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Novel Method for the Determination of Haemoglobin Phenotypes by Cyclic Voltammetry using Glassy Carbon Electrode

M. Ogunlesi[1,], W. Okiei[1], A. S. Akanmu[2] and T. Popoola[1], K. Okafor[1], and O. Akore[1]*

[1] Chemistry Department, University of Lagos, Lagos, Nigeria
[2] Department of Haematology and Blood Transfusion, College of Medicine, University of Lagos, Lagos, Nigeria
[*]E-mail: mayogunlesi@yahoo.com

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Haemoglobin is the oxygen transporter within the red blood cell. The oxygenation reaction is accompanied by release or uptake of protons depending on the pH. The electroactive nature of the reaction between haemoglobin and oxygen renders the binding study suitable for investigation by electrochemical method. Haemoglobin samples prepared from blood samples of known phenotypes were diluted with 0.2M acetate buffer, pH 5.0, to give stock solutions of concentration 2 x 10^{-5} mol. Fe/dm^3. Aliquots were drawn and diluted with the buffer and cyclic voltammetry was carried out under nitrogen using glassy carbon/Ag, AgCl/Pt electrode system. 1.4 x 10^{-7} mol.Fe.dm^{-3}. The values of peak current obtained were in the range of 3.1 – 3.6 µA for HbA, 1.7 – 2.2 µA for HbAS, 1.2 – 1.6 µA for HbS and 2.5 – 3.00 µA for HbAC in the fixed range of concentrations of the haemoglobins. These values were used to identify the phenotypes of several haemoglobins successfully. The values of the peak current are sufficiently distinct to allow for rapid determination of these phenotypes. The method is simple and faster than the conventional electrophoretic method and removes the need to use standards once the characteristic values of peak current have been established. The good correlation between peak current and concentration also provides an accurate determination of the concentrations of haemoglobin samples.

Keywords: Phenotypes, cyclic voltammetry, glassy carbon electrode

1. INTRODUCTION

Haemoglobin is the oxygen transporter in mammalian blood. It is a tetrameric molecule made up of two subunits α and β, identical in pairs. The iron in the heme group is the active centre for

oxygenation [1]. The reaction between deoxygenated haemoglobin and oxygen is reversible and occurs according to the pH dependent equation shown below [2, 3].

$$Hb + nO_2 \rightleftharpoons (HbO_2)_n + xH^+ \quad (n = 2.8)$$

At pH values of about 6, **x** is positive reaching a maximum around pH 7, thus oxygenation at pH values in this range is accompanied by release of protons. This phenomenon is the alkaline Bohr effect. For pH values below 6, **x** is negative, thus oxygenation is accompanied by uptake of protons from the medium and this phenomenon is the acid Bohr effect. The value of the number of moles of protons associated with alkaline Bohr effect of various mammalian haemoglobins stripped of 2, 3-diphosphoglycerate varies over a small numerical range. However, in the case of the acid Bohr effect of vertebrate the range is much appreciable. This large range in the value of **x** could make it possible to use this value to distinguish haemoglobins to some extent. In fact the values of the acid Bohr effect at pH below 6 have been reported to be dependent on the nature of the vertebrate haemoglobins [4, 5, 6]. The Bohr effect is also expressed mathematically as

$$\frac{d \log p½\, O_2}{dpH} = - \Delta h^+$$

where $p½\, O_2$ is the oxygen pressure when haemoglobin is half saturated with oxygen and Δh^+ is the number of mole equivalent of hydrogen ion produced when one mole equivalent of oxygen reacts with haemoglobin. The hydrogen ions arise from the effect of oxygenation of haemoglobin on the pK of ionizing groups on the protein [7, 8].

Beetlestone et al measured the moles of protons accompanying oxygenation, that is the Bohr effect, by direct titrimetric method [4]. The normal adult haemoglobin is labeled A. However, there are variants found in the black race, namely the homozygous S, C and the heterozygous AS, AC, SC and SF. Haemoglobin S is the most common haemoglobin variant found in the United States followed by haemoglobin C. Approximately 2 – 3% of African American in the United States are AC heterozygotes [9].

Homozygous S gives rise to sickle-cell anaemia; the red cells from such patients sickle in vitro with reduced oxygen concentration. Fibrous precipitate is formed when a concentrated solution of sickle cell haemoglobin is deoxygenated and electron micrographs of fibers of deoxy HbS have been obtained [10].

Haemoglobin C is an abnormal haemoglobin in which there is reduced plasticity of the red blood cells [11, 12].

HbC is the second variant to be described after HbS. It is a prevalent variant in West African Sub-region [13]. The highest prevalence occurs in Northern Ghana where about 22% of the population carries HbC gene. It is also prevalent in South Western Nigeria where up to 8-10% of the population possess the gene. The prevalence is, however, low in areas east and north of the Niger where the gene prevalence is as low as 1-2%.[14, 15]. HbC is a soluble haemoglobin unlike HbS; however, it has a higher oxygen affinity and is also capable of erythrocyte cytoplasmic precipitation resulting in HbC

crystals that make the red cell subject to effector cell removal. Thus homozygous inheritance of the C gene may not present with feature of sickling syndrome but patient may present with mild anaemia [13].

In heterozygous, subjects, about 28-44% of the total haemoglobin is HbC and no anemia develops. In homozygotes, nearly all the haemoglobin is in the HbC form and there is mild hemolytic anemia.

Unlike HbA and HbF that may prevent deoxygenated HbS tactoid formation, HbC when co-inherited with HbS coprecipitates with HbS and the patients do present with sickling syndromes although in a milder form when compared with HbS inheritance [16]

Haemoglobins A, S and C differ only in the amino acid present at the 6th position of the β-chain, thus only two amino acid differences occur in a tetramer. The first eight amino acids in the β-chain of the three haemoglobins are listed below

β-chain	1	2	3	4	5	**6**	7	8
HbA	Val	His	Leu	Thr	Pro	**Glu**	Glu	Lys
HbS	Val	His	Leu	Thr	Pro	**Val**	Glu	Lys
HbC	Val	His	Leu	Thr	Pro	**Lys**	Glu	Lys

The position β6 in normal haemoglobin, HbA, is occupied by glutamate which is negatively charged at physiological pH, while valine at the corresponding position in HbS is neutral and lysine at the corresponding position in HbC is positively charged. [17, 18].

Position β6 is at the surface of the molecule and hence interacts with solvent [19]. The amino-acid substitutions in the abnormal haemoglobins S and C indicate that the net charge on each of them will differ and also differ from that on haemoglobin A; specifically the net charge on HbA is 2 units less positive than that of HbS while HbC is 2 units more positive than that of HbS.

The values of the isolelectric point for oxyhaemoglobins A, S and C are 6.87, 7.09 and 7.45 respectively and this is the expected trend from their net charges [16].

Haemoglobins A, S and C have different electrophoretic mobility arising from the differences in net charge on the molecules.

These differences in electrophoretic mobility is the basis of the determination of genotype in several laboratories. In a typical experimental determination, samples of haemoglobin under study are spotted on cellulose acetate paper medium. Various standard haemoglobins usually A, S and C are applied on the medium as well. The electrophoresis is carried out using Tris-EDTA-Borate buffer at pH 8.4 under a voltage of 250 volts and a runtime of 15-20 minutes after which the spots would have moved sufficiently and the phenotypes are determined by comparing the positions of the spots with those of the standards after staining with Ponsceau S dye and destaining with 5% glacial acetic acid in 3 washes. It is pertinent to note that the standards, have to be included in the samples for every electrophoretic determination of phenotypes.

The heterozygous haemoglobins AS, AC, SC and SF would each give two spots on electrophoresis corresponding to the constituent haemoglobins. Haemoglobin AS contains a maximum of 50% of HbS [18].

More than 900 variants of human haemoglobins are known and the common methods used for analysis or determination of phenotypes to identify them include conventional electrophoresis on cellulose acetate, chromatographic assays, HPLC retention times, capillary electrophoresis and the recently introduced technique applying mass spectrometry (MS). The application of MS is reported to improve the sensitivity of haemoglobin analysis while the combination of MS with electrophoretic and chromatographic methods is declared optimal for the detection of variants [20, 21, 22].

Since the oxygen binding reaction is electroactive it should be possible to investigate it by electrochemical methods. The direct electrochemical reaction of bonded oxygen in bovine oxyhaemoglobin was examined for the first time on a bare glassy carbon (GC) electrode by Chen et al [23] using cyclic voltammetry (CV).

The study demonstrated that the reaction involved the bonded oxygen not the heme iron, that is, the reaction was the electrochemical reduction of HbO_2. The peak potential was pH dependent and the peak current attained a maximum value in 0.2M acetate buffer at pH 5.0. It was also observed that surfactants such as hexadecyltrimethylammonium bromide promoted the electrochemical reaction and the peak current was found to be linearly proportional to the concentration of HbO_2.

Following the findings of Chen et al [23], it would thus be expected that the peak current that would be obtained for HbA, S and C at pH 5.0 would differ significantly because of the differences in their net charge and thus the values of the peak current obtained during the electroreduction reaction could be used to identify them.

The aim of this study is to measure the values of peak current for human haemoglobins of various phenotypes, namely A, S, C, AS, AC, SC and SF accompanying the electrochemical reaction and apply the results as a tool for the determination of phenotypes. In this report, we present the results of the values of peak current obtained for some of these haemoglobins in 0.2M acetate buffer, pH 5.0 using bare glassy carbon (GC)/Ag, AgCl/Pt electrode system.

Cyclic voltammetry is an electrochemical procedure in which electroactive species in solution at a certain initial potential are subjected to a linear alteration in potential up to a limiting value (switching potential) after which the direction of the potential scan is reversed and linear alteration in potential is applied until the initial value is attained. Thus species are oxidized in the first scan and reduced on the reverse scan. The peak current can be a measure of various parameters such as concentration of analyte or rate of electron transfer between the electroactive species and the working electrode.

In the cyclic voltammetric studies on bovine haemoglobins in 0.2 M acetate buffer at pH 5.0 using bare glassy electrode, it is pertinent to note that Chen et al [23] obtained the maximum value of peak current at pH 5.0 while Beetlestone et al [4] obtained appreciable value of Δh^+ at pH 5.3 suggesting that Δh^+ may be responsible for the peak current.

2. EXPERIMENTAL PART

2.1. Preparation of Haemoglobin Samples

Whole blood samples of known phenotypes A, S, AS and AC were obtained from the Haematology Laboratory of the Lagos University Teaching Hospital (LUTH), Lagos between August 2007 and June 2008. The haemoglobin samples were extracted within three days of drawing the blood samples. The blood was centrifuged (10,000 r.p.m.) in the cold for five minutes, washed three times with double volume cold saline (9.5g NaCl/dm^3) with centrifugation in between washings, till the supernatant was colourless after which the packed cells were lysed by shaking with ice-cold water of equal volume. After centrifugation, the haemoglobin was filtered off on sterile gauze and the concentration (mol.Fe/dm^3) was determined using Drabkin's solution, measuring the absorbance at 540nm and using extinction coefficient value of 10.9×10^3. The visible spectra of the samples were obtained and confirmed to correspond to that of HbO_2. The haemoglobin was diluted with cold 0.2M acetate buffer, pH 5.0 prepared with de-ionized water to give stock solutions of concentrations in the range $1.4 - 2 \times 10^{-5}$ mol. Fe/dm^3.

2.2. Cyclic Voltammetric Analysis

The method is similar to the procedure used by Chen et al [23].

The voltammetric measurements were carried out with a BASI-Epsilon Potentiostat/Galvanostat. A single compartment cell with a three-electrode configuration was used. The working electrode was made of glassy carbon while silver/silver chloride served as the reference electrode. A platinum electrode served as the auxiliary (counter) electrode. Before each experiment, the glassy carbon electrode was polished with 0.05 µm alumina and 0.01µm alumina sequentially, till a mirror-like surface was obtained. It was then placed in a freshly-prepared pyrana solution for 10 minutes after which it was thoroughly rinsed with de-ionized water.

Preliminary experiments in our laboratory had shown that the value of the peak current attained a maximum value at pH 5.0 for haemoglobins A, AS, S and AC in agreement with the observation of Chen et al [23] in the study on bovine haemoglobin hence the cyclic voltammetry was carried out at this pH.

The acetate buffer ($10cm^3$) in the cell to which 20 µl of the stock haemoglobin solution was added was purged with nitrogen for 10 minutes. Cyclic voltammetry was carried out using the following operating conditions: initial potential 300 mV, switching potential -400 mV, final potential 300 mV, scan rate 20 mV/s, number of segments 2, Quiet time zero, full scale current 10 µA. The peak current for each voltammogram was recorded at -220 mV. The experiment was repeated with addition of 50µl and 70 µl of haemoglobin sample to each 10 cm^3 portion of the buffer.

Whole blood samples of undisclosed phenotypes were also collected and processed as before to determine the values of peak currents and their phenotypes were inferred from the data on values of peak currents obtained previously.

The experiments were carried out at 20^oC in an air-conditioned environment.

3. RESULTS AND DISCUSSION

This study is the first report of analysis of some human haemoglobin variants by electrochemical reactions using cyclic voltammetry.

The values of the peak current at pH 5.0 in 0.2 M acetate buffer for haemoglobins of known phenotypes A, AS, S and AC at various concentrations are presented in Table 1. The ranges of haemoglobin concentrations for these phenotypes and the corresponding ranges of values of the peak current are presented in Table 2. The values of peak current for twenty haemoglobin samples of undisclosed phenotypes are presented in Table 3. Five samples each of HbA, AS and S are presented but only three of HbAC in Table 1 due to non-availability. The voltammograms of the acetate buffer as well as haemoglobin A (3.8×10^{-8} mol.Fe.dm^{-3}) are shown as I and II in figure 1. Figure 2 shows the overlay of the voltammograms of HbA, S, AS and AC at 1.4×10^{-7} mol.Fe.dm^{-3}.

Table 1. Values of Peak Current for haemoglobins of various and known phenotypes at specified concentrations

Haemoglobin A

S/N	Concentration ($x10^{-8}$) mol.Fe/dm^3	Peak current (µA)	Concentration ($x10^{-8}$) mol.Fe/dm^3	Peak current (µA)	Concentration ($x10^{-8}$) mol.Fe/dm^3	Peak current (µA)
1	**3.87**	3.142	**9.68**	3.304	**13.55**	3.618
2	**3.67**	3.096	**9.16**	3.289	**12.83**	3.573
3	**4.22**	3.151	**10.55**	3.342	**14.77**	3.628
4	**4.13**	3.149	**10.32**	3.339	**14.45**	3.598
5	**3.94**	3.107	**9.86**	3.308	**13.80**	3.621
	Haemoglobin AS					
1	**4.13**	1.729	**10.32**	1.934	**14.45**	2.203
2	**3.93**	1.701	**9.84**	1.910	**13.77**	2.189
3	**3.94**	1.711	**9.86**	1.918	**13.80**	2.192
4	**3.98**	1.720	**9.94**	1.921	**13.92**	2.200
5	**4.00**	1.726	**10.02**	1.930	**14.03**	2.206
	Haemoglobin S					
1	**4.06**	1.286	**10.16**	1.592	**14.22**	1.601
2	**4.07**	1.301	**10.17**	1.584	**14.23**	1.624
3	**4.08**	1.308	**10.21**	1.596	**14.29**	1.625
4	**3.90**	1.202	**9.74**	1.542	**13.64**	1.599
5	**4.06**	1.284	**10.16**	1.599	**14.22**	1.605
	Haemoglobin AC					
1	**4.01**	2.508	**10.02**	2.712	**14.03**	2.911
2	**4.11**	2.552	**10.28**	2.802	**14.39**	2.996
3	**3.98**	2.497	**9.94**	2.708	**13.92**	2.901

Table 2. Ranges of haemoglobin concentrations and the corresponding values for the Peak current

Phenotype				
A	Concentration range (x 10^{-8}) mol.Fe/dm^3	3.67 – 4.22	9.16 – 10.55	12.83 – 14.77
A	**Peak current range** (μA)	***3.096 – 3.149***	***3.289 – 3.342***	***3.573 – 3.628***
AS	Concentration range (x 10^{-8}) mol.Fe/dm^3	3.93 – 4.13	9.84 – 10.32	13.77 – 14.45
AS	**Peak current range** (μA)	***1.701 – 1.729***	***1.910 – 1.934***	***2.189 – 2.206***
S	Concentration range (x 10^{-8}) mol.Fe/dm^3	3.90 – 4.08	9.74 – 10.21	13.64 – 14.29
S	**Peak current range** (μA)	***1.202 – 1.308***	***1.542 – 1.599***	***1.599 – 1.625***
AC	Concentration range (x 10^{-8}) mol.Fe/dm^3	3.98 – 4.11	9.94 – 10.28	13.92 – 14.39
AC	**Peak current range** (μA)	***2.497 – 2.552***	***2.708 – 2.802***	***2.901 – 2.996***

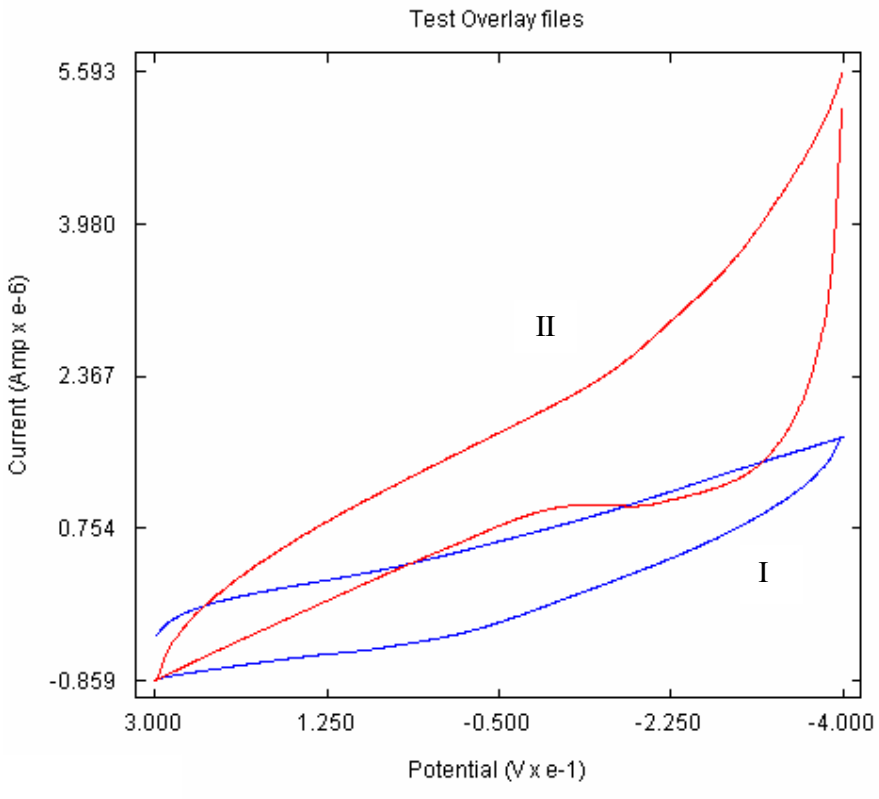

Figure 1. Voltammogram of (I) 0.2M acetate buffer, pH 5.0 and (II) haemoglobin A (3.8 x 10^{-8} mol.Fe.dm^{-3})

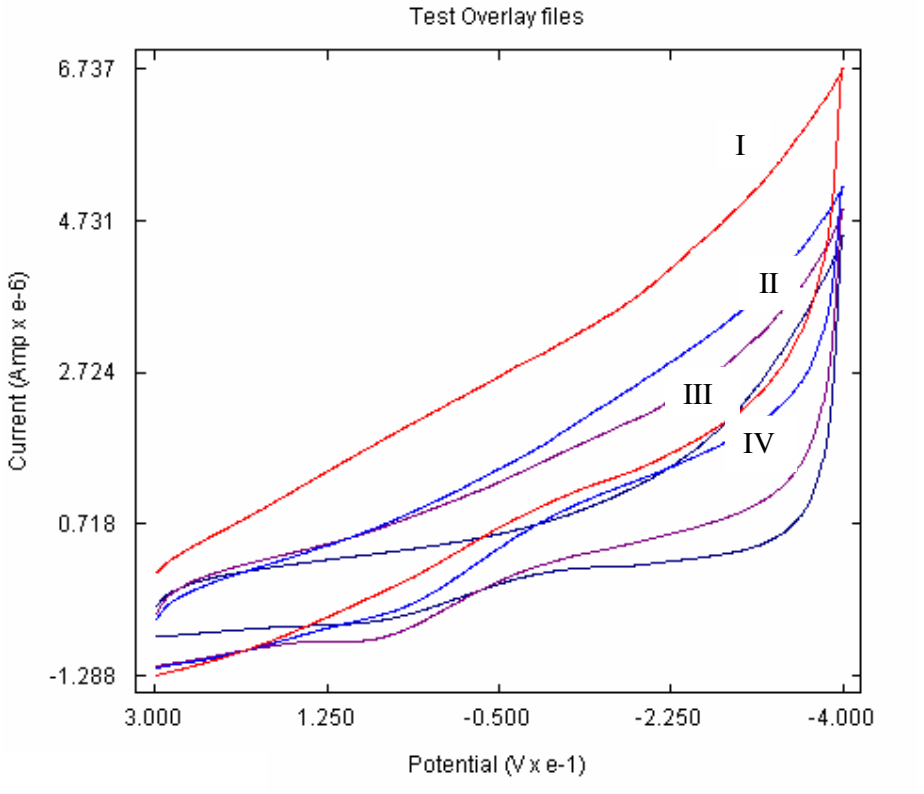

Figure 2. Overlay of the voltammograms of haemoglobins of different phenotypes (1.4 x 10^{-7} mol.Fe.dm^{-3}). (I). HbA, (II). Hb AC, (III). HbAS and (IV). HbS.

The values of peak current are distinct for the various haemoglobin genotypes and the genotypes of the blind samples presented in Table 3 were predicted with 100% accuracy using the values in Tables 1 and 2.

It is pertinent to note that of the 1045 blood samples screened in Lagos University Teaching Hospital (LUTH) Laboratory in the first half of 2008, 74% was HbA, 20% HbAS, 2% AC, 4% SS and 1% SC. The values of peak current for the four haemoglobin phenotypes A, S, AS and AC presented in this report would thus permit the determination of the phenotypes of 99% of the laboratory samples. Similar studies on haemoglobins SC, CC and SF will enhance the usefulness of the CV data obtained for determination of almost 100% phenotypes of haemoglobins among the black race. These phenotypes are rare as will be seen from the LUTH data. Plots of peak current against concentrations are shown for some of the haemoglobin samples in figures 3-6. It should be noted that the linear concentration dependence of the peak current observed by Chen et al [23] is confirmed in this study. However, a perfect correlation would not be expected due to methaemoglobin presence, variations in the concentrations of HbCO, and the presence of SOx, NOx, S^{2-} and H_2S in some of the samples as these would affect the HbO_2 concentration since the concentrations determined by Drabkin's procedure indicate mol.Fe/dm^3 while the value of the peak current depends on mol. Fe (HbO_2)/dm^3.

These results show that some haemoglobin phenotypes can be determined successfully by cyclic voltammetry. The advantages of this method over the conventional electrophoresis procedure

include rapidity, non-exposure of operators to corrosive chemicals such as staining and destaining reagents and elimination of the need to carry out CV on standard haemoglobins repeatedly once the initial sets of values have been obtained. This is borne out by the observation that these experiments were carried out over a period of ten months using the same electrodes and the values of peak current for the standards did not deviate significantly.

Table 3. Values of Peak Current for Haemoglobin samples of Undisclosed Phenotypes

S/N	Conc. ($x10^{-8}$) mol.Fe/ dm^3	Peak current (μA)	Conc. ($x10^{-8}$) mol.Fe/ dm^3	Peak current (μA)	Conc. ($x10^{-8}$) mol.Fe/ dm^3	Peak current (μA)	Predicted Genotype	Genotype Declared by Haematology Laboratory
1	**4.06**	3.163	**10.15**	3.345	**14.21**	3.641	**AA**	AA
2	**4.03**	3.099	**10.06**	3.269	**14.09**	3.602	**AA**	AA
3	**4.19**	3.187	**10.46**	3.351	**14.65**	3.662	**AA**	AA
4	**4.00**	3.079	**10.01**	3.267	**14.01**	3.543	**AA**	AA
5	**4.09**	3.007	**10.24**	3.312	**14.33**	3.534	**AA**	AA
6	**4.16**	1.741	**10.39**	1.922	**14.55**	2.217	**AS**	AS
7	**4.21**	3.189	**10.52**	3.373	**14.73**	3.674	**AA**	AA
8	**3.88**	1.273	**9.70**	1.565	**13.59**	1.616	**SS**	SS
9	**4.12**	1.716	**10.30**	1.928	**14.43**	2.196	**AS**	AS
10	**4.01**	3.173	**10.02**	3.326	**14.03**	3.627	**AA**	AA
11	**4.21**	1.319	**10.52**	1.599	**14.74**	1.656	**SS**	SS
12	**4.05**	2.512	**10.13**	2.806	**14.19**	2.975	**AC**	AC
13	**3.93**	1.701	**9.84**	1.913	**13.77**	2.145	**AS**	AS
14	**4.07**	3.124	**10.18**	3.308	**14.25**	3.611	**AA**	AA
15	**4.22**	3.108	**10.55**	3.277	**14.77**	3.561	**AA**	AA
16	**3.99**	1.702	**9.98**	1.911	**13.97**	2.009	**AS**	AS
17	**3.95**	2.913	**9.88**	3.276	**13.82**	3.503	**AA**	AA
18	**4.20**	3.184	**10.50**	3.333	**14.71**	3.637	**AA**	AA
19	**4.03**	3.003	**10.08**	3.267	**14.11**	3.515	**AA**	AA
20	**4.13**	3.149	**10.34**	3.307	**14.47**	3.614	**AA**	AA

In haemoglobin AS, it has been observed that not more than half of the haemoglobin is S [18]. Thus, it is of interest to know if the peak current for HbAS is about the mean value of those of HbA and HbS at similar concentrations. Table 4 shows the mean values of peak currents for HbA and HbS Hb(AS *calculated*) as well as those for HbAS, (AS *observed*). Inspection of Table 4 shows that assuming a maximum percentage of 50% of HbS in HbAS, the calculated values of the peak current are much higher than those obtained experimentally for HbAS, that is, HbAS *observed*. Hence the values of peak current are not additive, an indication that the two haemoglobins A and S do not exist independently of each other in the red blood cell, thus suggesting that there are intermolecular forces between the quaternary structures of the tetramers in them and possibly other heterozygous haemoglobins.

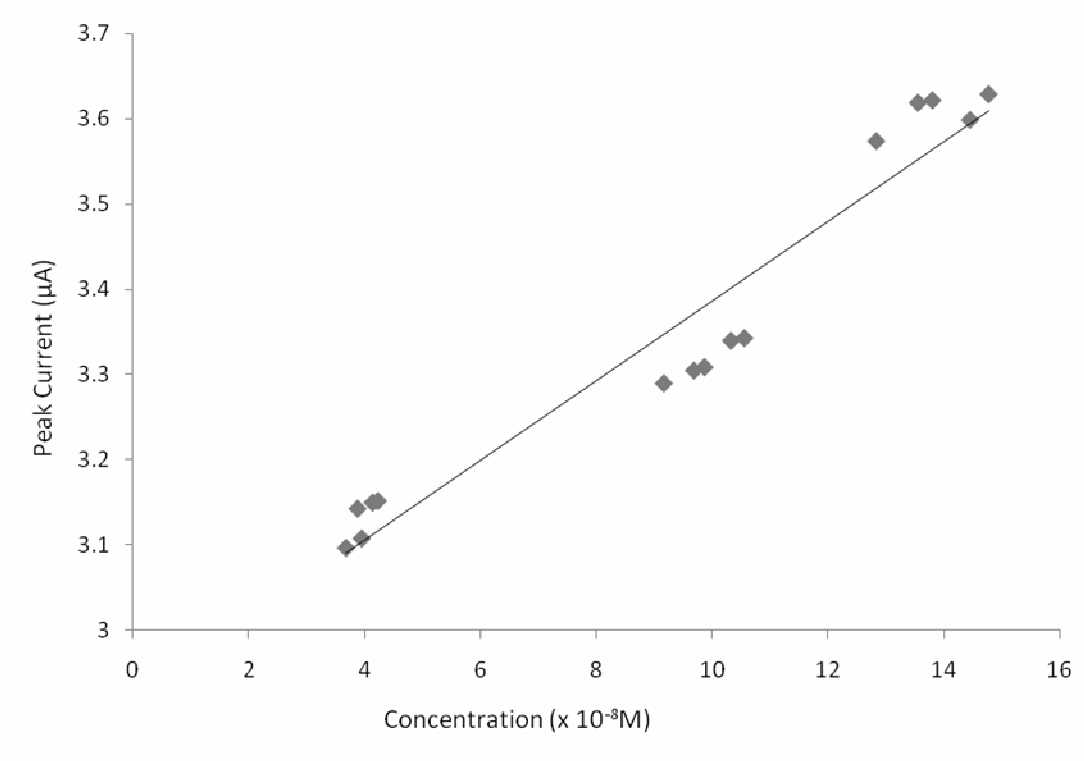

Figure 3. Plot of peak current against concentration (mol.Fe/dm^3) for HbA

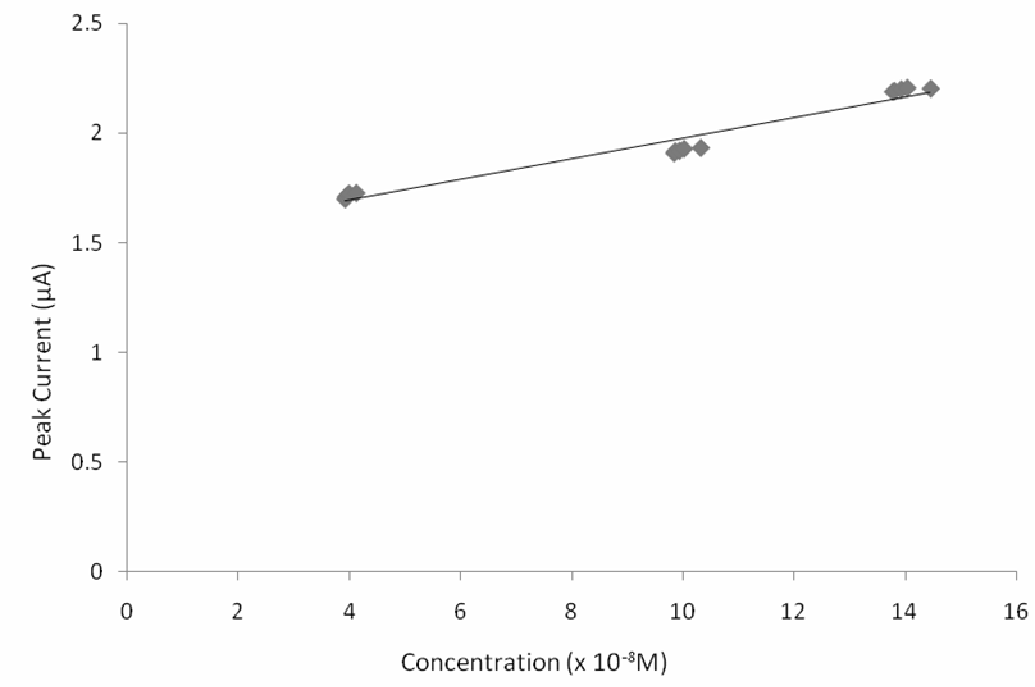

Figure 4. Plot of peak current against concentration (mol.Fe/dm^3) for HbAS

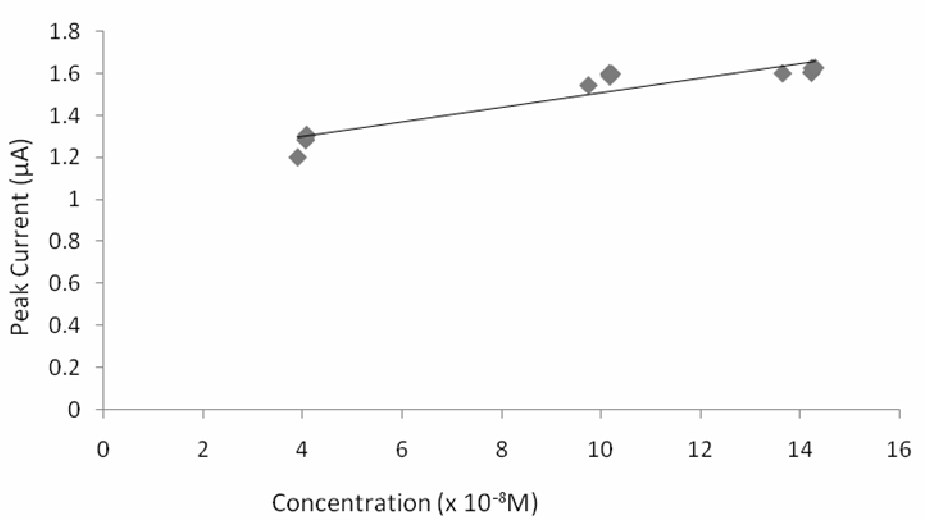

Figure 5. Plot of peak current against concentration (mol.Fe/dm^3) for HbS

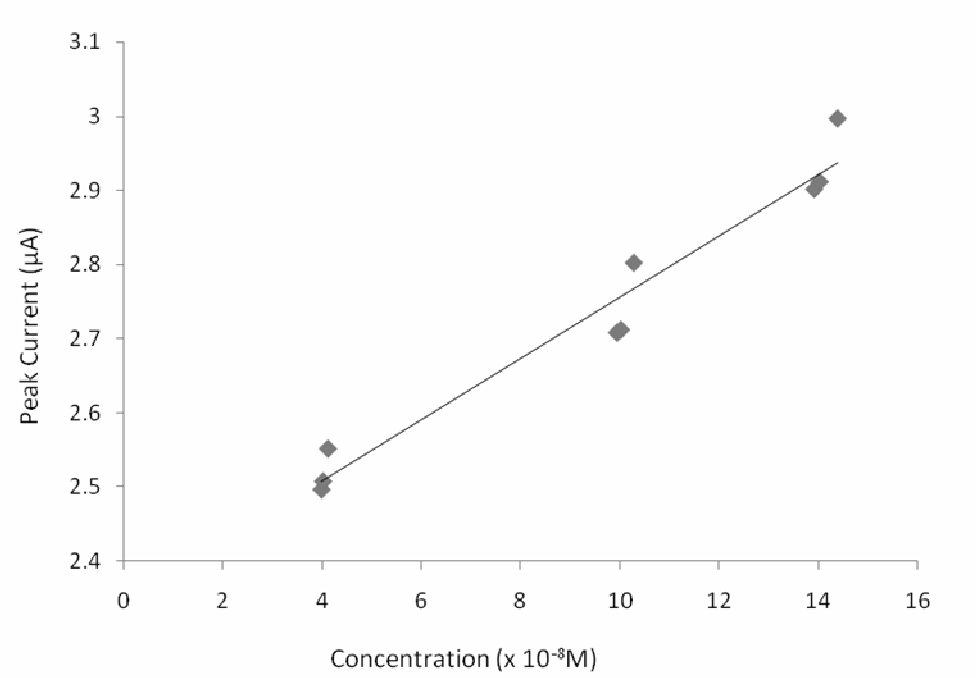

Figure 6. Plot of peak current against concentration (mol.Fe/dm^3) for HbAC

Indeed there is evidence that the α-chain of haemoglobin has a higher affinity for β^A-chain than for β^S-chain [24] perhaps due to differences in the charge of the two chains [25]. As such in an individual with AS genotype, haemoglobin S proportion is always lower than 50% and may be as low

as 30% although the exact proportion varies from one individual to another [26]. If one assumes that the proportion of HbS in people who have HbAS is 30% and that of HbA is 70% and these assumptions were used to estimate peak current instead of the 50% used earlier, the values of peak current that is Hb (AS *calculated*) will even be further away from the observed peak current.

Thus the values of the peak current for haemoglobins C, SC and SF cannot be obtained from calculations and need to be determined in order to enhance the usefulness of results of this study.

It would be of interest to predict the success of this method in the identification or analysis of over 900 haemoglobin variants identified considering the range of values of the peak currents obtained. Chen et al [23] reported an increase in the values of the peak current in the presence surfactants such as hexadecyltrimethylammonium bromide. Thus the addition of detergents of similar nature would appreciably increase the range of values of the peak current and thus afford the identification of several haemoglobin variants.

In a study of the electrochemical behaviors of neutral red on single and double stranded DNA electrode, cyclic voltammetric technique was also identified as a useful tool because of its high sensitivity, small dimensions, low cost and wider dynamic range [27]. Cyclic voltammetric technique was also used in studying the direct electrochemistry and electrocatalysis of myoglobin on Mb/MWNTs/Cs/GCE [28].

Table 4. Calculated and Observed Values of Peak current for Haemoglobin AS

Phenotype	Concentration range ($x\ 10^{-8}$) mol.Fe/dm^3	3.67 – 4.22	9.16 – 10.55	12.83 – 14.77
A	**Peak current range**	**3.096 – 3.149**	**3.289 – 3.342**	**3.573 – 3.628**
S	Concentration range ($x\ 10^{-8}$) mol.Fe/dm^3	3.90 – 4.08	9.7 4 – 10.21	13.64 – 14.29
	Peak current range	**1.202 – 1.308**	**1.542 – 1.599**	**1.599 – 1.625**
AS (observed)	Concentration range ($x\ 10^{-8}$) mol.Fe/dm^3	3.93 – 4.13	9.84 – 10.32	13.77 – 14.45
	Peak current range	**1.701 – 1.729**	**1.910 – 1.934**	**2.189 – 2.206**
AS (calculated)	Concentration range ($x\ 10^{-8}$) mol.Fe/dm^3	3.79 – 4.15	9.45 – 10.38	13.24 – 14.53

	Peak current range	**2.149 – 2.229**	**2.416 – 2.470**	**2.586 – 2.627**

4. CONCLUSIONS

The results of the study indicate the novelty, rapidity and simplicity of cyclic voltammetric measurements as an analytical tool in the analysis of haemoglobins and determination of some haemoglobin phenotypes.

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