1972

The Hemoglobin of Tubifex tubifex

Lalitchandra Vrajlal Vora
Eastern Illinois University

PAPER CERTIFICATE #2

TO: Graduate Degree Candidates who have written formal theses.

SUBJECT: Permission to reproduce theses.

The University Library is receiving a number of requests from other institutions asking permission to reproduce dissertations for inclusion in their library holdings. Although no copyright laws are involved, we feel that professional courtesy demands that permission be obtained from the author before we allow theses to be copied.

Please sign one of the following statements.

Booth Library of Eastern Illinois University has my permission to lend my thesis to a reputable college or university for the purpose of copying it for inclusion in that institution's library or research holdings.

Sept. 6, 1972
Date

I respectfully request Booth Library of Eastern Illinois University not allow my thesis be reproduced because _____

_____

_____

_____ _____

Date Author

The Hemoglobin of Tubifex tubifex

(TITLE)

BY

Lalitchandra Vrajlal Vora
B.Sc. (1969)
Gujarat University
Ahmedabad, India

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

Master of Science

IN THE GRADUATE SCHOOL, EASTERN ILLINOIS UNIVERSITY
CHARLESTON, ILLINOIS

1972
YEAR

I HEREBY RECOMMEND THIS THESIS BE ACCEPTED AS FULFILLING
THIS PART OF THE GRADUATE DEGREE CITED ABOVE

9/2/72
DATE

ADVISER

9/6/72
DATE

The Hemoglobin of Tubifex tubifex

BY

Lalitchandra Vrajlal Vora
B.Sc. (1969)
Gujarat University
Ahmedabad, India
June, 1969

Submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Chemistry
at the
Graduate School
of
Eastern Illinois University

CHARLESTON, ILLINOIS
1972

The Hemoglobin of Tubifex tubifex

Thesis Approved

Dr. C. D. Foote, Thesis Advisor

Dr. R. H. Karraker

Dr. R. L. Keiter

DEDICATION:

to my mother and father

ACKNOWLEDGEMENT

The author expresses his sincere appreciation to Dr. C.D. Foote for suggesting the problem and for providing guidance, inspiration and assistance throughout the investigation.

The author gives thanks to other members of the faculty and graduate students for their active interest and help.

Special thanks to Ina Leung, from Hong Kong, for typing this thesis.

TABLE OF CONTENTS

LIST OF FIGURES

ABSTRACT

Title of Thesis: The Hemoglobin of Tubifex tubifex

Lalit V. Vora, Master of Science, 1972

Thesis Directed By: Dr. C. Dan Foote

The hemoglobin of Tubifex tubifex is found in solution in the blood of the worm in cells as in vertebrates. The binding of oxygen to the hemoglobin can be followed spectrophotometrically. A peak of absorbtion at 540 nm is fairly constant under different conditions, while a peak at 575 nm disappears from the spectrum when oxygen is removed. The hemoglobin contains heme, non-heme iron and protein.

Binding of oxygen to the hemoglobin was found to be dependent on the concentration of the hemoglobin, at low concentrations (4.16×10^{-6} M), oxygen could not be removed from the hemoglobin. Only at the higher concentrations (9.72×10^{-6} M and above) could the oxygen removed. Direct measurement of the Bohr effect (by titration in a NaCl solution) gave a very small, essentially negligible value. Acrylamide gel electrophoretic analysis of reaction mixtures containing hemoglobin and parachloromercuricbenzoate provided no evidence for the reaction between the two. The latter two observations are consistant with the conclusion that this hemoglobin does not contain any free sulfhydryl groups.

The iron : heme ratio was found to be 5:1. Acrylamide gel electrophoresis and DEAE column chromatography provided evidence of the presence of two proteins in these preparations. One of the peaks from the column exhibited absorption at 540 nm and is most likely a hemoprotein, while the other did not absorb at 540 nm and is potentially an iron containing non-hemeprotein.

Introduction:

Blood contains somewhat less than 15 g of hemoglobin per 100 ml. Hemoglobin has the power to attach oxygen molecules and to hold them in a loose chemical combination known as oxyhemoglobin. As the blood passes through a capillary network in the thin air sacs of the lungs, oxygen enters into a loose chemical combination with hemoglobin (oxyhemoglobin) and is carried to the tissues. There, as the blood passes through tissue capillaries, the hemoglobin loses oxygen to the tissues and is then referred to as reduced hemoglobin. Hemoglobin not only functions directly to carry oxygen to the tissues, but indirectly to carry carbon dioxide away from the tissues (10).

Hemoglobin oligomers have been found in amphibians, reptiles, mice and men. The vertebrate hemoglobins occur in cells. These hemoglobins have been shown to polymerize by forming intermolecular disulfide bridges and belong to the characteristic vertebrate. In most cases hemoglobin particles appear hexagonal (3).

The hemoglobin of Tubifex tubifex (Tu Hb) is typical of invertebrate hemoglobins, occuring in free solution and having a molecular weight range from 4×10^5 to 3×10^6, which depends on the form of the hemoglobin and which is a greater molecular weight than that of vertebrate hemoglobin (1).

When the protein is prepared by the method suggested by W. Scheler (9), followed by dialysis to remove ammonium sulfate, the resultant aqueous solution contains an oligomeric hemoglobin which is in equilibrium with the various monomers, dimers etc. The oligomer : monomer ratio is highest in distilled water, diminishing with increasing ionic strength in solution of sodium chloride and sodium phosphate. According to Nakajima and Braunitzer (3), tubifex hemoglobin appears cylindrical.

According to Nakajima and Braunitzer (3), the spectrum of tubifex hemoglobin is quite similar to that of human hemoglobin. Although tubifex hemoglobin has the absorption spectrum of a typical hemoglobin, it more closely resembles the hemocyanins of arthropods and mollusks as an association-dissociation system (1,7). The most stable oxyhemoglobin in salt solution of high ionic strength (.15-1.0M) seems to be of molecular weight 1.2 or 3.0×10^6 (1).

This study was planned with several purposes: to see the equilibrium of oxygen with tubifex hemoglobin, to measure the 'Bohr effect'; to study subunit character; as well as to measure the iron and heme content of tubifex hemoglobin. In the work reported here, the tubifex hemoglobin has been prepared by the method suggested by W. Scheler (9).

Oxygen Equilibrium:

The amount of oxygen (or carbon dioxide) the blood will hold is inversely proportional to the partial pressure of oxygen (or carbon dioxide) in the blood. An increase in either the carbon dioxide pressure or acidity of blood will favor the dissociation of oxyhemoglobin. A simplified equation for the binding of oxygen by hemoglobin is:

$$Hb + O_2 \rightleftharpoons HbO_2 \qquad \text{I}$$

On exposure to molecular oxygen, ferrous hemoglobin binds the gas in a ratio of one oxygen molecule per heme iron unit. The binding of oxygen by hemoglobin is reversible and may be studied by variations in the UV-VIS spectrum (6). The reaction of hemoglobin with oxygen is complex.

Bohr Effect:

It has been shown that among terrestrial mammals the smaller the animals the higher is the oxygen pressure ($P_{\frac{1}{2}}$) at which the whole blood is half saturated with oxygen. Thus the blood is adapted to deliver oxygen to the tissues at a higher pressure in smaller animals (2).

One of the most intriguing phenomena associated with the function of hemoglobin is its reversible dissociation of protons which accompanies its binding of oxygen. This oxygen linked ionization,

which is commonly referred to as 'Bohr effect', results in the liberation of about 2.7 protons per mole, when human hemoglobin is oxygenated at physiological pH (4). Hemoglobin molecules have four iron atoms and it must take up four molecules of oxygen when saturated, as shown by the following equation:

$$H_{2.7} + 4O_2 \rightleftharpoons Hb(O_2)_4 + 2.7H^+ \qquad \text{II}$$

A different 'Bohr effect' occurs in the region of pH 5.50, at which oxygenation is accompanied by absorption of one proton per mole (4).

The primary differences between bloods lies in the sensitivity of their hemoglobins to changes in pH. As the 'Bohr effect', it is well known that high carbon dioxide pressure or decreased pH tends to dissociate oxyhemoglobin and promotes gas exchanges in the tissues. In a series of terrestrial mammals ranging in size from elephant to mouse, the smaller the animals the greater is the sensitivity of the oxygen equilibrium of its hemoglobin to pH. This pH sensitivity predicts the fact that certain acid groups of hemoglobin are functionally linked to the oxygenation process in such a way that they become more acidic when oxygen is bound. The results indicate that the smaller the mammals the greater is the number of protons discharged from its hemoglobin when the molecule is oxygenated.

According to Austin Riggs (2), the number of mercuric ions

bound to the hemoglobin is directly proportional to the number of sulfhydryl groups in the protein. The extent of the 'Bohr effect' plotted against the ions of Hg^{+2} bound by hemoglobin produces a straight line as shown in Fig. 1, thus the 'Bohr effect' is directly proportional to the free sulfhydryl groups of the hemoglobin. In other words, with a hemoglobin that exhibits no 'Bohr effect', we expect to find no or very few sulfhydryl groups. In passing note that myoglobin (muscle hemoglobin) exhibits no or very small 'Bohr effect', apparently because there are no or very small number of sulfhydryl group in that heme protein.

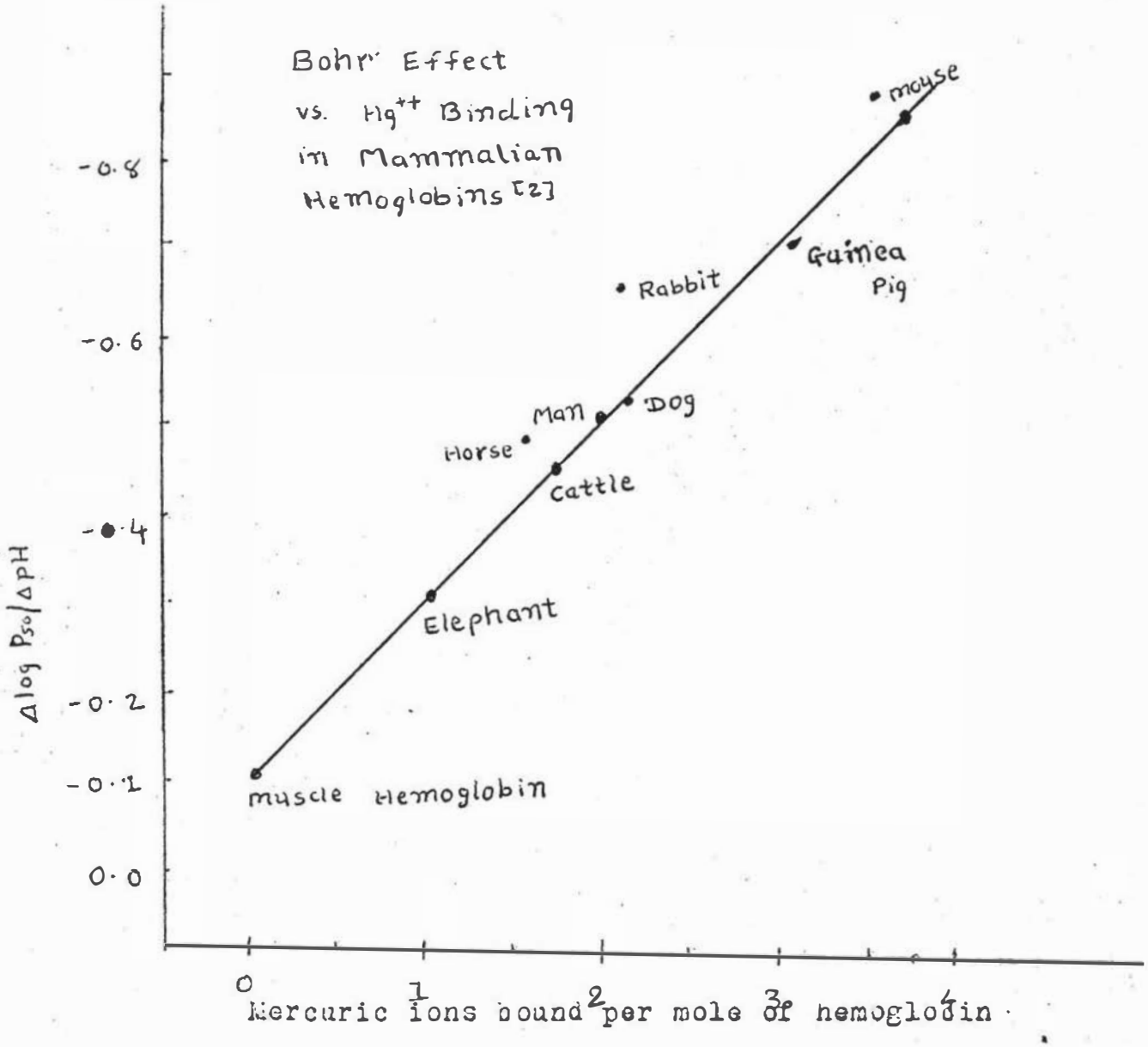


Fig.1. The relation between the magnitude of the Bohr - effect and the number of mercuric ions which can be bound per hemoglobin molecules.

Sub-unit Nature:

Human hemoglobin has molecular weight of 67,000, and has four sub-units, two alpha and two beta, each weighing about 18,000. Tubifex hemoglobin has a molecular weight of about 3,000,000, but sub-unit weights are not known accurately. According to J. Russell (1), these molecular weights vary from 4×10^5 to 3.0×10^6, depending on the solvent, as mentioned before.

Two modern methods for study of protein are Disc electrophoresis and DEAE-cellulose column chromatography. These are the two methods that we have used in our study of tubifex hemoglobin.

Iron-Heme Content:

Human hemoglobin is composed of a pigment, heme, containing iron, and a protein, globin. Human hemoglobin contains about 0.338 percent of iron (15). Each molecule of hemoglobin contains four atoms of iron and four hemes.

According to W. Scheler and L. Schneiderat (13) iron : heme ratio in tubifex hemoglobin is about 3 : 1 and according to Nakajima and Braunitzer (3), this ratio could be much higher.

Materials and Methods:

Preparation of Tubifex hemoglobin:

The tubifex worms were found in the banks of streams polluted by city waste treatment plants. The worms were little thicker than hair and about 2" long. The worms were washed until the rinse water appeared free from debris and the worms looked clean. Sufficient distilled water to cover them thoroughly was then added. They were then frozen and thawed at least three times. After the last thawing, the worms were centrifuged at 5000 RPM for thirty minutes. This was to separate the body debris of the worms from the crude hemoglobin solution.

The supernatent was then treated with 16 gms of ammonium sulfate per 100 ml. of solution. The sample was then centrifuged at 5000 RPM for thirty minutes and the supernatent was again saved.

Enough ammonium sulfate was added to cause a precipitate of hemoglobin to appear. The resulting mixture was again centrifuged at 5000 RPM for thirty minutes. This time, the supernatent was discarded. The resultant pinkish precipitate was dissolved with a minimum of appropriate sodium chloride or buffer solution.

This solution was then dialyzed against sodium chloride or buffer solution, depending on the solvent for the ammonium sulfate precipitate of hemoglobin.

Measurement of Concentration:

The concentration of hemoglobin was obtained by measurement of absorbance at 540 nm, using a molar absorbtivity of 7.20×10^5 $1\ mole^{-1}cm^{-1}$. This molar absorbtivity was calculated from the extinction coefficient of 1.2×10^4 $cm^{-1}Val^{-1}$ at the same wavelength as given by Nakajima and Braunitzer (3). In this extinction coefficient, Val stands for equivalents per liter, where one equivalent is one mole of heme or 50,000 gms of hemoglobin. In our molar absorbtivity, one mole is taken as 60 equivalents or 3,000,000 gms of hemoglobin (9).

Oxygen Equilibrium:

The precipitate of tubifex hemoglobin was dissolved in 0.1 M sodium chloride solution and was dialyzed against 0.1 M sodium chloride for twenty four hours.

Oxygen gas was passed on the surface of tubifex hemoglobin solution through the train of wash bottles (except oxygen acceptor reagent bottle), as shown in Fig. 2, for thirty minutes. The UV-VIS spectrum was taken on a Beckman DB-G Spectrophotometer, Fig. 3, After the nitrogen gas was passed through the same train of wash bottles but including oxygen acceptor reagent bottle, for an hour to an hour and half. The spectrum was again taken, as shown in Fig. 4. Care was taken during transfer of the anaerobic sample to exclude oxygen from the air.

Measurement of the 'Bohr effect':

A forty ml. graduated cylinder (cut off 250 ml. graduated cylinder) was fitted with a rubber stopper through which were inserted a pair of Beckman calomal electrodes and two narrow glass tubes as shown in Fig. 2. The flask which contained 15 to 20 ml. of 9.30×10^{-6} M tubifex hemoglobin solution, was stirred gently and continuously using a magnetic stirrer. In this way the solution was equilibrated with oxygen or nitrogen by blowing the washed gas over the surface of the protein solution. The calomal electrodes were connected to a Coleman metrion IV pH meter. Additions of acid or alkali were made from a syringe microburette fitted with a disposable pipette through polyethylene tubing sufficient to pass through the glass tube and under the surface of the protein solution.

At the beginning of each experiment, the tubifex hemoglobin solution in 0.1 M sodium chloride was brought to the desired pH (usually 7.40), under oxygen. Any addition of reactants were usually made at this stage, and the pH was readjusted to 7.40 if necessary. Thereafter the null-point setting on the high sensitivity of the pH meter was left unchanged. The tubifex hemoglobin was now equilibrated with nitrogen gas, the pH being kept constant by addition of 0.1 M HCl until no further change took place.

Complete deoxygenation takes about an hour to an hour and half. Deoxygenation was monitored spectrophotometrically. This measurement was always followed by the reoxygenation of the solution which requires only 15 to 20 minutes, and the determination of the amount of 0.1 M sodium hydroxide necessary to maintain the pH at 7.40. Reoxygenation was also followed spectrophotometrically. The equivalents of acid or alkali added divided by the moles of hemoglobin gives the Bohr effect in protons per mole.

Disc Electrophoresis:

Polyacrylamide gel electrophoresis of tubifex hemoglobin solutions was carried out by the method suggested by Brewer and Ashworth (8).

Reaction with PCMB:

The carbon monoxide gas was passed for 15 to 20 minutes on the surface of 7.8×10^{-6} M tubifex hemoglobin, which gives HbCO. 1 ml of 3.34 mg/ml solution of PCMB was added to the 9.0 ml of protein (HbCO) solution and was kept for overnight in refrigerator (5). Standard diagram is shown in Fig 13.

DEAE-Cellulose Column:

10 g DEAE cellulose was washed with distilled water and then 1.0 M NaOH solution, followed by a washing with distilled water

until it was no longer responsive to litmus paper. Then, the cellulose was washed with 0.1 M HCL in acetone and rinsed with distilled water until again neutral to litmus. It was then washed wih .02 M KH_2PO_4 buffer until rinse was at the same pH as that of the buffer.

The cellulose was then stirred into a slurry and carefully poured into the column. After the desired height of the cellulose was attained, the desired (.02 M KH_2PO_4, pH - 8.0) buffer solution was run through the column until the pH of the effluent was equal to the pH of the buffer entering the column.

40 mg of protein was carefully poured on the top of the DEAE cellulose, and was covered with about same volume of .02 M KH_2PO_4 solution. The buffer was changed to 0.1 M KH_2PO_4 + 1 M NaCl solution after the column was run about an hour.

Each 5 ml. fraction was collected in test tube, was analyzed at 280 and 540 nm on Beckman DB-G Spectrophotometer.

Determination of the heme content:

The following recipe suggested by E. Antonini and M. Brunoni (11), has been used in our experiment: 1 ml. of a solution of a heme protein, having an absorbance at 0.50 - 0.01 at 540 nm, is mixed with 3 ml. of a solution of pyridine in NaOH (pyridine 100 ml., normal NaOH 30 ml., water to 300 ml.), oxidized pyridine-hemochromogen which is a fairly stable compound is formed

quite rapidly and the solution can be used a few minutes later for the spectroscopic assay. The spectra between 600 and 450 nm is recorded on Beckman spectrophotometer DBG, just after addition of solid sodium dithionite ($Na_2S_2O_4$), which yields the relatively unstable reduced pyridine hemochromogen. The complete transfer of heme from protein to pyridine was checked by determining the absorbancy at the maximum (557 nm) and at the minimum (525 nm).

Determination of iron:

2.0 ml. tubifex hemoglobin plus 6.0 ml. concentrated HNO_3 and 2.0 cc. $HClO_4$ (1 : 1 dilution) was heated until color of solution turned pale yellow and white fumes of $HClO_4$ started to come off. After that solution was diluted with Fe free water, so dilute sample contains 10 to 200 microgram of Fe per 10 ml. One ml. of hydroxylamine hydrochloride and pH was adjusted to 4.5 with sodium acetate. After the adjustment of the pH, 1 ml. of the O-phenanthroline reagent was added and mixture was diluted to 25 ml., and after 10 to 15 minutes extinction at 500 nm was determined.

A standard curve was constructed by carrying samples of a standard Fe solution through the same procedure.

Results:

The oxygen equilibrium was followed by observing changes in the light absorption in the range from 520 to 620 nm. In particular, the peak at 575 nm was due to oxyhemoglobin. This conclusion is derived from the disappearance of this peak when nitrogen gas is passed across the surface of the hemoglobin solution.

15 ml of 1.16×10^{-5} M hemoglobin was put into the graduated cylinder shown in Fig. 2, oxygen was passed across the surface for an hour, and the spectrum was taken, as shown in Fig. 3. Nitrogen gas was now passed across for an hour and a half and the spectrum again recorded, as shown in Fig. 4. The peak at 575 nm in Fig. 3 has essentially disappeared from the spectrum in Fig. 4. The cycle of oxygen and nitrogen was repeated (as shown in Fig. 5 and 6) to show the reversibility of the process.

A lower concentration of hemoglobin was carried through the same procedure, omitting the final nitrogen flushing. The spectra in Fig. 7, 8, 9 show partial loss of the peak at 575 nm.

A very low concentration, 4.16×10^{-6} M, was run through the same procedure. The spectra in Fig. 10, 11, 12 provide no evidence of loss of peak at 575 nm, and hence no evidence for loss of oxygen from the oxyhemoglobin.

When a very low concentration, about 4.16×10^{-6} M was run through the same procedure, oxygen was passed for an hour and spectrum was taken as shown in Fig 10. After that nitrogen gas was passed for an hour to an hour and half and spectrum was taken as shown in Fig 11, which did not show any change.

Bohr Effect:

The precipitate of hemoglobin was dissolved in 0.1 M NaCl solution and was dialyzed against 0.1 M NaCl solution for twenty four hours; pH was adjusted 7.21. 25 ml of 5.49×10^{-4} M hemoglobin was taken in graduated cylinder as shown in Fig 2, and nitrogen gas was passed for an hour. The change to pH 7.34 was recorded. Added acid to maintain pH constant at 7.21 as about 0.05 ml. From that it can be calculated that 0.38 moles of hydrogen ion were released per mole of hemoglobin. This amount is best considered insignificant.

Sub-Unit Nature:

Disc Electrophoresis:

The Disc electrophoresis of the original hemoglobin and HbCO plus PCMB mixture yielded same results. There were two bands; one was diffuse and in the middle of the gel and one small and sharp all the way to the bottom of the gel. This would be expected if the hemoglobin did not contain free sulfhydryl groups.

DEAE Cellulose Column:

The concentration of the original hemoglobin was found to be 42 mg/ml. The concentration of the sample from DEAE cellulose chromatography was about 0.52 mg/ml. The percent recovery was about 12 percent.

The absorbances at 540 nm and 280 nm are plotted for each sample in Fig 14.

When the sample was first applied with 0.02 M potassium phosphate buffer at pH-8, a band of color was seen to move down the column. When the buffer was changed to 0.1 M, pH-7.15 potassium phosphate buffer in 1.0 M sodium chloride solution, the band became more diffuse. More color remain on the DEAE cellulose column after detectable amounts of protein had ceased to come off, as it is indicated by the low percent recovery.

There are two peaks at 280 nm., but only one peak at 540 nm as shown in Fig 14.

Iron and Heme Content:

The iron and heme analyses were done on a solution containing 6.25×10^{-6} M hemoglobin.

The iron content was determined to be on the average 1.04×10^{-3} m moles Fe/ml, which is about 0.32 percent. This result was confirmed by Atomic Absorption. Heme content was determined to be on the average 20×10^{-5} m moles Heme/ml.

This gives an iron : heme ratio of about 5 : 1.

Discussion:

The absorbance peaks at 540 and 575 nm, which can be seen in Fig. 4-13, are characteristic of oxyhemoglobin and are superimposable on the spectrum given by W. Scheler (9), indicating that the protein isolated was indeed the same as isolated by W. Scheler. The peak at 540 nm is a fairly constant peak for hemoglobin under different conditions, while the peak at 575 nm disappeared when nitrogen gas was passed through, as shown in Fig. 5. Here environment should be anaerobic.

Fig. 5 shows the complete disappearance of the peak at 575 nm. The protein concentration was about 1.3×10^{-5} M. Fig. 9 shows partial disappearance of peak at 575 nm. The protein concentration was about 9.72×10^{-6} M. Fig. 12 does not shown any change in spectrum. The protein concentration was 4.16×10^{-6} M.

From this result one can conclude that oxygen binding is concentration dependent. Increasing the protein concentration will favor the dissociation of oxyhemoglobin.

The addition of the alkali or acid during reversible deoxygentation was very small. This indicates that there is very small 'Bohr effect'; and agrees with the observation that the hemoglobin did not react with PCMB. Both of these are consistent with the conclusion that this hemoglobin has no or very few free sulfhydryl groups. This result

is different from that of vertebrate hemoglobins. From this we conclude that invetebrate hemoglobin polymerizes through some other interaction than sulfhydryl groups (1).

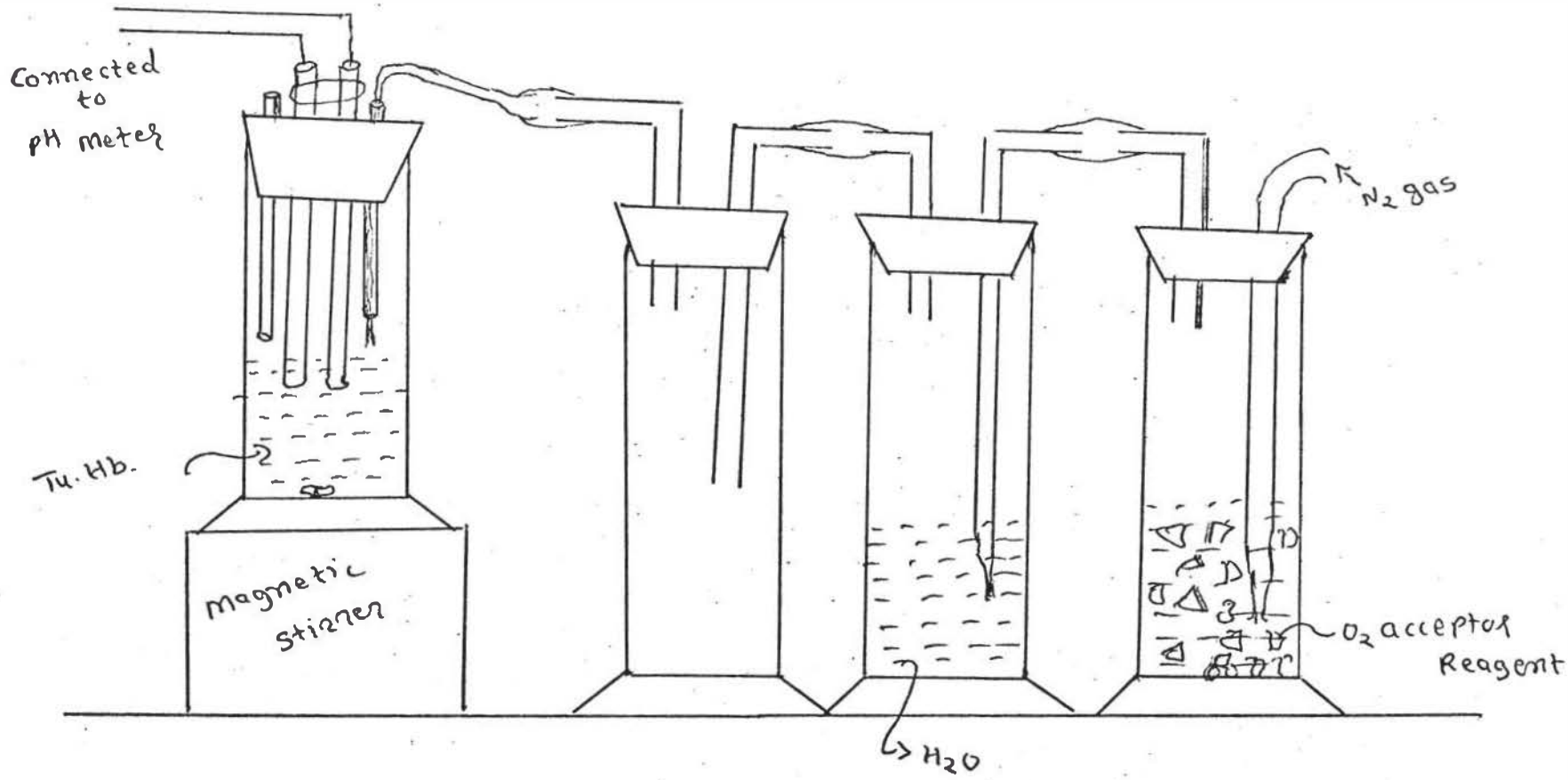
During Disc gel electrophoresis, we obtained two bands. This agrees with DEAE cellulose chromatography which also gave two peaks as shown in Fig. 14. Here we did not have enough protein from the DEAE cellulose column to run gel electrophoresis, and to check the result. One of these protein peaks is most likely a heme-protein, while the other is most likely a non-heme-protein.

According to Powers (14), if there is no Bohr effect, the protein should move towards the cathod for vertebrate hemoglobins. This means that acrylamide gel may not be the best analytical tool for tubifex hemoglobin, since it measures only anodic movement.

The vertebrate hemoglobins all have an Fe:: Heme ratio of about 1, hemerythrin has no heme (7), while tubifex hemoglobin has a ratio of Fe : Heme greater than one. This makes function of iron in tubifex hemoglobin interesting.

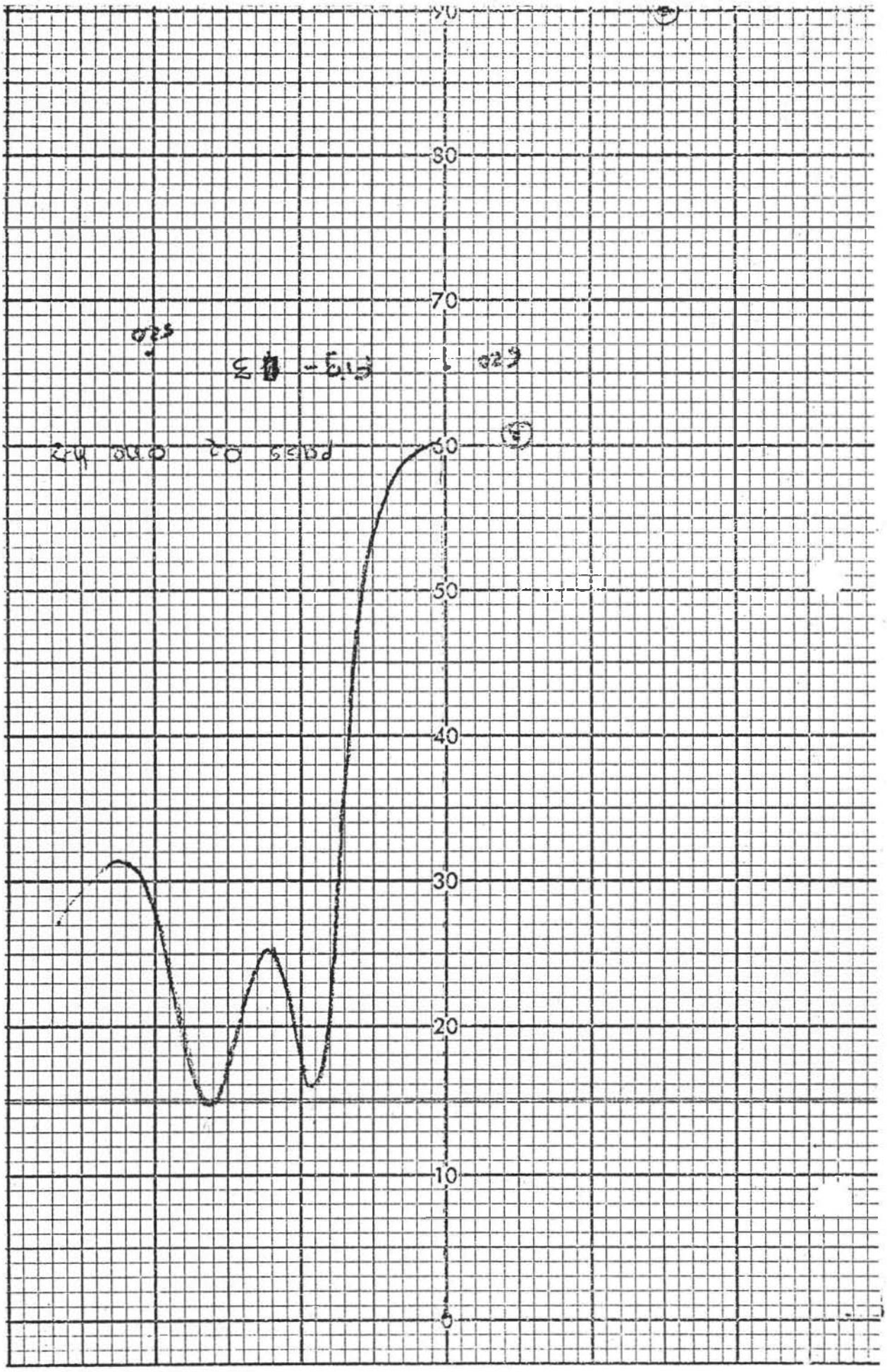
The ratio of Fe:Heme is about 5:1, which is close to 3:1 obtained by W. Scheler and L. Schneidart (13) but does not agree with the data obtained by Nakajima and Braunitzer (3). This means that some one does not have pure hemoglobin. It will be interesting to

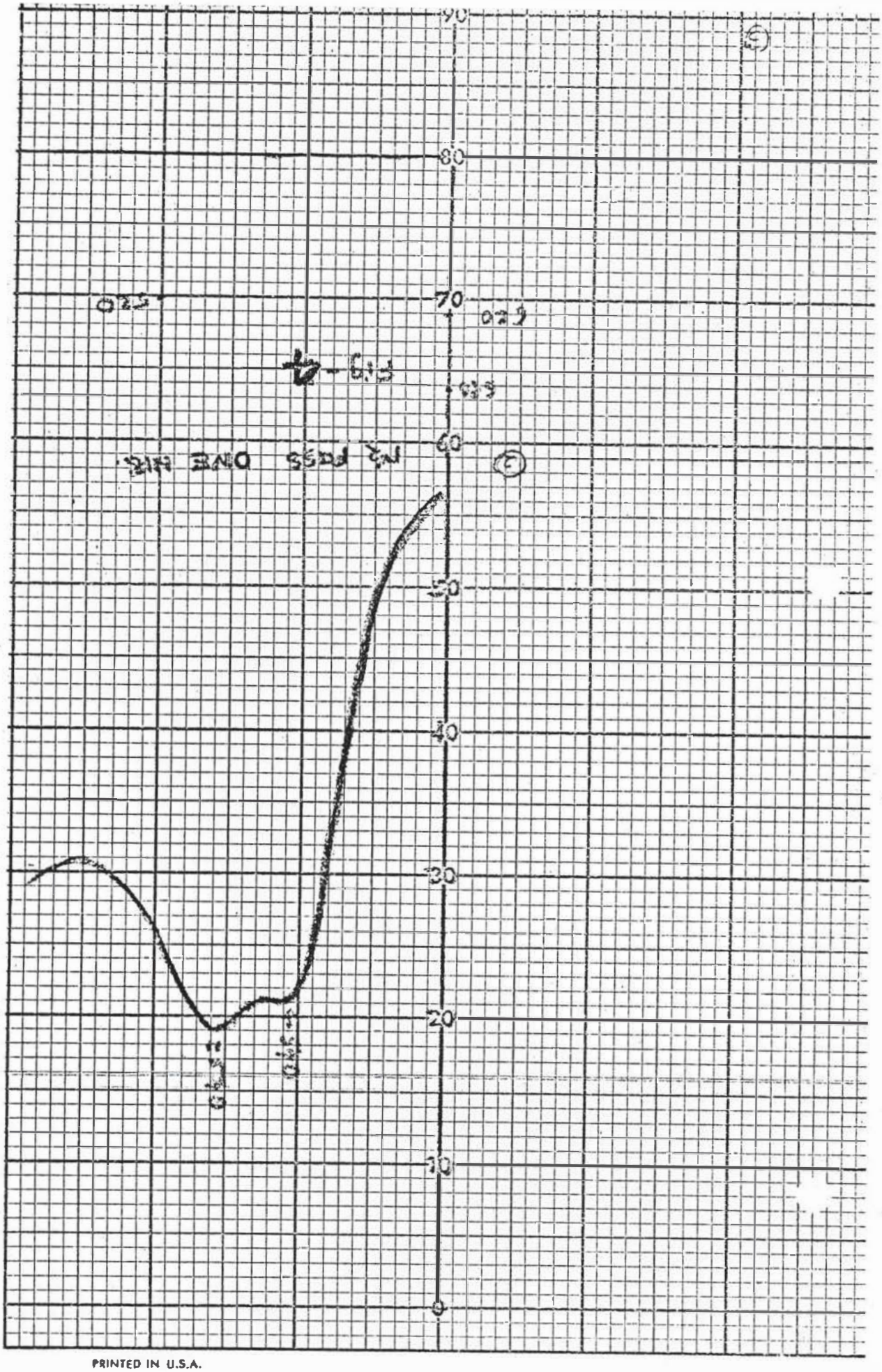
prepare hemoglobin, by some other methods and run starch gel electrophoresis suggested by Powers (14), and check the result.

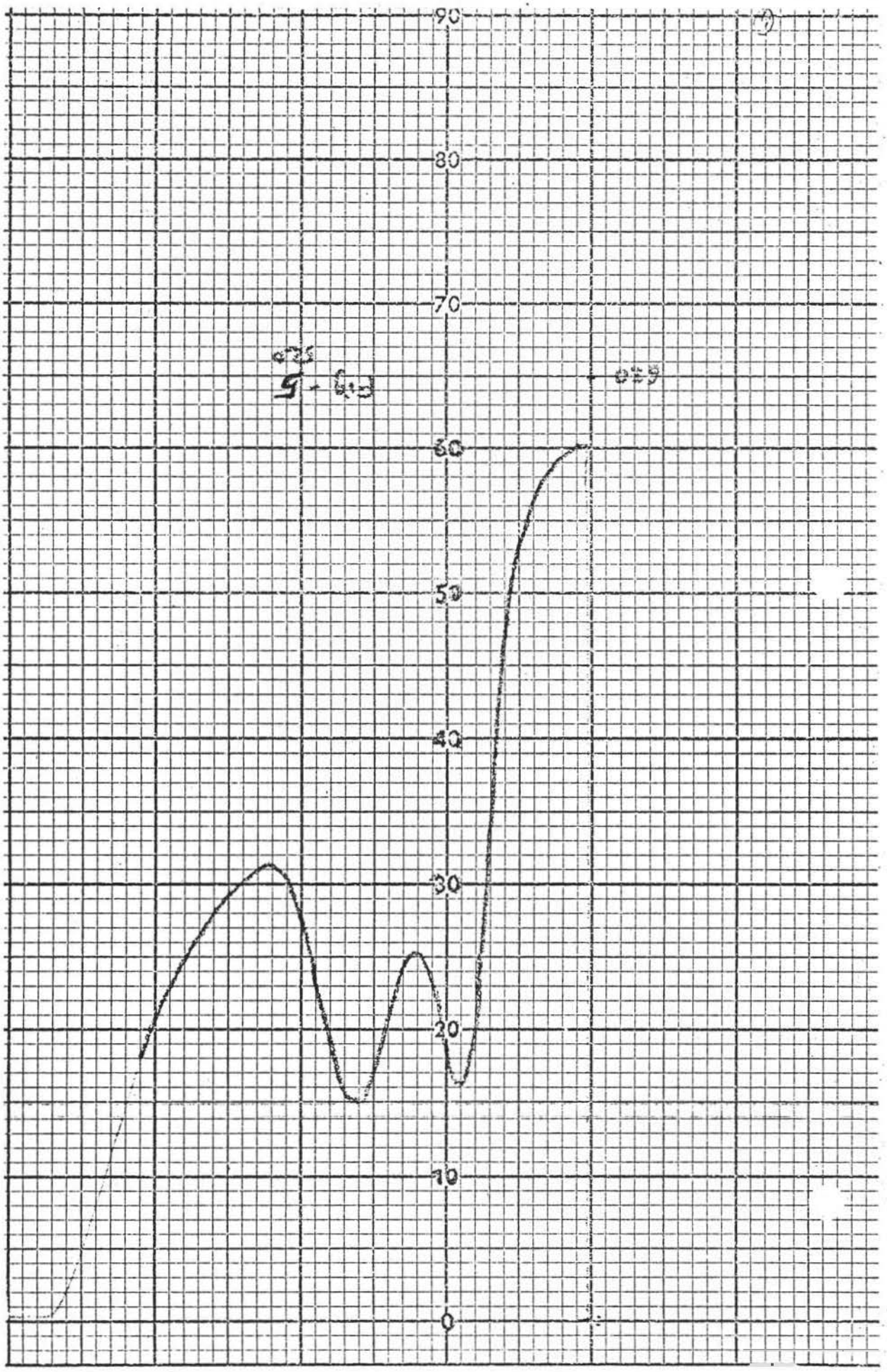


Bohr - effect'

Fig. 2






90
80
70
60
50
40
30
20
10
0

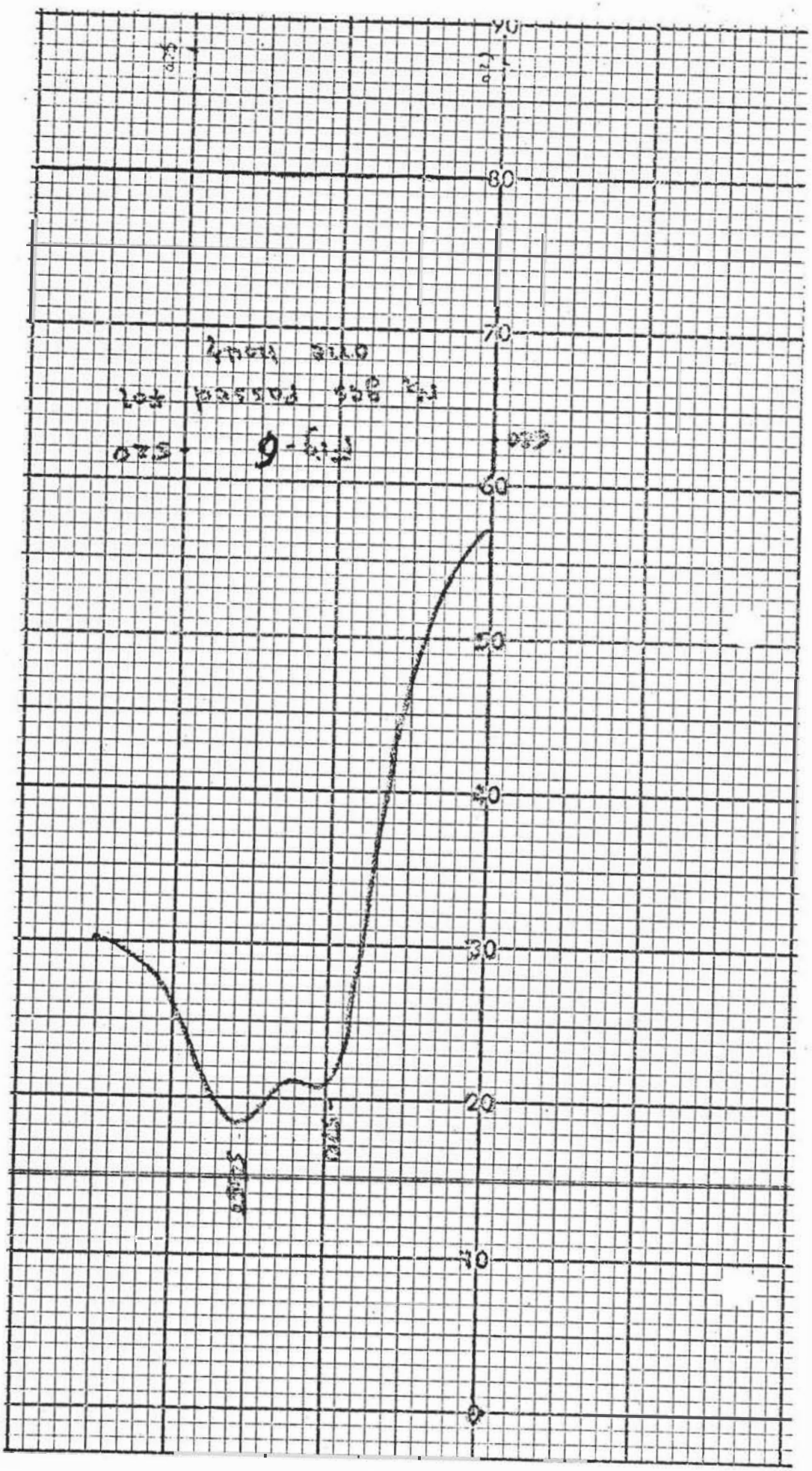

70
80
70
60
50
40
30
20
10
0

Fig. 7

Fig. 8.

Fig. 09.

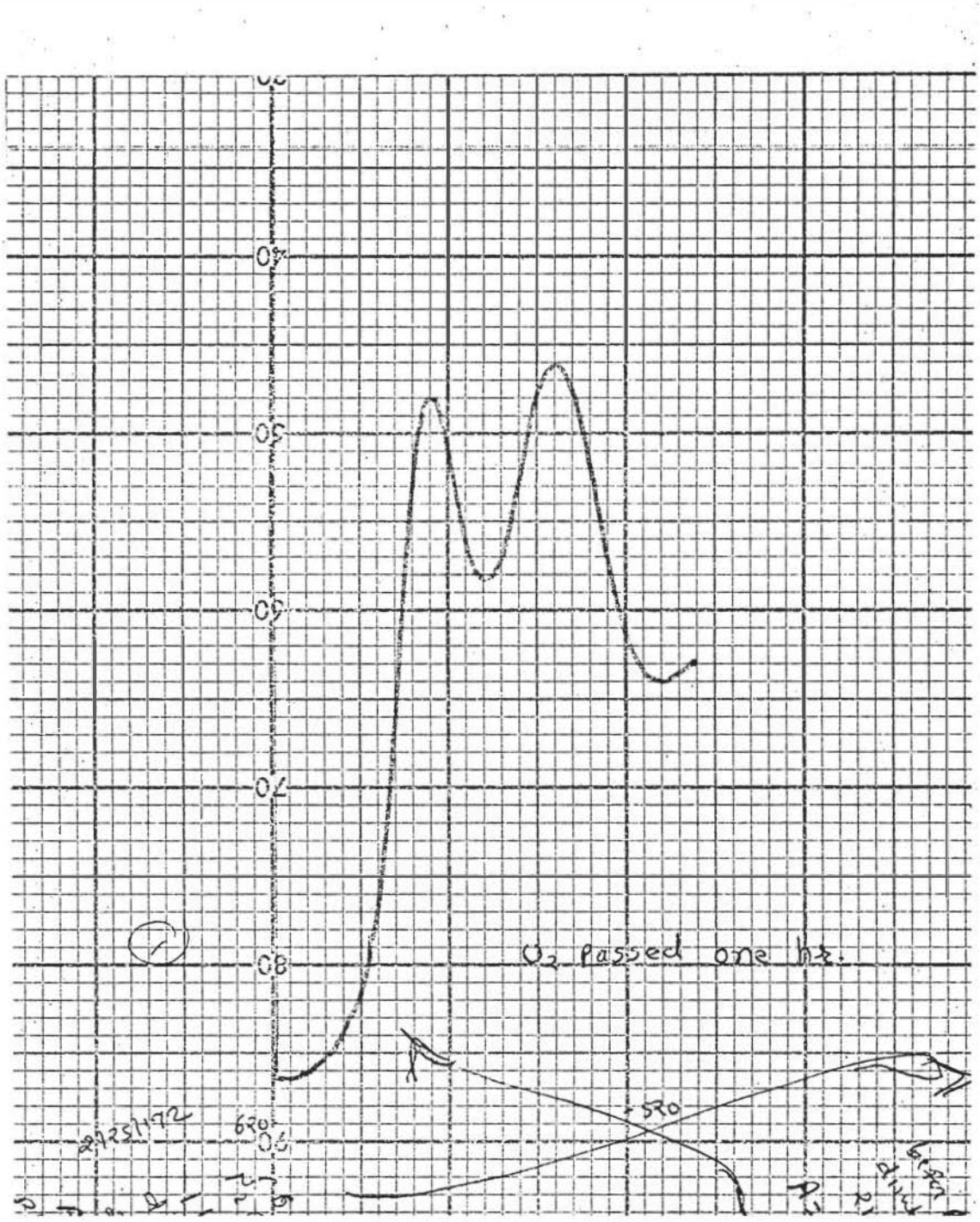
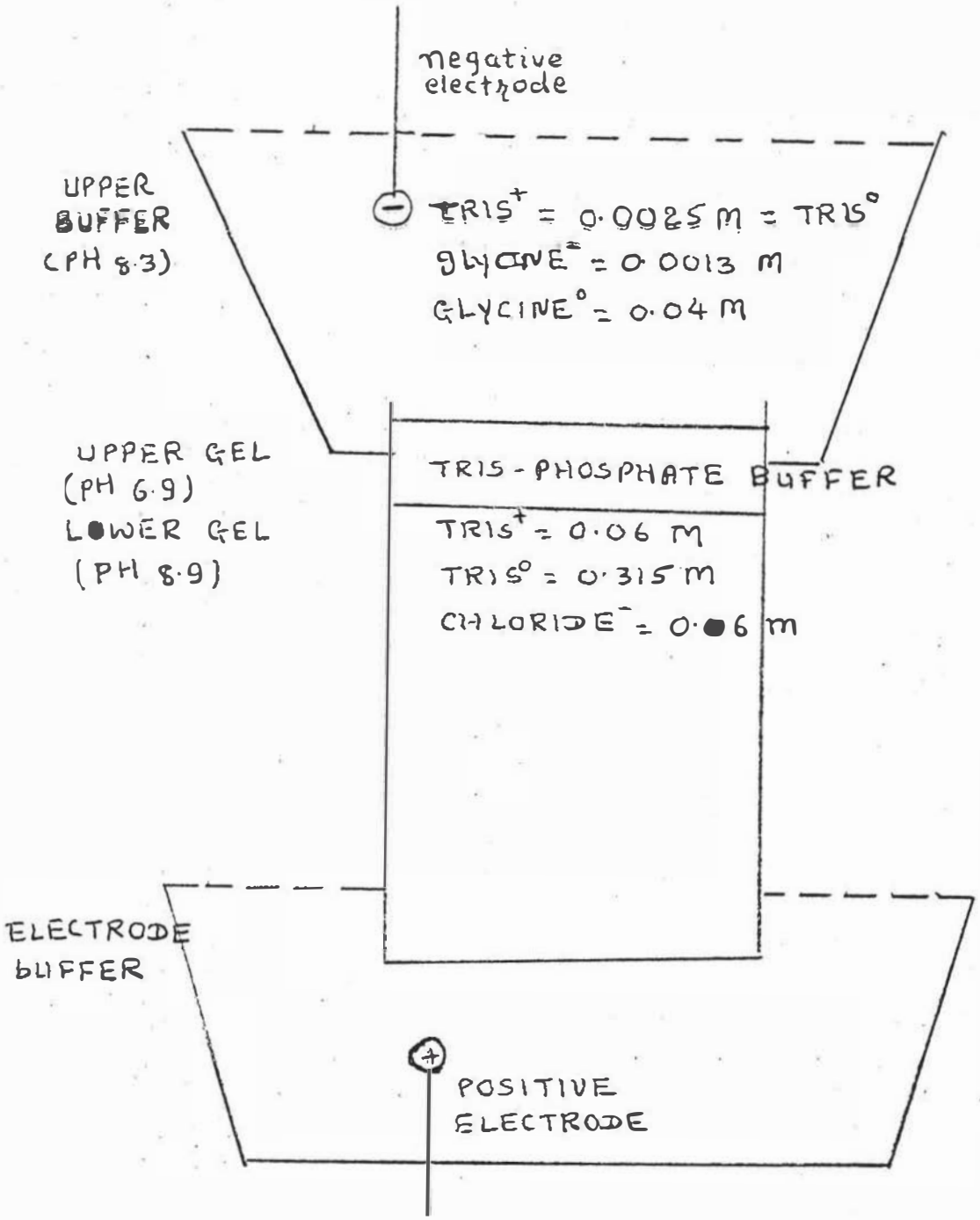


Fig. 10.

20
40
50
60
70
80
90
100
½ passed
1½ hr
620
820
21251-72
BECKMAN PART NO. 101283

Fig. 11

Fig. 12



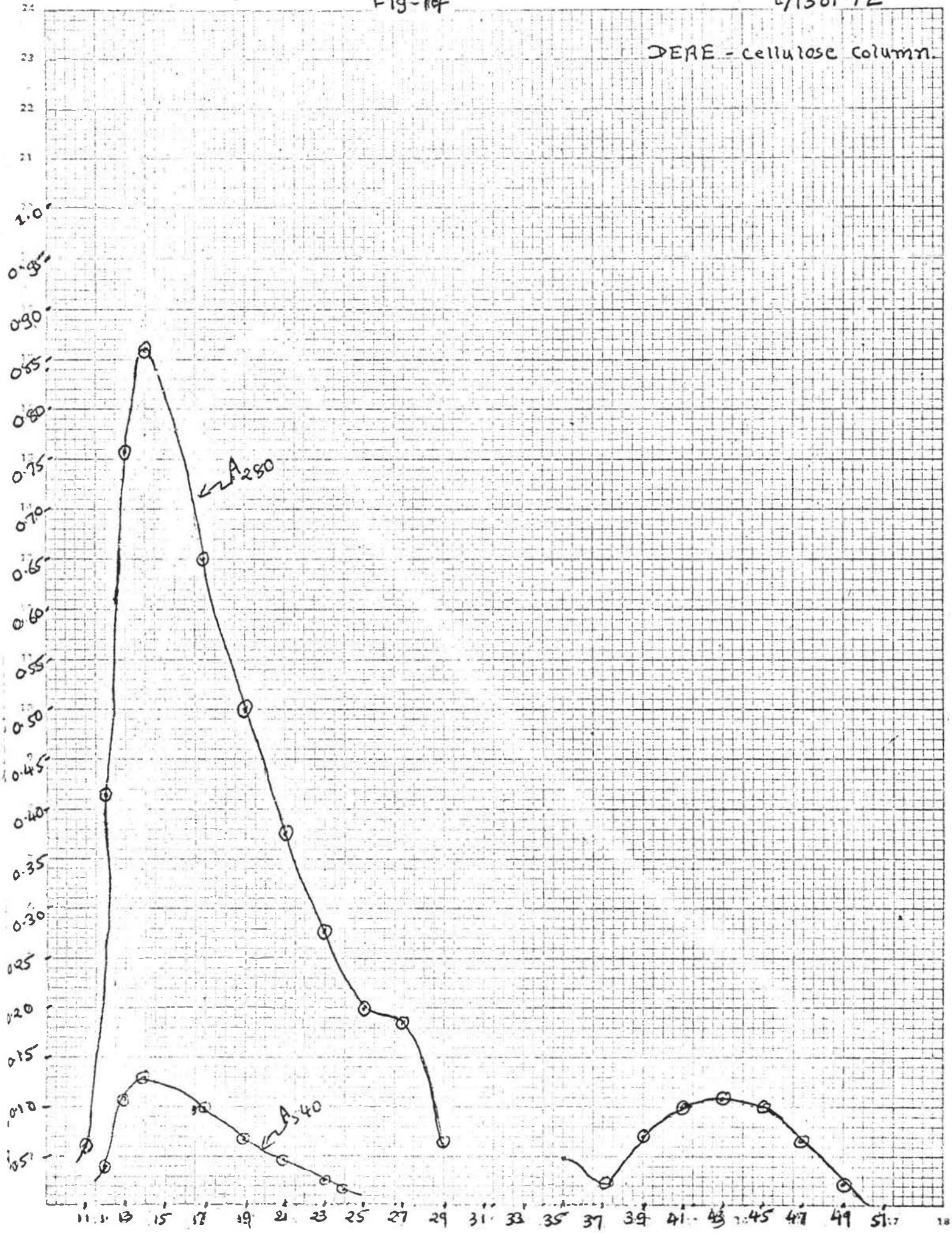
IONIC COMPOSITION OF THE "STANDARD" SYSTEM

Fig. 13

Fig-14

7/30/72

DERE - cellulose column.



Bibliography:

(1) J. Russell and J.M. Osborn, Nature 220, 1125-1127 (1968).

(2) Austin Riggs, Nature 183, 1037-1038 (1959).

(3) Nakajima H. and G. Braunitzer, Hoppe-Seyler's Z. Physiol. Chem. 348, 1-8 (1967).

(4) Reinhold Benesch and Ruth E. Benesch, Journal of Biological Chemistry 236, 405-410 (1961).

(5) Enrico Bucci and Clara Fronticelli, Journal of Biological Chemistry, 240, PC551-PC551 (1965).

(6) Eraldo Antononi, Science 158, 1417-1421 (1967).

(7) G.L. Klippenstein, Biochemistry 11, 372-379 (1972).

(8) John M. Brewer and Raymond B. Ashworth, Journal of Chemical Education 46, 41-45 (1969).

(9) W. Scheler, Biochemische Zeitschrift 332, 366-375 (1960).

(10) R.M. Decoursey, The Human Organism, 289-290 (1961) Second Edition, McGraw-Hill, New York, Publisher.

(11) E. Antonini and M. Brunoni, Hemoglobin and Myglobin In Their Reactions with Ligands, 10-11. Amsterdam, North-Holland Pub. Co.(1971)

(12) Methods in Ensymology III. Edited by Sidney P. Colowick and Nathan O. Kaplan, 1017-1018. Academic Press Inc., Publishers, New York (1957).

(13) W. Scheler and L. Schneiderat, Acta Biol. Med. Germ, $Band_3$, Seite 558-597 (1957).

(14) Dennis A. Powers, Science 177, 360-362 (1972).

(15) George H. Bell, J. Norman Davidson and Harold Scarborough, Text Book of Physiology and Biochemistry, Sixth Ed. 418-420. Academic Press Inc., New York (1965).

Vita

Name:	Vora Lalitchandra Vrajlal
Permanent Address:	c/o M.K. Vora & Co. Tobacco-Merchant Main Bazar, Dhoraji (Gujarat), India
Degree and Date to be Conferred:	M.S. (Chemistry) November, 1972
Date of Birth:	June 27, 1949
Place of Birth:	Dhoraji, India
Secondary Education:	Matriculation from the Shri B.H. School Dhoraji, India, March 1964

Collegiate Institution Attended	Date	Degree	Date of Degree
Gujarat University	1965 1969	B.Sc.	June, 1969
Eastern Illinois University	1970 1972	M.S.	November, 1972

Major:	Biochemistry
Minor:	Physics

Positions Held	Date	Institution
Graduate Assistant	Sept. 1970- May 1971	Eastern Illinois University
Graduate Assistant	Dec. 1971- May 1972	Eastern Illinois University