A New Chemiluminescent Triazine Reagent

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Abstract: A new chemiluminescent reagent 7-(4,6-dichloro-1,3,5-triazinylamino)-4-methyl-coumarin (DTMC) was synthesized by linking 7-amino-4-methylcoumarin to cyanuric chloride at 0-5 °C, and with it a novel chemiluminescence method was developed for the determination of hydrogen peroxide. The selectivity of this method is high, and most of the transition metal ions have no effect on the determination of H_2O_2.

Keywords: 7-(4,6-dichloro-1,3,5-triazinylamino)-4-methylcoumarin, chemiluminescent reagent, hydrogen peroxide.

The determination of hydrogen peroxide is of great importance in biochemistry, environmental fields and clinical control[1,2]. Chemiluminescence method is commonly used in the determination of hydrogen peroxide because of its low detection limit and wide dynamic range that can be achieved with relatively simple instrumentation. Up to now, however, established systems for analytical purposes fall into one of a limited number of types and these systems lack selectivity due to the possible interferences from catalyzing actions of many metal ions[3,4]. Therefore, it is necessary to develop new types of chemiluminescent reagents, which possess high selectivity. In this work, DTMC was synthesized by treating cyanuric chloride with 7-amino-4-methylcoumarin, and chemiluminescence was observed in the reaction of DTMC with hydrogen peroxide. Based on this, a new chemiluminescent method was developed for the determination of H_2O_2.

Experimental

To a test solution containing 2.0 mL of DTMC stock solution (3.0×10^{-3} mol/L), 10.0 mL of 0.1 mol/L Na_2HPO_4-NaOH buffer (pH = 11.4) was added, and the final volume adjusted to 50 mL with water. The test solution was prepared daily. The above test solution (2.0 mL) was pipetted into a 5 mL tube, and then 25-100 μL of H_2O_2 solution was injected automatically. At the same time chemiluminescent spectra were recorded or chemiluminescence intensities were measured.

Results and Discussion

DTMC reacts with H_2O_2 in alkaline medium to emit light with a wavelength of 448 nm. Several possible factors affecting chemiluminescent intensity were studied. In this work, 0.1 mol/L Na_2HPO_4-NaOH (pH = 11.4) and 1.2×10^{-4} mol/L of DTMC were chosen for the following experiments.

Under the optimum conditions, the peak height signals of chemiluminescence are directly proportional to the H_2O_2 concentration over a wide linear range. The log-log calibration plot was linear from 1.0×10^{-7} mol/L to 4.0×10^{-4} mol/L H_2O_2, and the regression equation was determined to be: log I (chemiluminescence intensity) = 0.898 log C (H_2O_2, mol/L) + 7.402, n = 12, r = 0.9997. The precision was checked by repeated determinations (n=10) using 1.0×10^{-6} mol/L H_2O_2 test solution. The relative standard deviation was 4.9%. The detection limit for H_2O_2 was 4.0×10^{-8} mol/L (S/N = 3).

The well-known liquid phase chemiluminescence reactions based on the luminol or other chemiluminescence reagents in the presence of a catalyst often suffer from the interferences of transition metals[4]. In this system, most of the metal ions have no effect on the chemiluminescence signal. The results of interference tests are summarized in **Table 1**. The tolerance limit was estimated with a ±5% relative error. These data show that the present method has high selectivity, superior to those based on luminol. Its real application is in progress.

Table 1 Effect of diverse ions on the determination of 1.0×10^{-6} mol/L H_2O_2

Ions	Maximum tolerance ratio of ion to H_2O_2 (molar ratio)
K^+, Ca^{2+}, Mg^{2+}, HCO_3^-, Cl^-, NO_3^-, SO_4^{2-}	>2000
Cr^{3+}, Cr(VI)	200
Ce^{3+}, Ce^{4+}, V(V), Mn^{2+}, Fe^{3+}, Tb^{3+}, Pb^{2+}	40
Cu^{2+}, Ni^{2+}, Hg^{2+}, Zn^{2+}	10
Co^{2+}, Ag^+	4

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