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# Fluorometric Determination of Cyanide by the Reaction with Pyridoxal

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Cyanide produces a fluorescent compound from pyridoxal (PAL) by a catalytic action. The velocity of the reaction could be measured by measuring the fluorescence intensity. For a concentration of cyanide below $10^{-5}$M, the apparent initial velocity of the reaction was maintained for 60 min at a concentration of $6\times10^{-4}$M of PAL, and the initial velocity was proportional to the concentration of cyanide. A simple and sensitive fluorometric method for the determination of cyanide was devised based on the above findings. Cyanide was treated with PAL at pH 7.5 and the fluorescence intensity was measured at pH 10.

It is known that pyridoxal (PAL) is oxidized with oxygen by a catalytic action of cyanide in slightly alkaline solution to 4-pyridoxolactone (PiAL) which is a highly fluorescent compound.[2] The velocity of the reaction was thought to be in proportion to the concentration of cyanide under a suitable condition and it was expected that cyanide might be determined by measuring the initial velocity of the reaction by means of fluorometry.

In the present work, factors affecting on the reaction velocity were studied, and a simple and sensitive method for the determination of cyanide was devised.

## Experimental

PAL and PiAL were synthesized from pyridoxine according to the method of Harris, *et al.*[3] KCN was the preparation of Junsei Chemical Co. The reaction of cyanide with PAL was carried out in a phosphate buffer (pH 7.5) at 50°. Colorimetric determination of cyanide was carried out according to BrCN–benzidine method.[4] Excitation and emission spectra, and fluorescence intensity were measured in a Hitachi fluorescence spectrophotometer MPF 2.

## Result

### Excitation and Emission Spectra of PAL and PiAL

Excitation and emission spectra of $5\times10^{-4}$M PAL and of the reaction product of $10^{-6}$M PAL with KCN, that is PiAL, were measured at pH 10. As shown in Fig. 1, both excitation and emission maximum of PAL were in shorter wavelength than those of PiAL, and the fluorescence intensity of PiAL was much stronger than that of PAL. So PAL was thought not to interfere the fluorometric determination.

### Relation between PAL Concentration and Reaction Velocity

PAL in various concentrations as specified in Fig. 2 was heated with $10^{-5}$M KCN in 0.2M phosphate buffer (pH 7.5) at 50° for 60 min. After cooling, 1 ml of 0.2M $Na_2CO_3$ was added

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[2] V. Bonavita, *Arch. Biochem. Biophys.*, **88**, 366 (1960); S. Takanashi, Z. Tamura, A. Yoshino and Y. Iidaka, *Chem. Pharm. Bull.* (Tokyo), **16**, 758 (1968); N. Oishi and S. Fukui, *Arch. Biochem. Biophys.*, **128**, 606 (1968).

[3] S.A. Harris, D. Heyl and K. Folker, *J. Am. Chem. Soc.*, **66**, 2088 (1944).

[4] F.D. Snell and C.T. Snell, "Colorimetric Methods of Analysis," 3rd ed., Vol. 2, D. Van Nostrand Co., New York, N.Y., 1958, p. 863.

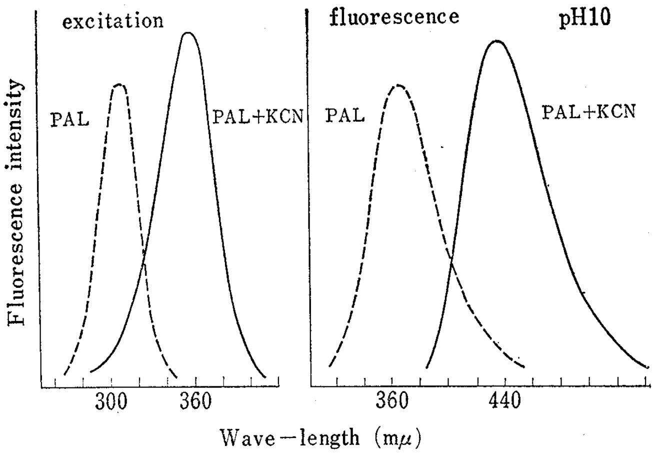

Fig. 1. Excitation and Emission Spectra of PAL and PiAL

------: PAL ($5\times10^{-4}$M) in 0.2M phosphate buffer (pH 7.5) was adjusted to pH 10.
——: A mixture of $10^{-6}$M PAL and $10^{-5}$M KCN in 0.2M phosphate buffer (pH 7.5) was heated at 50° for 60 min and then adjusted to pH 10.

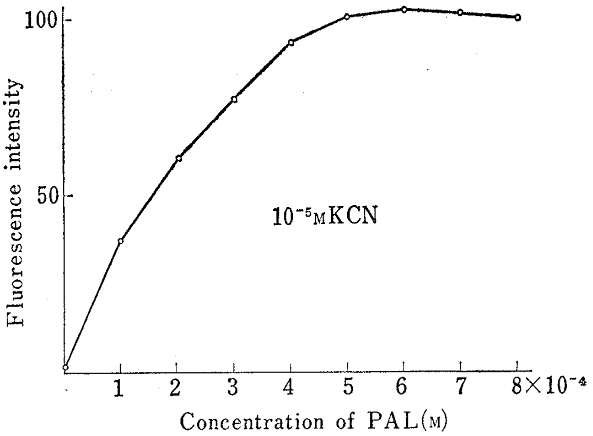

Fig. 2. Relation between PAL Concentration and Reaction Velocity

PAL was heated with $10^{-5}$M KCN in phosphate buffer (pH 7.5) at 50° for 60 min and the mixture was adjusted to pH 10.

to each 5 ml of the reaction mixtures to adjust pH 10 and fluorescence intensity at 432 mμ, excited at 356 mμ, was measured. The fluorescence intensity, that is the apparent reaction velocity, was almost constant at a concentration over $5\times10^{-4}$M of PAL. But a decrease of the fluorescence intensity was observed over $8\times10^{-4}$M. The optimal concentration of PAL was $6\times10^{-4}$M.

### Relation between Reaction Time and Reaction Velocity

Aliquots of the reaction mixtures of $6\times10^{-4}$M PAL with $10^{-5}$M or $10^{-6}$M KCN were taken at intervals of 15 min and the fluorescence intensity was measured at pH 10. The reaction velocity was constant till 60 min after the reaction was initiated (Fig. 3). Hence it is necessary to stop the reaction within 60 min to measure the initial velocity.

### Calibration Curves for Cyanide

KCN in various concentration as specified in Fig. 4 was made to react upon $6\times10^{-4}$M PAL for 60 min and the fluorescence intensity was measured at pH 10. As shown in Fig. 4, the calibration curves showed linearity from 1 to 50 mμ mole of KCN.

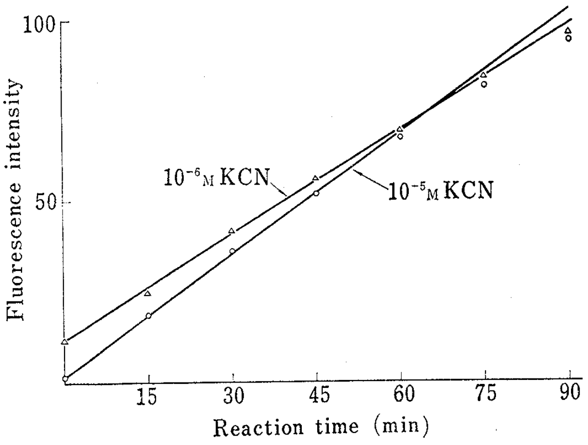

Fig. 3. Relation between Reaction Time and Reaction Velocity

PAL ($6\times10^{-4}$M) was heated with $10^{-5}$M (—○—) or $10^{-6}$M (—△—) KCN in 0.2M phosphate buffer (pH 7.5) at 50° and the mixtures were adjusted to pH 10.

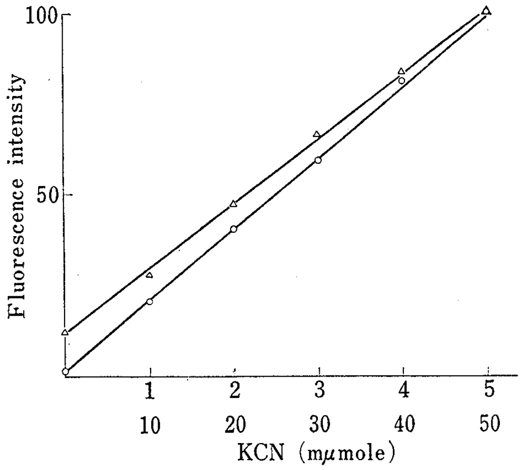

Fig. 4. Calibration Curves for Cyanide

Mixtures of KCN and $6\times10^{-4}$ M PAL in 0.2M phosphate buffer (pH 7.5) were heated at 50° for 60 min and then adjusted to pH 10.
—△—: 1—5 mμ mole, —○—: 10—50 mμ mole

## Method of Determination

**Reagent** Phosphate buffer, 0.2M, pH 7.5

$Na_2CO_3$ solution, 0.2M

PAL solution, $6 \times 10^{-3}$M; PAL hydrochloride (122.4 mg) is dissolved in 100 ml of water and the solution is stored below 5° with protection from light.

Standard KCN; KCN is dried to constant weight and tested for its purity by titration with $AgNO_3$ according to the Liebig–Deniges method.

**Procedure** One ml of a sample is taken in a brown test tube. For a blank test, another aliquot of the sample is acidified with acetic acid (pH 6), through which air is passed for 20 min, and 1 ml of the solution is taken. A sample, which is coloured or contains some interfering substances, is acidified and nitrogen gas is passed through the solution for 20 min. The gas including HCN is then led into two 5 ml portions of 0.1N NaOH successively and HCN is collected in them. The cyanides in NaOH solutions are combined and 1 ml of the solution is taken. In this case, 1 ml of 0.1N NaOH is used as a blank. Besides these solutions, 1 ml of the standard KCN solution and water for a standard blank are taken in test tubes respectively. To each test tube are added 3.5 ml of the phosphate buffer and 0.5 ml of the PAL solution. All the test tubes are heated at 50° for 60 min. After cooling, 1 ml of the $Na_2CO_3$ solution is added to each test tube and the fluorescence intensity is measured at 356 mμ for excitation and at 432 mμ for emission. The value obtained from the sample is compared with that from the standard.

## Discussion

Though PAL itself a weakly fluorescent compound, the excitation and emission maximum of PAL were found to differ from those of PiAL. When PAL concentration was raised from $10^{-4}$M to $8 \times 10^{-4}$M, no increase of blank fluorescence was observed in the fluorometry of PiAL. Therefore the fluorometry is not interfered by PAL.

It has been found that hydrogen peroxide is formed simultaneously during the reaction of PAL with cyanide.2) The oxidation of cyanide by hydrogen peroxide was thought to be negligible under the condition of the present method. But the decrease of the reaction velocity at higher concentration of PAL or at longer reaction time might be caused by the oxidative effect or decrease of oxygen.

In the present method, blank fluorescence reached to 50% when 0.7 mμmole of cyanide was determined. On the other hand, 7 mμmole of cyanide corresponded to 0.08 of optical density in the colorimetric method by BrCN–benzidine. These values were thought to be limit of determination respectively, hence the fluorometric method is about 10 times as sensitive as the colorimetry, and the former is much simpler than the latter.