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RAPID AMPEROMETRIC DETERMINATION OF (L+)–LACTATE USING IMMOBILIZED LACTATE OXIDASE

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ABSTRACT

The enzyme lactate oxidase was immobilized on a perioxide-sensing electrode for the rapid and repetitive determination of lactate in aqueous solution and in normal pooled human serum. The immobilized enzyme is extremely stable if stored at 0°C when the probe is not being used. Lactate concentrations as low as 2×10^{-7} M can be detected. The calibration curve is linear in the concentration range of 5×10^{-7} M to 3×10^{-4} M at ph 7.50 and the method described is selective for L (+) – lactate.

INTRODUCTION

The analysis of lactate is important in the food, beverage, and cosmetic industries. Its analysis in biological samples is also of importance. Enzymes have been used to an increasing extent in these analyses, in general, because of the selectivity, simplicity, comparable sensitivity, and relatively low cost of the enzymes used.

A number of the enzymatic methods have utilized lactate dehydrogenase which requires a coenzyme for its activity, while others have used lactate oxidase in conjunction with the Clark Oxygen Electrode. L-lactate and lactate dehydrogenase have been determined sequentially in the same reaction mixture (Mizutani et al., 1983). In another study the flow injection analysis technique was used for the analysis of L-lactate (Yao et. al., 1982). In enzyme analysis, substrate recycling results in high sensitivity and this has been used in conjunction with an enzyme thermistor to determine L-lactate (Scheller et. al., 1985). The use of biocatalysts other than purified enzymes has become quite popular in chemical analysis. A biosensor using permeabilized yeast for the determination of L-lactate has been reported (Vinke et al., 1985)

In this work, the enzyme lactate oxidase was used in conjunction with an amperometric electrode to detect hydrogen peroxide, a product of the enzyme-catalyzed oxidation of L(+)-lactate, and relate it to the concentration of L(+)-lactate in the sample solution via a calibration curve.

EXPERIMENTAL SECTION

Apparatus

All measurements of current were made using the YSI-Clark Model 2510 Oxidase Probe™ and the YSI Model 25 Oxidase Meter (Yellow Springs Instrument Co., Yellow Springs, OH). The Fisher Model 4512BF strip chart recorder was used to mark current traces. A Fisher Model 80 water bath circulator was used to control the temperature of a 50-ml double-jacketed glass sample cell at 25.0 ± 0.1° C.

Materials

Lactate oxidase (EC 1.1.3.X) from *Pediococcus* species, normal pooled human serum, and lithium lactate were purchased from Sigma Chemical Co., St. Louis, MO. Phosphate buffer solutions of 0.10 M concentrations were prepared in distilled deionized Millipore water. Only analytical reagents were used in the study. Collagen membranes were obtained from YSI and activated chemically according to established methods (Coulet et al., 1974) while cellulose acetate membranes were prepared according to established procedures.

Preparation of the Enzyme Probe

An ultrafine cellulose acetate membrane was first placed over the Oxidase Probe™. Then lactate oxidase (5 units) was pipetted onto the center of this membrane, over the circular platinum anode of the probe. The enzyme layer was then covered with a one-inch diameter of the chemically activated collagen membrane which was held in place with an "O" ring according to the manufacturer's instructions. The probe was then conditioned in a pH 7.50 phosphate buffer for about 2 hours or until a steady background current was obtained at the probe.

RESULTS AND DISCUSSION

The analysis of lactic acid using the enzyme lactate oxidase is based on the enzyme-catalyzed reaction shown below:

$$\text{Lactate} + O_2 + H_2O \xrightarrow[\text{(EC 1.1.3.X)}]{\text{lactate oxidase}} \text{Pyruvate} + H_2O_2$$

In the above reaction, the recently commercially available enzyme lactate oxidase (as yet unclassified by the Enzyme Commission), catalyzes the oxidation of lactate to pyruvate and hydrogen peroxide. The hydrogen peroxide is oxidized at the platinum anode of the Oxidase Probe™ producing a current that is proportional to the concentration of lactate in the sample solution.

The purpose of the cellulose acetate membrane is to minimize the effects of reducing agents such as ascorbic acid in the sample solution from reaching the probe. Previous studies have shown this membrane to be very effective at screening ascorbic acid

from interfering with the response of the amperometric probe (Srinivasan et al., 1983). Collagen membrane was used to immobilize the enzyme because it is a general purpose membrane with the distinct advantage of providing a texture to which exzymes can directly bond after its surface has been chemically activated.

The analysis time was significantly reduced as a result of the immobilization of the enzyme on a probe. In this case, the enzyme is highly concentrated and converts only the substrate which diffused through the membrane from the bulk solution. Once a stable background current was obtained at the probe, it was offset on the meter and a calibration curve could be obtained in less than 10 minutes.

The pH profile of immobilized lactate oxidase is shown in Figure 1, where maximum enzyme activity occurs at pH 7.50. Subsequent experiments were carried out at this pH value and all measurements were carried out at 25°C.

The calibration curve for L (+) – lactate (added as the lithium salt) is shown in Figure 2, where the logarithm of the molar concentration of L (+) – lactate is plotted against the logarithm of the current. The upper limit of the linear region of this curve was limited by the meter which measured currents up to 100 nA. The enzyme was selective only for the L (+) – isomer of lactic acid.

Precision studies were carried out using this enzyme probe, to ascertain its stability and reproducibility. The results are shown in Table I, where aqueous samples were studied. These results show that immobilized lactate oxidase is quite stable and it is suitable for the quantitation of lactate in aqueous samples. The probe was useful for a period of over 18 days before it was necessary to replace the enzyme layer.

The application of the immobilized enzyme in the analysis of L (+) – lactate in human serum was undertaken by carrying out recovery studies of lactate added to various volumes of diluted (1 + 20) normal pooled human serum. Sample volumes varied from 5 ml to 50 ml. The measured concentrations were compared to the true values as shown in Table II. The results of this study indicate that such a probe could be a useful tool for the rapid analysis of lactate in aqueous and serum samples. Samples more concentrated than 15 mg l^{-1} were diluted before analysis.

The method of analysis of lactate studied in this work is simple, convenient, fast, and reliable. It could therefore be adapted for the routine analysis of lactate in the clinical laboratory.

Table 1. Precision studies and relative errors in random Lactate assays at pH 7.50 and 25°C.

Lactate Concentration (mg l^{-1})		Relative error (%)	Within-run precision[a] (%)
Taken	Found		
0.21	0.20	−4.8	± 3.1
0.45	0.44	−2.2	± 2.6
1.05	1.07	+1.9	± 1.5
2.24	2.22	−0.9	± 1.0
4.61	4.63	+0.4	± 1.0
10.50	10.54	+0.4	± 1.0

[a]RSD calculated from eight determinations

Table 2. Recovery Studies of Lactate in normal Pooled Human Serum.

Average Lactate added (mg l^{-1})	% Recovery
1.23	99
2.51	99
6.16	101
9.80	101
12.20	98

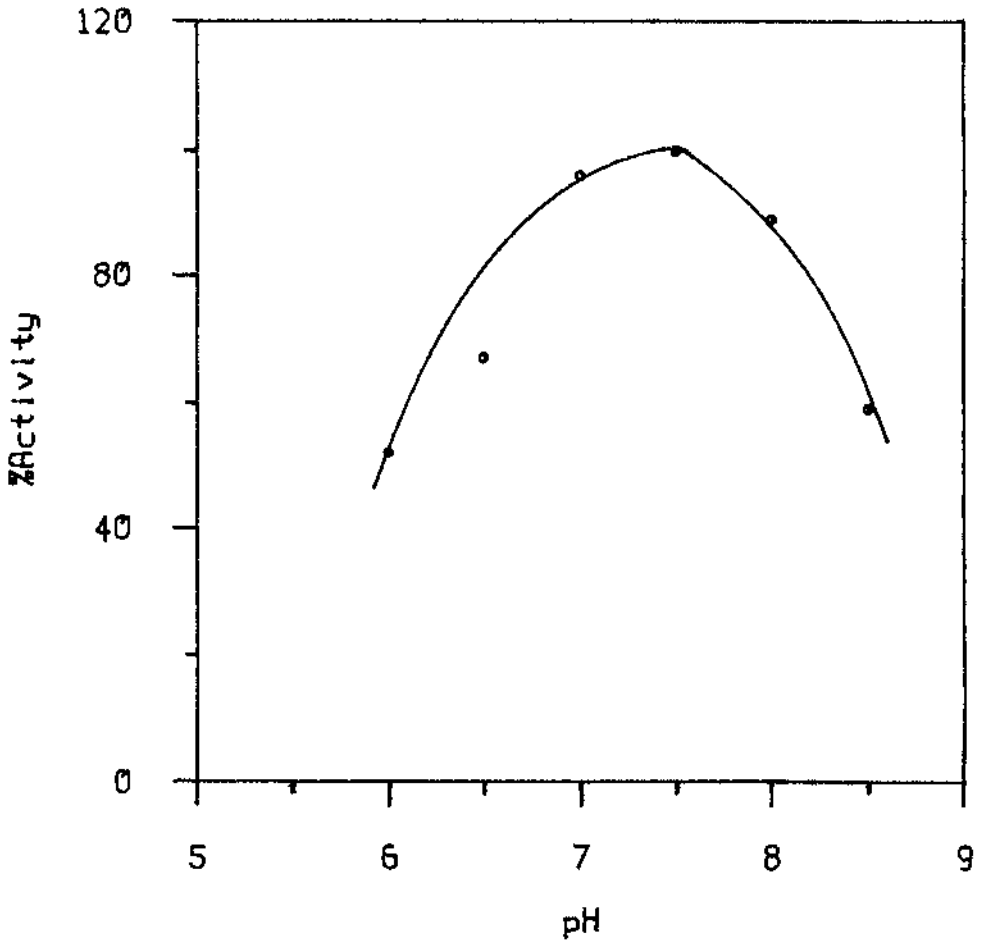

Fig. 1. pH profile of immobilized lactate oxidase at 25°C.

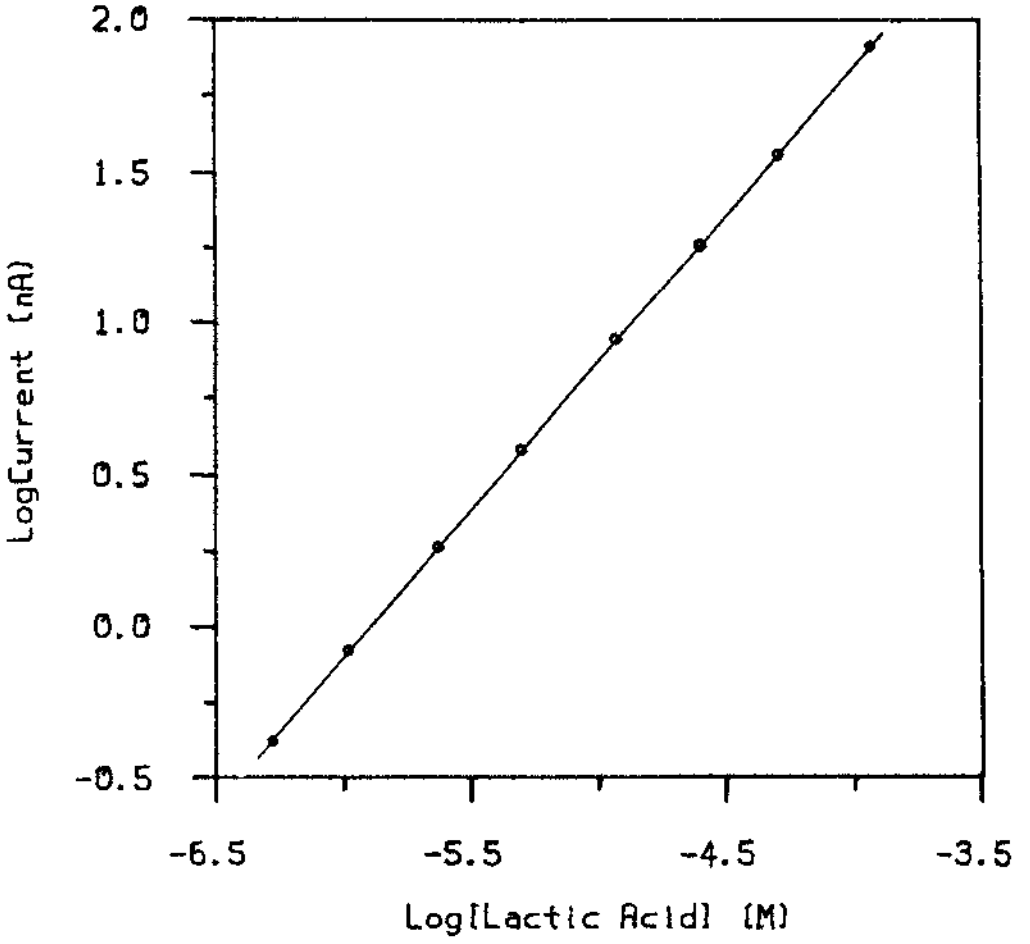

Fig. 2. Typical calibration curve for lactate with immobilized lactate oxidase at pH 7.50 and 25°C.

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