Flow-Injection Chemiluminescence Determination of Formaldehyde with a Bromate-Rhodamine 6G System

Baoxin LI, Meilin LIU, Zhujun ZHANG,[†] and Chunli XU

Department of Chemistry, School of Chemistry and Materials Science, Shaanxi Normal University, Xi'an, 710062, P. R. China

In this paper, a novel flow-injection chemiluminescence (CL) system for the determination of formaldehyde is described. It is based on a strong enhance effect of formaldehyde on the weak CL emission of the reaction between potassium bromate and rhodamine 6G in a sulfuric acid medium. A possible mechanism for this CL reaction is proposed. A CL calibration graph was linear in the range of 0.8–200 $\mu\text{g l}^{-1}$ and the detection limit was 0.3 $\mu\text{g l}^{-1}$ (3σ). The relative standard deviation was less than 3% for 10 $\mu\text{g l}^{-1}$ formaldehyde ($n = 11$). The method has been applied to determine formaldehyde in the air samples.

(Received April 8, 2003; Accepted August 27, 2003)

Introduction

Formaldehyde is ubiquitous in the environmental as a result of its use in various materials and processes. For example, it is used in and is emitted from plywood, decoration board and adhesives. Other sources include cosmetic products, tobacco smoke, ozone generators, carpeting, special clothes and fire place exhaust.¹ Formaldehyde is also a biological consequence of lipid peroxidation following oxidative stress both physiologically and chemically induced.² At a concentration slightly greater than 100 ppbv in ambient air, formaldehyde can cause watery eyes, itching skin, burning sensations in mucous membranes (eyes), nausea, difficulty to breath, and so on.³ Formaldehyde is also a probable human carcinogen according to the United States Environmental Protection Agency.¹ It is obvious that formaldehyde is one of the most important air pollutants. The levels of outgassed formaldehyde need to be checked regularly in order to maintain safe levels. Hence, it is of great concern to develop a rapid and sensitive method to measure formaldehyde.

There are various available methods for the determination of formaldehyde, including gas chromatography,^{1,4} high-performance liquid chromatography,^{5–7} polarography,⁸ amperometry,⁹ conductometry,¹⁰ mass spectrometry,¹¹ fluorometry^{12,13} and spectrophotometry.^{14–17} Most methods for the detection formaldehyde require a chemical reaction of formaldehyde with various reagents to form colored derivatives, which can be observed spectrophotometrically. Most methods use the reaction of formaldehyde with 2,4-dinitrophenylhydrazine (DNP) to form hydrazone.¹⁸ However, DNP reacts with most aldehyde and ketones, and the DNP derivatization reaction takes 1 h. Another derivatization reaction with Nash reagent¹⁹ takes 12 min at 51°C, and the detection is not exceedingly sensitive.¹¹

Flow-injection chemiluminescence (CL) analysis is known to be one of the most powerful techniques that promises high sensitivity, a wide linear range and simple instrumentation, and has been successfully applied to assay formaldehyde. Maeda *et al.*²⁰ reported a CL method for the determination of formaldehyde based on the reaction of gallic acid, H_2O_2 and formaldehyde in an alkaline medium. Takeuchi *et al.*²¹ converted formaldehyde into a fluorescent derivative and then determined the derivatized formaldehyde by using a bis(2,4,6-trichlorophenyl)oxalate flow-injection CL system. Lu *et al.*²² found an inhibition effect of formaldehyde on the lucigenin- $\text{NaClO}-\text{H}_2\text{O}_2$ CL system and applied the CL system to an analysis of formaldehyde. In this work, we found that potassium bromate can directly oxidize rhodamine 6G (Rh6G) in a sulfuric acid medium to produce weak CL, and that formaldehyde can strongly enhance weak CL emission. Based on this phenomenon, a novel CL flow system for the determination of formaldehyde is presented. The proposed method was proved to be rapid, simple, sensitive (detection limit 0.3 $\mu\text{g l}^{-1}$) and suitable for automatic and continuous analysis. The interference of the method is significant only when there are large amounts (~ 100 -fold excess) of acetaldehyde. The method has been successfully applied to the determination of formaldehyde in samples of room air.

Experimental

Apparatus

The flow system used in this work is shown in Fig. 1. A peristaltic pump (Zhejiang Xiangshan Shipu Haitian Electronic Instrument Plant, China) was used to deliver all flow streams at a flow rate of 2.5 ml min^{-1} . PTFE tubing (0.8 mm i.d.) was used as connection material in the flow system. The flow cell was a flat spiral-coiled colorless glass tube (1.0 mm i.d.; total diameter of the flow cell, 3 cm, without gaps between loops) and placed close to the window of the photomultiplier tuber (PMT). A mixed solution (150 μl) of the sample and Rh6G was injected

[†] To whom correspondence should be addressed.
E-mail: zj18@hotmail.com or libx29@hotmail.com

Fig. 1 Schematic diagram of CL flow system for formaldehyde determination. a, sample; b, Rh6G; c, H_2SO_4 ; d, KBrO_3 ; P, peristaltic pump; V, injection-valve; F, CL reaction flow cell; W, waste; D, detector; PC, personal computer.

by a six-way valve into the carrier stream (H_2SO_4), and then merged just prior to reaching a flow cell with a stream of potassium bromate. The CL signal produced in the flow cell was collected by a CR-105 PMT (Hamamatsu, Japan, operated at -800 V) of the ultra-weak Chemiluminescence Analyzer (Institute of Biophysics, Chinese Academy of Sciences, Beijing). The signal was recorded using an IBM-compatible computer, equipped with a data acquisition interface. The data acquisition and treatment were performed with BPCL software running under Windows 95. The concentration of the sample was quantified by the relative CL intensity.

A CH-5 air sample obtained from Hubei Environmental Protection Scientific Research Instrument Plant was used for sample collection.

Reagents

All reagents were of analytical grade, and the water used throughout was deionized and double distilled.

A 1 mg ml^{-1} formaldehyde stock standard solution was prepared by diluting 2.5 ml of 37% formaldehyde solution (Xi'an Chemical Reagent Plant, Xi'an, P. R. China) to 1 liter with water and standardized by the iodimetry method.²³ Formaldehyde working solutions were prepared daily from the stock solution by further dilution just before use. A potassium bromate solution (0.3 mol l^{-1}) was prepared by dissolving the required amount of potassium bromate in water and diluting to the mark. Rh6G was obtained from Merck, and sulfuric acid was purchased from Xi'an Chemical Reagents Factory.

Results and Discussion

Mechanism of the CL reaction

Chemi-excitation of fluorophores is an well-investigated area of CL with numerous applications, in which the product of the CL reaction is first excited during the reaction; then the excited product can then easily transfer its energy to a fluorophore intentionally added to the CL system so as to excite the fluorophore.²⁴⁻²⁹ Nevertheless, there are examples in which the fluorophore reacts with an oxidant to excite the unoxidized molecules of the fluorophore, which emit radiation.^{30,31}

In order to explain the possible CL reaction mechanism, a series of experiments were performed. First, when other oxidants, such as KMnO_4 , Ce(IV) , *N*-bromosuccinimide and hypochlorite, were used to oxidize Rh6G, formaldehyde, and a mixture of Rh6G and formaldehyde, respectively, no CL emission was observed; only Br_2 oxidized Rh6G (or the mixture of Rh6G and formaldehyde) to produce a strong CL signal. Second, it was found that Br^- had a similar enhancing ability as formaldehyde for the weak CL signal between KBrO_3 and Rh6G. Third, the experimental results showed that the reaction

Fig. 2 Effect of the KBrO_3 concentration on the CL intensity. Formaldehyde, $2 \times 10^{-6}\text{ g l}^{-1}$; Rh6G, $1 \times 10^{-4}\text{ mol l}^{-1}$; H_2SO_4 , 2 mol l^{-1} ; flow rate, 2 ml min^{-1} .

of KBrO_3 and Rh6G was slow, and that Br_2 could very quickly oxidize Rh6G to be decolorized.

In this CL system, KBrO_3 can oxidize Rh6G to produce a weak CL signal. KBrO_3 is not fluorescent, and the products of the reaction were also found to be non-fluorescent; only Rh6G is a fluorogenic compound. KBrO_3 can react with formaldehyde to produce Br_2 and CO_2 .³² Based on the above experimental results, the possible mechanism of the CL reaction may be attributed to the following reactions in its simplest form:

Where Red is the reduced form, Ox is the oxidized form and $h\nu$ is light emission.

Because reaction (1) is very slow³² in the absence of formaldehyde, KBrO_3 oxidized Rh6G to produce a weak CL signal in this flow system. When formaldehyde was added to this system, reactions (2) and (3) were very fast. Because the Br_2 generated *in situ* in the flow system was a nascent oxidant,³³ reaction (4) rapidly occurred to emit a the strong CL signal.

Effect of the KBrO_3 concentration on the relative CL intensity

In the absence of KBrO_3 , no CL emission was observed. This suggests that dissolved oxygen can not oxidize formaldehyde and Rh6G to produce CL. The proposed method is based on a CL reaction of KBrO_3 , formaldehyde and Rh6G in an acid medium. As a CL oxidant, the concentration of KBrO_3 would influence the CL intensity. The effect of the KBrO_3 concentration on the CL signal was investigated in the $0.001 - 0.30\text{ mol l}^{-1}$ range. The results are shown in Fig. 2. It can be seen that the CL intensity increased with increasing KBrO_3 concentration. For the KBrO_3 concentration higher than 0.20 mol l^{-1} , the CL intensity almost remained constant. Considering its solubility, the optimal concentration of KBrO_3 was 0.20 mol l^{-1} for subsequent studies.

Effect of the flow rate on the CL intensity

The flow rate is a key factor in flow-injection analysis, which

Fig. 3 Effect of the flow rate on the CL intensity. Formaldehyde, 2×10^{-6} g l $^{-1}$; Rh6G, 4×10^{-4} mol l $^{-1}$; H₂SO₄, 2 mol l $^{-1}$; KBrO₃, 0.2 mol l $^{-1}$.

Fig. 4 Effect of the H₂SO₄ concentration on the CL intensity. Formaldehyde, 2×10^{-6} g l $^{-1}$; Rh6G, 4×10^{-4} mol l $^{-1}$; KBrO₃, 0.2 mol l $^{-1}$; flow rate, 2 ml min $^{-1}$.

not only depends on the analytical efficiency, but also on the sensitivity of the system. The effect of the flow rate on CL intensity was investigated in the 0.5–3.5 ml min $^{-1}$ range for each stream (Fig. 3). The result showed that the CL intensity increased with increasing flow rate in the range of 0.5–2.0 ml min $^{-1}$, probably because this CL reaction is a fast process, or because of more efficient mixing. However, above a flow rate of 2.0 ml min $^{-1}$, the CL intensity declined, probably because higher flow rates with a shorter contact time resulted in an insufficient CL reaction. Thus, a flow rate of 2.0 ml min $^{-1}$ for each stream was selected as being optimum.

Effect of the Rh6G concentration on the CL intensity

Different fluorescers, such as fluorescein, fluorexon, RhB and Rh6G, were investigated in this CL system. The experiment showed that only when Rh6G was used in the CL system was a strong CL signal for determining of formaldehyde obtained. A possible reason is that Rh6G is the best energy receptor in this CL system. Thus, Rh6G was selected as the fluorescer in the CL system. The effect of Rh6G concentration in the range of 1×10^{-3} – 1×10^{-6} mol l $^{-1}$ was investigated. The result showed that the CL intensity increased with increasing Rh6G concentration. However, while the Rh6G concentration was higher than 4×10^{-4} mol l $^{-1}$, the reproducibility of the CL intensity was unacceptable. Therefore, 4×10^{-4} mol l $^{-1}$ Rh6G was chosen for further work.

Effect of an acid media

Because KBrO₃ has a high redox potential only in an acidic media, the acidic media was used as the CL reaction media. The influence of different acid media (such as H₃PO₄, HNO₃, HAc, H₂SO₄ and HCl) was studied through the same flow manifold as mentioned above. When H₂SO₄ was the reaction

Table 1 Results of an analysis of formaldehyde in air after using an oil stove

Sample	Proposed method ^a / mg m $^{-3}$	Spectrophotometry ^a / mg m $^{-3}$	Difference, %
No. 1	0.097 ($\pm 2.5\%$)	0.102 ($\pm 3.6\%$)	–5.1
No. 2	0.256 ($\pm 1.7\%$)	0.247 ($\pm 3.0\%$)	+3.5
No. 3	0.139 ($\pm 2.4\%$)	0.145 ($\pm 3.4\%$)	–4.3
No. 4	0.305 ($\pm 2.0\%$)	0.311 ($\pm 3.5\%$)	+2.0

a. Average of three replicates ($\pm$ R.S.D).

media, the strongest CL intensity was obtained. Sulfuric acid, like in vast majority of CL systems involving Ce(IV) or MnO₄ $^{-}$, was then selected as the optimum. Furthermore, the effect of the H₂SO₄ concentration was studied. The result is shown in Fig. 4. The CL intensity increased with increasing the H₂SO₄ concentration in the range of 0.0–3.0 mol/l, probably because the redox potential of KBrO₃ increased with increasing sulfuric acid concentration. However, above a concentration of 3.0 mol l $^{-1}$, the CL intensity declined. Then, the 3.0 mol l $^{-1}$ H₂SO₄ was chosen as the optimum media.

Performance of the system for formaldehyde measurements

Under the optimum conditions described above, the calibration graph of the emission intensity (*I*) versus the formaldehyde concentration was linear over the range 0.8–200 μ g l $^{-1}$, and the detection limit was 0.3 μ g l $^{-1}$ (3 σ). The regression equation was $I = 73 + 28.5 \times C$ (*C*: formaldehyde concentration, μ g l $^{-1}$) with a correlation coefficient of 0.9962 (*n* = 9). The relative standard deviation was less than 3% for 10 μ g l $^{-1}$ formaldehyde (*n* = 11).

Interference study

The influence of foreign species was investigated by analyzing a standard solution of 20 μ g l $^{-1}$ formaldehyde to which increasing amounts of interfering species were added. The tolerable concentration ratios with respect to 20 μ g l $^{-1}$ formaldehyde for interference at the 5% level were: over 1000 for K⁺, Na⁺, NH₄⁺, Al³⁺, CO₃²⁻, NO₃⁻, Mg²⁺, Ca²⁺, Ni²⁺, Zn²⁺ and uric acid; 500 for glucose, oxalic acid, methanol, ethanol and acetic acid; 100 for acetone, acetaldehyde, propionaldehyde, butyraldehyde, Fe³⁺, Cl⁻ and benzaldehyde; 50 for Br⁻ and NO₂⁻; 5 for S²⁻ and SO₃²⁻, respectively. The results showed that the proposed method has good selectivity; only S²⁻ and SO₃²⁻ exhibited negative interference, possibly because S²⁻ and SO₃²⁻, as strong reducers, can also react with KBrO₃ and Br₂.

Sample analysis

To investigate the applicability of the proposed method to real samples, the present CL flow system was applied to the assay of formaldehyde in room air after using an oil stove. After using an oil stove in the room (3.6 m width $\times$ 6 m depth $\times$ 2.7 m height), formaldehyde was absorbed from an air sample over a 30 min sampling period at a sample rate of 0.5 l min $^{-1}$ into 10 ml H₂O. The results are given in Table 1, which were proved by a *t*-test (level of significance, 0.05) to show that the results with the present method agreed with those obtained by the spectrophotometry.²³ Samples No. 1 and No. 2 were collected after using the same oil stove, and samples No. 3 and No. 4 were from a different oil stove.

The proposed method was also applied to determine formaldehyde in the air of a newly decorated room air. Under different temperature and humidity, formaldehyde was absorbed from the air sample over a 20 min sampling period at a sample

Table 2 Result of an analysis of formaldehyde in air of a newly decorated room

Sample	Temperature/ °C	Relative humidity, %	Formaldehyde concentration ^a /mg m ⁻³
No. 1	8	50	0.294
No. 2	8	70	0.456
No. 3	28	50	0.680
No. 4	28	70	0.722

a. Average of three replicates.

rate of 0.5 l min⁻¹ into 10 ml of H₂O. The results given in Table 2 show that the formaldehyde concentration in the room air increased, while the temperature and humidity of the room increased.

Conclusions

To the best of our knowledge, only three studies used KBrO₃ as a CL oxidant,³⁴⁻³⁶ though they belonged to the same CL system of BrO₃⁻-SO₃²⁻-steroids and detected BrO₃⁻,³⁴ steroids³⁵ and bile acids,³⁶ respectively. In this paper, one novel CL reaction using KBrO₃ as an oxidant is reported. Based on this CL reaction and combined with a flow-injection analysis, a novel CL flow system was proposed for trace amounts of formaldehyde. The proposed method offers advantages of simplicity, rapidity and selectivity, and has been applied to the analysis formaldehyde in environmental air samples.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (Grant No. 20175039) and Youth Foundation of Huo Yingdong (Grant No. 81013).

References

1. S. B. Jones, C. M. Terry, T. E. Lister, and D. C. Johnson, *Anal. Chem.*, **1999**, 71, 4030.
2. D. Bagchi, M. Bagchi, E. A. Hassoun, J. Kelly, and S. J. Stohs, *Toxicology*, **1995**, 95, 1.
3. E. B. Rietz, *Anal. Lett.*, **1980**, 13, 1073.
4. T. Dumas, *J. Chromatogr.*, **1982**, 247, 298.
5. S. B. Jones, C. M. Terry, T. E. Lister, and D. C. Johnson, *Anal. Chem.*, **1999**, 71, 4030.
6. Y. V. Heyden, A. N. M. Nguyet, M. R. Detaevernier, D. L. Massart, and J. Plaizier-Vercammen, *J. Chromatogr. A*, **2002**, 958, 191.
7. J. J. Michels, *J. Chromatogr. A*, **2001**, 914, 123.
8. Z. Q. Zhang, H. Zhang, and G. F. He, *Talanta*, **2002**, 57, 317.
9. B. Winter, K. Kamman, and Z. Fres, *Anal. Chem.*, **1989**, 334, 670.
10. S. V. Dzyadevych, V. N. Arkhypova, Y. I. Korpan, A. V. El'skaya, A. P. Soldatkin, N. Jaffrezic-Renault, and C. Martelet, *Anal. Chim. Acta*, **2001**, 445, 47.
11. S. Kato, P. J. Burke, T. H. Koch, and V. M. Bierbaum, *Anal. Chem.*, **2001**, 73, 2992.
12. T. Sakai, S. Tanaka, N. Teshima, S. Yasuda, and N. Ura, *Talanta*, **2002**, 58, 1271.
13. M. I. H. Helaleh, M. Kumemura, S. Fujii, and T. Korenaga, *Analyst*, **2001**, 126, 104.
14. L. Gamizgracia and M. D. L. Decastro, *Analyst*, **1999**, 124, 119.
15. R. R. Mlksch, D. W. Anthon, L. Z. Fanning, C. D. Hollowell, K. Revzan, and J. Glanville, *Anal. Chem.*, **1981**, 53, 2118.
16. A. P. Altshulter, D. L. Miller, and S. F. Slega, *Anal. Chem.*, **1991**, 33, 621.
17. A. Afkhami and M. Rezaei, *Microchem. J.*, **1999**, 63, 243.
18. J. Ramer, M. L. Holland, D. P. Wiesler, and M. Novotny, *Anal. Chem.*, **1984**, 56, 962.
19. T. Nash, *Biochem. J.*, **1953**, 55, 416.
20. Y. Maeda, X. C. Hu, S. Iton, M. Kitano, N. Takenaka, H. Bandow, and M. Munemori, *Analyst*, **1994**, 119, 2237.
21. K. Takeuchi, H. Takahashi, S. Kutsuna, and T. Ibusuki, *Bunseki Kagaku*, **1993**, 42, 439.
22. X. U. Lu, M. G. Lu, and F. Yin, *Anal. Lett.*, **1992**, 25, 2075.
23. J. Cui, Q. Wang, and H. Wang, "Monitoring Gaseous Pollutants", **1997**, Chemical Industry Press, Beijing, 523 - 547.
24. A. A. Al-Tamrah, A. Townshend, and A. R. Wheatey, *Analyst*, **1987**, 112, 883.
25. J. L. Burguera and M. Burguera, *Anal. Chim. Acta*, **1988**, 214, 429.
26. I. I. Koukli and A. C. Calokerinos, *Anal. Chim. Acta*, **1990**, 236, 463.
27. X. Zhang, W. R. G. Baeyens, G. Van der Weken, A. C. Calokerinos, and K. Nakashima, *Anal. Chim. Acta*, **1995**, 303, 121.
28. F. A. Aly, N. A. Alarfaj, and A. A. Alwarthan, *Talanta*, **2001**, 54, 715.
29. B. Li, Z. Zhang, L. Zhao, and C. Xu, *Anal. Chim. Acta*, **2002**, 459, 19.
30. L. P. Palilis and A. C. Calokerinos, *Anal. Chim. Acta*, **2000**, 413, 175.
31. G. N. Chen, J. P. Duan, and Q. F. Hu, *Anal. Chim. Acta*, **1994**, 292, 159.
32. S. Wang, L. Du, and A. Zhang, *Chin. J. Anal. Chem.*, **2000**, 28, 628.
33. X. Zheng, M. Yang, and Z. Zhang, *Acta Chimica Sinica*, **2001**, 59, 945.
34. J. C. G. E. da Silva, J. R. M. Dias, and J. M. C. S. Magalhães, *Anal. Chim. Acta*, **2001**, 450, 175.
35. A. B. Syropoulos, E. G. Sarantonis, and A. C. Calokerinos, *Anal. Chim. Acta*, **1990**, 239, 195.
36. I. M. Psarellis, N. T. Deftereos, E. G. Sarantonis, and A. C. Calokerinos, *Anal. Chim. Acta*, **1994**, 294, 27.