Journal of Health Science, **46**(2) 146—148 (2000)

Determination of Acetaldehyde Using Immobilized Aldehyde Dehydrogenase in a Flow System and Application to Analysis of Acetaldehyde Content in Liquors

Hisakazu Mori*

Kyoritsu College of Pharmacy, 1–5–30, Shibakoen, Minato-ku, Tokyo 105–8512, Japan

(Received September 14, 1999; Accepted November 28, 1999)

The quantity of acetaldehyde was determined using an apparatus containing a reactor with immobilized aldehyde dehydrogenase in a flow line. NADH formed by an enzymatic reaction was fluorometrically detected. The optimal concentration of NAD^+ in the carrier was determined. Various buffer types were examined as a carrier medium. When the pH of the carrier was 7.8, great peak areas due to NADH were observed for buffers of phosphate, pyrophosphate, HEPES, PIPES–(piperazine-*N*,*N*'-bis(2-ethanesulfonic acid)) and triethanolamine, compared with that for Tris buffer. In the pH range from 7.0 to 8.0, the peak area due to NADH increased with the increase of pH in the case of phosphate buffer, in contrast to the case of Tris buffer in which peak area decreased with the increase of pH. When the carrier composed of phosphate buffer (0.1 M, pH 7.8) was used, the calibration curve for acetaldehyde was linear in the range of 0.2—10 μM (r=0.9994). Detection limit (S/N=3) was 0.1 μM. Relative standard deviation of peak area at 2 μM was 2.6 % (n=7). The sampling rate was 40 samples h^{-1}. This method was applied to the analysis of aldehyde in several liquors, and aldehyde content determined by the method agreed with that determined by a commercially available test-kit method.

Key words —— acetaldehyde, aldehyde dehydrogenase, immobilized enzyme, flow injection analysis, liquor

*To whom correspondence should be addressed: Kyoritsu College of Pharmacy, 1–5–30, Shibakoen, Minato-ku, Tokyo 105–8512, Japan. Tel.: +81-3-5400-2671; Fax: +81-3-3434-5343; E-mail: mori-hs@kyoritsu-ph.ac.jp

INTRODUCTION

Immobilized enzymes have been used as an enzyme reactor in a flow system or as an enzyme electrode. We earlier applied immobilized enzymes in reactors to determine components in drinks: glucose,[1] sucrose,[2] both glucose and sucrose,[3] and both D- and L-lactic acid.[4]

In the present work, the determination of acetaldehyde was studied using immobilized aldehyde dehydrogenase(AlDH) in a flow system. Acetaldehyde is an intermediate in ethanol fermentation, and its content in liquor may affect the taste or flavor. Thus, the present method was applied to determine the content of acetaldehyde in liquors. The enzymatic reaction utilized is shown below; NADH formed by this reaction was detected fluorometrically.

$$CH_3CHO + NAD^+ + H_2O \xrightarrow{AlDH} CH_3COOH + NADH + H^+ \quad (1)$$

MATERIALS AND METHODS

Materials —— AlDH from yeast was purchased from Boehringer Mannhein (Germany), and NAD^+, 25% aqueous solution of glutaraldehyde and aminopropyl glass (500 Å pore size, 200—400 mesh), from Sigma Chemical Co. (U.S.A.).

Enzyme Immobilization —— AlDH was immobilized as described below. To aminopropyl glass beads (0.4 g) in 3.6 ml of 0.1 M phosphate buffer (pH 10.0) was added 0.4 ml of a 25% aqueous solution of glutaraldehyde. The mixture was bubbled with N_2 gas for 1 h at room temperature. After the activated aminopropyl glass was filtered and washed with 100 ml water, 3.2 ml of 0.05 M phosphate buffer (pH 6.0) and 20 units of AlDH were added. The mixture was stirred with a shaker for 20 h at 4℃, then BSA was added to become 1%(w/v) and it was shaken for 4 h. Enzyme-immobilized glass beads were filtered and washed with 100 ml of 0.05 M phosphate buffer (pH 6.0) and then with 100 ml of water. They were stored in 3.2 ml of 0.05 M phosphate buffer (pH 6.0). Three percent of the activity of the AlDH initially applied was found in immobilized enzyme. The enzyme-immobilized glass beads thus prepared were packed into a stainless steel column (8 cm, i.d. 2 mm).

Apparatus —— A schematic diagram of the apparatus used in the present study is shown in Fig. 1. The enzyme reactor(ER) containing immobilized AlDH

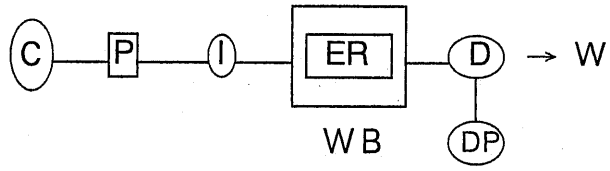

Fig. 1. Apparatus for Determination of Acetaldehyde

C, carrier reservoir; P, pump; I, sample injector; ER, enzyme reactor; D, spectrofluorometer; DP, data processor; WB, water bath; W, waste.

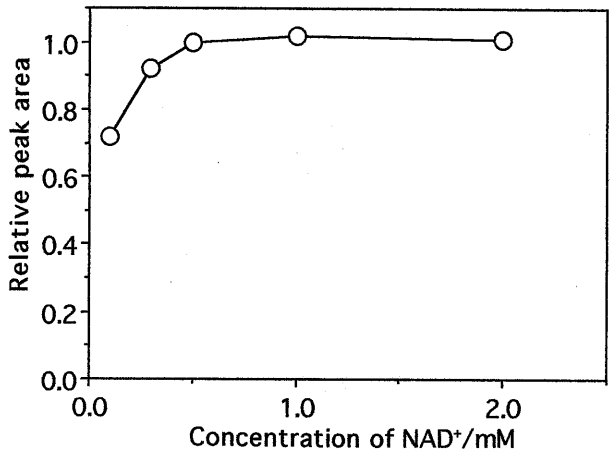

Fig. 2. Dependence of Peak Area upon NAD^+ Concentration in Carrier

Ordinate indicates the peak area relative to that at 0.5 mM NAD^+. Values were obtained from the average of triplicate determinations. Carrier used was 0.1 M Tris buffer (pH 7.8) containing NAD^+, 0.1 M KCl and 10 mM 2-mercaptoethanol. Flow rate of the carrier was 0.5 ml min^{-1}. Temperature of the water bath in which ER was immersed was 30℃. Concentration of aldehyde injected was 50 μM.

was immersed in a water bath (WB) thermostated at 30℃. The carrier used was 0.1 M sodium phosphate buffer(pH 7.8) containing 0.5 mM NAD^+, 0.1 M KCl and 10 mM 2-mercaptoethanol, and this was delivered at a rate of 0.5 ml min^{-1} by a Shimadzu LC-10AD pump (P). NADH formed by the enzymatic reaction was fluorometrically detected (λ_{ex}, 340 nm; λ_{em}, 460 nm) by a Hitachi F-1050 spectrofluorometer (D) with a 12 μl flow cell. The peak area was obtained by a Hitachi D-2500 data processor (DP). The sample injection volume was 50 μl.

RESULTS AND DISCUSSION

Carrier solution contains potassium ion(KCl), 2-mercaptoethanol and NAD^+. Potassium ion is needed[5] for the activation of AlDH. The effect of the concentration of NAD^+ in the carrier upon the peak area due to NADH was

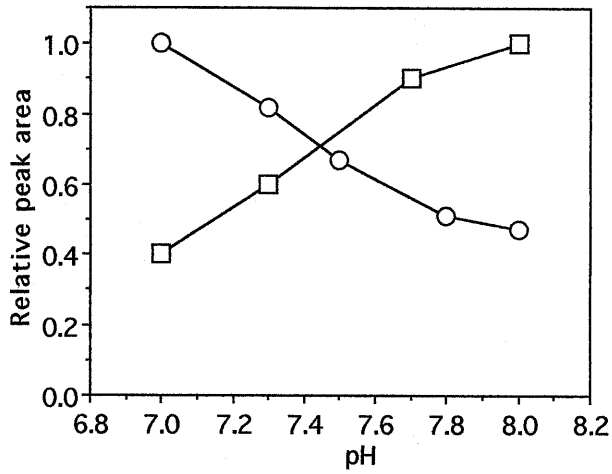

Fig. 3. Dependence of Peak Area upon pH of Carrier

—○—, carrier composed of Tris buffer; —□—, carrier composed of phosphate buffer. Ordinate indicates the peak area relative to that at pH 7.0 for Tris buffer and that at pH 8.0 for phosphate buffer. Values were obtained from the average of triplicate determinations. Carrier used contained 0.5 mM NAD^+, 0.1 M KCl and 10 mM 2-mercaptoethanol. Other conditions were the same as those described in the legend of Fig. 2.

Table 1. The Effect of Buffer Type (0.1 M, pH 7.8) as a Carrier Medium upon the Peak Area due to NADH

Buffer	Relative peak area
Tris	1
Phosphate	6.7
Pyrophosphate	6.1
HEPES	6.5
PIPES	6.0
Triethanolamine	6.8

Values are the average of triplicate determinations. Carrier contains 0.5 mM NAD^+, 0.1 M KCl and 10 mM 2-mercaptoethanol. Other conditions were the same as described in the legend of Fig. 2.

examined. As shown in Fig. 2, NAD^+ concentrations of more than 0.5 mM afforded an almost constant peak area. Consequently, a concentration of 0.5 mM was used in subsequent experiments.

Table 1 shows the effect of buffer type (0.1 M, pH 7.8) as a carrier medium upon the peak area of NADH. All buffers other than Tris buffer afforded great peak areas. These results were in contrast to that in the case of soluble AlDH; the activity in Tris buffer (0.1 M, pH 7.8) was 1.7 times higher than that in phosphate buffer (0.1 M, pH 7.8). In Fig. 3 are shown the dependence of the peak area upon pH in Tris buffer and phosphate buffer. Peak area increased with the increase of pH in the case of phosphate buffer, as was

Table 2. Aldehyde Content in Some Liquors (mM)

	Proposed method	F-kit
Wine(white)	1.3	1.4
Wine(red)	1.1	—a)
Sake1	0.37	0.35
Sake2	0.36	0.34
Shōchu	0.01	—b)

Values are average of triplicate determinations. The samples for analysis were prepared by diluting original liquor with the carrier 1000 times for wine, 200 times for sake and 50 times for shōchu.

a) Accurate measurement could not be made due to a color change observed when wine(red) was mixed with F-kit buffer.

b) Accurate measurement could not be made due to the small quantity of acetaldehyde contained.

expected from the reaction (1), while the peak area decreased with the increase of pH in the Tris buffer. However, with respect to soluble AlDH in Tris buffer, the activity at pH 8.0 was 3.6 times higher than that at pH 7.0. Thus, immobilized AlDH showed unusual character in Tris buffer. The reason for this effect is unclear.

Phosphate buffer (0.1 M) was used as the carrier medium for the following experiments. The pH selected as that of the carrier was 7.8, being not as alkaline for the maintenance of the stability of the immobilized enzyme. The calibration curve obtained under the condition was linear ($r=0.9994$) in the acetaldehyde concentration range from 0.2 to 10 μM. The detection limit ($S/N=3$) was 0.1μM, and the relative standard deviation of the peak area at 2μM was 2.6% ($n=7$). The sampling rate calculated from the peak width at the base line was 40 samples h^{-1}.

The present method was applied to analysis of the content of acetaldehyde in liquors. Table 2 lists the results of analysis, compared with those obtained by a commercially available test kit (F-kit, Boehringer Mannheim), and showed good agreement. As the present method has high sensitivity, it could be applied to the sample which could not be analyzed by the F-kit method.

The peak area for 7 μM acetaldehyde was almost constant during the analysis of 50 samples including standard samples for a calibration curve. After storage of the enzyme reactor for 90 d at 4℃ in the carrier(pH 7.8) composed of Tris buffer, the activity of the enzyme in the reactor dropped to 54% of its initial activity.

REFERENCES

1) Mori H., Ishida M., Okamoto S., *J. Health Sci.*, **45**, 126—129 (1999).
2) Kogure M., Mori H., Ariki H., Kojima C., Yamamoto H., *Anal. Chim. Acta*, **337**, 107—111 (1997).
3) Mori H., Kogure M., Kuroda Y., Nissato M., Tamura Y., Watanabe N. and Yamamoto H., *Anal. Lett.*, **32**, 1531—1541 (1999).
4) Mori H., *Anal. Lett.*, **32**, 1301—1312 (1999).
5) Black S., *Meth. Enzymol.*, **1**, 508—511 (1955).