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Short communication

Analysis of cystine in human blood for monitoring of cases of burns

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Abstract

Various diseases cause abnormalities in the composition of blood and urine by increase or decrease of concentrations of its constituents or through the appearance of new compounds therein. The analysis of blood samples obtained from a healthy individual and burns patients, for its cystine contents has been done successfully using direct current poloragraphy (DCP), differential pulse polarography (DPP) and differential pulse anodic stripping voltammetry (DPASV). The observed data was analysed statistically. The standard deviation and coefficient of variance of the data proved high reliability and accuracy of the method. In Bredicka Cobaltous solution (0.001 M $CoCl_2 + 0.1$ M $NH_4OH + 0.1$ M NH_4Cl) at pH 8.5 ± 0.02, cystine present in blood samples produces two step catalytic hydrogen reduction wave with peak potential (E_P) values equal to -1.22 and -1.46 V vs SCE. The height of the second wave was found to be proportional to cystine concentration. Blood samples obtained from a healthy individual and those from cases of burns having different percentages (16, 13, 9 and 6%) were analysed. Monitoring of the recovery of a patient (burn case 58%) after different time intervals during the treatment period was also done, till the patient was relieved from the hospital. The method has proved to be convenient and less time consuming for the clinical purpose.

Keywords: Cystine analysis; DPP; Monitoring; Cases of burns

1. Introduction

Blood consists of many types of proteins and other constituents [1]. The composition of blood as regards to its constituents content for a healthy individual is fixed. But, various diseases cause abnormalities in the composition of blood by increase or decrease of concentration of its constituents through appearence of new compounds therein. Cystine is one of the important constituents of amino acid protein. It helps in maintaining structure of the protein molecule by forming bridges between peptide chains through its 'S–S' bond. The normal concentration of cystine is 8–11 mg/100 ml of human blood [2].

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However, their concentration undergoes a change due to various diseases [3].

The determination of organic compounds in blood samples is a challenging analytical task because the medical diagnostic conclusions will

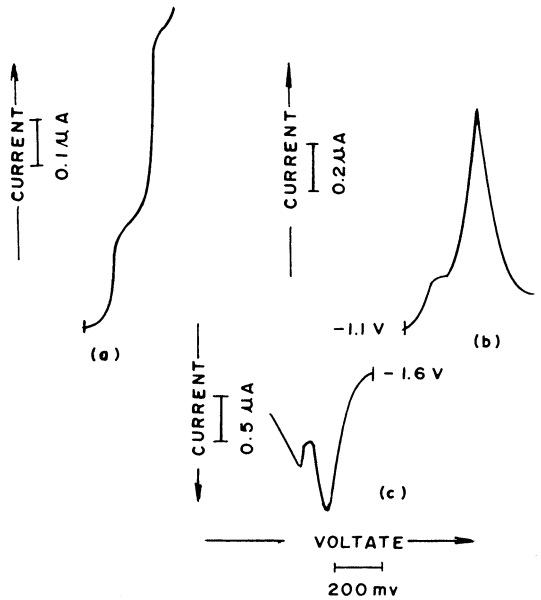

Fig. 1. (a) Direct current polarogram; (b) differential pulse polarogram; and (c) differential pulse anodic stripping voltammogram for cystine content of blood sample in 0.1 M $NH_4OH + 0.1$ M $NH_4Cl + 0.001$ $CoCl_2$ at pH 8.5 ± 0.02 obtained from a healthy individual.

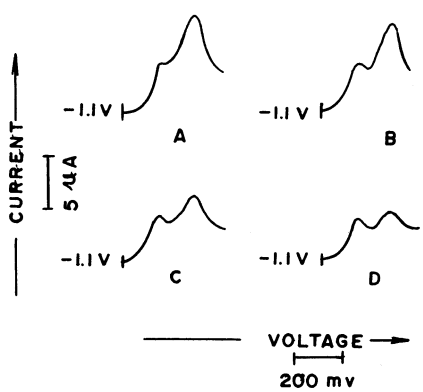

Fig. 2. Differential pulse polarogram for cystine content of blood sample of burn cases (A-16% burns, B-13% burns, C-9% burns, D-6% burns) in 0.1 M $NH_4OH + 0.1$ M $NH_4Cl + 0.001$ $COCl_2$ at pH 8.5 ± 0.02.

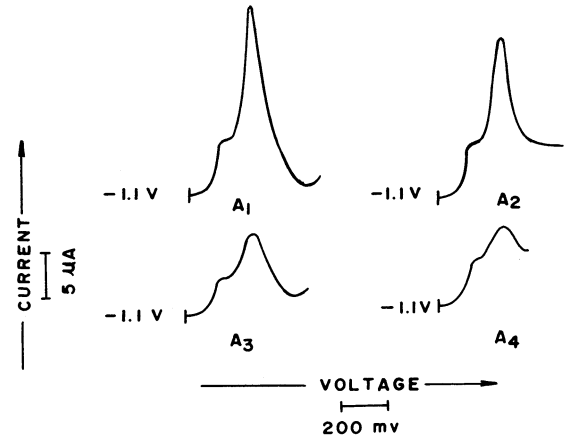

Fig. 3. Differential pulse polarogram for cystine content of blood sample in 0.1 M $NH_4OH + 0.1$ M $NH_4Cl + 0.001$ $CoCl_2$ for burn case (58% burns). A_1—2 days, A_2—7 days, A_3—22 days, A_4—53 days of undergoing treatment after injury.

depend basically and virtually on the analysis [4]. The work requires suitable methods and analysts with expertise, skill and imagination, able to design for the particular analysis in a particular sample, comprehensive analytical with sufficiently low accuracy risks. Polarography has achieved widespread appeal in the analysis of organic and inorganic substances because of relative simplicity and specificity of the method.

A survey of relevant literature reveals that the polarographic methods have been successfully employed for the analysis of various organic compounds like glucose, albumin, cholesterol, pyruvic acid and cystine etc. in blood [5–11]. The polarographic behaviour of cystine, its qualitative as well as quantitative determination in blood samples of healthy individuals and those suffering from burns have been reported in the present paper.

2. Experimental

2.1. Apparatus

Polarographic measurements were made on an Elico (Hyderabad, India) Pulse polarograph model CL-90 coupled with X–Y polarocard LR-108. The three electrode system consisted of a

dropping mercury electrode (DME) as a working electrode, a coiled Platinum wire as an auxiliary electrode and a saturated calomel electrode (SCE) as a reference electrode. pH measurements were made on a Systronic (India) digital pH meter model 335.

2.2. Chemicals and reagents

All the chemicals used were of Anala R/BDH grade. Stock solutions of Cobaltous chloride (0.01 M), ammonium chloride and ammonium hydroxide (1 M) were prepared by dissolving their requisite amount in double distilled water.

2.3. Sampling

One of the authors (JS) was selected as a healthy individual for blood sampling purpose. All the blood samples having different percentages of burns were procured from the Tili, district hospital, Sagar (M.P.), India. The blood samples were collected by veinupuncture of antecubital vein with the help of polyolefine disposable syringes with type of stainless steel (to be used once only). One millilitre of sodium citrate (3.5%) solution, as anticoagulent was taken in the syringe before collection of the sample.

2.4. Preparation of analyte and recording of polarogram

One millilitre of blood sample was taken in a polarographic cell along with 0.001 M $CoCl_2$, 0.1 M NH_4OH and 0.1 M NH_4Cl (Bredicka cobaltous solution) and deaerated by passing pure nitrogen gas through it for 10 min and its pH was measured before recording polarogram. Replicate

Table 1
Voltammetric monitoring of blood sample (obtained from a case of burns) (58%) for their cystine content

Period after injury	Method used	Added	Found[a]	Percentage recovery	S.D.[b]
After 2 days	DCP	–	0.604	–	–
		0.566	1.160	99.1	0.03
	DPP	–	0.605	–	–
		0.566	1.165	99.4	0.02
	DPASV	–	0.605	–	–
		0.566	1.167	99.6	0.02
After 7 days	DCP	–	0.360	–	–
		0.280	0.640	99.5	0.02
	DPP	–	0.363	–	–
		0.280	0.644	99.6	0.01
	DPASV	–	0.363	–	–
		0.280	0.644	99.6	0.01
After 22 days	DCP	–	0.192	–	–
		0.141	0.331	99.3	0.03
	DPP	–	0.193	–	–
		0.141	0.332	99.4	0.03
	DPASV	–	0.193	–	–
		0.141	0.332	99.4	0.03
After 53 days	DCP	–	0.081	–	–
		0.07	0.150	99.6	0.01
	DPP	–	0.081	–	–
		0.07	0.150	99.6	0.01
	DPASV	–	0.081	–	–
		0.07	0.152	99.7	0.02

[a] Amount found in mg/ml.
[b] Standard deviation mg/ml.

Table 2
Voltammetric analysis of blood samples obtained from a case of burns (different percentages) for their cystine content after 2 days of injury

Period after injury (%)	Method used	Added	Found[a]	Percentage recovery	S.D.[b]
16	DCP	–	0.154	–	–
		0.15	0.301	99.0	0.01
	DPP	–	0.154	–	–
		0.15	0.302	99.3	0.01
	DPASV	–	0.154	–	–
		0.15	0.302	99.3	0.02
13	DCP	–	0.146	–	–
		0.15	0.294	99.3	0.03
	DPP	–	0.147	–	–
		0.15	0.296	99.6	0.01
	DPASV	–	0.146	–	–
		0.15	0.295	99.6	0.02
9	DCP	–	0.132	–	–
		0.15	0.280	99.2	0.02
	DPP	–	0.132	–	–
		0.15	0.280	99.2	0.03
	DPASV	–	0.134	–	–
		0.15	0.283	99.6	0.03
6	DCP	–	0.098	–	–
		0.075	0.172	99.4	0.02
	DPP	–	0.098	–	–
		0.075	0.172	99.6	0.01
	DPASV	–	0.098	–	–
		0.075	0.172	99.6	0.04

[a] Amount found in mg/ml.
[b] Standard deviation mg/ml.

analysis of the analyte was done to obtain statistical data.

3. Result and discussion

In Bredicka (0.001 M $CoCl_2 + 0.1$ M $NH_4OH + 0.1$ M NH_4Cl) solution cystine produces a characteristic catalytic wave [10]. This complex showed two well defined polarographic waves/peaks with $E_{1/2}/E_P$ values equal to -1.22 and -1.46 V vs SCE in DCP, DPP and DVASV mode corresponding to the reduction of cobaltus–cystine complex and the catalytic hydrogen wave, respectively. The height of the second peak is proportional to the cystine concentration.

In spite of abundant literature the mechanism responsible for the polarographic current of cystine and protein has not been explained in detail [11]. However, it is an established fact that cystine contains a sulphohydryl group. The sulphohydryl group has a strong tendency to combine with Co. The cobaltous ion from the chelates with cystine [12–14]. These chelates decrease the over voltage resulting from the evolution of hydrogen in buffered solution since the catalytic waves are observed at a potential at which all the cobalt closes to the electrode has already been reduced.

Fig. 1 shows D.C. polarogram, D.P. polarogram and D.P.A.S. voltammogram of cystine content of blood obtained from a healthy individual.

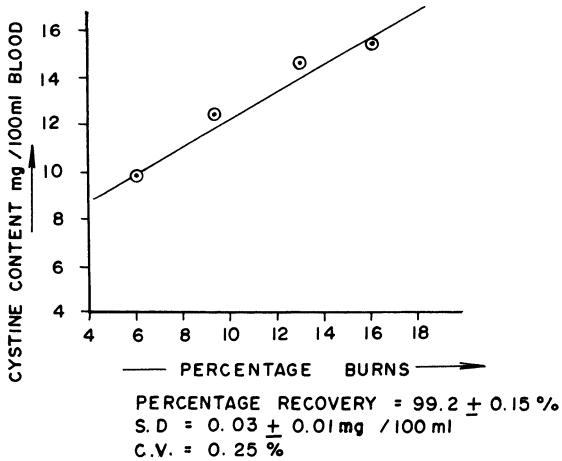

Fig. 4. Cystine content of blood sample as a function of percentage burns.

On performing polarographic quantitative analysis on the above sample the cystine content of the sample was found to be 8.5 mg/100 ml of blood, which is also the standard value for healthy individuals as per the literature. Fig. 2 a, b, c, are the DP polarograms of cystine content of blood samples which were collected form patients suffering from different percentages of burns. Fig. 3 A_1, A_2, A_3 and A_4 show DP polarograms of cystine content of blood sample for a burn case (58%) after different time intervals i.e. 2, 7, 22 and 53 days of undergoing treatment after injury (Tables 1 and 2).

Some synthetic samples of varying concentrations of cystine were prepared and polarograms of each synthetic sample were recorded under identical experimental conditions as discussed earlier. The results showed no change in E_P values.

3.1. Minimum tried detection limit of DPP

The minimum tried detection limit of DPP method for cystine content is 0.002 μg/dl.

3.2. Quantitative analysis of the sample

Each blood sample was spiked using external spiking method and the quantitation of cystine of each sample was done. The results have been presented in Figs. 4 and 5. The percentage recovery is always over 99%. The statistical data i.e. the calculated standard deviation of the data shows

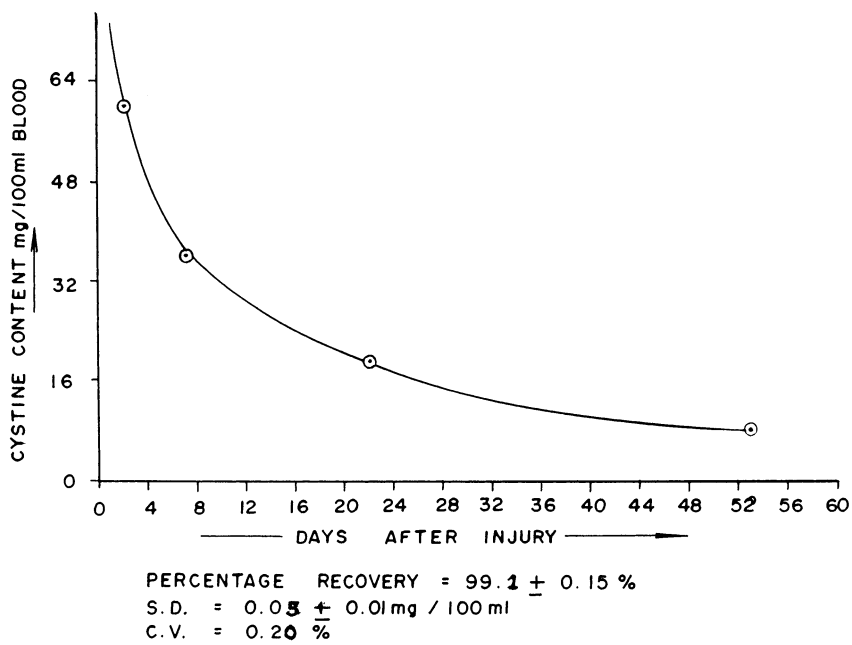

Fig. 5. Monitoring of cystine content of blood samples of a patient with burns (58%) during the course of treatment.

the reliability of the procedure. It is quite clear from Fig. 3 that the cystine content of the blood sample increases with the percentage of burns i.e. for 6, 9, 13 and 16% of burns the cystine content is 0.098, 0.132, 0.146 and 0.154 mg/ml of blood, respectively, the statistical data i.e. percentage recovery ($99.1 \pm 0.15\%$), standard deviation (0.03 ± 0.01/ml) and coefficient of variance (0.25%) reveals the reliability of observed data. Thus the data shows that the cystine content of blood depends on the degree and the area of burns.

On monitoring the cystine content of 58% burns case at regular time intervals after injury and during treatment (Fig. 5) it is seen that after 2, 7, 15 and 53 days the cystine content of blood is 0.604, 0.363, 0.193 and 0.081 mg/ml, respectively with percentage recovery $= 99.1 \pm 0.15$, standard deviation $= 0.05 \pm 0.01$/ml and coefficient of variance is 0.20%. It could be concluded that initially the increase in polarographic activity of cystine was several times higher as compared to the normal value. It may be explained as due to deproteinisation of cystine during burns and after treatment it gradually decreases.

4. Conclusion

On the basis of ongoing discussion and the data presented in Figs. 3 and 4 it could be concluded that the discussed procedure is highly sensitive, accurate, precise, rapid and economic. The procedure may be recommended for monitoring of cystine content of blood samples obtained from cases of burns and also for diagnostic purposes.

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