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Effect of Arsenious and Arsenic Acids on the Chemiluminescence of Luminol in the Absence of Hydrogen Peroxide and Its Analytical Application

Hiroyuki SAKAI,† Terufumi FUJIWARA, and Takahiro KUMAMARU*
Department of Chemistry, Faculty of Science, Hiroshima University, 1-3-1, Kagamiyama, Higashi-Hiroshima 724
† Laboratory of Inorganic Materials, Railway Technical Research Institute, 2-8-38, Hikari-cho, Kokubunji, Tokyo 185

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Synopsis. Arsenious and arsenic acids caused an increase in the chemiluminescence of luminol even in the absence of hydrogen peroxide. Based on the finding, a new flow injection chemiluminescence method was developed for determination of arsenic(III,V). Applicability to biological materials containing arsenic is also demonstrated.

For the determination of inorganic arsenic(III,V) in water samples, a spectrophotometric detection based on the molybdenum blue reaction$^{1)}$ has been used in conjunction with a flow injection analysis (FIA) technique,$^{2,3)}$ in which the preoxidation of arsenic(III) to arsenic(V) was required. On the other hand, in recent years, development of sophisticated instrumental FIA techniques like hydride generation (HG)-flame atomic absorption spectrometry$^{4,5)}$ and HG-inductively coupled plasma atomic emission spectrometry,$^{6)}$ capable of sensitive and selective determination, has been achieved. Unfortunately, these analytical methods require complex and expensive apparatus. Chemiluminescence (CL) provides a valid detection system for FIA.$^{3,7)}$ In addition to high sensitivity and wide dynamic range, simple and inexpensive instrumentation is generally one of the main advantages associated with CL detection systems. However, the lack of CL reactions has limited their application to the determination of anions by FIA.$^{8)}$ Some methods for the determination of arsenic by using solution-phase$^{9)}$ and gas-phase$^{10,11)}$ CL reactions have been reported although the CL reactions were not applied to FIA.

In general, a relatively high baseline CL emission results from luminol oxidation by hydrogen peroxide. In this work, we found that even when hydrogen peroxide is not present, a CL emission was produced by mixing an acidified solution of arsenic(V) with a basic solution of luminol. A method based on this CL reaction allows us to carry out the arsenic determination without the need for an oxidizing reagent such as hydrogen peroxide. Such CL measurement would be intrinsically sensitive to low levels because the change in CL emission against more or less negligible background signal is measured.$^{12)}$ This CL reaction is so simple that an additional advantage of the present CL detection system comes from the use of a single reagent stream for the FIA technique. Moreover, the same behavior in the effect on the luminol CL was also observed for arsenic(III). This implies that, where the oxidation state of the arsenic is less important, the present CL emission is preferable to be used for the determination of total arsenic without any chemical conversions such as the oxidation of arsenic(III) to arsenic(V) for the molybdenum blue method and the reduction of arsenic(V) to arsenic(III) for the HG technique$^{13)}$ in sample pretreatments.

Some metal ions such as iron(II),$^{14)}$ cobalt(II), and copper(II)$^{12)}$ have been also reported to cause CL from luminol in the absence of hydrogen peroxide although the CL reaction is relatively more specific. Oxoanions of arsenic(III,V) can be readily converted into molecular acids as in the case when the weak acid anions are acidified.$^{15)}$ Ion-exclusion chromatography is particularly useful for separating such molecular species from ionic materials.$^{16)}$ Thus, the chromatographic separation technique combines the advantage of its ability to remove such interfering metal ions. Arsenic(III,V) eluted in the molecular acid form would be suitable for the proposed CL detection.

Experimental

Reagents and Standards. Luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) was purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI, USA) and used without further purification. Other chemicals of analytical-reagent grade and a 1000 $\mu g\,cm^{-3}$ standard of arsenic(III) in 10 $mmol\,dm^{-3}$ hydrochloric acid were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan) and were used as received. All solutions, except for eluent, were prepared with water collected from an Advantec Toyo (Tokyo, Japan) Model GSU-901 water purification system. The water used in the eluent preparation was of high performance liquid chromatography (HPLC) grade and was obtained from Kanto Chemical Co., Inc.

The CL reagent solution of 0.80 $mmol\,dm^{-3}$ luminol in 3.0 $mmol\,dm^{-3}$ potassium hydroxide was prepared daily with 30 $mmol\,dm^{-3}$ potassium hydroxide and water. The resulting solution was allowed to stand for ca. 3 h before use. The pH of the reagent solution was 11.1. A 1000 $\mu g\,cm^{-3}$ arsenic(V) standard was made by dissolving sodium arsenate in water. Working standard solutions of arsenic(III) and arsenic(V) were prepared by serial dilutions of the respective 1000 $\mu g\,cm^{-3}$ standards with water before use.

Instrumentation and Procedures. The FIA system was essentially the same as that reported previously$^{17,18)}$ but without the oxidizing reagent of hydrogen peroxide. A Tosoh (Tokyo, Japan) Model CCPD pump was used to drive the CL reagent uniformly through the flow system. The dilute (0.75 $mmol\,dm^{-3}$) perchloric acid solution (pH 3.2) used as an eluent was driven via a Tosoh Model HLC-803D pump unit for ion chromatograph with a 200-mm×4.9-mm i.d. cation-exchange column (SCX1, Yokogawa Analytical Systems, Musashino, Japan). A 100-mm^3 sample was inserted into the stream of perchloric acid using an injection valve. The acid and CL reagent streams were driven through the system both at the constant flow rate of 1.0 cm^3 min^{-1}, where PTFE tubing (0.5 mm i.d.) was used. When the column effluent from the ICE was mixed directly with the reagent stream in a coiled flow cell (70 mm^3) mounted in front of a photomultiplier tube of a Niti-on (Funabashi, Japan) Model LF-800 bio-chemiluminescence monitor, CL signals were produced. The pH of the resulting mixture was 10.5. The CL signals were recorded with an ordinary strip-chart recorder. The peak height of the analytical CL signal was read against the reagent blank like before.[18)]

Results and Discussion

Arsenious or Arsenic Acid-Luminol Chemiluminescence System. In our previous work,[18)] it has been reported that molecular acids such as silicic acid, phosphoric acid and carbonic acid, present in acidic media, caused an increase in the CL emission from luminol oxidation by hydrogen peroxide in a basic solution. In this work, on the other hand, it was observed that even in the absence of hydrogen peroxide in the basic solution of luminol, an increased CL emission was produced when the luminol solution was mixed with an acidified solution of arsenate ion. In acid solution, the oxoanion will be present in the form of a molecular acid, i.e. arsenic acid, and the oxoacid is a moderately strong oxidizing agent.[19)] On the other hand, almost the same CL behavior was also obtained for an acidic solution of arsenite ion which is an anion of weak acid.[15)] In the observed enhancement of the CL emission, the molecular acids of arsenic(III,V) may play an important role like a catalyst. Similarly, an increased CL emission results from metal-catalyzed oxidation of luminol by dissolved oxygen alone present in the basic solution.[12,14,20)] However, the involvement of arsenious or arsenic acid in the CL reaction process could not be explained at this time. The CL system is simple and relatively more specific as no addition of hydrogen peroxide is required.

Optimization. In our previous work on the determination of silicate,[18)] almost complete removal of the potential interfering metal ions with ion-exclusion chromatographic (ICE) separation was confirmed. Thus, the present CL detection system was combined with an ICE separation technique using dilute perchloric acid as an eluent. In order to obtain a reasonable analysis time, a perchloric acid concentration of 0.75 mmol dm^{-3} was recommended.

Of much importance is the optimization of the CL detection system to be combined with the ICE separation. The luminol CL reaction in the basic medium is affected by the eluent concentration, because the acidic eluent would bring a change in the pH of the luminol basic solution. The results for the optimization of potassium hydroxide concentration are presented in Fig. 1. Both the maximum CL intensities for arsenic(III) and arsenic(V) occurred at the potassium hydroxide concentration of 3.0 mmol dm^{-3} which was chosen as optimal for this system. Figure 2 shows the CL intensity as a function of luminol concentration. The optimum luminol concentration obtained was 0.80 mmol dm^{-3}. Around the concentration, the CL intensities for arsenic(III) and arsenic(V) reached equally their maxima.

Under the optimum conditions, when a 100-mm^3 sample was injected in this FI-CL system, the same detection limits (DL) of 100 ng cm^{-3} were obtained for arsenic(III) and arsenic(V), where the DL is defined as the concentration of the analyte for which the CL signal is three times higher than the noise level of the baseline. A calibration graph for the determination of arsenic(III) was linear from 500 ng cm^{-3} to 15 μg cm^{-3}, almost identical to that of arsenic(V). The relative standard deviations of 6.1 and 6.9% were obtained for five replicate measurements of 3.0 μg cm^{-3} arsenic(III) and arsenic(V), respectively.

Determination of Arsenic in Biological Reference Materials. The FI-CL method was applied to the determination of arsenic in the certified reference

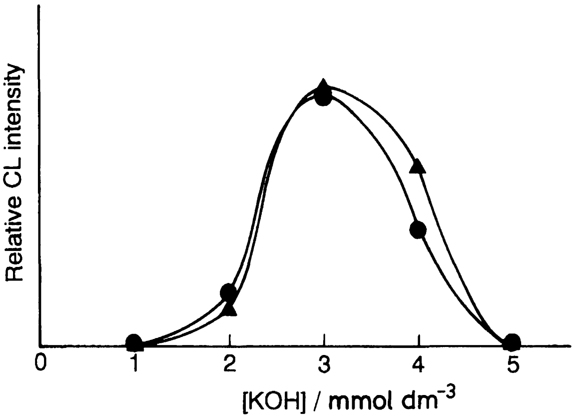

Fig. 1. Effect of potassium hydroxide concentration on CL intensity. [As(III)]=10 μg cm^{-3} (●). [As(V)]=10 μg cm^{-3} (▲). [Luminol]=0.80 mmol dm^{-3}.

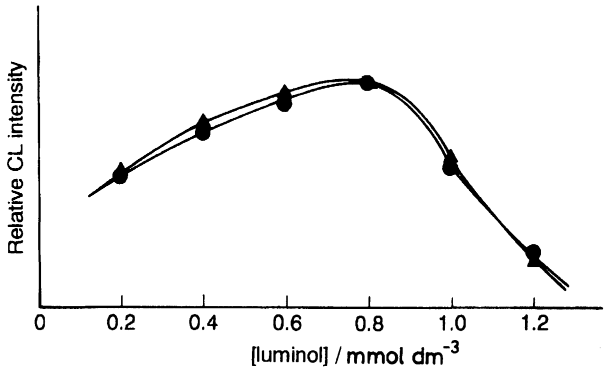

Fig. 2. Variation of CL intensity with luminol concentration. [As(III)]=10 μg cm^{-3} (●). [As(V)]=10 μg cm^{-3} (▲). [KOH]=3.0 mmol dm^{-3}.

Table 1. Determination of Arsenic in Biological Reference Materials

Sample	Found μg cm^{-3}	Certified value μg cm^{-3}
Mussel (NIES CRM No. 6)	8.9, 9.0	9.2±0.5
Sargasso (NIES CRM No. 9)	107, 112	115±9

materials (CRMs), No. 6 and No. 9, supplied by the National Institute for Environmental Studies (NIES), Environmental Agency of Japan. The procedure for the sample digestion using the double vessel method was the same as in the literature.[21] FI-CL determination of arsenic in the sample solutions prepared was conducted directly using the calibration graph obtained under the optimum conditions. The results are given in Table 1. The data are in good agreement with the certified values for the respective samples.

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