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Electrochemical Derivatization of Thiamine in a Flow Injection System—Application to Thiamine Analysis

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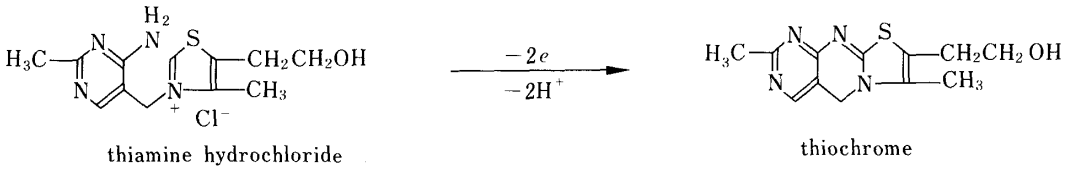
A novel technique using on-line electrochemical derivatization of thiamine is described which can enhance considerably both the sensitivity and specificity of detection. Material injected is electrolyzed in a flow electrolytic cell which may induce a reaction yielding highly fluorescent or ultraviolet (UV)-absorbing products. The electrolyzed eluent then passes into a suitable detector.

An example of the use of this technique is in the detection of thiamine which was found to form a highly fluorescent product, thiochrome, by electrochemical oxidation in the presence of sodium hydroxide. In the present investigation, we used such a flow injection method to determine thiamine content specifically in some pharmaceuticals.

Keywords——thiamine electrochemical derivatization; thiamine fluorescence; flow injection; thiochrome

Specific and highly sensitive detection methods using various chemical derivatization reagents have been developed for use in high performance liquid chromatography (HPLC).[1] Recently, derivatization techniques utilizing enzymatic[2] or photochemical[3] reactions have also been reported. However, little work has been done on electrochemical derivatization systems.

It is well known that thiamine (vitamin B_1) is converted to a highly fluorescent product, thiochrome, by chemical oxidation using cyanogen bromide or potassium ferricyanide in the presence of sodium hydroxide (Fig. 1), and several methods have been reported for measuring thiamine based on this reaction.[4] However, these methods require a considerable amount of cyanogen bromide or potassium ferricyanide, which present disposal problems. The methods are also complicated and time-consuming.



thiamine hydrochloride — thiochrome

Fig. 1. A Possible Oxidation Reaction of Thiamine

During the course of an investigation of the electrochemical properties of thiamine, it was found that thiamine was converted to a highly fluorescent product, thiochrome, by electrochemical oxidation. The present investigation was undertaken to develop a simple and specific flow injection analysis (FI) for thiamine using the electrochemical oxidation reaction.

Experimental

Chemicals and Reagents——Thiamine hydrochloride was obtained from Wako Pure Chem. (Osaka, Japan).

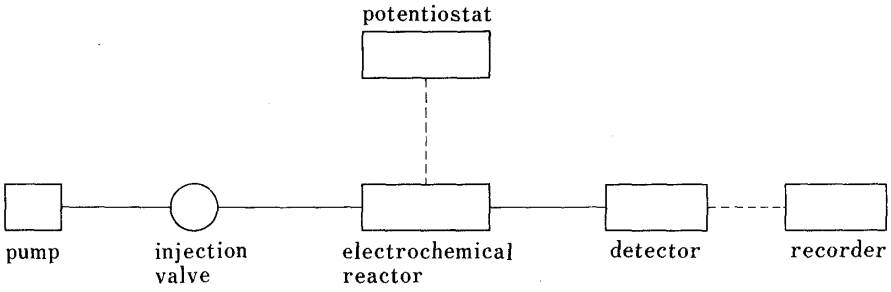


Fig. 2. Instrumental Setup for FI with an Electrochemical Reaction Detector

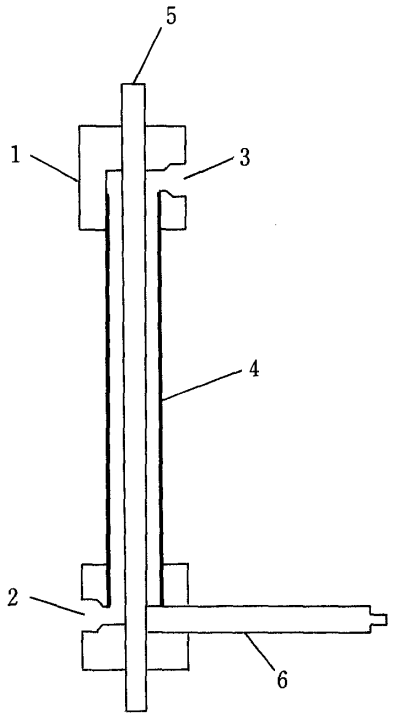


Fig. 3. Schematic Diagram of the Electrochemical Reactor (ECR)

1) Teflon blocks, 2) inlet, 3) outlet, 4) glassy carbon working electrode, 5) glassy carbon auxiliary electrode, 6) Ag/AgCl reference electrode.

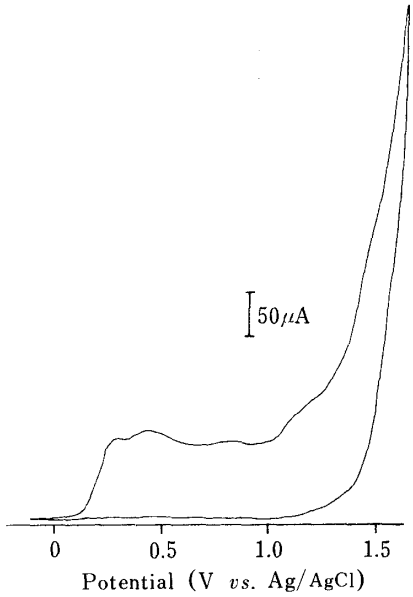


Fig. 4. Cyclic Voltammogram of Thiamine (10 mM)

In methanol containing 1% sodium hydroxide; glassy carbon anode (area = 0.071 cm^2); voltage sweep rate, 50 mV/s.

Sodium hydroxide and sodium perchlorate were of analytical grade (Kanto Chemical Co., Inc., Tokyo, Japan). Methanol was of HPLC grade (Wako Pure Chem., Osaka, Japan). Water used was distilled and deionized. A standard solution of thiamine was prepared by dissolving it in methanol at 10 μg/ml.

Flow Injection System—— Figure 2 shows the flow diagram of the analytical system. The system consists of a HPLC pump (110A, Altex, Berkeley, CA, U.S.A.), an injector with a 100 μl sample loop (Rheodyne, Berkeley, CA, U.S.A.), an electrochemical reactor, a potentiostat (HA-101, Hokuto Denko, Tokyo, Japan), a ultraviolet (UV) detector (UVIDEC-100-II, JASCO, Tokyo, Japan), a fluorescence detector (FP-110, JASCO, Tokyo, Japan), and a dual pen recorder (U-228, Nippon Denshi Kagaku, Kyoto, Japan). The UV detector was set at 254 nm, and the fluorescence detector was set at a 375 nm excitation wavelength and a 430 nm emission wavelength.

Electrochemical Reactor (ECR)——The ECR (Fig. 3) consisted of a glassy carbon pipe (10 cm × 0.5 cm i.d.) as a working electrode and a glassy carbon rod (15 cm × 0.3 cm o.d.) as a counter electrode (Tokai Carbon, Tokyo, Japan). Both ends of the pipe were fitted with Teflon blocks. An Ag–AgCl reference electrode (Yanaco, Kyoto, Japan) was inserted into one of the blocks. The pipe is connected to the anode and the rod to the cathode, respectively. The reference electrode is connected to a reference terminal of the potentiostat.

Results and Discussion

A cyclic voltammogram of thiamine is shown in Fig. 4. The oxidation wave of thiamine

was found to be +0.4 V *vs.* Ag/AgCl at a glassy carbon electrode. The result indicates that thiamine is oxidized at this potential.

The absorbance and fluorescence spectra of the electrochemical oxidation product of thiamine gave a 375 nm maximum absorbance wavelength and a 430 nm emission wavelength, which is in good agreement with those of thiochrome. The thin layer chromatogram of the electrolyzed product also supported the conversion of thiamine to thiochrome. These results clearly demonstrate that thiamine is converted to thiochrome through the electrochemical reaction.

This electrochemical oxidation reaction of thiamine was applied to an FI analytical system. The ECR was connected to the system. If thiamine is electrochemically oxidized while passing through the ECR, the product, thiochrome, can be detected by a fluorescence detector. Figure 5 shows sample peaks using UV and fluorescence detectors (connected in series). When no potential was applied to the ECR, only a UV detector signal was found. On

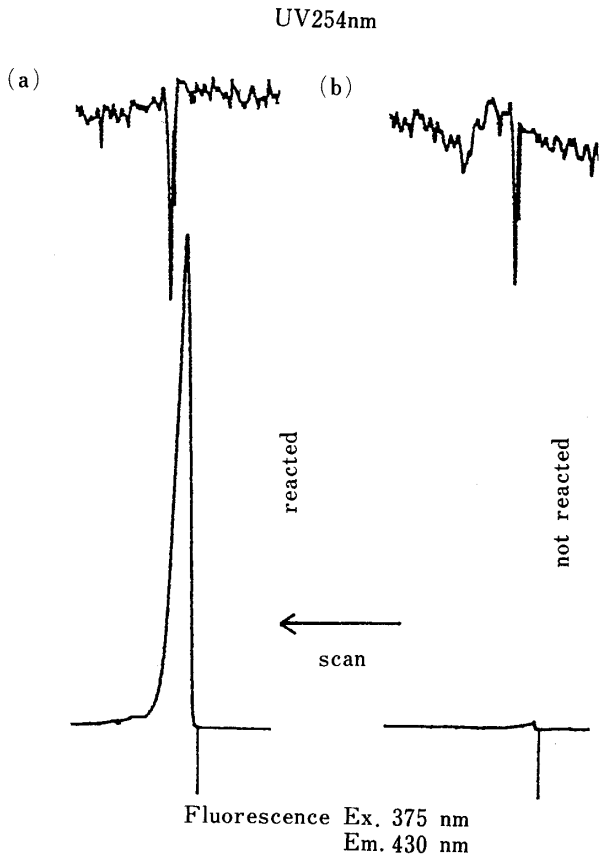


Fig. 5. Electrochemical Detection of 10 ng of Thiamine

(a) with potential applied to the ECR, (b) without potential applied to the ECR.

Conditions: mobile phase, 90% methanol–water (0.05 M $NaClO_4$) containing 1% sodium hydroxide; flow rate, 0.5 ml/min; applied potential, +0.4 V *vs.* Ag/AgCl; detection, UV detector set at 254 nm (upper) and fluorescence detector set at Ex. 375 nm, Em. 430 nm (lower).

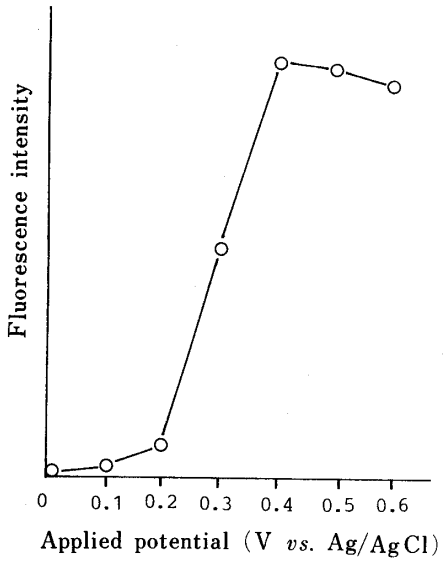


Fig. 6. Effect of Applied Potential to the ECR

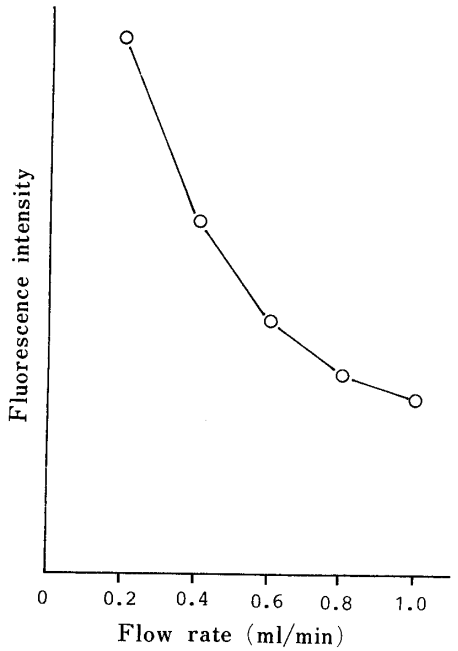


Fig. 7. Effect of Flow Rate

the other hand, when the potential was applied, a fluorescence detector signal also appeared.

The relationship between the applied potential to the ECR and the intensity of the fluorescence signal is shown in Fig. 6. The fluorescence signal appeared at +0.3 V and was found to be maximum at +0.4 V. This value coincides with the oxidation potential of thiamine determined from the cyclic voltammogram. Below this potential, no fluorescence signal was found, because electrochemical reaction of thiamine did not occur at such potential. On the contrary the signal decreased gradually at higher potential. The decrease may indicate that the product is reoxidized, or the hydroxide ion is also oxidized and a duplicate reaction occurs, at a higher potential than +0.4 V. From these results, it was concluded that the most suitable potential for conversion of thiamine to thiochrome was +0.4 V *vs.* Ag/AgCl.

Figure 7 shows the relationship between the flow rate and the intensity of the fluorescence signal. The signal became stronger as the flow rate decreased. This indicates that the intensity of the fluorescence signal depends on the reaction time in the ECR. On the basis of the analytical time and the broadening of the signal, a flow rate of 0.5 ml/min was selected under these conditions.

For the conversion of thiamine to thiochrome by chemical oxidation, a high concentration such as 15% sodium hydroxide is required. Under the conditions of electrochemical oxidation of thiamine, sodium hydroxide is also needed. The relationship between the sodium hydroxide concentration and the intensity of the fluorescence signal is shown in Fig. 8. In the absence of sodium hydroxide, the peak signal is not observed. In the presence of sodium hydroxide, the peak signal increases depending on the concentration of sodium hydroxide, but the concentration of sodium hydroxide required in the ECR method was not as high as that in the chemical oxidation method. Since a higher concentration of sodium hydroxide was not necessary to the detection system, 1% sodium hydroxide was used.

Figure 9 shows the relationship between water content in the mobile phase and the intensity of the fluorescence signal. The fluorescence signal reached maximum at 10% water content in methanol. The signal intensity decreased as the water content increased and the peak was not detected at 100% water. This is because the fluorescence intensity of thiochrome in water is as low as about one-third of that in methanol; it is also possible that the electrochemical oxidation reaction of thiamine to thiochrome does not proceed in water.

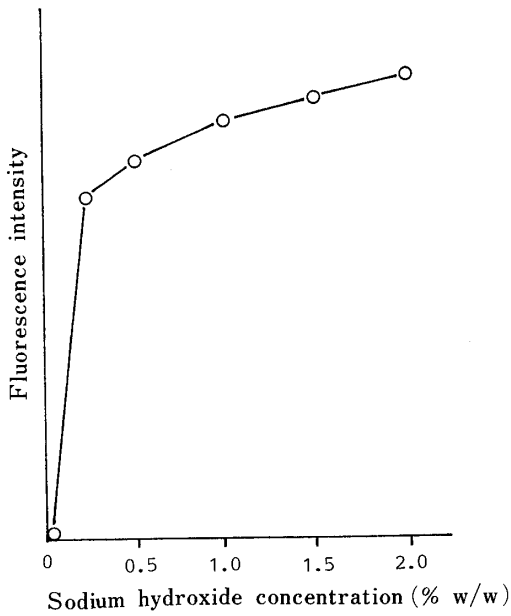


Fig. 8. Effect of Sodium Hydroxide Content in the Mobile Phase

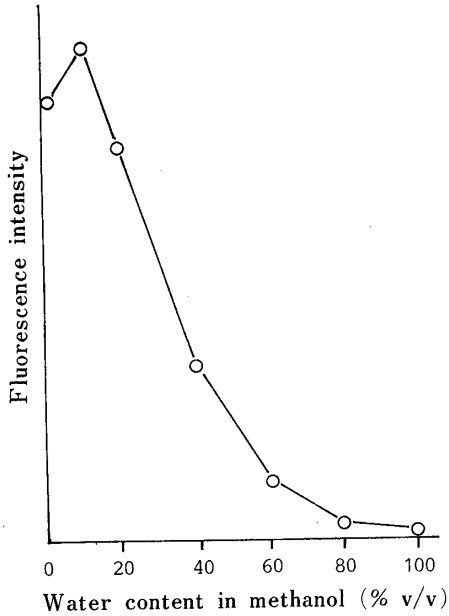


Fig. 9. Effect of Water Content in the Mobile Phase

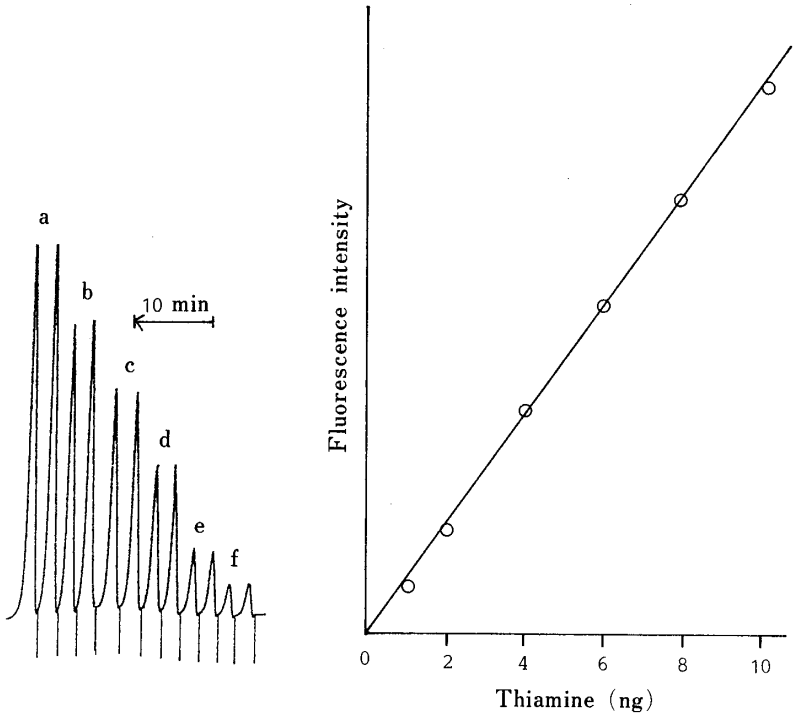


Fig. 10. Continuous Flow Injection Profile for Thiamine (in Amounts of 10, 8, 6, 4, 2, and 1 ng) and the Calibration Curve

TABLE I. Comparison between the Conventional Fluorimetric Method, the HPLC Method, and the ECR–Fluorescence Method for Determination of Thiamine in Some Pharmaceutical Preparations

Multivitamin sample	Thiamine content (mg)			
	Label	Conventional	HPLC	ECR
A Capsule	10/cap	10.90	10.91	11.27
B Capsule	10/cap	10.89	10.85	11.04
C Tablet	5/tab	6.02	5.75	5.55
D Tablet	5/tab	5.85	5.97	5.80
E Syrup	1/ml	1.40	1.30	1.24
F Syrup	1/ml	1.24	1.31	1.33

Prescription.
Capsule: vitamins A, B_1, B_2, C, E.
Tablet: vitamins B_1, B_2, B_6, biotin, nicotinamide, calcium pantothenate.
Syrup: vitamins A, B_1, B_2, B_6, B_{12}, D_3, K_1, nicotinamide, panthenol, arginine glutamate.

The continuous FI profile and the calibration curve of thiamine are shown in Fig. 10. The calibration curve showed good linearity in the concentration range of 1—100 ng of thiamine and the coefficient of variation of the peak height was 1.2% for 10 ng of thiamine. From these results, this ECR–fluorescence method was found to be effective for the quantitation of thiamine.

Table I shows the values obtained for several different vitamin preparations by the conventional fluorimetric method,[5] HPLC method,[6] and our ECR–fluorescence method. The values determined by the ECR–fluorescence method were in good agreement with those of the other two methods. This result indicates that this ECR method specifically detected thiamine without interference by other pharmaceuticals. In addition, our method is advantageous in

that direct injection of thiamine is possible, and no column separation step is necessary.

The present investigation has demonstrated that the ECR–fluorescence method permits simple, rapid, and specific analysis of thiamine. By this method, as little as 1 ng of thiamine could be detected within three minutes per sample. Further improvements of the ECR may both increase the sensitivity and reduce the analysis time.

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