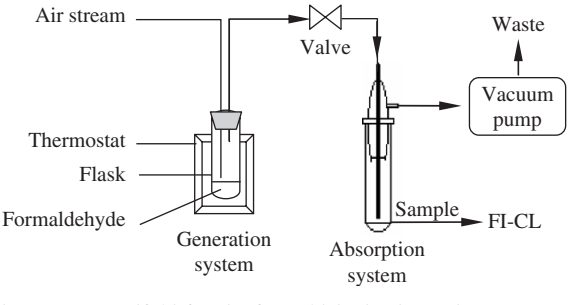

Figure 3. Manifold for the formaldehyde absorption apparatus.

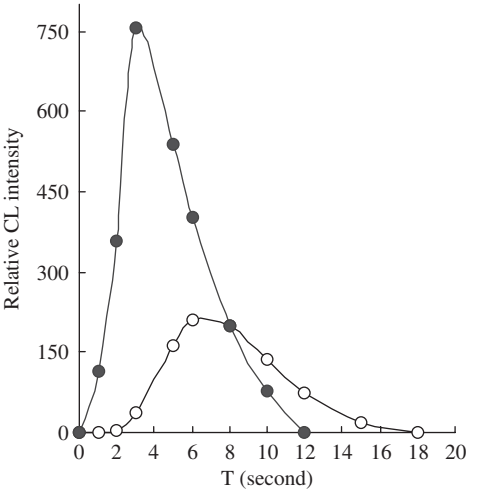

Figure 4. Kinetic CL intensity–time profile in static system. (○): Mb–luminol; (●): Mb–formaldehyde–luminol. Luminol: 5×10^{-5} mol L^{-1}; Mb: 5.0×10^{-8} mol L^{-1}; sodium hydroxide: 0.05 mol L^{-1}; formaldehyde: 1.0×10^{-11} mol L^{-1}.

3. Results and discussion

3.1 *CL intensity–time profile*

The CL intensity–time curves in different reaction systems were tested with the static method. As shown in figure 4, a CL signal was produced by the reaction of luminol and Mb, which reached a maximum at 6 s after the mixing of reactants and became extinguished within 18 s thereafter. In the presence of 1.0×10^{-11} mol L^{-1} of formaldehyde, the time period of CL process was shortened to 12 s, and the CL signal reaches its peak in only 4 s, giving a maximum intensity 2.0-fold higher than that in the absence of formaldehyde. The CL intensity–time profile with the flow system was also tested. As shown in figure 5, the CL intensity varied with the concentration of formaldehyde.

3.2 *Effect of luminol, Mb, and sodium hydroxide concentration*

The effect of luminol concentration was investigated over the range of 5.0×10^{-6} to 1.0×10^{-4} mol L^{-1}. It was found that the CL intensity increased steeply with increments in luminol concentration up to 5.0×10^{-5} mol L^{-1}, above which it decreased slightly. Therefore, 5.0×10^{-5} mol L^{-1} was selected for the analysis.

As regards the concentration of Mb, the intensity rose drastically when the concentration was increased to 5.0×10^{-8} mol L^{-1}, and then decreased slowly from a higher concentration. Thus, 5.0×10^{-8} mol L^{-1} was the optimum concentration used in subsequent experiments.

Owing to the nature of the luminol CL reaction, which is more favoured under basic conditions, sodium hydroxide was introduced into the manifold through a flow line to improve the sensitivity of the system. A series of different concentrations of sodium

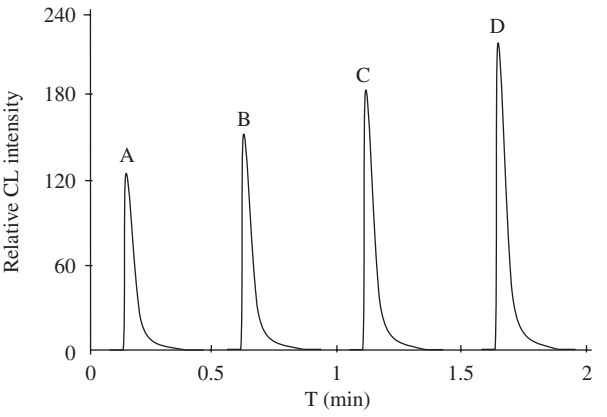

Figure 5. CL intensity–time profile. A: CL intensity in the absence of formaldehyde; B: CL intensity in the presence of 10 pmol L^{-1} formaldehyde; C: CL intensity in the presence of 100 pmol L^{-1} formaldehyde; D: CL intensity in the presence of 1000 pmol L^{-1} formaldehyde. Luminol: 5.0×10^{-5} mol L^{-1}; Mb: 5.0×10^{-8} mol L^{-1}; sodium hydroxide: 0.05 mol L^{-1}.

hydroxide (0.01, 0.025, 0.05, 0.1, and 0.5 mol L^{-1}, respectively) was studied. The concentration of sodium hydroxide *versus* CL intensity plot reached a maximum of about 0.05 mol L^{-1}, and this concentration was employed in subsequent experiments.

3.3 *Effect of flow rate, length of reaction tube, and sample volume injected*

The signal-to-noise ratio was greatly influenced by the flow rate. A rate of 2.0 mL min^{-1} was chosen as a suitable condition with superior sensitivity, precision and reducing reagent consumption. The length of the mixing tube was also adjusted to yield maximum light emission in the CL cell. It was observed that a 16.0 cm mixing tube afforded the best results with regards to sensitivity and reproducibility. Accordingly, 16.0 cm was then selected as the optimum length of mixing tube.

The influence of sample volume injected on CL was examined. By determining a series of injected volumes (30, 50, 70, 100, 150, and 200 μL), it was found that the intensity increased steeply when the injected volume was increased to 100 μL, and then the CL intensity was steady. Thus, the injected volume of 100 μL was selected as the optimum volume injected.

3.4 *Performance for determination of formaldehyde*

Under the selected conditions given above, the calibration graph of CL increment (ΔI) *versus* formaldehyde concentration was linear over the range of 1.0×10^{-12} to 3.0×10^{-9} mol L^{-1}, and the regression equation for formaldehyde is $\Delta I_{CL} = 7.6352\, C_{formaldehyde} + 118.45$ ($r^2 = 0.9998$) with the detection limit of 3.0×10^{-13} mol L^{-1} (3σ). The RSDs were 4.57, 3.49, 2.78, and 1.01% for 5.0×10^{-12}, 1.0×10^{-11}, 5.0×10^{-11}, and 5.0×10^{-10} mol L^{-1} formaldehyde, respectively.

3.5 *Interference studies*

The interference of foreign species was tested by analysing a standard solution of formaldehyde into which increasing amounts of interfering analyte were added. The tolerable concentration of foreign species with respect to 5.0×10^{-11} mol L^{-1} formaldehyde for interference at 5.0% level was less than 1.0×10^{-3} mol L^{-1} for Cl^-, NO_3^-, Ac^-, I^-, SO_4^{2-}, PO_4^{3-}, BrO_3^-, borate, amylum, glucose, and oxalate; 3.0×10^{-4} mol L^{-1} for NH_4^+, Mg^{2+}, Ca^{2+}, methanol, and ethanol; 1.0×10^{-6} mol L^{-1} for urea; 5.0×10^{-7} mol L^{-1} for urea; and 1.0×10^{-8} mol L^{-1} for Cu^{2+}, Zn^{2+}, Ni^{2+}, Cr^{3+}, and Fe^{2+}/Fe^{3+}, respectively.

4. Applications

4.1 *Determination of formaldehyde in air samples*

Following the procedure described in the section 2, formaldehyde can be determined in air samples with the absorption system designed as shown in figure 2. Samples are collected for 5–10 min at a flow rate of 300 mL min^{-1}. The samples are collected inside the isolated Chemical Lab containing formaldehyde vapour. The recovery studies were performed on each of the analysed samples by adding a known amount of formaldehyde to the sample before the recommended treatment. The experimental results are shown in table 1. The method was verified by the recoveries, which were 95.9–105.4%.

4.2 *Determination of formaldehyde at different temperatures*

Formaldehyde solutions (37%) were kept at temperatures of 291.5, 301.0, 310.0, and 315.0 K, respectively. The formaldehyde in air was absorbed by water using the apparatus shown in figure 3, with a flow rate of 3 mL min^{-1} for 5 min, and the collection efficiency of formaldehyde used in this apparatus was 95% [12]. After appropriate dilution, the proposed CL method was used to determine formaldehyde directly. The concentration of formaldehyde was 75.2 mg m^{-3} at a temperature of 291.5 K, which increased gradually to 315 K. The results are summarized in table 2, with

Table 1. Determination of formaldehyde in air samples.[a,b]

Sample	Added (pmol L^{-1})	Found (pmol L^{-1})	RSD (%)	Recovery (%)	Content (mg m^{-3})
1	0	26.1	2.56	105.4	0.39
	50	78.8	3.12		
2	0	21.2	1.79	95.9	0.32
	70	88.3	2.68		
3	0	103.0	3.47	97.3	0.31
	70	171.1	3.06		
4	0	99.8	2.53	101.6	0.30
	100	201.4	2.98		

[a]The average of five determinations.
[b]The collection efficiency of HCHO in used this apparatus was 95%.

Table 2. Determination of formaldehyde in air samples under different temperature.[a]

Temperature (K)	Added (pmol L^{-1})	Found (pmol L^{-1})	RSD (%)	Recovery (%)	Content & vapour pressure ($mg\,m^{-3}$/kpa)
291.5	0	50.1	3.93	98.8	75.2/63.19
	50	99.5	3.47		
301.0	0	77.0	3.92	102.1	115.5/98.85
	50	128.1	3.65		
310.0	0	93.2	2.87	95.0	139.8/118.24
	50	140.7	2.26		
315.0	0	103.6	2.73	107.9	157.5/131.38
	50	157.6	1.90		

[a]The average of five determinations.

recoveries of 95.0–107.9% and RSDs of less than 4.0%. The linear relationship between formaldehyde vapour pressure and temperature was $P_v = 2.8407T_k - 761.73$, and a correlation coefficient of 0.9917 ($n = 7$).

4.3 *Determination of formaldehyde in spiked human serum samples*

The serum samples supplied by the Hospital of Northwest University were spiked before determination. To prepare the spiked samples, known quantities of formaldehyde (5.0×10^{-6}, 1.5×10^{-5}, 2.5×10^{-5}, or 3.5×10^{-5} mol L^{-1}) were spiked into 0.1 mL of serum. After homogenization, the spiked samples were diluted 5.0×10^5-fold, and then the resulting samples were determined directly by the CL method. The results are listed in table 3, and the method was verified by the recoveries, which were 87.6–108.3%.

4.4 *Possible mechanism*

The possible mechanism of the formaldehyde-enhanced Mb–luminol CL reaction was discussed using the static CL and UV-Vis methods, and the results are shown in table 4. Based on the fact that the absorption spectra of Mb–formaldehyde was the same as that of Mb(Fe(III)), with the characteristic λ_{max} located at 409 nm [13], it was suggested that Mb, still in the ferric state, viz. Mb(Fe(III)), could bind with formaldehyde through the remaining iron coordination position on the distal face of haem [14], in accordance with the literature [15]. As shown in figure 4, the optical activity of the Mb–formaldehyde complex for the luminol–Mb CL system, could give a maximum intensity of 2.0-fold in the presence of 1.0×10^{-11} mol L^{-1} of formaldehyde than in its absence, thus greatly improving the quantum efficiency. As a more quick and efficient electron-transfer process occurs between Mb(Fe(III)) and luminol, its time period for the CL process was also shortened from 18 to 12 s, and the CL signal reached its maximum in 4 s. Under the aforementioned experimental conditions, the CL emission spectrum was obtained using an F-4500 spectrofluorimeter, and the results showed that the maximum CL emission wavelength was 425 nm, which suggested that the possible emission species was excited 3-aminophthalate. Therefore, the specific binding of Mb(Fe(III)) with formaldehyde

Table 3. Results of determination for formaldehyde in spiked human serum.[a]

Sample no.	Added (pmol L^{-1})	Found (pmol L^{-1})	RSD (%)	Recovery (%)	*t*-Test ($t_{0.05,4} = 2.78$)	Content 1×10^{-12} (mol L^{-1}/1.0 mL) In sample	Spiked
1	0	9.7	4.21	105.7	1.26	4.87	5.0
	10.0	20.3	3.87				
2	0	10.1	4.24	113.2	0.82	5.04	5.0
	30.0	44.8	3.51				
3	0	10.0	3.66	97.8	0.06	5.01	5.0
	50.0	58.9	2.54				
4	0	30.6	3.82	102.3	1.10	15.32	15.0
	10.0	40.9	2.75				
5	0	29.8	3.33	108.3	0.48	14.88	15.0
	30.0	62.2	2.77				
6	0	30.9	3.36	87.6	1.65	15.43	15.0
	50.0	74.7	2.12				
7	0	49.7	3.12	108.2	0.40	24.85	25.0
	10.0	60.5	2.11				
8	0	51.7	3.14	94.0	2.07	25.84	25.0
	30.0	79.9	2.06				
9	0	51.8	2.65	95.2	2.66	25.91	25.0
	50.0	99.4	1.77				
10	0	68.6	2.74	104.5	1.51	34.29	35.0
	10.0	78.3	2.16				
11	0	68.5	2.52	96.3	1.77	34.23	35.0
	30.0	116.6	1.81				
12	0	70.8	2.01	89.2	1.08	35.38	35.0
	50.0	115.4	1.09				

[a]The average of five determinations.

Table 4. Results of different reaction systems by UV-Vis and static CL methods.[a]

Types of reaction system	UV-Vis (λ_{max} nm^{-1})	CL[b] (T_1 s^{-1})	CL[c] (T_2 s^{-1})
$Mb(Fe^{III})$	409	–	–
$Mb(Fe^{II})$	414	–	–
$Mb(Fe^{III}) + HCHO$	409	–	–
$Mb(Fe^{III}) +$ luminol	414	6	18
$Mb(Fe^{III}) + HCHO +$ luminol	414	3	12

[a]The concentrations of Mb, HCHO and luminol were 1.0×10^{-5}, 1.0×10^{-5} and 1.0×10^{-7} mol L^{-1}, respectively.
[b]The time period of CL reached its maximum.
[c]The time period of complete CL process.

could lead to a more quick and efficient electron transfer process between Mb and luminol catalysing the CL reaction. The whole process is outlined in figure 6.

5. Conclusions

The CL method presented here, combined with the FI technique, offers several advantages, including instrumental simplicity, a high sampling efficiency, reduction in

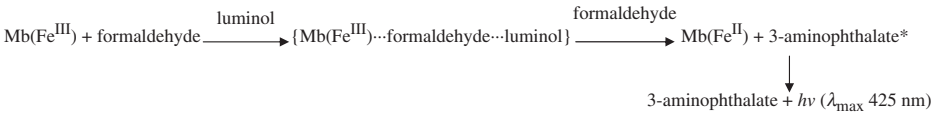

Figure 6. Schematic possible mechanism of the CL reaction.

consumption of reagents, analytical sensitivity, and selectivity, over existing methods. The satisfactory performance in an assay of formaldehyde in air and human serum, and at different temperatures from 291.5 to 315.0 K, demonstrated that the method was practical and suitable not only for quality-control analysis but also for complex biological samples, thus indicating possible use in clinical research.

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