

[Chem. Pharm. Bull.]
29(12)3763-3764(1981)

Fluorometric Determination of Cyanide and Thiocyanate by König Reaction

A fluorometric method for the determination of cyanide and thiocyanate was developed according to König reaction, which is generally used for their colorimetric determination.

Keywords—cyanide; thiocyanate; fluorometry; chloramine T; pyridine; barbituric acid; König reaction

Numerous colorimetric methods for the determination of cyanide and thiocyanate based on König reaction have been reported.¹⁻³⁾ There are also the fluorometric methods for the determination of cyanide with *p*-benzoquinone,⁴⁾ nicotinamide⁵⁾ and pyridoxal.⁶⁾

During the study on the colorimetric method based on König reaction with pyridine, barbituric acid and chloramine T as reagents, we found that the product formed from cyanide and thiocyanate was an intensely fluorescent compound. The excitation and emission maximum wavelength of the fluorescence from this compound are at around 583 nm and 607 nm, respectively (Fig. 1).

This paper describes a new fluorometric method for the specific and sensitive determination of cyanide and thiocyanate. A recommended procedure for general use is as follows:

0.1% Chloramine T solution; dissolve 0.1 g of chloramine T in 100 ml of redistilled water.

Pyridine-barbituric acid reagent; mix 30 ml of pyridine, 3 g of barbituric acid, 6 ml of concentrated hydrochloric acid and 164 ml of redistilled water.

Acetate buffer solution; 0.3 M acetate buffer adjusted to pH 5.0.

To 0.5 ml of an aqueous sample in a reaction tube with 10 ml capacity, add 2.0 ml of the acetate buffer and 0.5 ml of 0.1% chloramine T solution, and mix well. Within 1 min, add 0.5 ml of the pyridine-barbituric acid reagent, mix and 6 to 15 min later, measure the fluorescence intensities of the test solution and blank at Ex. 583 nm and Em. 607 nm. Prepare a reagent blank by treating 0.5 ml of H₂O in the same manner.

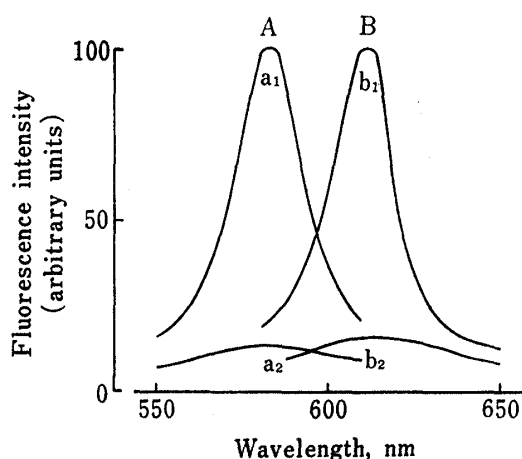


Fig. 1. Excitation and Emission Spectra of the Final Reaction Mixture of Cyanide

A: excitation spectra. B: emission spectra.
a₁ and b₁: a portion (0.5 ml) of 2.5 μM cyanide solution was treated by the standard procedure.
a₂ and b₂: reagent blank corresponding to a₁ and b₁

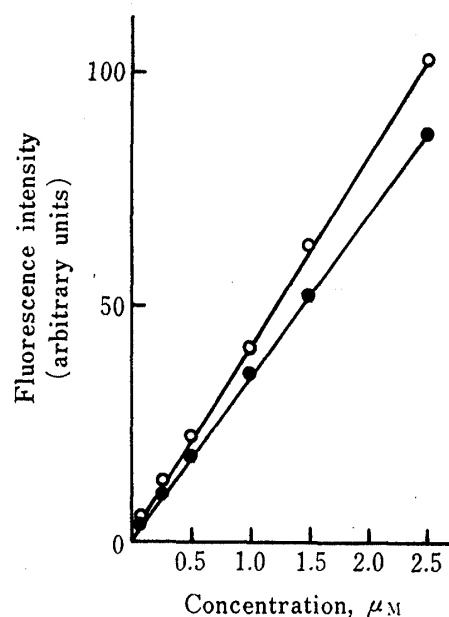


Fig. 2. Calibration Curves for Cyanide and Thiocyanate

—●—: cyanide. —○—: thiocyanate.

The calibration curves of cyanide and thiocyanate were linear in the range of 0.05 to 2.5 μM , respectively (Fig. 2).

Although this fluorometric method is the same procedure as that of the colorimetric method based on König reaction, in practical point of view, it is 10—20 fold more sensitive than the colorimetric methods.¹⁻³⁾ Therefore, it may be possible to use this method for the microdetermination of cyanide and thiocyanate in various kinds of samples. Application of the present method to biological materials is now under study. Moreover, it may be employed to the detection for high performance liquid chromatography. A more complete investigation will be reported in the near future.

References and Notes

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Received October 6, 1981