Application of a Flow Injection System in Wine Analysis

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A flow injection system with an immobilized lactate dehydrogenase enzyme reactor was constructed and characterized. The system was successfully used to determine the free lactic acid content of Hungarian red and white wines.

INTRODUCTION

The specificity of the molecular recognition–transformation process has led to the widespread use of enzymes as analytical reagents in food analysis. Enzymes in immobilized form can be employed repeatedly or continuously. Much attention has recently been devoted to the determination of biologically important substances and food components through the use of biospecific sensors or analyzers based on minireactors (Lowe, 1985; Ho, 1988). Flow methods, such as flow injection analysis, can readily be automated, and they have therefore become very important in the past decade (Carr et al., 1980; Ruzicka and Hansen, 1981; Lundback and Olsson, 1985). Bioluminescent or chemiluminescent detection of the reactions has promoted practical applications (Wienhausen and Deluca, 1982; Kurkijarvi et al., 1982; Kricka and Thorpe, 1986).

The present paper reports the application of a flow injection system using immobilized lactate dehydrogenase for determination of the lactic acid content of wines.

MATERIALS AND METHODS

Apparatus. An LKB 2238 Uvicord SII instrument (Bromma, Sweden) with a flow-through cell (10 μL) was used to monitor the increase in absorbance at 365 nm. The outlet tubings were made of Teflon (length 20 cm; i.d. 1.2 mm). The output signal was recorded with an OH-814/1 potentiometric recorder (Radelkis, Budapest, Hungary). The carrier stream was delivered by an LKB 2132 microperspex peristaltic pump. The sample volume was 100 μL.

Procedure. The lactate dehydrogenase activity was determined spectrophotometrically by following the increase in absorbance at 365 nm. In the activity assay of the soluble enzyme, the reaction mixture (3 mL) contained 810 mM glycine, 330 mM hydrazine hydrate, 2.7 mM NAD, and 4 mM lactate (Mattenheimer, 1970). The reaction was initiated by the addition of 100 μL of lactate dehydrogenase (1–1.5 units). The pH of the reaction mixture was 9.5. The rate of reaction was calculated from the appearance of NADH at 25 °C.

In the case of immobilized lactate dehydrogenase activity measurements, 50–100 mg of immobilized enzyme was suspended and swollen in 4 mL of glycine buffer containing the same components mentioned above and was stirred for an appropriate time at 25 °C. Then the enzyme was filtered off quickly (a few seconds), and the amount of NADH was determined spectrophotometrically in the filtrate.

Reagents. Pig muscle lactate dehydrogenase (L-lactate:NAD$^+$ oxidoreductase, EC 1.1.1.27) immobilized by covalent binding to a polyacrylamide bead support having carboxylic functional groups activated by water-soluble carbodiimide according to the method of Kotormán et al. (1986) was a commercial product of Reanal Laboratory Chemicals (Budapest, Hungary). The specific enzyme activity was 90 units/g of solid material. One unit of enzyme catalyzes the formation of 1 μmol of pyruvate from lactate per minute at 25 °C and pH 9.5. All other chemicals were Reanal products of reagent grade.

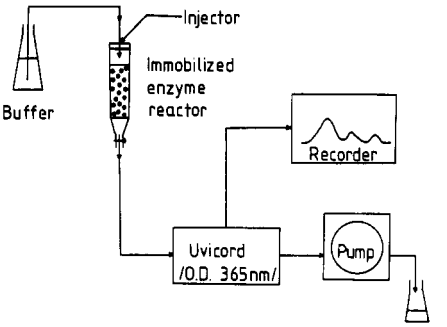

Figure 1. Schematic diagram of the flow injection system.

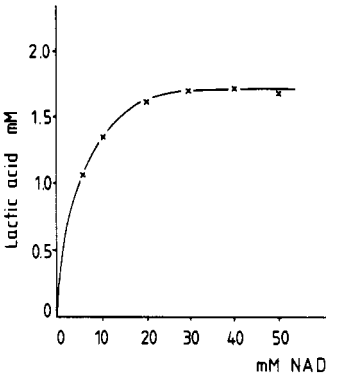

Figure 2. Effect of NAD$^+$ concentration on lactic acid conversion. Lactic acid was concentration 1.7 mM; flow rate was 100 mL/h.

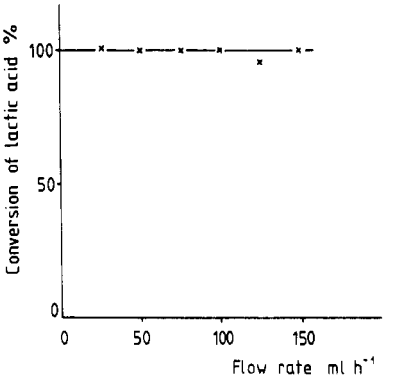

Figure 3. Effect of flow rate on lactic acid conversion. The carrier stream (1.0 M glycine/0.4 M hydrazine, pH 9.5) contained 1.7 mM lactic acid and 34.0 mM NAD$^+$.

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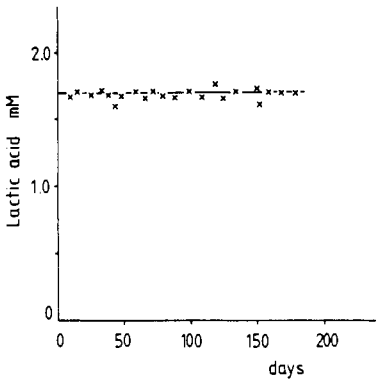

Figure 4. Operational stability of immobilized lactate dehydrogenase bioreactor (NAD^+/lactate ratio 20:1).

Table I. Determination of Lactic Acid Contents of Hungarian Wines

wine brands	lactic acid, mM	
	soluble LDH	immobilized LDH
Debröi	8.44 ± 0.31	8.48 ± 0.17
Egri muskotály	14.29 ± 0.15	14.15 ± 0.18
Pinot noir	15.01 ± 0.16	14.96 ± 0.06
Szürkebarát	15.05 ± 0.47	15.05 ± 0.13
Pusztamérgesi	15.73 ± 0.04	15.76 ± 0.01
Rajnai	15.90 ± 0.13	15.94 ± 0.22
Bikavér	16.36 ± 0.43	16.48 ± 0.11
Szegedi muskotály	20.09 ± 0.29	19.63 ± 0.07
Csongrádi cabernet	21.15 ± 0.25	21.26 ± 0.21

RESULTS AND DISCUSSION

The diagram of the flow injection system is shown in Figure 1. The most important component of the system is a column reactor with a bed volume of 4.5 mL (i.d. 0.9 cm), containing 14.1 units of immobilized lactate dehydrogenase. The equilibrium of the reaction catalyzed by lactate dehydrogenase at neutral pH is strongly in favor of lactate, but at alkaline pH in the presence of excess NAD and hydrazine the oxidation of lactate is complete (Mattenheimer, 1970). Accordingly, 1 M glycine/0.4 M hydrazine buffer (pH 9.5) was used as carrier stream. The dependence of the conversion of lactic acid to pyruvate on the NAD concentration in the reaction mixture is shown in Figure 2. It can be seen that 100% conversion requires an approximately 20-fold excess of NAD compared to lactate. In the range of 20–150 mL/h, the lactate conversion was independent of the flow rate (Figure 3). The operational stability of the enzyme reactor was tested under the above optimized conditions. Each day, 20 samples of a 1.7 mM solution of lactic acid were measured, and aliquots were withdrawn from the reactor to determine the residual activity. The immobilized lactate dehydrogenase proved to be very stable (Figure 4).

The flow injection system described here was used in wine analysis to determine free lactic acid in wines. Samples of Hungarian red and white wines were diluted 10–20-fold and were injected into the enzyme reactor. For the lactic acid a linear calibration curve could be produced up to 2.5 mM. The detection limit was 0.05 mM.

For comparison, the lactic acid content was measured with soluble lactate dehydrogenase, too. The results listed in Table I show the good agreement and reproducibility. The values are calculated as an average of 15 measurements for each wine brand.

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Registry No. Lactate dehydrogenase, 9001-60-9; lactic acid, 50-21-5.