TRACE ANALYSIS OF CYSTINE AND PYRUVIC ACID IN BLOOD SAMPLES FOR DIAGNOSTIC PURPOSES

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ABSTRACT

Direct current and differential pulse polarographic methods have been developed for the qualitative as well as quantitative analysis of two important compounds, viz. cystine and pyruvic acid in blood.

Cystine produces a catalytic hydrogen wave with $E_{1/2}/E_p$ = –1.65V/ –1.70V vs SCE in Brdicka Cobaltous solution (0.1 M NH_4Cl, 0.1M NH_4OH and 0.001M $CoCl_2$) at pH 8.5. Pyruvic acid produces a well defined reversible two-electron reduction wave at pH 3.1 in 0.01M NH_4Cl and 0.005M NH_4OH with $E_{1/2}/E_p$ = –0.90 V/–1.08V vs SCE. The height of wave/ peak is found to be proportional to the concentration of the compound in the solution.

The concentration of cystine and pyruvic acid is definite in normal healthy blood (cystine 0.016 mg/ml and pyruvic acid 0.28 mg/ml). However, their concentration undergoes a change due to various diseases. The developed method has been standardized to examine blood of patients suffering from ulcer and cancer for their cystine content and it was found that blood samples of the cancer patient under study contains 0.90 mg/ml and that of an ulcer patient contains 0.76 mg/ml of cystine. Blood of patients suffering from heart diseases was examined for pyruvic acid content and it was found that the blood of an inferior wall ischemia patient contained 3.84 mg/ml of pyruvic acid. However, after angioplasty, the pyruvic acid content was found to be 1.62 mg/ml.

INTRODUCTION

Polarography has achieved widespread appeal in the analysis of organic and inorganic substances because of the relative simplicity and specificity of the method. Any substance, either molecular or ionic, that can be reduced or oxidized at the dropping mercury electrode can be analysed by polarographic technique. In some cases substances that cannot be reduced or oxidized directly at the DME can be determined indirectly.

In organic and biochemistry, polarographic and voltammetric techniques are important tools in the analysis of biological samples, especially in body fluids. Of these body fluids, blood in particular is a very important biological sample for the purpose of analysis of various compounds, because of its easy and convenient availability from a large group of people. Biopsy materials from other organs of diseased persons are only available to a very limited extent. Blood can be sampled with a comparatively low risk of analytical contamination.

Blood has a very definite composition. Various diseases cause abnormalities in the composition of blood by the increase or decrease of the concentration of its constituents or through the appearance of new compounds therein. Diagnosis of different diseases depends mainly on the convenient and accurate analysis of blood.

The disulfide, cystine, is an important structural unit of proteins. It plays an important role in determining the gross shape of molecules and serves as a cross-link between protein chains /1/. The concentration of cystine in normal blood is 0.8-5.0 mg/100ml. Diseases in which the cystine level varies in blood are cancer /2/, stomach ulcer /3/, etc. Similarly, pyruvic acid is a keto-acid which plays an important part in the metabolism process. Its concentration in a normal healthy individual is almost negligible. There is an increase in the level of pyruvic acid in blood in patients suffering from heart disease.

Polarographic methods have been successfully employed for the analysis of various organic compounds like glucose /4/, albumin /5/, cholesterol /6/, cystine /7/, pyruvic acid /8/, etc., in blood. Of these, two important compounds, viz., cystine and pyruvic acid, have been selected for the present study. Their polarographic behavior and qualitative as well as quantitative determination in blood samples of healthy individuals, and those suffering from various diseases, have been reported in this paper.

EXPERIMENTAL

Instrumentation

All the polarograms/voltammograms were recorded on an Elico (India) DC polarograph, model CL357 and an Elico (India) pulse polarograph, model CL-90, respectively, coupled with an x-y polarocard model LR-108. The polarographic cell consists of an electrode assembly having a dropping mercury electrode (working electrode), a saturated calomel electrode (reference electrode), and a coiled platinum wire electrode (auxiliary electrode). A Systronics digital pH meter-335 was used for the pH measurements.

Chemicals and Reagents

All the solutions were prepared in doubly distilled water. The chemicals used were of Anala R/BDH grade. pH adjustments were made using dilute solutions of HCl, NaOH, NH_4OH, acetic acid or buffers wherever necessary.

0.01M solution of cobalt(II) chloride and pyruvic acid, 1M solution of NH_4Cl, NH_4OH were prepared by dissolving a requisite quantity of the compound/salt in doubly distilled water. 0.01M solution of cystine (Fluka) was prepared by dissolving a requisite quantity of the compound in 0.5 ml of concentrated HCl. The volume was then made up to 100 ml with distilled water. 25% solution of sodium chloroacetate, 10% solution of sodium tungstate and 0.1N solution of H_2SO_4 were prepared by dissolving their requisite quantity in distilled water.

Blood samples were supplied from Hement Pathology Sagar, UTD Dispensary Sagar and the Government Engineering College Dispensary, Jabalpur. Blood was collected over 10% EDTA solution as anticoagulant.

Determination of Cystine

A known concentration of cystine solution was taken in a polarographic cell containing Brdicka solution (10 ml of 0.1M $CoCl_2$, 10 ml of 1M NH_4OH and NH_4Cl each). The total volume of the test solution was made up to 100 ml with distilled water. The pH of the test solution was adjusted to 8.5. The analysis was carried out no later than fifteen minutes after the addition of a fresh solution of cobaltous chloride, since divalent cobalt is easily oxidized to trivalent state in ammoniac medium. In the preparation of the analyte,

ammonia was added last to prevent the precipitation of cobalt as hydroxide. The solution was deaerated with purified H_2 gas. Polarograms were recorded keeping the initial potential set to –0.8 volts. Polarograms of whole blood were recorded under similar experimental conditions.

Determination of Pyruvic Acid

A known concentration of pyruvic acid acid was taken in the polarographic cell, containing 5 ml of 0.1M NH_4OH and 10 ml 0.1M NH_4Cl. The total volume of the solution was made up to 100 ml with distilled water. Purified H_2 gas was bubbled through the test solution for 10 minutes before recording the polarograms. Polarograms were recorded at pH 3.1 keeping the initial potential set to –0.85V. For the determination of pyruvic acid in blood samples, 2 ml of blood sample was taken in a flask. 0.5 ml of 25% sodium chloroacetate was added to it. The solution was treated with 24 ml of 0.1N H_2SO_4 and 3 ml of 10% sodium tungstate. The protein thus precipitated was separated by filtration. Clear filtrate was used as analyte.

5 ml of this solution was transferred to a polarographic cell, and the polarograms were recorded under similar experimental conditions to those discussed above.

The concentration of cystine and pyruvic acid were determined in the unknown samples by calibration and standard addition method.

RESULTS AND DISCUSSION

The calibration curve for cystine, obtained under the above stated experimental conditions, was not linear but approached a limiting value. The concentration of cystine at which the height of the wave reaches a limiting value is characteristic for cystine and is known as a crossing effect. Due to the crossing effect, calibration plots for cystine are plotted at different ranges of cystine concentration.

Cystine produces a catalytic hydrogen wave with a characteristic round maxima with $E_{1/2}/E_p$ = –1.65V/–1.70V in 0.001M $CoCl_2$, 0.1M NH_4OH and 0.1M NH_4Cl at pH 8.5 (Fig. 1). DP polarograms are depicted in Fig. 2. Cystine forms a complex compound with divalent cobalt. In the complex there is a coordination bond between the cobalt and the –SH group. This

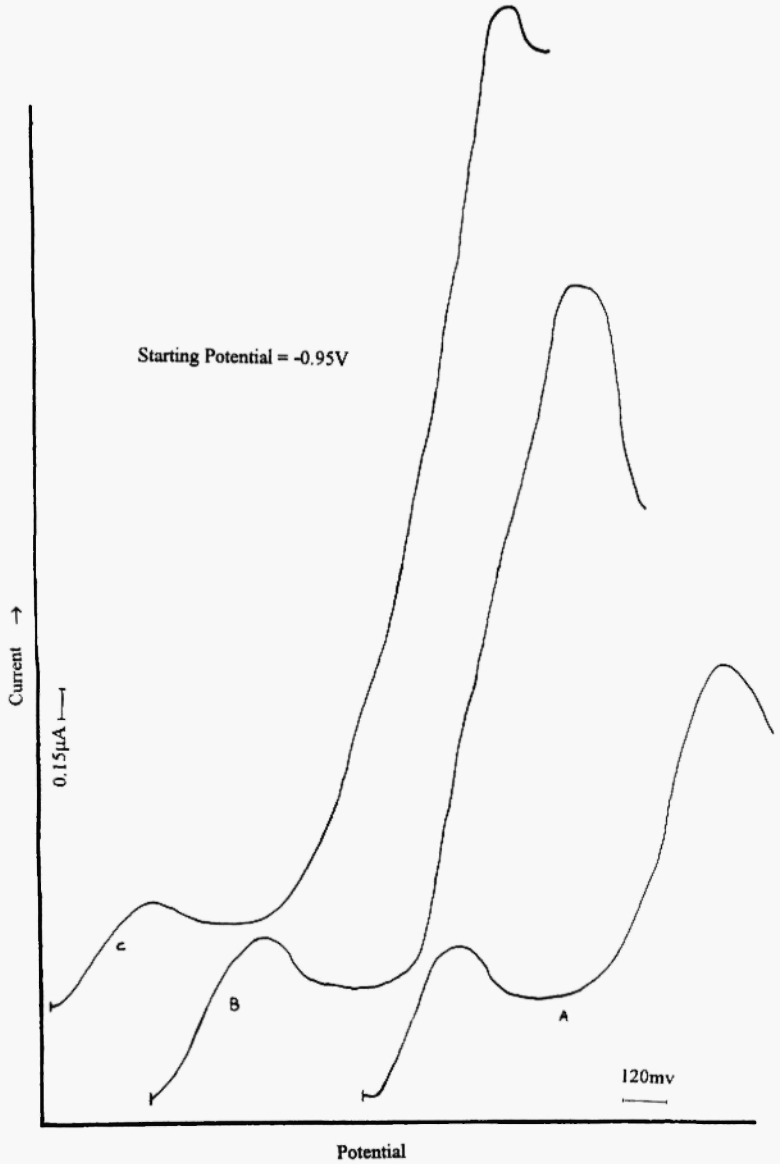

Fig. 1: DC polarograms of cystine in 0.1M NH_4OH, 0.1M NH_4Cl and 0.001M $CoCl_2$ solution at pH 8.5 showing effect of concentration. (A) 0.24 mg, (B) 0.6 mg, (C) 1.2 mg (per 100 ml of analyte).

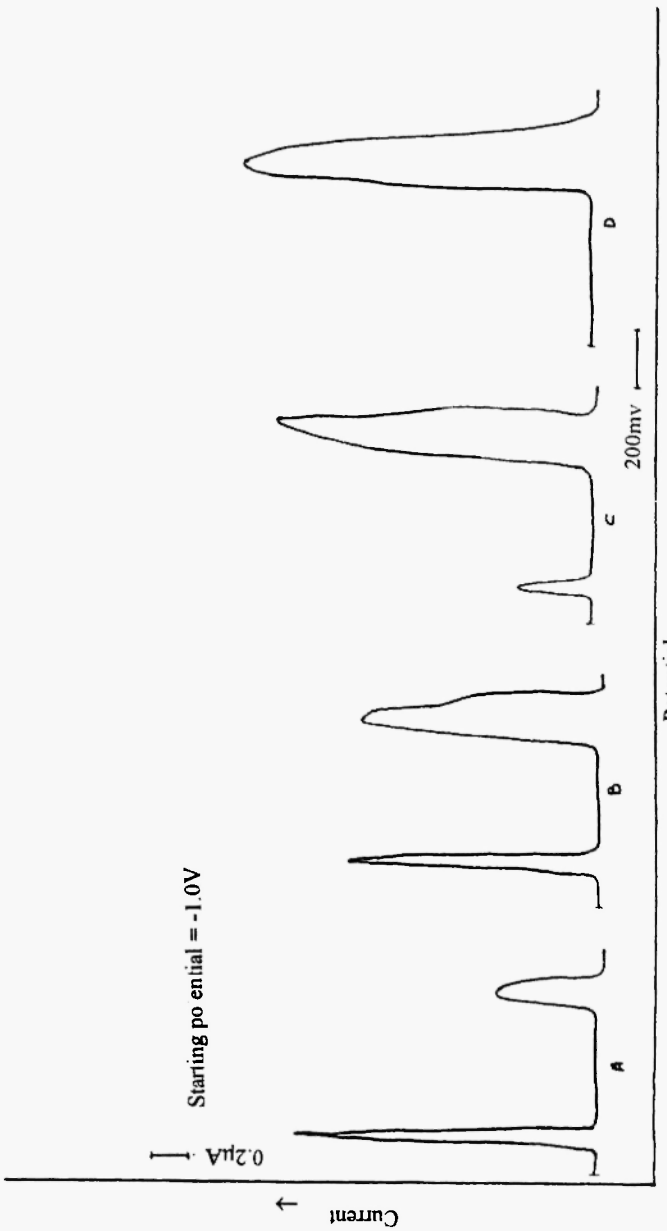

Fig. 2: DP polarograms of cystine in 0.1M NH_4OH, 0.1M NH_4Cl and 0.001M $CoCl_2$ solution at pH 8.5 showing effect of concentration. (A) 0.24 mg, (B) 0.48 mg, (C) 0.72 mg, (D) 0.96 mg (per 100 ml of analyte).

bond weakens the bond between sulfur and hydrogen in the –SH group and the deposition of hydrogen at the dropping mercury is facilitated /9/.

The authors have used the polarographic method in cancer and ulcer diagnosis by taking a definite amount of fresh carcinoma and ulcer blood and adding it to an ammoniac cobaltous solution. On recording its polarogram it was observed that the concentration of cystine increases in patients suffering from the above diseases. The concentration of cystine in normal blood was found to be 0.016 mg/ml, whereas in the case of cancer patients it was 0.9 mg/ml, and in ulcer patients0.76 mg/ml.

Pyruvic acid is easily reduced at the DME surface under the above-mentioned set of experimental conditions. It gives a well-defined reversible reduction wave at pH 3.1 with $E_{1/2}/E_p$ =–0.90V/–1.08V vs SCE. Under these conditions pyruvic acid is reduced to lactic acid at the DME,

$$\underset{\text{Pyruvic acid}}{CH_3COCOOH} \quad \Leftrightarrow \quad \underset{\text{Lactic acid}}{CH_3(CH)OHCOOH}$$

The number of electrons involved in the electrode process was found to be two as calculated by the measurement of $E_{3/4} - E_{1/4}$ and the plot of E vs log i/id-i. The wave peak height was found to be proportional to the concentration of pyruvic acid in the solution. The DC and DP polarograms of pyruvic acid are shown in Figures 3 and 4.

The concentration of pyruvic acid has been determined in the blood filtrates of healthy individuals and of patients suffering from heart ailments. The results have been depicted in Table 2. The table shows that concentration of pyruvic acid in blood samples of healthy individuals is almost negligible (0.028 mg/ml). However, it increases drastically in cases of heart ailments. It was found to be 3.72 mg/ml in cases of heart patients. However, the concentration was found to come down to 1.62 mg/ml of blood after angioplasty.

The final analysis results of cystine and pyruvic acid contents in blood samples of healthy and diseases individuals have been depicted in Table 3. The statistical analysis of data has been given in Tables 1 and 2. The value of relative mean deviation, standard deviation and coefficient of variance, which were below 0.1, 0.03 and 1.2 respectively, vouch for the reliability of the data. The percentage recovery was more than 95% in each case.

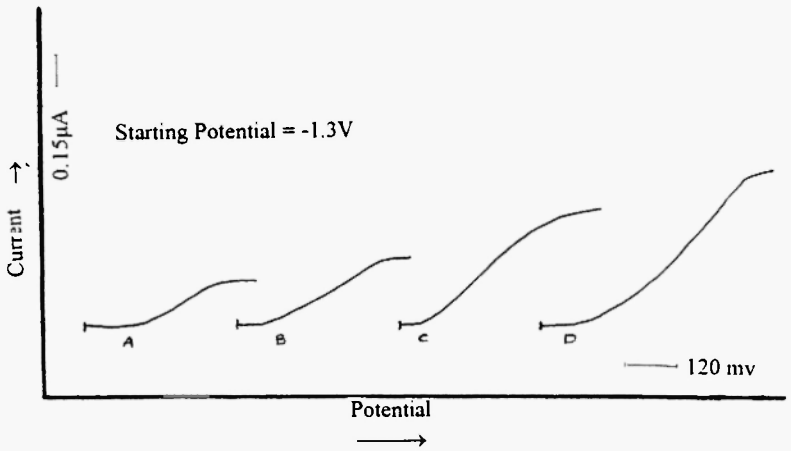

Fig. 3: DC polarograms of pyruvic acid in 0.1M NH_4Cl and 0.005M NH_4OH solution showing effect of concentration. (A) 2.2 mg, (B) 4.4 mg, (C) 6.6 mg, (D) 8.8 mg (per 100 ml of analyte).

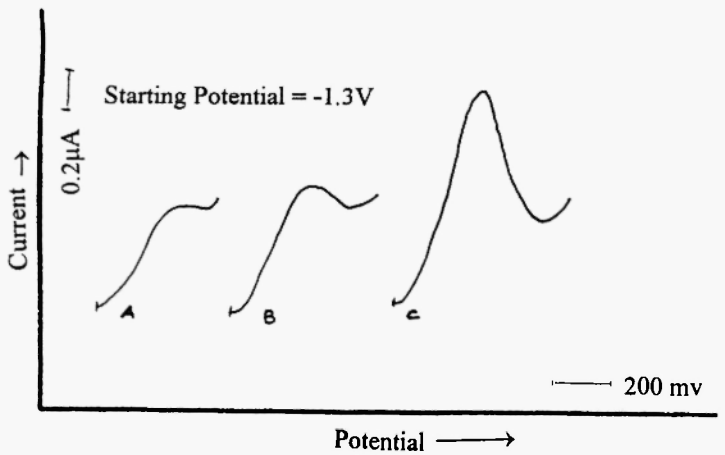

Fig. 4: DP polarogram of pyruvic acid in 0.1M NH_4Cl and 0.005M NH_4OH solution at pH 3.1 showing effect of concentration. (A) 1.38 mg, (B) 2.6 mg, (C) 4.4 mg (per 100 ml of analyte).

Table 1
Results* of blood samples for their cystine content
(mg/ml of blood)

Blood Sample	Parameter	DCP		DPP	
		Added	Found	Added	Found
Healthy individual	Amount	–	0.016	–	0.016
		0.024	0.036	0.024	0.039
	%R	95.0		97.5	
	RMD	0.1		0.05	
	SD	0.03		0.01	
	CV	11.5		4.1	
Cancer patient	Amount	–	0.94	–	0.90
		1.2	2.11	1.2	2.06
	%R	98.5		98.0	
	RMD	0.03		0.01	
	SD	0.03		0.01	
	CV	3.1		1.1	
Ulcer patient	Amount	–	0.80	–	0.76
		0.84	1.61	0.84	1.58
	%R	98.1		98.7	
	RMD	0.01		0.01	
	SD	0.01		0.02	
	CV	1.8		2.0	

*Average of four determinations
RMD = Relative mean deviation
SD = Standard deviation
CV = Coefficient of variance
%R = Percentage recovery

Looking at the sensitivity, accuracy and reliability of the results, the polarographic method of analysis can be recommended to clinics for diagnostic purposes.

Table 2

Results* of blood samples for their pyruvic acid content (mg/ml of blood)

Blood Sample	Parameter	DCP		DPP	
		Added	Found	Added	Found
Healthy individual	Amount	–	0.03	–	0.028
		0.03	0.058	0.03	0.057
	%R	96.6		98.2	
	RMD	0.005		0.004	
	SD	0.001		0.002	
	CV	1.8		2.6	
Inferior wall ischemia	Amount	–	3.83	–	3.84
		3.85	7.65	3.85	7.68
	%R	99.6		99.8	
	RMD	0.003		0.005	
	SD	0.01		0.02	
	CV	0.26		0.52	
After angioplasty	Amount	–	1.62	–	1.62
		1.65	3.25	1.65	3.26
	%R	99.3		99.6	
	RMD	0.007		0.01	
	SD	0.01		0.02	
	CV	0.06		1.23	

*Average of four determinations

RMD = Relative mean deviation

SD = Standard deviation

CV = Coefficient of variance

%R = Percentage recovery

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Table 3

Final analysis results of blood samples for their cystine and pyruvic acid content (mg/ml of blood)

Content	Blood Sample	Found	
		DCP	DPP
Cystine	Healthy	0.016	0.016
	Cancer	0.94	0.90
	Ulcer	0.80	0.76
Pyruvic acid	Healthy	0.03	0.028
	Inferior wall ischemia	3.83	3.84
	After angioplasty	1.62	1.62

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