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Chemiluminescence Determination of Benzoic Acid Using A Solid-Phase Verdigris Reactor

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Abstract: A new chemiluminescence flow system has been developed for sequential determina-tion of benzoic acid based on the reaction of the compound with copper carbonate entrapped in a solid-phase reactor. It was found that the unsaturated complex of Cu(II) and benzoic acid (1:1) has strong catalytic effect on the luminol-H_2O_2 chemiluminescence reaction. The calibration graph is linear over the range of 0.025 ~ 60 µg/mL of benzoic acid, with a relative standard deviation of less than 3.0 %, and the detection limit is 0.01µg·mL^{-1}. The proposed method was applied to the determination of benzoic acid content in different pharmaceutical formulations.

Keywords: Benzoic acid, chemiluminescence, solid-phase reactor, pharmaceutical formulations.

Benzoic acid is a common additive used widely as food preservative and plasticizer. It could promote severe reaction to allergic population even at low concentration level[1]. Routine methods for the assay of benzoic acid includes chromatographic[2-6], spectrophotometry[7,8], capillary electrophoresis[9-13] and electrochemical titration[14]. Stokes[15] *et al.* monitored airborne benzoic acid based on surface-enhanced Raman scattering techniques. There is also report on a microbial sensor using *pseudomonas* for benzoic acid and their derivatives in aqua[16]. Interest of using luminescence assay to determine trace and ultra-trace concentration of inorganic and organic species has increased in the recent years[17-19]. With the aid of time-resolved lanthanon-sensitized luminescence technique[20-22], benzoic acid was determined in food and pharmaceutical analysis.

In this paper, a simple and sensitive flow injection (FI) chemiluminescence (CL) method for determination of benzoic acid was established based on the complexometric dissolution of copper carbonate to form Cu(II)-benzoic acid unsaturated complex(1:1) and catalyzed luminol-H_2O_2 CL reaction. The concentration of benzoic acid is linear with the CL signal in the optimized conditions and the method has been applied to the determination of benzoic acid in pharmaceutical preparations with satisfactory results.

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Experimental

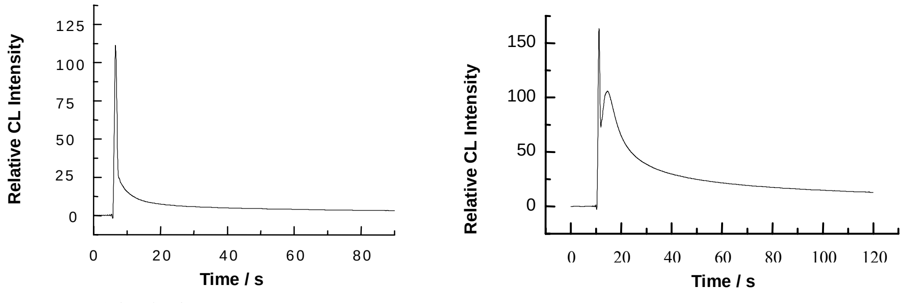
Materials and methods. All reagents used were of analytical-reagent grade. A perist-altic pump (Shanghai Meter Electromotor Plant, Model ND-15, 15 r/min) was used to generate flows at a rate of 2 mL·min^{-1} on each line. PTFE tubing (2 mm i.d.) was used in flow system. 200 μL reacted solution of benzoic acid in the solid-phase reactor was injected into the carrier stream using a six-way valve. The mixed solution of luminol-H_2O_2-benzoic-Cu(II) was passed into the CL cell and light signals was detected by a luminometer (Northwest Non-ferrous Geology Institute of China, Model GD-1) con-nected to a recorder (Shanghai Dahua Instrument and Meter Plant, Model XWT-206).

Preparation of solid-phase copper carbonate reactor. 30 mL $CuSO_4$ (0.33 g/mL) and 30 mL Na_2CO_3 (0.15 g/mL) solutions were mixed, stirred slowly and then filtered. The precipitate of copper carbonate was rinsed with doubly distilled water and dried at room temperature. Fiberglass was cut to about 1 mm in length; then it was washed with H_2SO_4 (10 %) and distilled water. The solid-phase reactor was made by encapsulating 0.1 g mixture of copper carbonate with fiberglass crumbs into a PTFE tubing (2 mm i.d., 3.0 cm in length), plugging with glass wool at both ends to prevent the loss of mixtures. The reactor could be reused for more than 300 times.

Results and Discussion

Time profiles of CL reaction. **Figure 1** (a, b) shows the CL time profiles of both the Cu(II)-luminol-H_2O_2 and Cu(II)-benzoic acid complex (1:1)-luminol-H_2O_2 reaction in a static system, respectively. It can be seen that the CL reaction of luminol-H_2O_2 catalyzed by Cu(II) present fast CL characteristic. The time interval from injecting needs 6.0 s then decays quickly. For Cu(II)-benzoic acid complex(1:1), the first peak is at 6.0 s, and the second is at 15 s, and then decays slowly.

Figure 1 Time profiles of CL reaction



(a) luminol-H_2O_2-Cu system
Cu(II): 0.1 μg/mL

(b) luminol-H_2O_2-Cu-benzoic acid system
Cu(II) : 0.1 μg/mL

Effect of luminol and H_2O_2 concentration. The CL intensity increased with the increase of luminol and H_2O_2 concentration. It was found that the maximal CL intensity could be obtained with 1.2×10^{-3} mol/L luminol and 5.0×10^{-4} mol/L H_2O_2, and these concentra-tions were used for following work.

Effect of sample acidity. The CL intensity was also affected by the acidity of sample solution. Lower pH resulted in a higher background due to the forming of Cu^{2+}. To maximize the signal-to-noise ratio, sample of pH 5.5 was used.

Effect of complex molar ratio. The effect of complex molar ratio ($M_{\text{benzoic acid}}$: M_{Cu}) on the CL intensity was tested with a static system. It was found that when the concentration of benzoic acid was lower than that of Cu(II), Cu(II) and benzoic acid formed a 1:1 unsaturated complex. The CL intensity increases with the benzoic acid concentration until the molar ratio ($M_{\text{benzoic acid}}$: M_{Cu}) was 1:1. When the benzoic acid concentration was greater than that, Cu(II) and benzoic acid was formed a 1:4 saturated complex and the CL intensity decreased with increasing benzoic acid concentration. It indicated that unsaturated complex of Cu(II)-benzoic acid had catalytic effect on the CL reaction and 1:1 unsaturated complex of Cu(II)-benzoic acid had the highest activity.

Effect of the reactor parameters. The influence of the reactor parameters, length and internal diameter, on the transient outputs was also studied. 4.0 cm PTFE tubing with 1.2 mm internal diameter was chosen as optimum.

Performance of the measurement. A series of standard solution of benzoic acid were determined under the optimized conditions to test the linearity of the calibration graph. The results are summarized in **Table 1**. At a flow rate of a 2.0 mL·min^{-1}, the deter-mination of benzoic acid can be performed in 2 minutes including sampling and washing, giving a throughput of about 30 h^{-1} with a relative standard deviation of less than 1.6%.

Table 1 Calibration graph of benzoic acid

Benzoic acid conc. µg/mL	Equation $\mathbf{Y} = a\mathbf{X} + b$	Corr. Coef. γ	RSD % n=5	DOL µg/mL
0.025~5.0	$\mathbf{Y} = 2.658\mathbf{X} + 7.52$	0.9986	3.0	0.01 (3σ)
5.0~60	$\mathbf{Y} = 0.172\mathbf{X} + 8.67$	0.9991	1.5	

Table 2 Results of benzoic acid in different pharmaceutical formulations

Samples	Found µg/mL	Added µg/mL	Total µg/mL	Recovery %	Found Content %	Label Content %
Aqua	3.33	4.0	7.12	94.8	0.095	0.1
Menthae	3.42	2.0	5.44	101.0	0.101	0.1
Prickly-heat	2.21	5.0	7.28	101.4	1.014	1.0
Powder	2.15	3.0	5.11	98.6	0.986	1.0

Interference study. The effect of foreign species was tested by analyzing a standard solution of benzoic acid, to which increasing amounts of interfering species were added . The tolerable limit of a foreign species was taken if it caused a relative error of less than 5%. It was found that with respect to 1.0 µg/mL benzoic acid, more than 8000 folds for methanol and ethanol, 1000 for chloromycetin, catechol, and glycerol, 500 for phenol, 100 for starch, and 20 for urea and glucose were tolerable.

Applications. Following the procedure described in experimental section, the proposed method was applied to the determination of benzoic acid in two different pharmaceutical preparations purchased from the local market. The results are listed in **Table 2**.

Conclusions

The proposed method offers a low detection limit and high selectivity to benzoic acid determination and is promising for measurement of benzoic acid in foods and drinks. The resulting manifold is suitable for the determination of benzoic acid in pharma-ceutical formulations with competitive precision and high sample throughput.

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