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CHEMILUMINESCENT DETERMINATION OF TRACES OF COPPER(II) BY FLOW INJECTION METHOD

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Chemiluminescence in conjunction with flow injection analysis is described for the determination of traces of copper(II) by means of the flavin mononucleotide-hydrogen peroxide-phosphate buffer system. This permits the determination of copper(II) more selectively than any other chemiluminescent system with a detection limit of 30 pg.

Great interest has recently been focused on the use of solution chemiluminescence(CL) for determination of certain species at trace levels because of the simplicity of the instrumentation, the low detection limit, and the short analysis time. Systems based on reagents such as luminol, lucigenin, lophine, or gallic acid have mainly been utilized for trace metals.[1)] Copper(II) down to sub-ppm levels can be determined by the catalyzed oxidation of these CL reagents in alkaline hydrogen peroxide solution, but it is subject to much interference due to lack of selectivity.[2)] This paper describes sensitive, selective, and rapid CL determination of copper(II) by flow injection method. It is based on the measurement of CL arising from the copper(II)-catalyzed oxidation of flavin mononucleotide(FMN, riboflavin-5′-phosphate) by hydrogen peroxide under nearly neutral conditions. The riboflavin-hydrogen peroxide system had been studied in connection with bacterial bioluminescence[3)]; the CL shows similar spectral with the fluorescence of riboflavin and is appreciably enhanced by the addition of copper(II), iron(II), or osmium(VIII). We noted this enhancement effect of copper(II) and attempted to combined with flow injection analysis.

A schematic diagram of the flow system is given in Fig. 1. The spiral flow cell is employed for D as shown in the previous paper.[4)] Teflon tubing(1-mm i.d.) is used for flow lines except the pump tubes and D. The emission is observed directly by a photomultiplier tube(Hamamatsu TV-R268) with no wavelength discrimination. For CL reagent, riboflavin was replaced by FMN to permit increasing solubility in water. The FMN solution is buffered with potassium dihydrogen phosphate-disodium hydrogen phosphate. Copper(II) and other species were injected by means of a 10-µl rotary valve injector(Kusano Sci.Instr.).

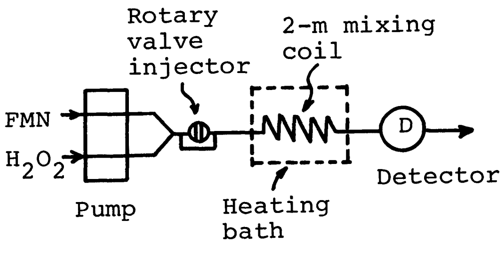

Fig. 1. Schematic diagram of the flow system.

The copper(II)-enhanced CL signal is dependent on the concentrations of reagents, the flow rates of reagent solutions, the pH of FMN solution, and the reaction temperature. Significant effect was observed in the reaction temperature because the speed of the CL reaction was considerably slow at room temperature; the signal increased by a factor of ca.60 at 60°C than at room temperature under the conditions specified below. Determination of the total flow rate in the system, which is closely related to the reaction temperature, is very important for flow injection analysis in sensitivity and rapidity; at too low or too high total flow rate, most of the CL reaction proceeds outside the flow cell, resulting in low signal or low sampling rate. The luminous oxidation of FMN proceeds best under nearly neutral conditions, in contrast that other CL systems as stated above emit light under alkaline conditions. The optimum conditions for the copper(II) determination ([FMN]:8×10^{-4}M, [H_2O_2]:1.4 M, pH of FMN soln.:6.0, reaction temp.: 60°C, flow rates of both reagent solns.:4.0 ml min^{-1}) provided a calibration graph with a slope of unity and a linear range of about 3 orders of magnitude, a detection limit of 30 pg(10 μl sample injection), and a sampling rate of 60 h^{-1}.

Most solution CL methods suffer from poor selectivity. For evaluation of the selectivity in the present system, various species were injected. The results are shown in Table 1, indicating that the FMN CL system is very selective for the copper(II) determination and chromium(III and VI) and iron(II) are only major enhancers providing signals less than 1 % of that for copper(II).

This preliminary results imply that ultratrace amounts of copper(II) can be rapidly determined by flow injection method with fewer interferences than any other CL system. Further studies on the effect of concomitant species are in progress along with application to real samples.

Table 1 Selectivity of the FMN CL system

Species[a]	Relative molar signal	Species[a]	Relative molar signal
Cu^{2+}	1000	$S_2O_3^{2-}$	0.2
$Cr_2O_7^{2-}$	9	CN^-	0.2
Fe^{2+}	8	MnO_4^-	0.1
Cr^{3+}	5	EDTA	0.1
S^{2-}	1	Mn^{2+}	0.09
Fe^{3+}	0.7	Mo^{6+}	0.07
Co^{2+}	0.6	$C_2O_4^{2-}$	0.07
SCN^-	0.5	citrate	0.07
Pb^{2+}	0.3		

[a] 10^{-3}M soln. of chloride or nitrate, or sodium or potassium salt except ammonium sulfate for Fe^{2+} and ammonium molybdate for Mo^{6+}, and 10^{-5}M soln. of nitrate for Cu^{2+}. Mg^{2+},Ca^{2+},Ba^{2+},Al^{3+},Sn^{2+},Ni^{2+},Ag^+,Zn^{2+},Cd^{2+} Hg^{2+},As^{5+},NH_4^+,NH_3,F^-,Cl^-,Br^-,I^-,CO_3^{2-},SO_3^{2-} SO_4^{2-}, $S_2O_8^{2-}$,NO_2^-,NO_3^-,$Fe(CN)_6^{3-}$,IO_3^-,ClO_4^-,$B_4O_7^{3-}$ acetate, and urea gave rise to no emissions.

References

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