

Polarographic Measurement of Lactate Dehydrogenase Activity in Human Serum “Clinical Application”

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ABSTRACT

The paper reports a sensitive and rapid method for the measurement of Lactate Dehydrogenase activity (EC 1.1.1.27) in human serum. The method is based on the polarographic measurement in the reduction of polarographic peak of NAD^+ via the enzymatic reaction of LDH with lactate and NAD^+ .

The method was successfully applied to measure LDH activity in serum of normal and diseased individuals after the removal of albumin by zinc acetate method.

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NAD^+

NAD^+

LDH

INTRODUCTION

Electrochemical methods have been successfully applied for the measurement of enzymatic reaction (Shram et. al., 1998, Sulaiman et. al., 1994). These methods have utilized polarographic or amperometric detection to measure the electroactive species generated or consumed by an enzymatic reaction with high sensitivity and accuracy.

Colour or turbid media do not affect the electrochemical measurement. Recently, we applied the differential-pulse polarographic method for measurement LDH activity in pure bovine

heart samples (Sulaiman *et al.*, 1997). The method is based on the polarographic measurement of the decrease in NAD^+ peak current with time.

The application of this method to serum samples gave unsatisfactory results due to the change in polarographic behavior of NAD^+ in the presence of albumin in serum, which interferes with NAD^+ peak current.

In the present work, we decided to remove albumin from serum sample.

Different methods have been reported to separate albumin from human serum such as the ammonium sulphate precipitation, the acetone freeze drying method (Colowick and Kaplan, 1963) and the zinc acetate method (Torro and Ackerman, 1975). The zinc acetate method has been successfully used and applied for LDH activity measurement in human serum.

EXPERIMENTAL

Reagents and Chemicals:

All reagents were of analytical-reagent grade and de-ionized water was used throughout.

LD-L20 (NAD^+ + Lactate) Kit:

The content of this kit was dissolved in 20 ml de-ionized water to give 50 mmol.L^{-1} lactate and 7 mmol.L^{-1} NAD^+ concentrations. The reagent kit (100 ml) was then taken for subsequent experiment. Zinc acetate (25 mmol) was prepared by dissolving 0.1372 gm of zinc acetate dihydrate (BDH) in 25 ml de-ionized water. (Sigma Diagnostics, 1994).

Pyrophosphate buffer solution ($0.155 \text{ mmol.L}^{-1}$) was prepared by dissolving 41.33 gm of anhydrous sodium phosphate in one liter de-ionized water. The buffer was adjusted to the required pH with concentrated NaOH solution.

Specimen collection and preparation:

Samples of human serum were obtained from routine clinical assays. The serum (200 μl) was treated with zinc acetate solution (250 μl) to precipitate albumin. The solution was filtered using whatman filter paper No.1, and then (20 μl) of the serum treated with zinc acetate filtrated was then taken for subsequent experiment.

Apparatus:

The polarographic measurements were carried out using (EG and G) PAR 384B computerized polarographic analyzer equipped with 301 static mercury drop and RE 0093 digital plotter.

A three-electrode system was used; the WE was dropping mercury electrode, the reference electrode was Ag/AgCl, KCl and the counter electrode was Pt wire. pH measurements were made using a WTW 410 pH meter. The temperature was controlled to $+0.1^\circ\text{C}$ using a radiometer VT S13-water thermostat.

Procedure:

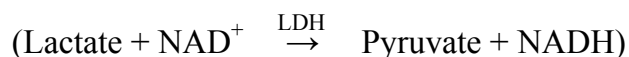
The differential-pulse mode was used with a 100 mV pulse, 1 S drop time and 5 mV. s^{-1} scan rate. The polarographic cell was thermostated at 37.5°C . The solution was de-aired by passing a slow stream of purified nitrogen gas for 40 S. to remove dissolved oxygen.

For polarographic measurement, the sample cuvette contained 4.9 ml of pyrophosphate buffer pH 9.01 and 100 μ l of (LD-L20) kit (NAD⁺ -Lactate) in a total volume of 5 ml. the differential-pulse polarogram was recorded between -0.2 and -1.4 V to give the peak current (Ip) of NAD⁺ and its peak potential (Ep) versus Ag/AgCl.

The reaction was initiated by the addition of 20 μ l of serum (free of albumin by treatment with zinc acetate). The solution was de-aired again for 60 S. and the polarogram recorded. The activity of the enzyme was determined during the first 1-6 min. by following the decrease in the current of NAD⁺ reduction peak appeared at -0.9 V versus Ag/AgCl.

RESULTS AND DISCUSSION

In recent paper , we successfully measured LDH activity in bovine heart from L \rightarrow P direction, (Sulaiman et al., 1997), via the measurement of continuous and regular consumption of NAD⁺ with time from the polarographic reduction peak at -0.88 V versus Ag/AgCl in phosphate buffer at pH 8.8 .



The application of this method to serum samples gave unsatisfactory results due to the interaction between NAD⁺ and albumin present in serum samples. The polarographic reduction peak of NAD⁺ appeared at more negative value (at -1.1 V versus Ag/AgCl). Therefore, it was decided to remove the albumin from serum samples by using zinc acetate method. Typical polarograms of 100 μ l of serum with the addition of 200,240 and 280 μ l of 25 mmol zinc acetate solution are shown in Fig. (1A-C). from this figure it is very clear that

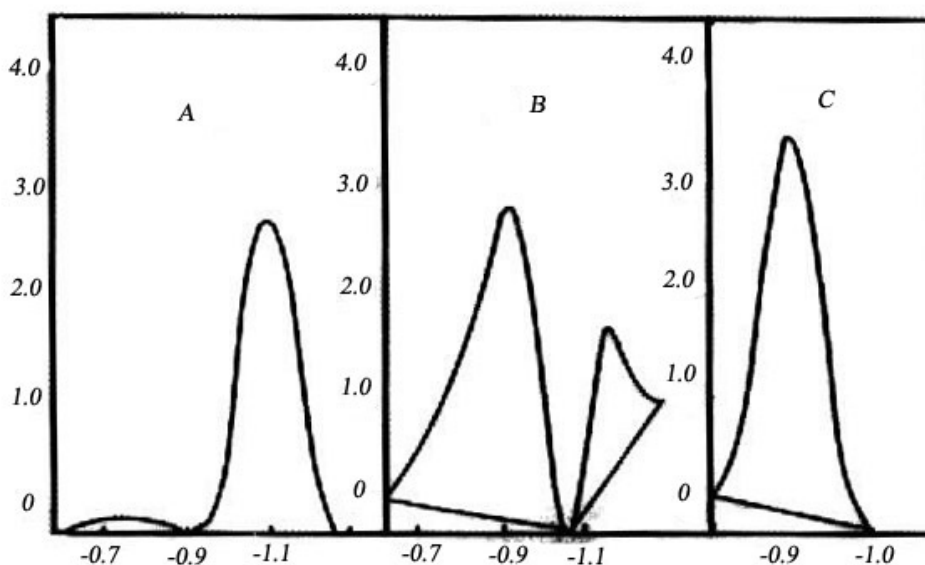


Fig. 1: Typical polarograms of NAD⁺ (related to LDH activity). Slowing the effect of increasing amounts of Zn(OAc)₂ on the removal serum Albumin. (A) 200 μ l, (B) 240 μ l, (C) 280 μ l of 25 mm Zn(OAc)₂.

the addition of 280 μl of zinc acetate is enough to remove all albumin from the serum. so that the reduction of NAD^+ is appeared at -0.88 V which represents the reduction of NAD^+ in pure aqueous solution in the absence of albumin and that at -1.1 V is completely disappeared (Hamdon, 1989) accordingly, the serum samples in the subsequent experiments where treated with 280 μl of 25 mmol of zinc acetate.

Optimization of conditions:

The effect of the amounts of zinc acetate treated serum:

In order to determine the optimum amount of zinc treated serum, a series of experiments were carried out. The amount of zinc acetate added were in the range of 5-20 μl to a polarographic cell containing 5 ml pyrophosphate buffer at pH 9.0 and 100 μl of LD-L20 kit (containing NAD^+ and lactate) at 37°C. The polarograms were then recorded and the activity of LDH measured as shown in Table (1). From these results it s clear that 20 μl of zinc acetate added to serum gave maximum activity.

Table 1: Effect of zinc acetate treated serum on LDH activity.

Amount of zinc acetate treated serum (μl)	LDH activity U/L
5	180.7
10	198.1
15	212.8
20	217.5

Effect of NAD^+ and lactate (LD-L20) kit:

The effect of NAD^+ + lactate concentration on LDH activity was studied by carrying out increasing amounts of (LD-L20) kit in a range of 50-200 μl . the maximum activity of LDH obtained using 100 μl of the (LD-L20) kit as indicated in Table (2).

Table 2: Effect of NAD^+ - lactate (LD-L20) on LDH activity.

Amount of (LD-L20) (μl)	LDH activity U/L
50	156.5
100	225.3
120	198.8
150	181.7
200	107.7

Clinical application of the method:

In order to access the applicability of the proposed method, the activity of serum LDH is measured in both male and female normal and diseased individual suffering from liver and heart diseases. The results are given in Table (3). It can be seen form the table that there are no significant differences of LDH activity between males and females in normal and diseased individuals.

These results are compatible with the results obtained by (Hughes, 1962; Hamdon, 1989) who found that LDH activity does not change with sex on the other hand it can also be

seen that serum LDH activity is significantly higher in heart than liver diseased subjects. These results are compatible with the results reported in the literatures (Hughes, 1962). More detail studies in this aspect are now under current investigations.

Table 3: Measurement of LDH activity in normal and diseased human serum.

Sample Type	Number of Samples	Sample Sex	LDH activity U/L	
			Mean	±S.D
Normal	12	Female	216.90	9.15
Normal	7	Male	224.10	2.46
Liver disease	5	Male	253.10	13.04
Liver disease	5	Female	245.80	12.20
Heart Disease	4	Male	383.71	69.20
Heart Disease	5	Female	378.28	40.50

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