THE APPLICATION OF MASS SPECTROMETRY TO BLOOD GAS ANALYSIS

by

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A thesis submitted for the degree of

Doctor of Philosophy

to

The University of Aston in Birmingham

January 1980

SUMMARY of thesis submitted for the degree of PhD in 1980
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This research project was concerned with applying the technique of mass spectrometry to the analysis of blood gases. A production model mass spectrometer was used for the experimental work.

The performance of a commercially available blood gas catheter was investigated and found to be unsatisfactory. This led to a fundamental study of membrane systems used with mass spectrometers and blood. In the course of this it became apparent that the analysis of oxygen from blood by this method was affected by phenomena concerned with boundary layer depletion, in particular a complicated non-linearity between the oxygen flux measured by the mass spectrometer and the partial pressure of the oxygen in blood. Such phenomena, although observed by others in the past, had not been systematically studied or satisfactorily explained.

A series of in vitro experiments was therefore devised to elucidate the situation. It was carried out using a number of membrane systems on whole blood, plasma, and haemolysed blood. A new theoretical model of oxyhaemoglobin dissociation in the depleted boundary level of blood was developed and found to explain the experimental observations qualitatively. An extension of this model yielded quantitative predictions that agreed with the results obtained by the experiments. The non-linearity effect was seen to be of significance for membrane systems with oxygen sampling rates of about 2.10^{-9} ml/s/torr and over.

The facts and ideas presented for the first time in this thesis have obvious implications for the feasibility of measuring the partial pressure of oxygen in blood by mass spectrometry employing membrane inlet systems, and should be taken into account if blood gas analysis by such means is to be a practical proposition.

Mass spectrometer; blood; oxygen; membrane.

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ACKNOWLEDGEMENTS

I would like to thank Professor Gordon Cumming for his support and encouragement both as my External Supervisor and as the Director of The Midhurst Medical Research Institute, at which the work described was carried out.

I am grateful to my University Supervisor Mr. C. G. Pearce for his help and advice throughout the period of the research. I am pleased to acknowledge the guidance of Dr. Francois, which has been of particular value to me in the preparation of this thesis.

The work was considerably facilitated by the involvement of Centronic, New Addington, Croydon, in terms of their general guidance and their practical assistance with the mass spectrometer and other equipment.

I would also like to thank the staff of The Midhurst Medical Research Institute for their collective and individual contributions to the research project. Particular thanks are due to Dawn Skinner for the time and trouble she has spent in typing this thesis.

Chapter 1

Introduction

There is often a need to measure the partial pressures or concentrations in blood of gases such as oxygen and carbon dioxide, anaesthetic agents, or tracer gases used in blood flow studies.

The partial pressure of a gas in a liquid is defined as that partial pressure in the gas phase with which it would be in equilibrium. However, the concentration of the gas in the liquid would, in general, be quite different from that in the gas phase. For example, a solution of argon in blood would have a much lower concentration of argon molecules than would the corresponding gas phase of argon, but the fact that at equilibrium there would be no net flow of argon between the two phases is expressed by the statement that the argon in each phase is at the same partial pressure.

Gases dissolve in the plasma of blood, and the dissolved concentration of a gas is directly proportional to its partial pressure, the constant of proportionality being the gas's solubility or Henry's Law coefficient. Therefore, measurements of partial pressure of biologically inert gases are readily interchangeable with measurements of concentration. The situation for oxygen and carbon dioxide in blood is more complicated, as these two gases are not merely dissolved in the plasma, but also involved in a series of reversible chemical combinations, some within the red blood cells.

This is necessary for the fulfilment of one of the basic functions of blood - the transport of oxygen from the lungs to the tissues of the whole body, and of carbon dioxide from the tissues back to the lungs.

The mechanisms involved in the transport of the respiratory gases are discussed further in Chapter 2. They cause the concentrations of oxygen and carbon dioxide in the blood not to be uniquely related to partial pressure. Measurements of partial pressure and concentration are therefore not simply interchangeable and each yields useful information about the body's blood gas status in different circumstances.

The blood gases, together with the blood pH, are normally kept within narrow limits by an automatic control system involving the lungs and the kidneys. In order to illustrate the significance of human blood gas values this control system will now be briefly described.

In the lungs, gas exchange occurs across the alveolar wall , 'arterialising' the capillary blood from its venous partial pressures (typically 40 torr oxygen, 46 torr carbon dioxide) until it reaches equilibrium with the gases in the alveoli (typically at a PO_2 of 100 torr and a PCO_2 of 40 torr). The composition of the alveolar air is largely governed by the rate of respiration - the rate at which gas is exchanged between the lungs and the air outside the body - and this provides one means of control of blood gases. For example, if the PCO_2 of the blood increases for some metabolic reason, the increase is detected by the respiratory centre in the brain, which in turn causes the rate of respiration to increase. This lowers the alveolar PCO_2, and consequently the arterial PCO_2, back towards their normal values.

This sequence of events would usually simultaneously increase the arterial PO_2.

Control of the blood pH close to the normal value of 7.40 is very closely linked to control of PCO_2 and PO_2. This is because the blood pH depends on a balance between the carbonic acid and the bicarbonate ion concentrations in blood. The former is produced by carbon dioxide dissolving in water in the blood, and is directly proportional to the blood PCO_2. The rate of respiration thus affects the blood pH indirectly as well as directly affecting the PCO_2 and PO_2.

The kidneys are involved in maintaining the blood acid/base balance by altering the rate at which they remove hydrogen ions and replenish the concentration of bicarbonate ions. For example, if the PCO_2 of the blood rises for some respiratory reason, causing the blood pH to fall, the kidneys detect this pH drop and compensate for it to some extent by increasing the rate of replacement of bicarbonate ions in the blood.

Values of PO_2 and PCO_2 found to be outside their normal ranges in spite of the body's control mechanisms must therefore be of some clinical significance. Disorders of blood gases and blood pH can be roughly classified in terms of acidosis, alkalosis, hypercapnia, hypocapnia and hypoxaemia. The effects can have a variety of causes and can interact to a considerable degree. For example, in a state of primary respiratory acidosis, the blood pH is initially lowered due to a high PCO_2, which in turn is caused by some impairment of normal gas exchange in the lungs. This impairment could be due to chronic lung disease, acute airways obstruction, or respiratory depression by cerebral injury or drugs. In general, the kidneys will tend to

compensate for the lowered pH by increasing bicarbonate ion concentration in the blood, but the PCO_2 will remain high.

Primary metabolic acidosis in contrast describes a state of low blood pH due to any one of a range of metabolic disorders, such as diabetic ketosis, renal disease, or the effects of drugs, vomiting, diarrhoea, or strenuous exercise. The increased acidity may be partially buffered in the body by the action of carbonic acid and bicarbonate, acting as a buffer pair. The corresponding increase in carbonic acid concentration causes extra carbon dioxide to be excreted by the lungs so tending to reduce PCO_2 and increase pH slightly.

Hypercapnia, a relatively high value of PCO_2, generally occurs with respiratory acidosis, just as hypocapnia may be accompanied by respiratory alkalosis. Hypoxaemia, a relatively low arterial PO_2, can indicate many types of lung disease. It is often, but not always, accompanied by hypercapnia and respiratory acidosis.

It can be seen then that measurements of the arterial blood gases can yield useful information about respiratory or metabolic disorders of the body.

There are two main areas of application for clinical blood gas analysis. One encompasses chest, renal and casualty units. These typically require measurements of PO_2, PCO_2, and pH to aid diagnosis and the monitoring of the effects of therapy. The measurement of bicarbonate ion concentration or total CO_2 content is sometimes useful too. In some of these cases, it may be necessary to measure gases other than the respiratory gases. For example, measurements of the partial pressure of nitrogen in the blood can be of diagnostic value in studies of ventilation/perfusion imbalance, and gases such as argon

or krypton may be involved in measurements of shunt or blood flow to specific organs.

The second area of application is in the intensive care wards, and during surgery. The care of the newborn with respiratory distress syndrome comes into this category, requiring constant monitoring of arterial PO_2 and PCO_2 to guide and control therapeutic procedures such as the adminstration of high oxygen breathing mixtures. For surgical patients, it may be necessary to measure oxygen saturation - the degree to which the blood's haemoglobin is saturated with oxygen - as well as PO_2 and PCO_2. During extracorporeal circulation, for example, it is particularly important to monitor the actual amounts of oxygen carried by the blood.

The analysis of blood gases, by whatever appropriate means are available, is therefore of importance, in clinical diagnosis and therapy as well as in physiological research. The historical development of methods for performing the various types of blood gas measurement in normal use is described in Chapter 3.

This particular research project was concerned with applying a mass spectrometer to the analysis of blood gases. Chapter 4 describes the mass spectrometer used in this work, with special reference to those aspects of its design and operation of relevance to blood gas analysis. Chapter 5 describes first a brief study of a currently available type of membrane covered blood gas catheter, and then preliminary experiments with polyethylene as a blood gas membrane. Chapter 6 comprises a detailed study of blood gas membranes, and Chapter 7 discusses the conclusions of the work. Appendix 1 consists of theoretical and experimental work on the flow dependence of signals

from gas consuming catheters. Appendix 2 describes some attempts at measuring trace concentrations of soluble gases in blood.

Chapter 2

Blood

This chapter summarises aspects of blood of particular relevance to the work to be described, specifically its gas carrying functions, its flow properties, and its responses to man-made objects either introduced to the blood stream in vivo or in contact with blood in vitro.

2.1 Gas Transport

Multicellular organisms need circulatory and chemical aids to achieve efficient gas transport. Mammals use the chemical haemoglobin in the blood to carry out much of this function by combining with oxygen from the alveoli of the lungs, delivering it to the tissues that need it, taking up waste carbon dioxide from the tissues, and delivering this back to the alveoli.

The haemoglobin molecule consists of four porphyrin groups, which contain iron, attached to a protein. At the alveoli oxygen diffuses into the blood under the influence of a partial pressure difference of about 60 torr acting across an alveolar wall thickness of about 1.5 μm. If the oxygen simply dissolved in the plasma, the concentration in solution would be directly proportional to the partial pressure, and also would be far too low to cope with the body's needs. In fact,

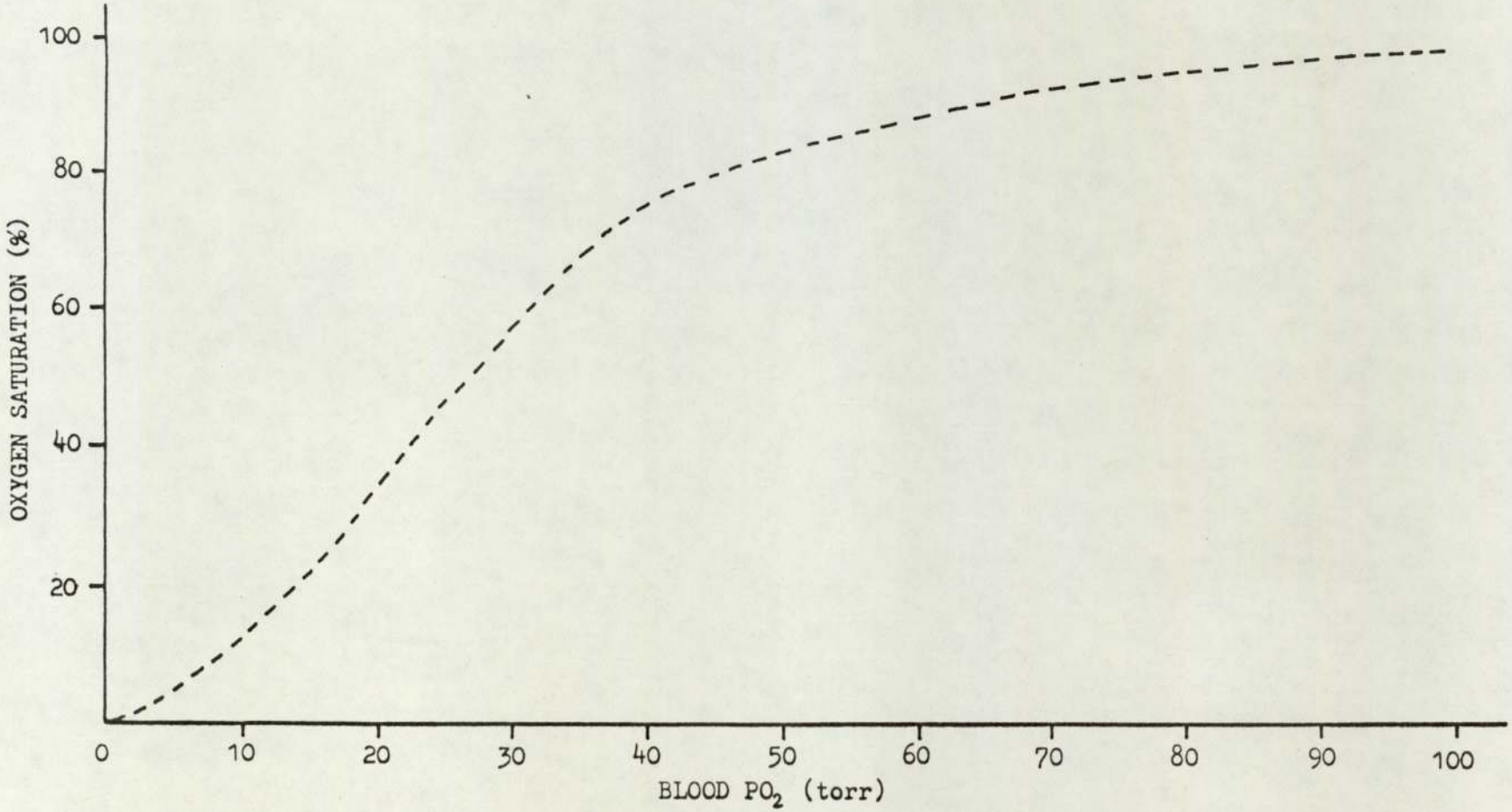
the dissolved oxygen undergoes chemical reactions with the haemoglobin present within red cells suspended in the plasma, so the oxygen concentration in the plasma drops, and more oxygen diffuses in from the alveoli. This process ensures that much more oxygen is taken up by the blood than simple solution could account for. The non-linear relationship between the amount of oxygen contained by the blood and the corresponding partial pressure is illustrated in Figure 1 as the oxygen dissociation curve of blood.

The final product of oxygenation is oxyhaemoglobin Hb_4O_8. In 1925, Adair hypothesised four stages to this reaction, each stage being reversible

$$Hb_4 + O_2 \rightleftharpoons Hb_4O_2$$
$$Hb_4O_2 + O_2 \rightleftharpoons Hb_4O_4$$
$$Hb_4O_4 + O_2 \rightleftharpoons Hb_4O_6$$
$$Hb_4O_6 + O_2 \rightleftharpoons Hb_4O_8$$

In vitro study of these reactions has yielded information on their equilibrium constants, and when these are inserted into a formula for theoretical oxygen saturation as a function of partial pressure, agreement with the experimentally derived relationship is found. Similar experiments have also shown that the rates of the reactions are sufficiently fast to account for the 97% degree of saturation of haemoglobin in the very short time (0.75s) that a red cell passing through a pulmonary capillary may be exposed to alveolar oxygen via the capillary wall and alveolar membrane. The pH of blood affects the polarisation state of haemoglobin, which alters its affinity for oxygen, and temperature affects the heat of combination between haemoglobin and oxygen, so both these factors affect the oxygenation

FIGURE 1 : Oxygen dissociation curve of human blood



reactions in blood, and in effect 'shift' the dissociation curve, as shown in Figure 2.

When the blood reaches the tissues, at the actively metabolising sites of which the partial pressure of oxygen may be as low as 1 torr, oxygen diffuses out of the plasma into the tissues. The plasma PO_2 drops, oxyhaemoglobin in the red cells dissociates, giving up oxygen to the plasma, so the plasma PO_2 tends to recover. More oxygen can then diffuse out of the plasma, and in this way the delivery of oxygen to the tissues is sustained as required. Thus, one vitally significant characteristic of haemoglobin is its ability to combine with oxygen at a high partial pressure, and yet readily reverse the process to give up oxygen at a low partial pressure.

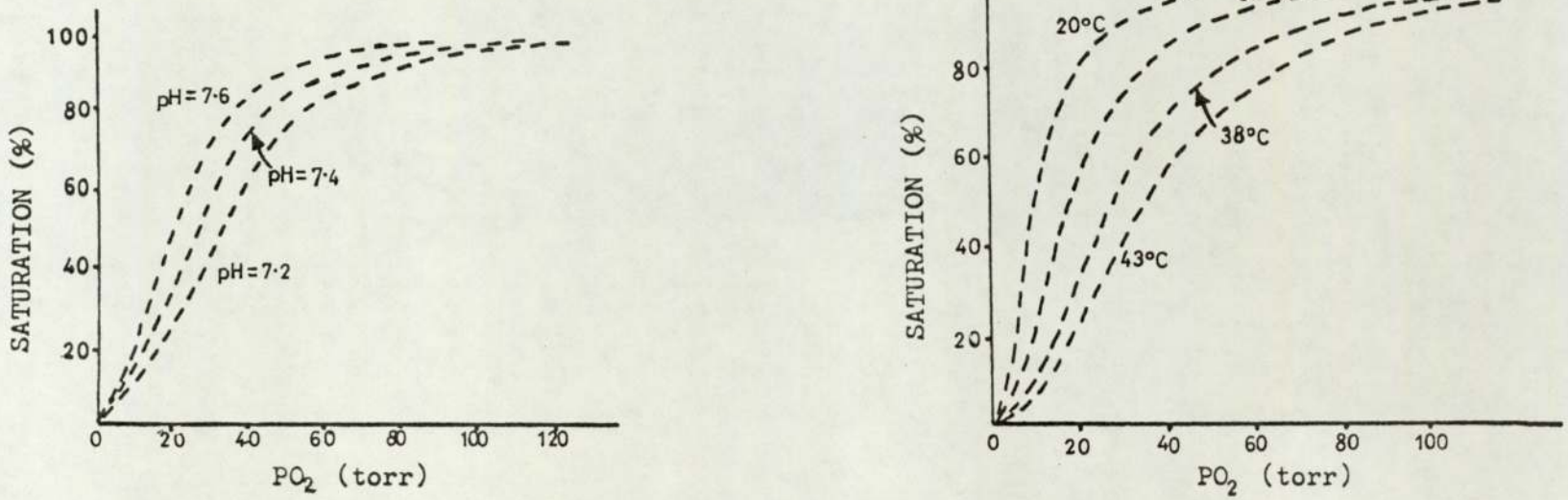
Haemoglobin is also involved in the transport of carbon dioxide, as follows

As carbon dioxide diffuses into the blood, and into the red cells themselves, it is hydrated to carbonic acid, the reaction being catalysed by carbonic anhydrase which is present in the cells. The acid dissociates spontaneously to H^+ ions and HCO_3^- ions, so the whole process is described by

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

The H^+ ions so formed are readily taken up by anionic sites on the haemoglobin molecule, such as the NH_2 groups of lysine, so the above equilibria are shifted to the right, and much more carbon dioxide is taken up by the blood than if the reaction products were allowed to accumulate. As more hydrogen ions are taken up by haemoglobin, the bicarbonate ions formed diffuse through the red cell membrane into the

FIGURE 2 : Effect of pH and temperature on O_2 dissociation curves



plasma, and to maintain electrical neutrality chloride ions, from sodium chloride in the plasma, diffuse back into the red cells. This phemomenon was first recognised in 1878 by Nasse, and is called the Chloride Shift.

A further complication is an interaction between the ability of haemoglobin to take up H^+ ions and its ability to combine with oxygen. It happens that at a 'normal' pH of 7.40 oxyhaemoglobin has a greater number of anionic groups than haemoglobin, so as more oxygen is given up to the tissues more H^+ ions must combine with anionic groups, and so more carbon dioxide is encouraged to dissolve. In a similar way, as more carbon dioxide enters the blood, causing H^+ ions to combine with haemoglobin, the ability of the haemoglobin to hold its oxygen decreases, and so more oxygen is encouraged to leave the blood and enter the tissues. This is known as the Bohr effect.

A small amount of carbon dioxide can react directly with haemoglobin via its amino groups, to form carbamino compounds, and is carried from the tissues in this form. This direct combination causes the oxygen dissociation curve to shift with the PCO_2 itself as well as the corresponding pH effects.

At the alveoli, the partial pressure gradient causes the carbon dioxide to diffuse out of the blood, so the haemoglobin has to give up some H^+ ions, which combine with bicarbonate ions in the plasma to form carbonic acid, which then dissociates to carbon dioxide and water. The carbon dioxide can then diffuse into the alveoli. This loss of carbon dioxide occurs much faster than if only diffusion occurred. The affinity of haemoglobin for oxygen increases with its loss of H^+ ions, and so oxygen is avidly taken up, and the whole cycle of gas transport is repeated.

2.2 Blood Flow

For the laminar flow of a Newtonian fluid through a cylindrical tube, the shear stress S is given by

$$S = \frac{4 Q \eta x}{\pi r^4}$$

where x is the distance of the point concerned from the axis of the tube, Q is the volume flow rate, r is the radius of the tube, and η is the fluid's viscosity. The shear stress is at a maximum at the walls of the tube where x=r, and a minimum at the axis, and for a given flow rate the stresses increase as tube diameter decreases. Also, the viscosity of such a Newtonian fluid is a constant, characteristic of the fluid, and independent of the velocity gradient developed.

The flow of blood through the vascular system conflicts in several ways with this simple picture. For one thing, about 80% of the vascular system consists of very small diameter channels - less than 180 μm - in which the resistance of flow is so high that the channels should be virtually impassable. In fact, experiments show that the viscosity of blood actually decreases in these small vessels, approaching the viscosity of plasma alone. In addition, a suspension of particles in a fluid would be expected to have a viscosity significantly higher than that of the fluid itself, and at volume fractions of about 45% to be several orders of magnitude higher. One physical reason for this is that the particles tend to rotate within the moving fluid, thus converting some of the translational kinetic energy of the smoothly flowing liquid into rotational energy. However, the viscosity of blood, which normally has a volume fraction

of suspended cells of about 45%, is only about twice that of pure plasma, and increases only slightly when the haematocrit is artificially raised to even 90%.

It is known that in the disease of sickle cell anaemia, the viscosity of the red cells is very high at low PO_2, and it is found that the overall blood viscosity is greatly increased. Similarly, if the plasma is artifically made hypertonic to increase the red cell viscosity, the overall blood viscosity increases.

These facts may be explained as follows. Theory shows that an emulsion (a suspension of liquid droplets) will have a viscosity very close to that of the carrier fluid if the viscosity of the droplets themselves is similar to that of the fluid. If the red cell membrane is considered as a sheathing around liquid contents of viscosity close to that of plasma, then it may be flexible enough to transmit shear stress so evenly that the contents of the cell (or 'droplet') share the flow characteristics of the plasma, rather than disturbing them, and so the overall measured viscosity is likely to be not much higher than that of the plasma. Also, this effect is likely to become more marked as the stresses on the cell membrane and so the velocity gradients in the fluid increase, i.e. in flow through narrower vessels. Any factor that increases internal red cell viscosity must increase the difference between cells and plasma, so lessening the similarity to an emulsion in favour of a suspension of solid particles - this explains the effects of sickle cell anaemia and hypertonicity.

Another experimental observation on blood flow is that the red cells tend to concentrate close to the axis of the blood vessels, leaving an annulus of plasma next to the wall, this 'axial stream' or

'skimming' effect being significant in blood vessels of diameter less than about 500 μm. This process lowers the viscosity of the blood as a whole, as the low viscosity plasma is situated in the area of maximum velocity gradient. It is achieved because the deformability of the red cells allows the shear stresses to force them to the axis of the tube. It is also this deformability that allows cells to dramatically streamline their shape to enter vessels of diameter less than 5 μm (i.e. less than the cell's original diameter) and flow through them with a viscosity only about 5% greater than that of plasma.

The driving forces for blood circulation are provided principally by the heart, but also by the thoracic and muscle 'pumps'. The major pressure drop in circulation occurs across the arterioles and capillaries, where the increase in resistance to flow caused by their narrow diameters much more than compensates for the corresponding drop in blood viscosity.

The heart produces almost square waves of blood pressure, but the elastic wall of the aorta converts this to a relatively small amplitude pulsation over a steady intermediate pressure i.e. it smooths the flow between systole (at about 120 torr) and diastole (at about 80 torr rather than zero). Arteries can change their diameters to control the distribution of blood flow, using smooth muscle as well as elasticity. In capillaries, which have diameters of 20 μm or less, the red cells may have to flow in single file, and even distort as mentioned above, to make up 'plug' flow. Flow can be completely blocked if the blood pressure within such vessels is so low that the tension in the vessel wall causes the vessel to collapse. The large

veins have no elasticity, and alter in diameter according to the flow, so venous pressure hardly alters even though flow changes may be considerable.

2.3 Blood responses to interference

Devices inserted into the bloodstream can disturb the system in three principal ways - by damaging the red cells, damaging blood vessel walls, or inducing clotting by the nature of their surfaces.

Clotting is the normal blood response to injury at a surface. A complex series of reactions, which may be summarised by four main steps, occurs. Platelet factors and other factors in the presence of the surface produce thromboplastin. Thromboplastin reacts with prothrombin to form thrombin. Thrombin reacts with fibrinogen to form fibrin. This fibrin collects on the surface trapping platlets and blood cells into a clot.

Under normal circumstances, the blood is naturally prevented from clotting by a number of controls including the laminar flow of blood, the smooth endothelial lining cells, and the plasmin enzyme system. Anticlotting agents that may be artifically introduced to the blood stream can be classified into two types. The heparin type are polyanions, and interfere with the production of thrombin and thromboplastin. They can be countered by chemicals like protamine sulphate. The coumarin type are electron donors, and they inhibit the formation of prothrombin in the liver. They can be countered by agents like vitamin K. Unrestricted use of such anticoagulants is inadvisable since the ability of blood to form clots is essential in case of a real injury, to avoid excessive loss of blood. Anticoagulants can also cause the loss of proteins and even whole red cells from the blood to the tissues, by increasing the permeability of blood vessel walls. Thus it is generally preferable to avoid inducing the clotting response if at all possible rather than to employ anticoagulants.

There is some evidence to show an electrochemical component in the mechanism of thrombosis. For example, when metal electrodes are placed in blood, the weight of thrombus deposited in a given time is directly proportional to the current flowing. The normal blood vessel wall is negatively charged on its inner surface. Injury, or a raised pH, can reduce the charge, and even make it positive. Both these influences are known to encourage clotting. Similarly, thrombogenic drugs are found to decrease the negative surface charge, while antithrombogenic drugs increase it.

Teflon, silicone rubber, polyvinylidene chloride, and Hypalon are amongst materials known to be more resistant to thrombus formation when treated chemically or electrically to induce a high negative charge density over the surface. However, glass, which is highly thrombogenic, also has a high negative surface charge, so charge density alone cannot be the critical parameter. It seems likely that the uniformity of the surface charge distibution is as important as its sign, when choosing materials for non-thrombogenic qualities.

Red cells make up about 45% of the total blood volume, and in many ways behave as if they were fluid drops bounded by flexible membranes. The cell membrane is coated with a mucoprotein, rich in sialic acid, which seems to 'proof' the cell to some extent against contact with other cells or vessel walls. Prolonged turbulence in the bloodstream can deform red cells, and make them behave as rigid particles rather than droplets. They may then block small capillaries, increase blood viscosity, clump together and damage vessel walls, and break up under shear stress, so encouraging fibrin formation, and clots.

Blood vessel walls are lined by endothelial cells, which are themselves further coated by a thin film, acting as a non-wettable barrier to contact with the blood. The composite wall is normally impermeable to molecules of molecular weight greater than 10,000. In spite of its high permeability to water, the hydrostatic pressure within the vessel acting outwards is 'balanced' by the colloid osmotic pressure of the plasma, which tends to draw water in. Turbulence in the bloodstream can damage the vessel walls, causing wetting of the endothelial cells by blood, leading to fibrin formation and so to clotting.

Devices inserted into the blood stream should thus be shaped to minimise turbulence, as well as being composed of materials chemically inert with respect to the blood, non-thrombogenic and sterilisable. Devices for in vitro use may not need to comply with such stringent requirements, especially as the blood samples themselves can be anticoagulated chemically after withdrawal.

Chapter 3

Historical Development of Blood Gas Analysis

3.1 General Background

Reproducible measurements of blood gases date from the 1830's, and the first methods used depended on the extraction of gases from discrete samples of blood by such means as evacuation over mercury, flushing with an excess of hydrogen, the application of high temperature, or saturation with carbon monoxide, usually followed by chemical separation and analysis. In 1897, Haldane found that potassium ferricyanide liberated oxygen from blood, and he developed a method for measuring the volume of oxygen liberated after saturation. Many variations on this theme followed. Then, from 1914 to 1927, Van Slyke developed several systems that combined vacuum extraction over mercury with chemical extraction and separation - the final, most accurate version measured the pressures of the liberated gases in a fixed volume, and then calculated the corresponding gas contents in terms of volume of gas per 100 volumes of blood. Even now, the manometric apparatus of Van Slyke and Neill (1924) is the primary standard for the measurement of blood oxygen content.

From 1872 another set of methods was developed for equilibrating a sample of blood with an introduced sample of gas (usually expired air) and then analysing the gas sample chemically, and so calculating the corresponding gas partial pressures in the blood.

In 1864, Stokes discussed the possibility of using the difference in colour between oxygenated and reduced haemoglobin to measure the degree of oxygen saturation of blood. This property has since been successfully used in transmission and reflectance oximeters to monitor oxygen saturation, at first qualitatively, but now modern spectrophotometers can measure saturation quantitatively and absolutely, in vitro and in vivo.

From 1959 onwards, gas chromatography has been applied to the measurement of blood gas contents after chemical or vacuum extraction of the gases from discrete blood samples.

At present, the most widely used methods of measuring partial pressures of the respiratory gases are electrochemical, employing polymer membranes to separate the blood from the sensing electrodes. These oxygen and carbon dioxide electrode systems have been adapted to suit particular applications, such as the continuous intra-arterial monitoring of the blood gases of premature babies, as well as being in routine use in hospital laboratories for the analysis of discrete in vitro samples. In view of their dominant position in present day blood gas analysis, the development of electrochemical systems is now considered in some detail.

3.2 Electrochemical Blood Gas Analysis

The basic principle behind most electrochemical measurements of PO_2 in liquids is that in an electric field between two electrodes oxygen molecules polarise and migrate to the cathode where they are reduced and so cause a flow of current. If the potential difference, which may be supplied from an external source or internally by galvanic action, acting across the electrodes is within a certain range, the magnitude of this current is independent of the potential difference, and proportional to the PO_2 of this liquid.

Initially, such electrode systems were not satisfactory for use in blood due, to a large extent, to contamination of the cathode by proteins and ions from the blood. Also, in vivo the application of an electric field to a conducting medium obviously involved hazards. To overcome these problems, membranes were introduced by Clark et al, first one of cellophane surrounding the cathode [Ref. 12], and then in 1956 [Ref.13] one of polyethylene surrounding both electrodes and a small amount of electrolyte. The latter arrangement, in which oxygen reduction occurs in the electrolyte, which reaches equilibrium with the blood through the membrane, has the advantage that the entire electrode system is chemically and electrically isolated from the blood, and such systems attracted much attention and development.

For example, in 1958 Severinghaus et al [Ref. 14] tried out different types of membrane with a Clark-type electrode and found that 0.001 inch thick polyethylene offered a useful compromise between the stability and fast response but corresponding depletion occuring with 0.001 inch thick teflon, and the low consumption but slow response and

instability of 0.00025 inch thick Mylar.

Experience led to modifications until, in 1965 [Ref.15], a reasonably typical PO_2 electrode could be described as follows - the cathode was a 25 μm diameter platinum wire sealed in glass with its tip exposed to a film of water with KCl and phosphate buffer. A potential difference of 0.7V was applied between the wire and an anode of silver coated with silver chloride, and a polypropylene membrane enclosed the whole assembly. Typical properties included a total response time of 30s, a sensitivity of 10^{-11} Amperes/torr, linearity in the physiological range of $\pm$ 1%, and drift less than 5% per hour. The ratio of the electrode's outputs for the same PO_2 in the blood phase compared to the gas phase was 1.0 below 100 torr, 0.985 above 100 torr.

The main areas of application in physiology for continuous PO_2 measurements have been intra-arterial monitoring, tissue gas analysis, and, more recently, transcutaneous analysis of arterial oxygen, especially for neonates.

An early attempt at continuous recording of PO_2 in vivo was reported by Kreuzer et al in 1958 [Ref.16]. They used a Clark-type electrode in a polyethylene catheter placed in the descending aorta of the dog, and made some comparisons with in vitro analyses of withdrawn samples. Typical problems encountered with such in vivo attempts were flow dependence, depletion leading to low blood/gas factors, instability, and zero drift, all of which complicate calibration and operation [Ref. 17 and 18].

In 1967 Schuler et al [Ref.19] published a theoretical analysis of membrane covered PO_2 electrodes as applied to continuous in vivo

blood gas monitoring. They derived equations for the dependence of diffusion layer thickness on liquid flow velocity, considering the effects of cathode diameter, liquid density and viscosity, the electrode's geometry, and membrane permeability among other factors. They concluded that to minimise response time while retaining sensitivity, it would be necessary either to use a number of small diameter cathodes together, or alter the geometry to a ring-shaped cathode. These suggestions were followed by some [Ref. 20 and 21] and found valid.

Shortly afterwards, various catheter designs were developed specifically for neonates [Ref. 22, 23 and 24] and were found useful for quantitative monitoring. Studies with commercially produced catheters were carried out - for example, Harris et al [Ref. 25] evaluated the performance of 50 IBC Clark-type electrodes (with separate anodes) in 48 acutely ill neonates. They found that in vivo recalibration was necessary every 17.5 hours, that flow dependence was minimal, but that the standard error for 999 comparisons with in vitro analyses was $\pm$ 18.8 torr. The error increased at high PO_2, and with elapsed time from calibration.

Many different membrane materials were tried, among them PTFE, PVC, silastic, Hydron, and polyethylene. Improvements were made, such as reducing response time to 3-4s so that breath by breath variations could be detected, or monitoring and correcting for temperature variations with an integral thermocouple [Ref.26], and indwelling PO_2 monitors have also been used in adults [Ref. 27 and 28], during anaesthesia, for example.

Local measurements of tissue oxygen tension are required in the

study of oxygen supply to organs, and have been attempted since 1942 [Ref. 29]. In some cases, membrane covered polarographic electrodes have been used to prevent contamination of the electrode surface, but in far more the requirements for high sensitivity and ease of insertion with minimal damage have led to the use of bare wire or needle electrodes with separate reference anodes. For example, Moss [Ref. 30] used bare platinum electrodes of overall o.d. 0.254 mm for quantitative measurements of intramyocardial oxygen tension in dogs.

One complication with tissue gas analysis is that measurements taken at a single place on an organ's surface or within its bulk cannot be considered as representative of the organ as a whole, and really it is the pattern of distribution of measured PO_2 through a region of tissue that is significant, so a number of separate measurements have to be made for each region of interest [Ref. 31]. In some situations this is impractical, and one or two results are assumed to represent an 'average' PO_2, as in measurements in the foetal scalp in the initial stages of delivery for example [Ref. 32].

Other problems include the effect of electrode oxygen consumption on the oxygen tension distribution being measured, and the particular importance of calibration, as in vivo independent checks are not generally possible. Nonetheless, stable in vivo readings that correlate well with repiratory and circulatory changes have been reported - for example in the skin of the human leg [Ref.33].

The possibility of measuring arterial blood gases without actually withdrawing a blood sample or even entering the vascular system is obviously an attractive one, and in 1951 Baumberger et al [Ref.34] tried to achieve this by immersing the subject's finger in a

hot buffer solution for a period of time and then measuring the PO_2 of the solution polarographically with dropping mercury and calomel electrodes. Some years later, the same principle of equilibrating the finger with a test solution was used [Ref.35] with more stable Pt and Ag/AgCl electodes, but in neither case were the results obtained considered to be quantitatively correct.

In 1967, Evans et al [Ref 36] used a Clark-type probe directly against the skin of the forearm , and found that the PO_2 measured was near zero on normal skin. The application of chemicals, or mechanical abrasion of the skin was found to increase the surface PO_2, but it was still about 43 torr below the estimated arterial levels. Later experiments [Ref 37] showed that the surface PO_2 altered considerably with local blood flow. Other similar attempts at transcutaneous monitoring used nicotinic acid derivatives, or heat to nearby areas for vasodilation and an in vivo calibration method was devised involving cutting off blood flow locally and detecting the onset of significant oxyhaemoglobin dissociation [Ref.38], but surface PO_2 was still low - typically 38 torr for adults and 52 torr for neonates.

The breakthrough occurred when it was realised that heating the PO_2 electrode itself could increase local perfusion to such an extent that the capillaries' PO_2 at their venous ends would still be virtually arterial and the increased oxygen consumption of the skin between the capillaries and the electrode would be insignificant, as would be the effect of physiological variations of blood flow [Ref. 39]. The net flow of oxygen diffusing out of the skin, although affected by a shift of the blood's oxygen dissociation curve, could then be a reasonable indication of arterial PO_2.

In 1973 Huch et al [Ref. 40] published results they obtained at the University of Marburg using a Clark-type probe with an inbuilt heater designed to keep skin temperature at 43°C. They used their probe on the forearm of 8 asthmatics in inhalation provocation tests,and found that quantitatively useful results of the same order of magnitude as arterial PO_2 could be achieved. In addition, the electrical energy supplied to the heater to keep the skin at 43°C was obviously a measure of total heat loss, and so, in practice, a measure of the local perfusion, so simultaneous PO_2 and perfusion monitoring was possible. This system was found to be useful for qualitative monitoring in neonates, foetuses during delivery, and mothers in labour [Ref. 41] after in vitro water calibration.

By 1974, some reasonably typical transcutaneous data could be presented [Ref. 42]. For a 12 μm teflon membrane, depletion caused errors of about 10%, in vivo delay time was 10s with a response time of 9s. Correlation with measured arterial PO_2 values in neonates was good, especially below 100 torr.

A similar comparative study of transcutaneous versus arterial PO_2 measurements in adults [Ref. 43] showed that accurate results were possible only if one or two in vivo reference PO_2 values were measured; with only in vitro water calibration, changes in arterial PO_2 were definitely detected but not quantitatively followed.

One anticipated problem was the effect that the vasoconstriction likely to occur in the severely ill might have in invalidating transcutaneous measurements, but it was found that clinically useful information could still be obtained unless gross circulatory disturbances were present [Ref. 44]. The effects of depletion could be

overcome by using a much less permeable membrane, for example the type used by Eberhard et al in 1975 [Ref. 45] but at the cost of a correspondingly increased response time. In fact, the 12 μm teflon membrane system developed at Marburg has generally drawn the most attention and been most widely applied. The use of transcutaneous monitoring has so far tended to be in neonatal care, partly because thinner skin is more easily vasodilated, but more importantly because of the particular difficulties and risks of invasive PO_2 monitoring in newborn infants, but with the benefits of such experience transcutaneous analysis is likely to increase in importance as a non-invasive option for blood gas monitoring generally.

The electrochemical measurement of PCO_2 in blood began with the development by Stow et al [Ref. 46] of a PCO_2 cell based on the fact that the pH of an aqueous solution is proportional to the logarithm of its carbon dioxide concentration. They used a rubber membrane to separate the blood sample being analysed from a film of water whose pH was measured using one glass electrode and one silver/silver chloride electrode. At equilibrium, this pH would be determined by the carbon dioxide that had diffused into the water from the blood via the rubber membrane, and this carbon dioxide concentration would be directly proportional to the PCO_2 of the blood.

Stability, sensitivity and response time were improved by replacing the rubber membrane with 0.001 inch teflon plus a layer of cellophane soaked in an electrolyte [Ref. 14]. Further modifications were carried out - by Hertz et al among others [Ref. 47] - and by 1965 a fairly typical PCO_2 cell consisted of a glass pH electrode in a thin film of water with KCl and $NaHCO_3$ held in spacing material bounded by

0.001 inch teflon, and a reference electrode of 0.1 N calomel. The voltage produced between the electrodes was proportional to the logarithm of the sample PCO_2. Typical problems in the use of such electrodes in discontinuous blood gas analysis were carbon dioxide consumption by non-measuring parts of the system, trapped air bubbles, temperature control and pinholes in the membrane.

The use of the PCO_2 electrode developed along similar lines to the PO_2 electrode. For example, it was applied to continuous intra-arterial monitoring - one study [Ref. 48] compared 100 results from an in vivo G.E. PCO_2 cell with those of an accurate in vitro analyser in the range 18-84 torr, and found the in vivo results not accurate enough for research applications or detailed clinical studies, but likely to be very useful for monitoring clinical trends, especially in neonates.

Tissue PCO_2 measurements and transcutaneous monitoring of arterial PCO_2 have also been tried, with similar degrees of success as for PO_2.

3.3 Blood Gas Analysis by Mass Spectrometry

There are many potential advantages in the application of mass spectrometry to blood gas analysis. One is its high sensitivity which means that accurate measurements may be possible from very small volumes of withdrawn blood, or even from blood in vivo without significant disturbance. Another is the very short response time of mass spectrometric analysis to changes of sample gas partial pressure. The intrinsic linearity of mass spectrometer output with respect to gas partial pressure suggests that high precision could be achieved, and as mass spectrometry is not limited to just one or two gas species, several dissimilar gases - respiratory, inert tracer, or anaesthetic - could be analysed simultaneously. Mass spectrometry could also offer the flexibility of performing continuous or discontinuous analysis, in vivo or in vitro.

The implementation of mass spectrometry for blood gas analysis depends on the means by which the gas to be measured is separated from the blood and presented to the mass spectrometer. One option is the complete extraction of gas from blood by chemical or physical means. This would yield measurements of gas contents rather than partial pressures. The main disadvantages of this method are that it is discontinuous and invasive - a blood sample needs to be taken for each analysis. Another possibility is to equilibrate the blood sample to be analysed with a relatively small volume of introduced gas such as expired air. At equilibrium, the bubble of gas is drawn into the inlet system of the mass spectrometer and analysed to give measurements of

blood gas partial pressures. In 1961, Strang et al [Ref. 49] reported on such a bubble equilibration system using a specially modified respiratory mass spectrometer.

The system was tested with samples of tonometered blood and found to give results agreeing with the tonometry gas partial pressures to within 6 torr for PCO_2 in the range 15-110 torr and within 5 torr for PO_2 in the range 15-100 torr. The time taken - about 10 minutes per analysis - and the degree of care and skill required as well as the disadvantages of it being discontinuous and invasive could explain why this bubble equilibration technique has not been widely adopted.

Virtually all the work done on applying mass spectrometry to blood gas analysis has involved the use of a polymer membrane as the interface between blood and the mass spectrometer. The principle of operation is that as the spectrometer's vacuum system maintains a vacuum on its side of the membrane, gases from the blood on the other side will dissolve in the membrane and diffuse through it, following the partial pressure gradient into the inlet of the mass spectrometer. Ideally, the rate at which molecules of each species diffuse through would be directly proportional to that gas's partial pressure in the blood, and so the corresponding output signals from the mass spectrometer would also be proportional to those partial pressures.

The first published account [Ref. 50] of work in this field was by Woldring et al in 1966. They tried several materials as membranes for catheters attached to a magnetic deflection mass spectrometer - the CEC model 21-610 - and rejected silicone rubber, neoprene, polyethylene and polyvinyl on grounds of low tensile strength, poor elasticity, and being unavailable in sufficiently thin sheets, and

polyvinylidene, Mylar, and Teflon as too impermeable. They chose a natural rubber membrane stretched over the end of a relatively impermeable polyethylene catheter for in vivo measurements of PO_2 and PCO_2. Calibration was carried out with the membrane wet, and heparin was administered to the bloodstream before the catheter was inserted into an artery of a cat. Readings taken during respiratory manoeuvres were 'verified' by conventional electrochemical blood gas analysis but no quantitative data was given on this. The time constant of the system was 10s (30s for full response), and the gas flow through to the mass spectrometer was 1 ml/24 hours. Two years later, a similar flexible catheter with a silicone rubber membrane 0.005 inches thick was used by Hass et al [Ref. 51] with an ion pumped mass spectrometer for following nitrogen washout and nitrous oxide uptake and washout in human arteries. In 1970 Wald et al [Ref. 52] successfully used a polymer membrane covered catheter for in vivo measurements of human cerebral blood flow, for which absolute calibration was not necessary.

Since these early attempts to use membrane covered catheters attached to mass spectrometers, there have been numerous published reports on the application of such catheters to continuous intra-arterial blood gas analysis. Membrane materials used have included silicone rubber, teflon, and polyethylene supported in various ways on catheters, usually of stainless steel, attached to magnetic or quadrupole mass spectrometers. There have been major differences in the types of results obtained, particularly as regards linearity of mass spectrometer oxygen output signal with respect to PO_2 in blood, and agreement with conventional ie. electrochemical blood gas analysis. Calibration procedures, in those cases where it

was carried out at all, differed greatly, as it was complicated by such factors as flow dependence, temperature dependence, and changes in membrane permeability according to the nature of the fluid being analysed - dry gas, wet gas, water, saline, and blood, for example, could all yield different steady state fluxes through the membrane for the same gas partial pressure. Some detailed theoretical work on the physical processes involved in membrane permeation was carried out - for example by Woldring in 1970 [Ref. 53] - but in general attempts to use membrane covered catheters were empirical and contradictory results were reported.

Brantigan et al [Ref. 54], for example, found that their stainless steel catheter with a diffusion membrane of heparinised silicone rubber gave in vivo readings, in the artery of a dog, agreeing with conventional blood gas analysis, and in vitro tests showed linearity for PO_2 and PCO_2 with identical response in water or blood. However, when Wald et al [Ref. 55] used a similar heparinised silicone rubber catheter, agreement of the human in vivo results with conventional blood gas analysis was poor, and when Lollgen et al [Ref. 56] carried out extensive in vitro tests on a similar system they found that the O_2 and CO_2 fluxes through the membrane differed between blood, serum, and water at the same partial pressure, and that the O_2 and CO_2 responses in static blood were not linearly related to partial pressure but depended to some extent on haemoglobin concentration.

The present state of development of membrane/mass spectrometer systems for continuous intravascular blood gas analysis can be gauged by considering four papers in this field published over the last four years. In 1976 Beste et al [Ref. 57] reported on the use of a silastic

covered catheter attached to a quadrupole mass spectrometer - the Centronic 200 MGA. They recorded PO_2, PCO_2, PN_2, PN_2O and PHe in the arteries and veins of a dog, and were satisfied with the results but gave no details of accuracy or linearity of the measurements. In fact the use to which they put the technique - studying blood supply to organs by means of uptake and washout curves - did not require absolute calibrations.

In 1978 J.S. Lundsgaard et al [Ref. 58] published details of a method for continuous in vivo PO_2 monitoring with an inbuilt calibration correction for the flow dependence of the oxygen signal. This correction was achieved by simultaneously monitoring the signal of an inert reference gas of constant partial pressure introduced to the inspired gas mixture. Variations in this reference signal being due to blood flow variations, an on-line computer was used to follow these variations and correspondingly alter the measured oxygen signals to compensate. Quantitative agreement of these corrected in vivo PO_2 measurements with reference analyses using a Clark electrode was very good, and the technique was envisaged as permitting the wider use of relatively highly permeable catheters, such as the silastic ones used in this case. The advantage in mass spectrometric analysis of highly permeable catheters - that the large gas fluxes allowed through to the analyser give rise to good signal to noise ratios and short response times - had previously been significantly offset by the correspondingly higher depletion of the sample and so flow dependence of the signals. Disadvantages of this method for eliminating flow dependence, however, were the need to add a fairly high concentration of a reference gas to the inspired mixture, the use of an alternative

method for an initial in vitro reference analysis of PO_2 (and similarly for any other gas it was desired to monitor), and the need for an on-line computing facility.

Pinard et al [Ref. 59] tried to minimise flow dependence in a different way - instead of silicone rubber they used a less permeable polyethylene membrane, thin enough to keep response times short, with a large enough exposed area to maintain reasonable sensitivity, but an overall low enough gas sampling rate to keep depletion, and so flow dependence, minimal. In vivo comparisons with polarographic analyses were satisfactory not only in arteries but in veins where blood flow velocities were very much lower.

In 1976 Brantigan et al [Ref. 60] published the results of investigations into a teflon covered catheter connected to a Perkin Elmer MGA 1100 mass spectrometer. They found that the response of the system to the PO_2 of flowing water was linear and identical to that in flowing blood, both being 5% lower than that for the same PO_2 in the gas phase, that the PO_2 in static water was reduced by 9% from that in gas, and that the PCO_2 response was identical in all phases. After calibration with humidified gases taking these factors into account, in vitro accuracies of better than 2% were achieved, and in vivo results agreed with electrochemical analyses with 'average differences' of only 3.5% for PO_2 and 5.0% for PCO_2. Flow dependence was insignificant in the range of normal arterial blood flows, and no thrombus formation was found after in vivo use for periods of 4 to 6 hours. In view of all these positive results it was decided to investigate this type of catheter, possibly confirming these findings, as the first objective of this project, and this work is described in

Chapter 5 of this thesis.

Membrane covered catheters pumped by mass spectrometers have been applied to monitoring tissue gases as well as blood gases. In 1969 Owens et al [Ref. 61] were the first to report on the use of a teflon covered catheter for intracerebral PO_2 and PCO_2 monitoring in dogs. Depletion being a great problem in tissue, teflon was chosen in preference to rubber in spite of its relatively long response time of about 6 minutes. The gas measurements, in terms of percentage change rather than with reference to any fixed calibration, were used in conjunction with EEG, cardiotachogram and blood pressure recordings to gain information on localised cerebral metabolism and so, indirectly, on cerebral blood flow. The technique has been further developed for intracerebral monitoring, tracing inert gases as well as respiratory ones, attempting more rigorous calibrations, and so on. Teflon and, to a lesser extent, silicone rubber were the materials most often chosen, but a recent paper [Ref. 62] by Seylaz et al described the use of a polyethylene covered catheter which had the advantage over teflon of short response times, and the advantage over silicone rubber of insignificant depletion of the tissue.

Applications for such intracerebral analysis include the study of brain tumours, monitoring the effectiveness of therapy for stroke patients, and studying the mechanism of action of various drugs. Tissue gas analysis within the myocardium has also been carried out, dating from initial work by Brantigan et al in 1972 [Ref. 63] performed with teflon catheters and a Medspect MS8 mass spectrometer. One particular advantage of mass spectrometry for such tissue gas analysis is that it gathers information over a large enough volume of

tissue for multiple measurements to be unnecessary, another is its ability to monitor several gases simultaneously, but its disadvantages include the damage inflicted on the tissue, and the slow response.

In vitro analysis of blood gases using mass spectrometry has been very limited as in general the advantage of analysing several gases simultaneously has been outweighed by the relative simplicity, cheapness, sensitivity, and fast response, of currently available, well established electrochemical in vitro analysers. One of the few reports of work in this field, by Millis et al [Ref. 64] described a system for PN_2 measurements, useful in the diagnosis of ventilation/perfusion imbalance. The system operated by using a constant flow carrier solution to transport injected blood samples past a silicone rubber membrane fixed over a chamber pumped by a Medspect mass spectrometer. The use for which this system was designed was one particularly suited to mass spectometric analysis, as the only real alternative to such analysis of blood nitrogen would involve gas chromatography which would be more complicated, probably less precise, and limited in accuracy by the precision to which the solubility coefficient of nitrogen in blood is known, as gas contents rather than partial pressures would actually be measured. For the same reasons, mass spectrometry could be the technique of choice for analysis of other non-respiratory gases - the so-called soluble gases such as halothane, acetone, diethyl ether, and so on. Some preliminary work on the feasibility of performing such measurements on the very low concentrations of soluble gases used in ventilation/perfusion studies is described in Appendix 2 of this thesis.

Trancutaneous measurement of blood gases using a mass

spectrometer was described by Delpy et al [Ref. 65] in 1975. They used a heated membrane covered probe similar to the oxygen probe used by Huch et al [Ref. 30] for neonatal PO_2 monitoring, but with the great advantage of being able to measure the partial pressure of carbon dioxide and other gases of interest as well as oxygen. No information on accuracy or reproducibility was given, but the preliminary results seemed promising.

Chapter 4

Description of Mass Spectrometer

The mass spectrometer used in the experiments to be described was the Centronic Medical Gas Analyser type 200 MGA. A copy of its specification is presented as Figure 3. The basic structure and operation of this instrument can be considered in three main parts - the inlet systems, the quadrupole head analyser, and the output system.

Figure 4 is a schematic diagram of the inlet systems. The respiratory inlet system, for monitoring in the gas phase, begins with either a flexible nylon capillary tube (of 0.25 mm id and 1.2 m length) or an electrically heated capillary tube for use in studies affected by changes in water vapour concentration. A rotary vacuum pump draws the sampled gas along the capillary from its entry, at atmospheric pressure, into the instrument and up to a molecular leak of sintered ceramic at which the gas pressure is about 5 torr. A small fraction of the sampled gas flows through this leak, emerging as a molecular beam at a pressure below 10^{-5} torr, the remainder of the gas being pumped away to waste by the inlet rotary pump. A Pirani gauge mounted close to the pump is used to monitor the pressure in this part of the inlet system - a low pressure reading due to a blockage in the capillary tube automatically lights a warning lamp on the front of the instrument. The pump speed and the dimensions of the tubing throughout the inlet system are chosen to minimise response and delay times to about 100 ms.

FIGURE 3 : Specification of 200 MGA

Mass Range:	2-200 amu
Resolution:	Up to unit resolution
Sensitivity:	0.03 mol. % (Argon)
Ion Source:	Twin filament switchability
Signal to noise:	Atmospheric argon 30:1
Linearity:	$\pm$ 1%
Stability:	$\pm$ 1% in A.S.C. mode for better than 24 hours.
Response time:	In respiratory mode using capillary inlet systems the response time is less than 100 ms.
Inlets:	Two connections for disposable capillary inlets or optional heated capillary. One connection for blood gas catheter.
Scanning capabilities:	Sequential scan allows the selection of any 8 gases. The total time for 8 channels sequentially scanned is 20 m.sec.
Signal output:	0 - 10V for 100% concentration. Individual outputs for use with oscilloscope or recorder.

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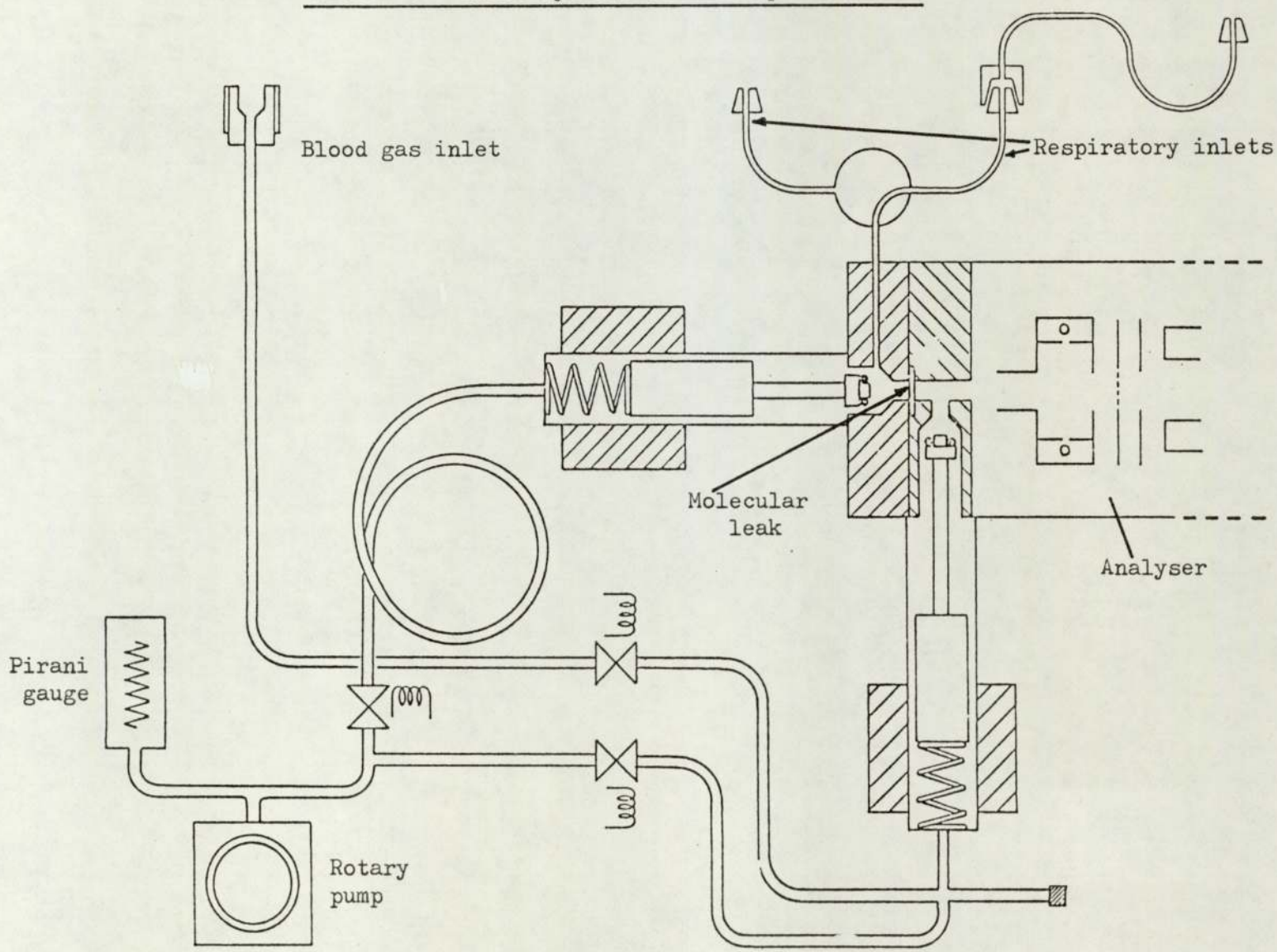
Monitor:	A single output D.V.M. is fitted for ease of calibration, mass selection and to indicate partial pressure in Torr or percent.
Vacuum shutdown:	Upon power failure instrument shuts down automatically.
Operational indicators:	Standby. Operate. Off. Fault warnings. High vac. Inlet. Filament. Sensitivity.
Mains:	230V 50 cycles, or 115V 60 cycles.
Weight:	Approximately 180 Kgms.
Size (cms):	62 x 61 x 113.

FIGURE 4 : Inlet systems of mass spectrometer



An alternative inlet system is provided for blood gas measurements, as the sampled gas fluxes are inevitably much lower. 2m of nylon coated stainless steel tubing connects the point of sample entry, which may be a polymer membrane covered catheter for example, to an inlet port on the body of the mass spectrometer. When the instrument is switched into its blood gas mode of operation, valves are automatically operated so that first the inlet rotary pump evacuates the tubing up to the sampling membrane until the pressure in the system, monitored by a Pirani gauge, drops to about 10^{-1} torr, and then all of the gas flowing into the system is allowed into the analyser part of the spectrometer. The molecular leak is thus completely bypassed in this mode of operation, so that full use is made of the limited sample available, but the vacuum in the analyser section is still maintained below 10^{-5} torr, so that gas flow within it is molecular.

The analyser section of the instrument is kept under vacuum by an air-cooled oil diffusion pump backed by a second rotary pump. The pressure in the analyser is continually monitored by a Penning gauge, and in the event of any significant rise in pressure the whole section is sealed off from the inlet and a warning lamp is lit on the front of the instrument.

As the sample gas molecules enter the analyser they are ionised by a beam of electrons emitted by a heated rhenium filament, and electrically focussed. The ionisation efficiency for a given emission current and electron energy differs for each gas species, but within each species the number of ions formed is directly proportional to the number of neutral gas molecules entering the ioniser.

The beam of ionised gas molecules is then directed into an electrostatic field produced between four accurately ground stainless steel rods mounted in a rectangular array. This quadrupole filter performs the actual separation of ions according to their mass to charge ratios, and the high speed at which separation can be achieved is the reason that such quadrupole mass spectrometers can be used for analysing a number of gases virtually simultaneously. In view of its central importance, the basic principles of quadrupole filtering will now be outlined.

Quadrupole Filtering

Consider four cylindrical rods precisely located in a rectangular array. DC voltages of equal magnitude but opposite sign are applied to opposite pairs of rods with a superimposed RF voltage Vo cosωt. If the rods were hyperbolic in cross section, the resulting electrostatic field, neglecting end effects, would be given (see Figure 5) by

$$\Phi = (V_1 + V_0 \cos \omega t) \frac{x^2 - y^2}{r_0^2} \qquad (1)$$

The field produced using rods of circular cross section is, in fact, a very close approximation to this. An ion of mass m and charge e within this field would be acted on by an electrostatic force with Cartesian components given by

$$F_x = m\ddot{x} = -e \frac{\partial \Phi}{\partial x} = -\frac{2ex}{r_0^2} (V_1 + V_0 \cos \omega t) \qquad (2)$$

$$F_y = m\ddot{y} = -e \frac{\partial \Phi}{\partial y} = +\frac{2ey}{r_0^2} (V_1 + V_0 \cos \omega t) \qquad (3)$$

$$F_z = m\ddot{z} = -e \frac{\partial \Phi}{\partial z} = 0 \qquad (4)$$

From equation 4, $\dot{z}$ is constant, so the velocity of the ion in the z direction remains unchanged by the electrostatic field.

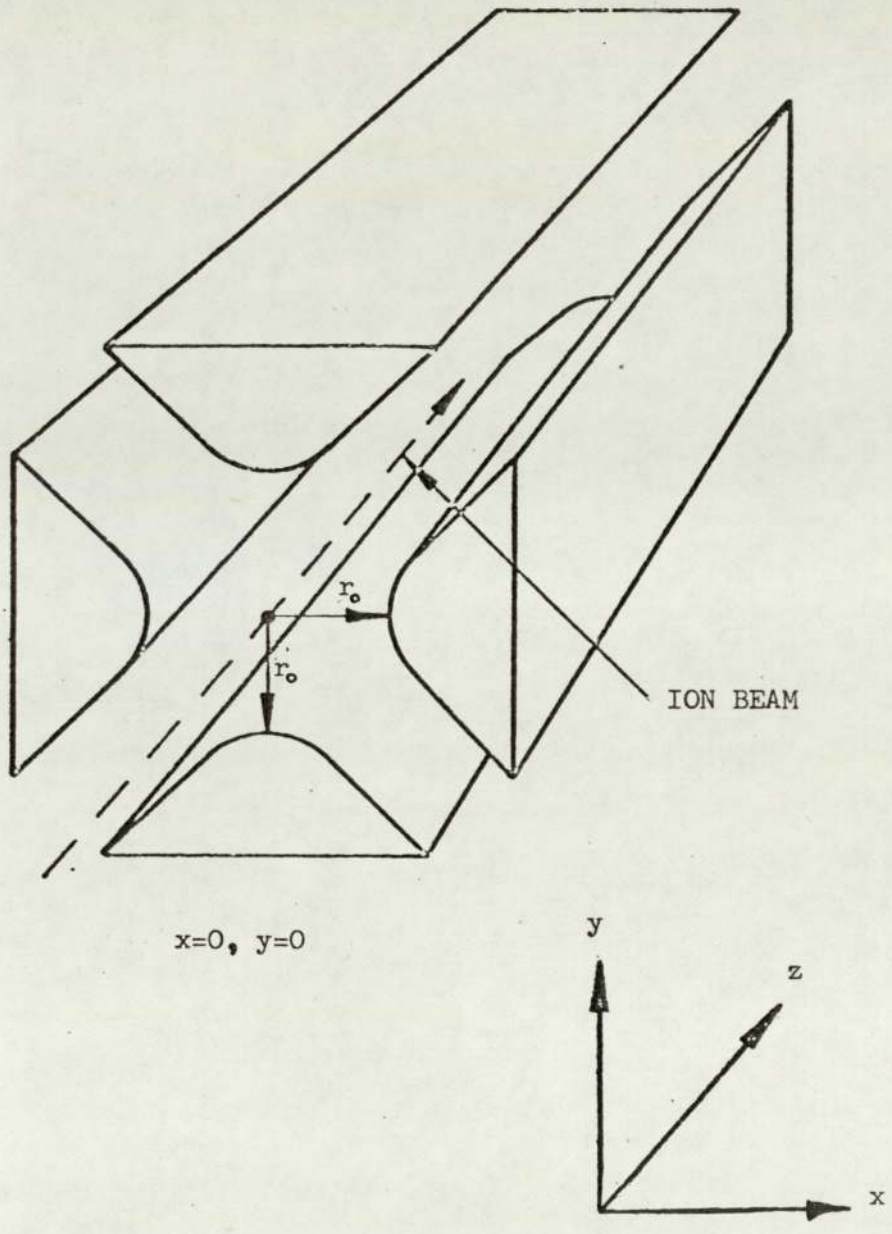


FIGURE 5 : Quadrupole filter

Simplify equations 2 and 3 with substitutions

$$\theta = \omega t$$

$$a = \frac{2eV_0}{mr_0^2\omega^2}$$

$$\beta = \frac{V_1}{V_0}$$

$$\longrightarrow \quad \ddot{x} + a\omega^2(\beta + \cos\theta)x = 0 \qquad (5)$$

$$\ddot{y} - a\omega^2(\beta + \cos\theta)y = 0 \qquad (6)$$

$$\ddot{z} = 0 \qquad (7)$$

Equations 5 6 and 7 describe the trajectory of the ion in this field. It can be shown that for most values of a and β this trajectory will cause the ion to collide with one of the rods or the walls of the analyser chamber, losing its momentum in the z direction and so never reaching the far end of the filter. However, for $\beta \approx 0.168$ there is a small range of values of a around the value 0.354 which will describe stable rather than unstable trajectories, so that the ion will pass through the filter and be detected at the far end.

Paul's diagram, given as Figure 6, shows these stable trajectories as corresponding to the intersection of the operating line, corresponding to a constant value of β, with an area representing possible trajectories stable in the x and y directions. Notice that it is conventional to choose the parameters a and q rather than a and β to represent the nature of the trajectories, where

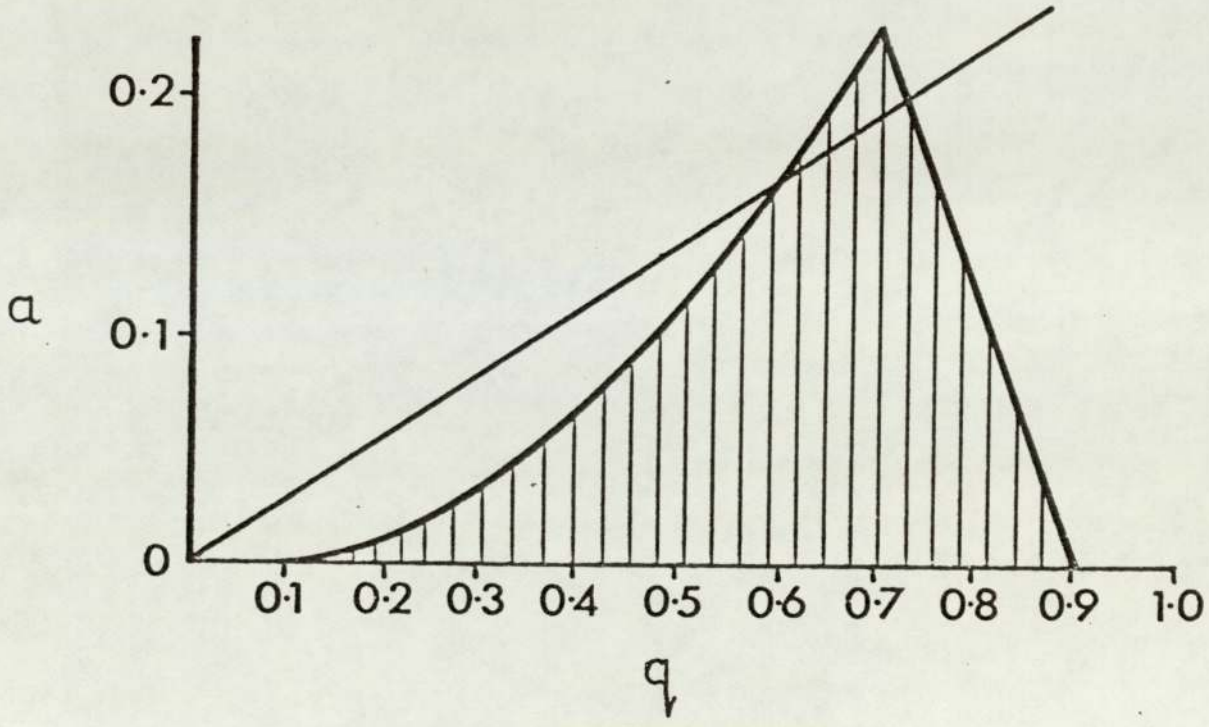
$$a = \frac{2eV_1}{mr_0^2\omega^2}$$

$$q = \frac{eV_0}{mr_0^2\omega^2}$$

These are related to a and β by the equations

$$a = a\beta$$

FIGURE 6 : Paul's diagram



and $$q = \frac{a}{2}$$

but the details of operation of the mass filter are slightly easier to understand in terms of a and β, so they have been chosen for this description.

Consider a stream of ions of different mass to charge ratios entering the field between the rods. If the DC and RF voltages applied to the rods are chosen to satisfy the condition for β ie. $V_1/V_0 \approx 0.168$ then only ions in the stream that satisfy the corresponding restriction on a will pass through the filter. These ions must fit the condition $a \approx 0.354$

$$\longrightarrow \quad \frac{m}{e} \approx \frac{2V_0}{0.354 r_0^2 \omega^2} \qquad (8)$$

Ions of mass to charge ratio given by equation 8 are thus selected by the filter; all the other ions are deflected out of the filter and are not detected. Equation 8 shows that the particular species of ion allowed through the filter depends on the amplitude and frequency of the RF voltage. Altering either of these would select a different ionic species from the stream entering the quadrupole.

The control of the quadrupole filter in this particular mass spectrometer can be effected in two different ways, known as the Spectrum and Peak Select modes of operation, each of which depends on altering the RF voltage amplitude, keeping its frequency constant.

The quadrupole filter operates in Spectrum mode by smoothly altering the amplitude of the RF voltage through a set range, with a correspondingly altering DC voltage, chosen to keep β at 0.168. The

species of ion allowed through the quadrupole is thus continuously altered through a corresponding range of m/e. This 'scan' of voltage is repeated every 0.02s in the MGA 200, producing a corresponding mass scan or spectrum of the ions entering the filter.

For the experimental work to be described, the mass spectrometer was used in Spectrum mode only occasionally - when adjustments to resolution were made for example, or for monitoring long term sensitivity changes - and in general it was operated in Peak Select mode.

The quadrupole operates in Peak Select mode by sequentially holding the RF voltage at each of a series of eight discrete levels, each level corresponding to a mass of interest. The DC voltage is of course simultaneously stepped along eight corresponding levels. Ions of the eight chosen mass to charge ratios thus successively pass through the filter to detection, and the cycle is continuously repeated.

Manual intervention to the operation of the quadrupole is restricted to altering the resolution, setting the mass range to be scanned in Spectrum mode, and choosing the eight ionic species to be analysed in Peak Select mode. The resolution is altered by making a small change to the value of V_1/V_0. This slightly alters the range of values around that of 0.354 that correspond to stable trajectories, and so in effect alters the spread of ionic masses allowed through the filter for a given value of Vo. The best resolution available with the MGA 200 corresponds to a spread of $\pm^1/_2$ amu. The mass range in Spectrum mode can be set anywhere between 1 and 200 amu, by altering the levels between which the RF voltage is swept in each cycle. In

Peak Select mode the masses are chosen by setting the values of Vo to be sequentially applied to the rods. These masses may be in the range 1-50 amu for the first four mass channels of the instrument, and 1-200 amu for the others.

As the ions of each gas species pass out of the quadrupole filter in turn, they are detected by an electron multiplier. Ions incident on the first dynode of this cause electrons to be emitted, these are accelerated to the second dynode, causing further secondary emission, and so on until at the final plate of the multiplier an electron current of about 2^{16} times the original ionic current is produced. The magnitude of the multiplication may be seriously reduced by any contamination of the surfaces of the beryllium/copper dynodes, so the multiplier is kept under high vacuum whenever possible. The stability of the multiplication is dependent on the stability of the voltage (up to 4 KV) applied across the series of dynodes to energise the system. The value of this voltage can be manually adjusted by an uncalibrated 'Sensitivity' control on the instrument.

In Peak Select mode, the signal from the electron multiplier consists of a repeated series of eight currents, each proportional to the corresponding ionic current leaving the quadrupole filter, and so proportional to the corresponding gas flux of that species entering the analyser. This gas flux is itself directly proportional to the rate of entry of that gas into the inlet system, which is determined by the partial pressure sampled.

The currents from the multiplier are then amplified, electrically smoothed and filtered, further amplified and then fed in parallel to eight sample-and-hold circuits. Each circuit holds an output voltage

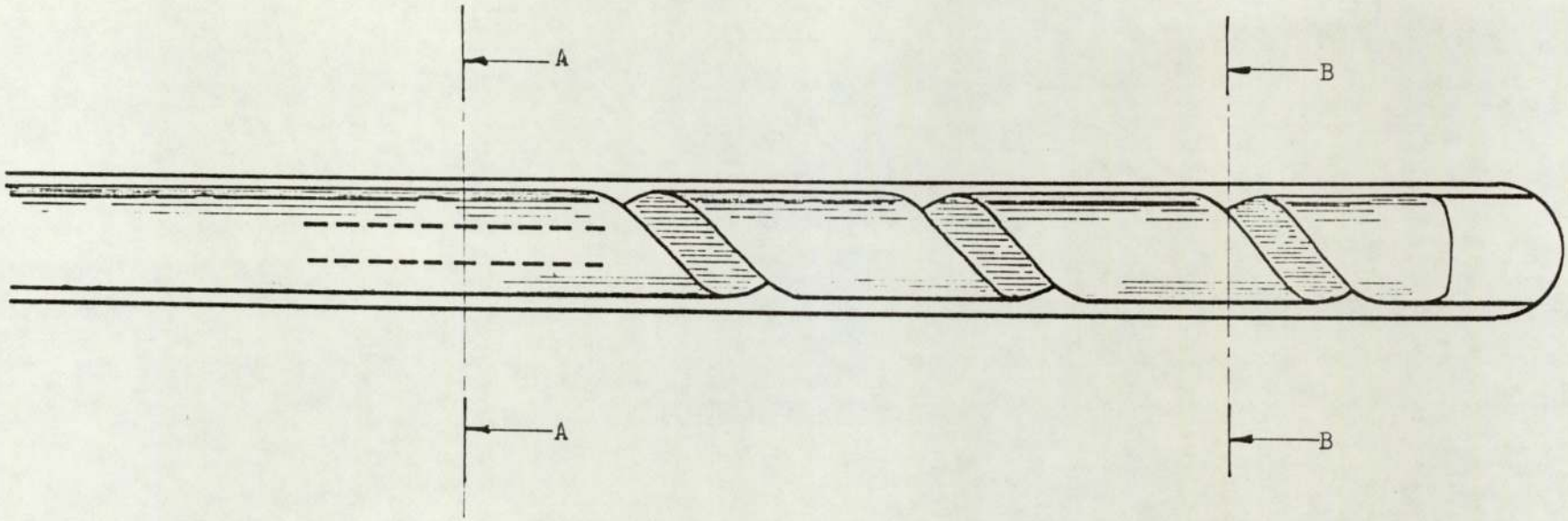
proportional to the input current representing a single ionic species, and this current is sampled 50 times a second, so the output voltage is updated at this rate. The voltages can then be independently amplified up to a maximum of 10V under manual control and are output continuously through an eight channel plug on the back panel of the mass spectrometer. In addition, a digital panel meter on the instrument can display any one of these output voltages, or an indication of the corresponding ionic mass, as an aid to tuning and calibration.

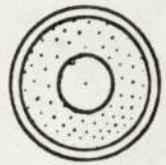
Chapter 5

Preliminary Experiments on Membrane Systems

5.1 Investigations of 'Spectra-cath' Blood Gas Catheters

The results obtained by Brantigan et al [Ref 60] using a membrane covered catheter with a mass spectrometer were described in Chapter 3 Section 3. Their system seemed to be quite satisfactory for continuous in vivo blood gas analysis, and it was decided to try out the same type of catheter with the 200 MGA as an initial approach to the present project. Figure 7 is a schematic diagram of such a catheter, manufactured by Sorenson Research Corp. It basically consists of a helically grooved stainless steel tube supporting a teflon membrane 0.002 in thick. The published work can be summarised as follows - after calibration of the system (mass spectrometer plus catheter) in a stream of gas heated to 37°C, in vitro tests showed linear responses for PO_2 and PCO_2 in water and blood whether static or flowing, although the absolute values of the oxygen signals were lower in liquids than in gases, and lower in static liquids than flowing ones. The temperature dependence was 2.0% per °C for oxygen and 1.6% per °C for carbon dioxide, and the in vitro 90% response times were 80s and 130s respectively. In vivo comparisons with electrode analyses showed 'average differences' of 3.5% for oxygen and 5.0% for carbon dioxide, with no problems of blood clotting around the catheter for up to 24 hours and negligible flow dependence.





SECTION
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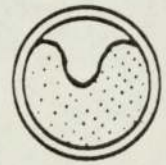

SECTION
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FIGURE 7 : Schematic diagram of 'SPECTRA-CATH'

In the experiments to be described the Sorenson catheter was attached to the blood gas inlet of the 200 MGA. Calibration was carried out in vitro using the calibration unit supplied with the catheter for heating humidified gas mixtures to 37°C and passing them via a bacterial filter over the teflon diffusion membrane of the catheter.

Temperature Dependence

The temperature of the air around the catheter was held successively at each of ten levels in the range 27°C to 40°C and the corresponding mass spectrometer oxygen signals were recorded. The results are shown in Figure 8 - the temperature dependence is seen to be linear. The factors by which readings at temperatures other than 37°C differed from the reading at 37°C were calculated and compared with correction factors supplied by the manufacturers. The results, shown in Table 1, agree closely.

Response Time

When step changes of gas partial pressure at 22°C were applied to the catheter, the average time taken by the mass spectrometer output voltages to cover 63% of the change between initial and final levels was found to be 84s for oxygen and 120s for carbon dioxide.

In Vivo Linearity

The calibrated catheter was passed into the aorta of a dog through the right femoral artery, and the signals for oxygen, carbon dioxide, and nitrogen were recorded. Three arterial blood samples were

FIGURE 8 : Temperature dependence of 'Spectra-cath' catheter

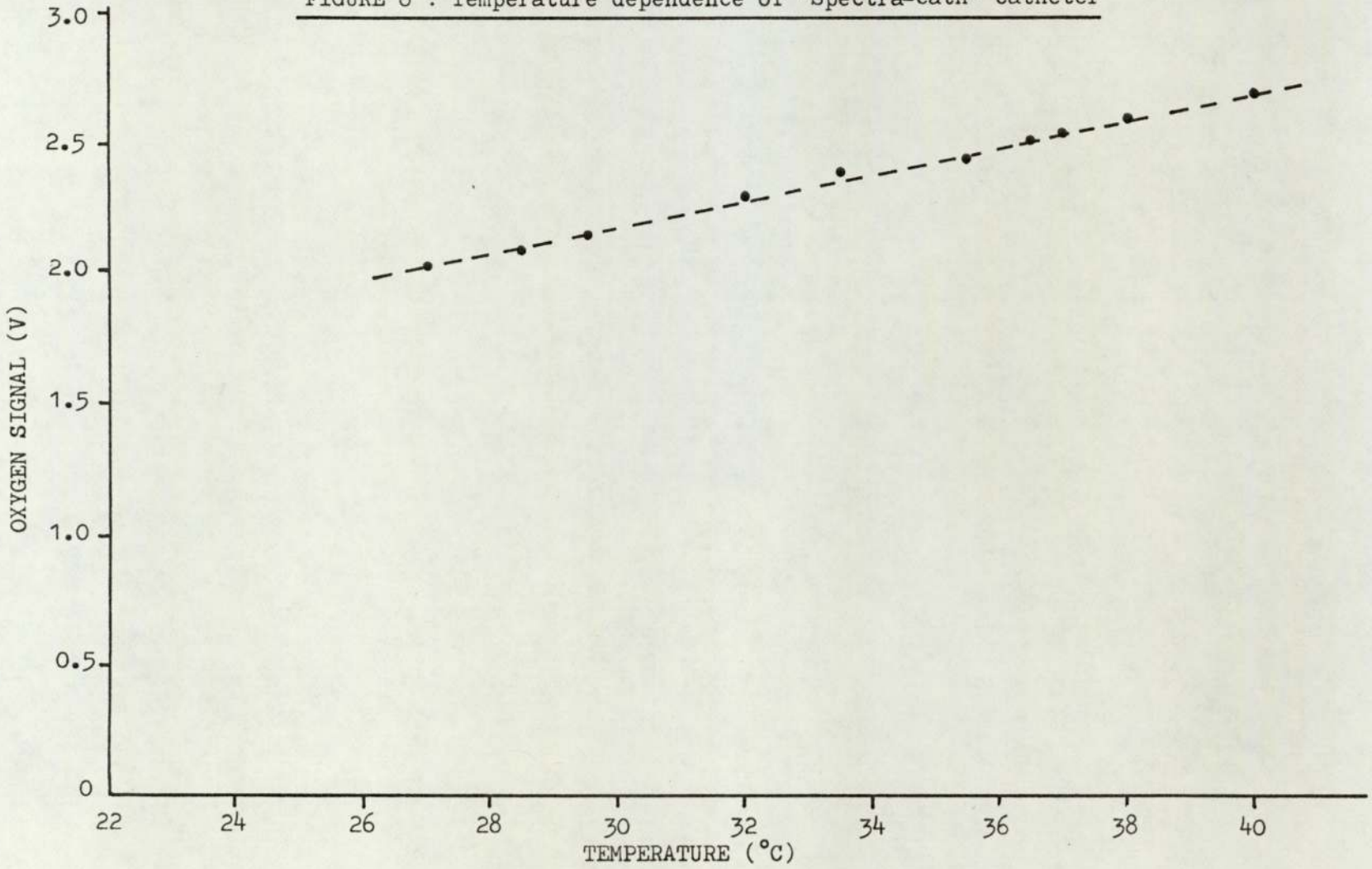


TABLE 1 Catheter temperature dependence

Temperature (°C)	30	32	34	36	38	40
Supplied Factor	0.854	0.895	0.937	0.979	1.03	1.08
Measured factor	0.86	0.90	0.94	0.98	1.01	1.05

TABLE 2 In vivo blood gas results

MS PO_2 (torr)	58.5	65	58.5
Corning PO_2 (torr)	73	77	84

MS PCO_2 (torr)	45	39	40
Corning PCO_2 (torr)	44	40	40

TABLE 3 In vitro blood gas results

MS PO_2 (torr)	120	110	221	182	44	55
Corning PO_2 (torr)	97	124	211	351	57	61

MS PCO_2 (torr)	36	43	36	43	50	30
Corning PCO_2 (torr)	42	42	30	42	44	37

taken at various times over the following hour, and analysed for PO_2 and PCO_2 by a standard electrochemical blood gas analyser - the Corning model 165. The mass spectrometer oxygen and carbon dioxide readings at the corresponding times were noted and compared with these - see Table 2. The results were within 2% in the case of PCO_2 but as the measurements spanned only 6 torr, no conclusions could be drawn as to linearity with respect to PCO_2. Corresponding PO_2 readings differed by up to 30% through a range of 11 torr, and therefore this experiment did not establish either accuracy or linearity of the Sorenson catheter with respect to PO_2 in vivo.

A series of in vitro experiments was carried out to compare the readings obtained from the catheter immersed in blood with the partial pressure readings obtained by analysing the tonometered blood with the Corning 165 blood gas analyser.

The first attempts to do this were unsuccessful as the output from the mass spectrometer fell sharply with time as soon as the catheter was placed in blood, and no plateau was reached where a representative reading could be taken. This effect was considerably reduced by magnetically stirring the blood during analysis, but was still occasionally a problem.

Another problem was found to be a loss of oxygen by the blood after equlibration with a gas mixture of high PO_2. To compensate for this, the equilibrated blood was split into three samples, one was immediately analysed by the Corning, the second was analysed by the mass spectrometer, and the third was simply kept alongside the second and analysed by the Corning after the mass spectrometer readings were taken. The two sets of Corning readings were averaged to correct for

any steady drift in time, and the results were compared with those of the mass spectrometer. From Table 3 it may be seen that the results differed in a thoroughly inconsistent way from one blood sample to another. The in vitro linearity of the Sorenson catheter with respect to PO_2 and PCO_2 was therefore not established.

Discussion

In view of the contradiction between the linearity results of these experiments and the published data, the procedures involved in the experiments were carefully re-examined, and it seemed possible that significant errors could have arisen in the handling of blood in the in vitro experiments. This could not explain the discrepancies in the in vivo experiment, but only one such experiment was successfully carried out, so its results may have been atypical. Some time after these experiments were performed it was learned that problems in the manufacture of the catheters had affected the permeability characteristics of one batch - it was not known whether the catheters used in this work were from this batch, but the possibility did exist. In fact the manufacture of the catheters was eventually suspended while designs modifications could be investigated, and no new catheters had been produced by the time this thesis was written. Considering the negative results of the experiments, and the lack of reproducibility that was found between individual catheters, it was decided not to pursue the investigation of these catheters further, but to turn instead to a more basic study of the processes involved in the permeation of membranes by blood gases. A membrane-holding device that could be attached to the inlet line of the mass spectrometer was

therefore constructed and used as an in vitro test system. This work is described in detail in the following section.

5.2 Investigation of Polyethylene as a Diffusion Membrane

A cell of perspex and brass was constructed in which a thin membrane could be supported between two chambers, one pumped by the mass spectrometer, the other able to be filled with chosen liquids or gases. The cell, shown in Figure 9, was attached to the blood gas inlet of the mass spectrometer. Polyethylene was chosen as a suitable membrane material to try initially, as it was easy to obtain in very thin films, could be handled without excessive precautions to avoid damage, and was relatively inert in blood. The properties that were chosen for study were linearity of gas flux through the membrane with respect to dry and wet gas partial pressures, temperature dependence, flow dependence, linearity for gases in water, and linearity for gases in blood.

Experiment 1

A clean dry polyethylene membrane of 0.001 inch thickness was inserted into the diffusion cell, and the cell was fully immersed in a water bath held at 37°C. Gas mixtures of known composition (certified to within 0.1% by the supplier, Air Products Ltd) were allowed to flow through the cell. The cell output for oxygen, carbon dioxide, and nitrogen, analysed by the mass spectrometer, was recorded for each mixture. The whole process was then repeated, this time passing the gas mixture through a humidifier, also at 37°C, so that the gases were saturated with water vapour before passing through the cell.

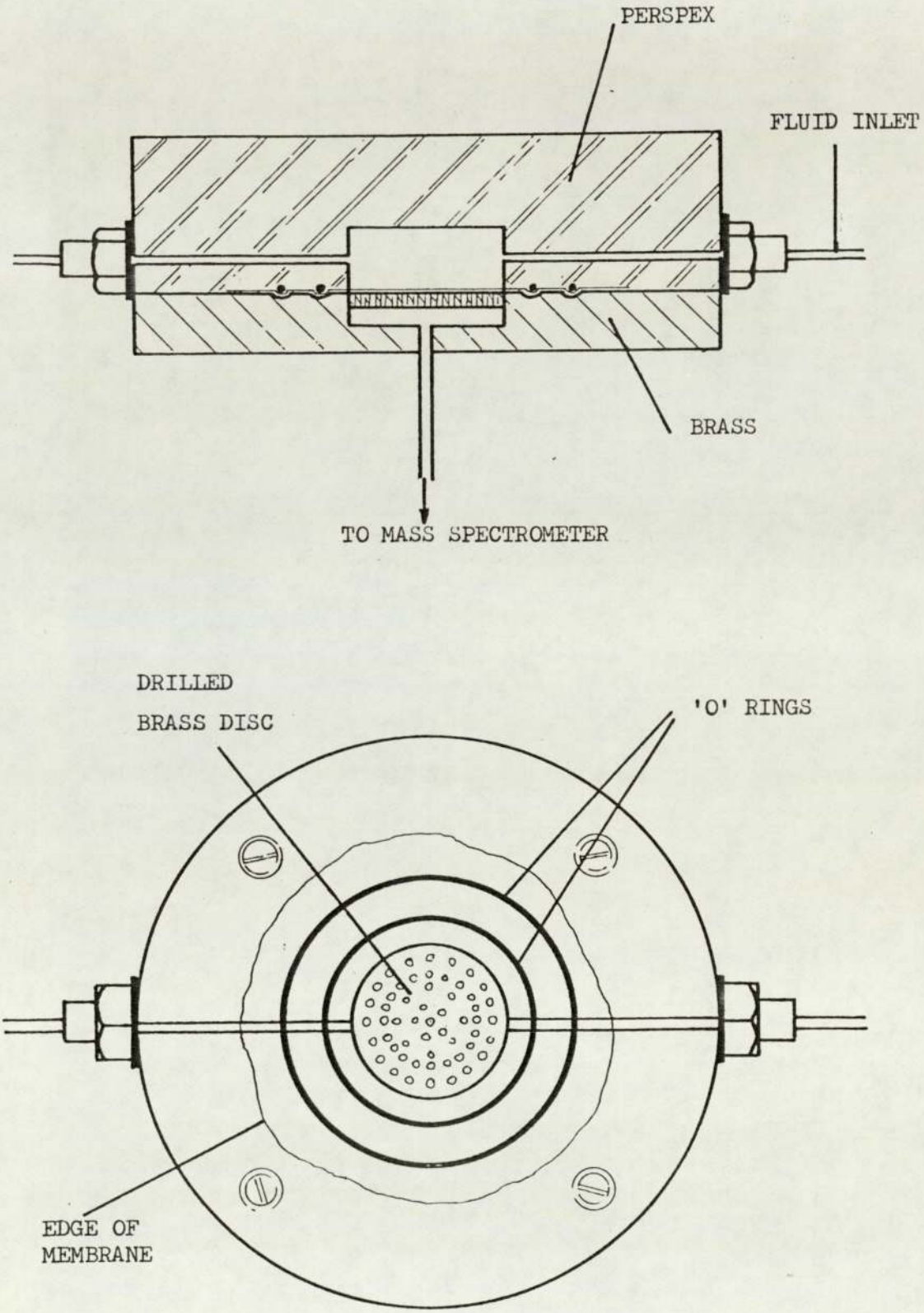


FIGURE 9 : Diffusion membrane test cell

Typical results are shown in Figure 10. The gas flux through the polyethylene was found to be linear with respect to partial pressures of oxygen, carbon dioxide, and nitrogen as dry or wet gases.

Experiment 2

The temperature dependence of the gas flux through the polyethylene membrane was studied by altering the temperature of the water bath in which the cell was immersed, while sampling a saturated gas mixture. The mass spectrometer output for oxygen, carbon dioxide and nitrogen was recorded at each temperature and the results are shown in Figure 11. Gas flux through the membrane is seen to increase smoothly with temperature in the range 26°C to 40°C. The specific temperature dependence at 37°C was approximately 5% per °C for oxygen and 4% per °C for carbon dioxide.

Experiment 3

A tonometer was constructed in which a small volume of blood or any other liquid could be swirled in a stream of humidified gas, until the gas tensions in the liquid were equal to those of the gas mixture. The accuracy of the tonometry could only be gauged by experiment, and the method chosen was as follows. 5ml of water was tonometered for 5 minutes with a constant gas flow of 1l/minute and then analysed by the Corning 165 for PO_2 and PCO_2. The procedure was repeated using the same liquid volume, gas flow and gas mixture for successively longer periods of tonometry, and the analyses were found to reach a plateau at about 12 minutes. Tonometry could therefore be considered as virtually complete after this time, and 15 minutes was chosen as a

FIGURE 10 : Cell response with dry and wet gases

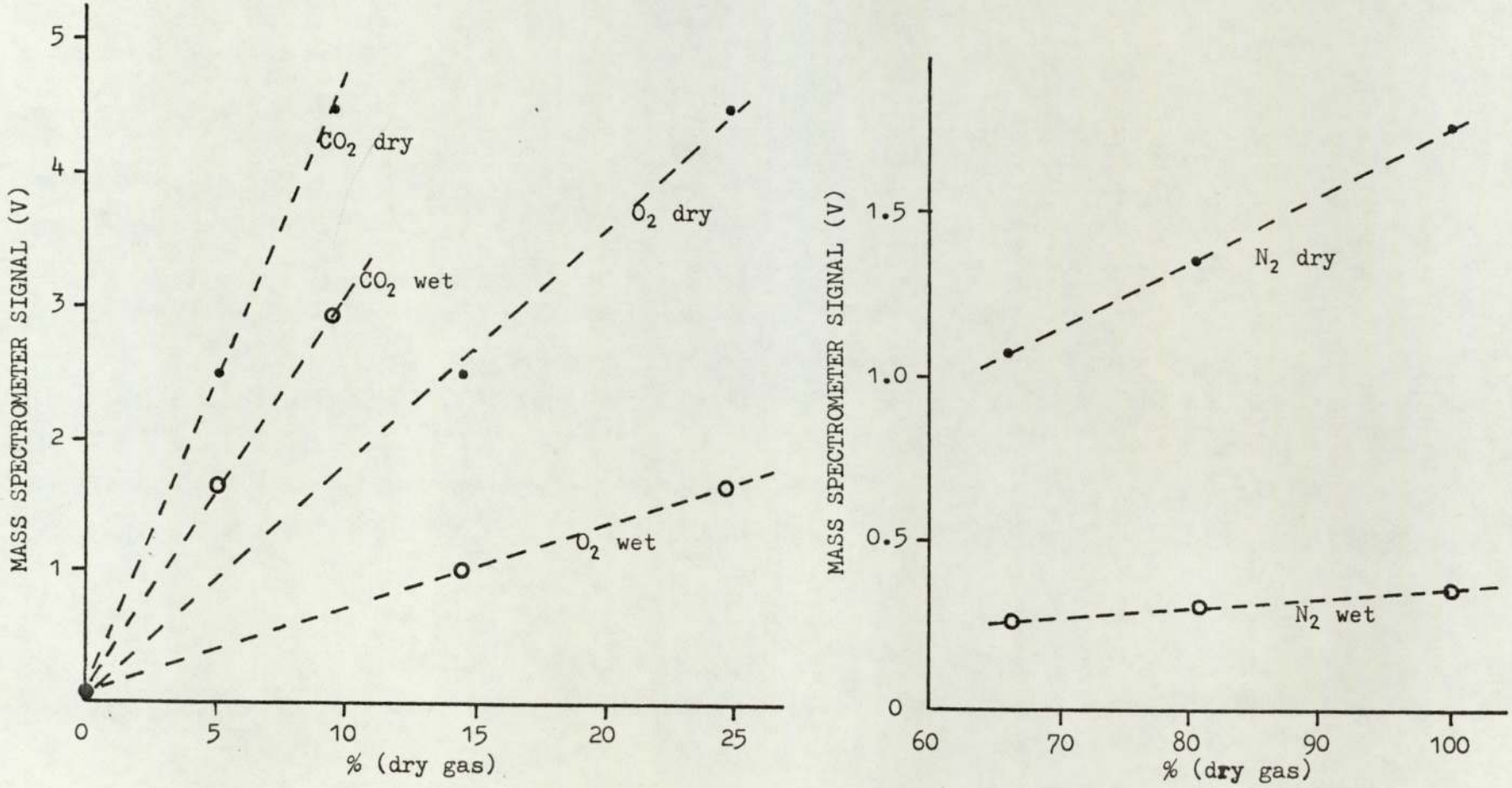
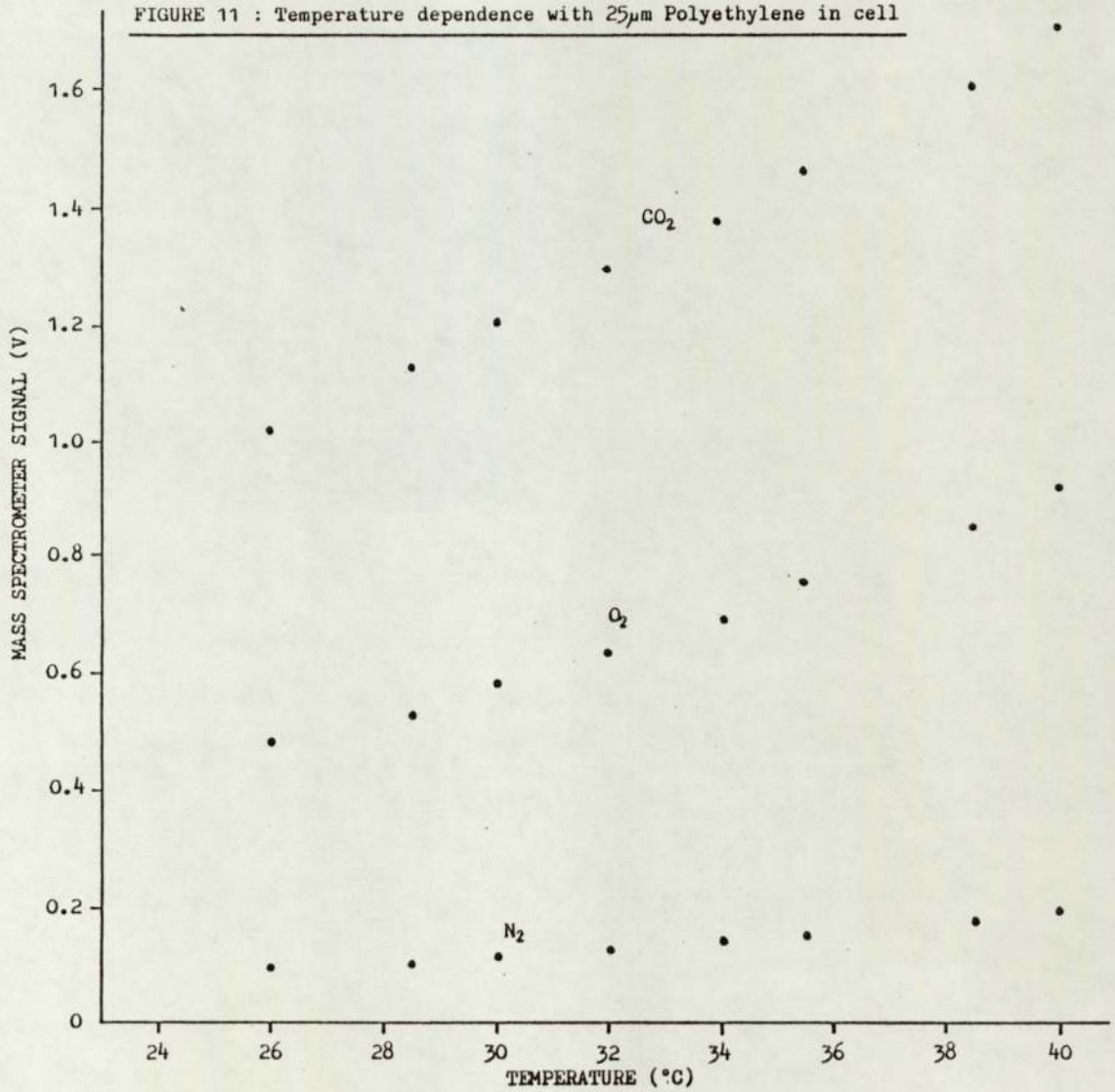


FIGURE 11 : Temperature dependence with 25μm Polyethylene in cell



safe tonometry period. Using the tonometer in this way, 5 ml samples of distilled water were equilibrated at 37°C with a series of known gas mixtures and passed through the cell by a peristaltic pump, the Watson-Marlow HR flow inducer type MHRE 200. A sample of the results is shown in Figure 12.

There were several sources of error in the measurements. One is the effect of temperature variation of the water bath in which the cell was placed - variations of up to 0.5°C were quite possible and these would cause signal changes of up to 2.5%. The accuracy of the tonometry could be taken as at least as good as the electrochemical analyser, which the manufacturer's data for the ranges covered in this work suggests as being better than 4%. Errors due to gas diffusion through tubing, air leaks, flow variations, and the analysis of the mass spectrometer itself were unlikely to amount to more than 1%. Assuming that all errors are normally distributed about the mean value, and given that the maximum error of the system is ±7.5%, the standard deviation overall can be estimated to be ± 2.5% of the measured values. Within these limits, the results showed that the flux to the mass spectrometer was linear with respect to partial pressures for these gases dissolved in water.

The flow rate of the water through the cell was held at a series of different values, while keeping the gas tensions in the water constant, and the oxygen output of the cell was recorded at each flow. The results are shown in Figure 13. It can be seen that the mass spectrometer output (and so the gas flux through the membrane) was a function of the flow past the membrane.

In order to explain the experimentally found flow dependence,

FIGURE 12 : Flowing tonometered water response

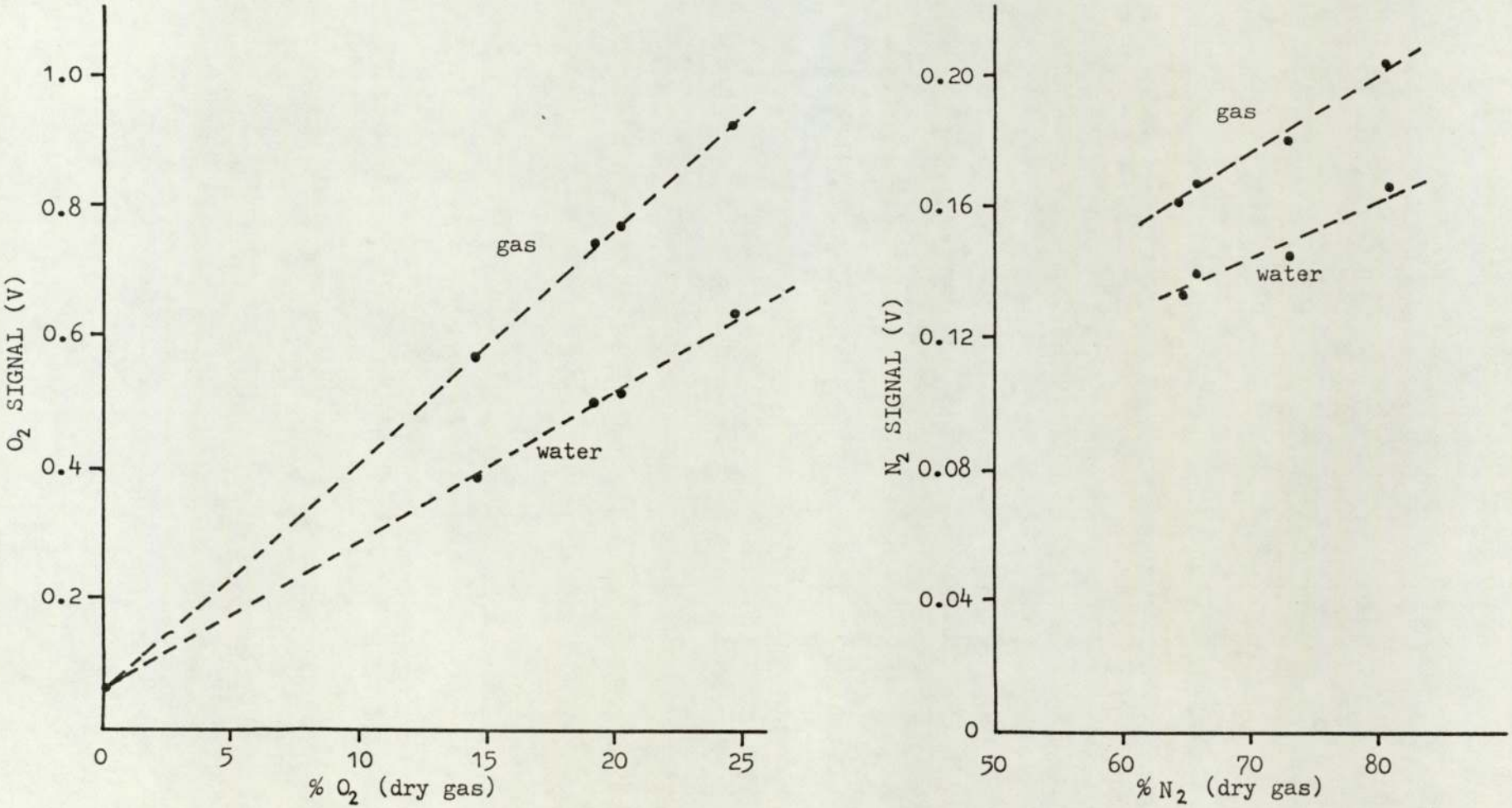
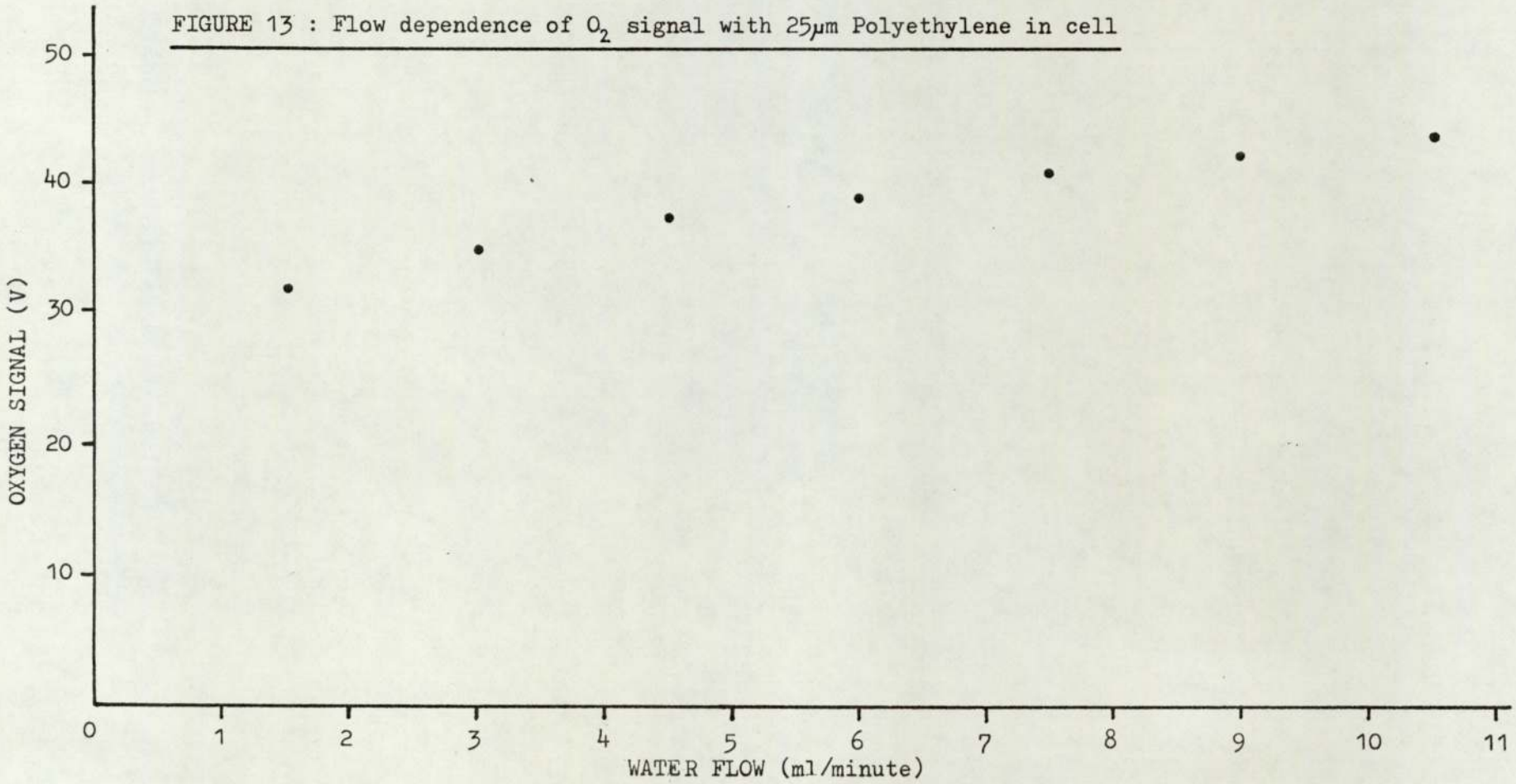


FIGURE 13 : Flow dependence of O_2 signal with 25μm Polyethylene in cell



the physical processes involved in the transmission of gas from the water through the membrane must be considered.

Consider a liquid such as water in equilibrium with a mixture of soluble gases and in contact with a gas permeable polymer membrane whose other surface is suddenly exposed to the vacuum of the mass spectrometer inlet line. Since the mass spectrometer maintains a vacuum on its side of the membrane while the liquid at the other side is at approximately atmospheric pressure, the gas molecules in the liquid must diffuse through under the influence of the difference in partial pressure, and the liquid immediately next to the membrane must gradually become depleted of gas. The falling gas concentration here causes gas from further away in the liquid to diffuse in, tending to restore the original concentration, but as gas is continually removed via the membrane, this is never quite achieved.

However, a steady state may be reached, when the supply of gas by diffusion through the liquid is equal to the consumption by the membrane/mass spectrometer system. In this steady state, the dissolved gas concentration next to the membrane can be significantly lower than in the bulk of the liquid, so the steady state mass spectrometer signal will be lower than would be directly representative of the bulk concentration.

This effect can be minimised by having enough stirring or directed flow in the liquid so that fresh gas molecules are continually brought to the depleted zone, and a steady state is reached more quickly and with a higher net flux through the membrane. Even with stirring however, it can be shown from hydrodynamic theory [Reference 66] that there must always be a depleted layer of finite

thickness in which the gas flux is almost entirely diffusive. This diffusion layer and the polymer membrane are effectively barriers in series to the gas flux. Figure 14 represents the steady state. To calculate the steady flux through the barriers, an expression for the permeability of a layered system is required.

Following Flynn et al [Reference 67] the diffusive resistance R_i of the i th layer is defined by

$$R_i = \frac{1}{P_i}$$

$$= \frac{h_i}{D_i K_i}$$

where P_i is the permeability of the i th layer

h_i is the thickness of the layer

D_i is the diffusivity of the gas species concerned in the material of the i th layer

K_i is the partition coefficient for the gas between the ith layer and the initial external phase

The diffusive resistance of a series of layers is the sum of the individual resistances.

$$R = \sum_i R_i$$

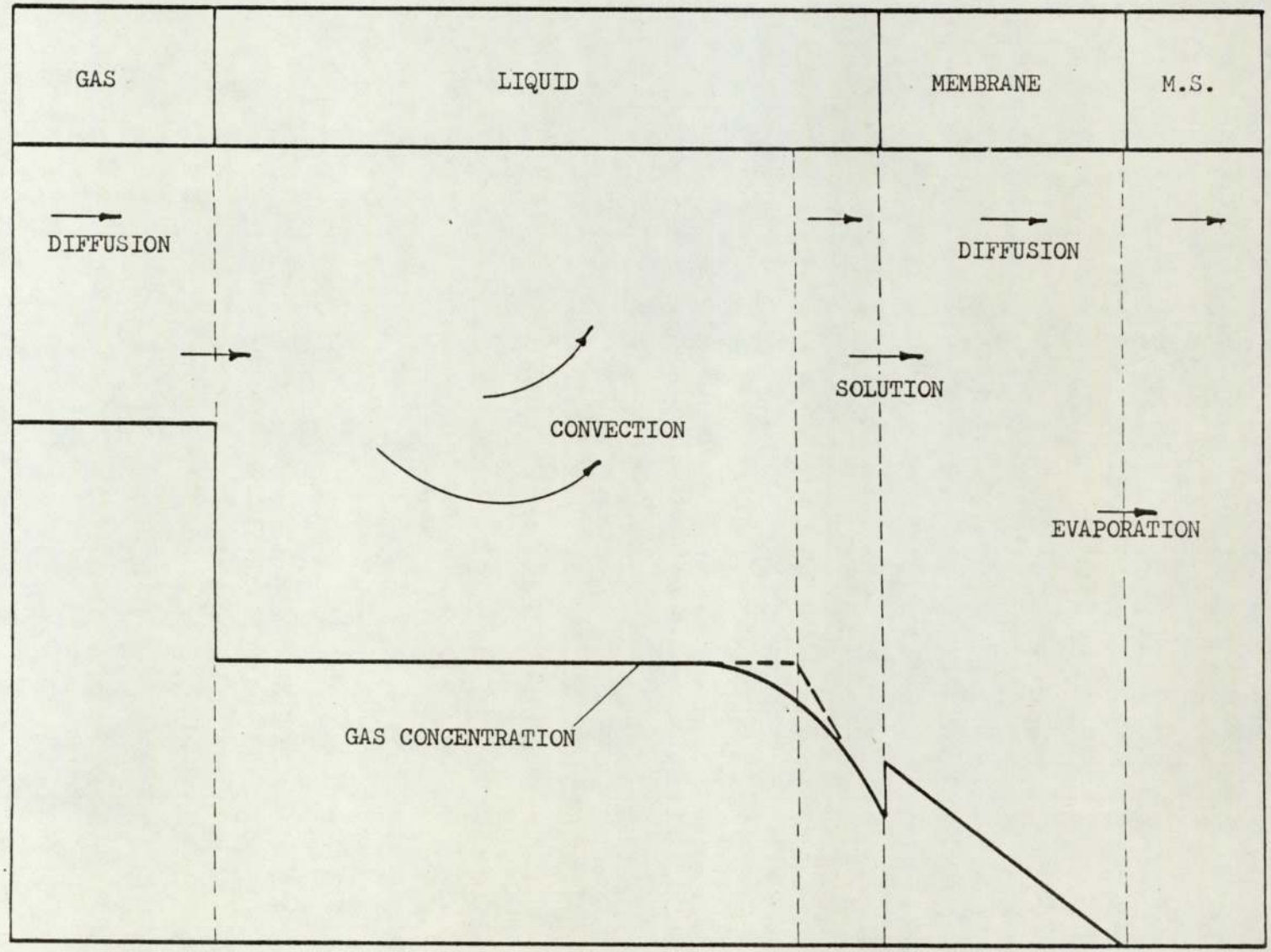
$$= \sum_i \frac{h_i}{D_i K_i}$$

So the permeability P_t of a series of layers is given by

$$P_t = \frac{1}{R}$$

$$= \frac{1}{\sum_i R_i}$$

FIGURE 14 : Generalised model of steady state depletion



For the special case of the liquid/membrane/vacuum system, as the vacuum affords no diffusive resistance to the gas flux, there are effectively only two barriers in series - the depleted layer of liquid, and the membrane itself - so the expression simplifies to

$$P_t = \frac{D_1 D_2 K_1 K_2}{h_1 D_2 K_2 + h_2 D_1 K_1}$$

Since the 'external' phase concerned is the same chemical liquid as the first barrier, the partition coefficient K_1 for the gas between the two is unity.

$$\therefore \quad P_t = \frac{D_1 D_2 K}{h_1 D_2 K + h_2 D_1}$$

where D_1 is the diffusivity of the gas in the liquid

D_2 is the diffusivity of the gas in the membrane

K is the partition coefficient for the gas between the membrane and the liquid

h_1 is the effective width of the depleted layer

h_2 is the membrane thickness

The steady state flux through the series of barriers to the mass spectrometer is given by

$$\frac{dm}{dt} = P_t \Delta C$$

where ΔC is the difference in gas concentration between the

bulk liquid and the vacuum. Taking the concentration on the mass spectrometer side of the membrane to be negligible

$$\frac{dm}{dt} = P_t \Delta C = \frac{D_1 D_2 K C}{h_1 D_2 K + h_2 D_1}$$

Assuming Henry's law, the bulk concentration $C = \sigma P'$ where P' is the partial pressure of the gas in the liquid and σ is the Henry's law solubility coefficient

$$\frac{dm}{dt} = \frac{D_1 D_2 K \sigma}{h_1 D_2 K + h_2 D_1} P'$$

The mass spectrometer signal S for the gas species concerned will be proportional to the mass flux entering via the membrane

$$S = \frac{A(D_1 D_2 K \sigma) P'}{h_1 D_2 K + h_2 D_1} \qquad (1)$$

where A is a constant for the mass spectrometer and the gas species concerned. It is clear from equation 1 that the steady state signal S depends on the thickness h_1 of the depleted liquid layer. Since this thickness itself depends, at least at low flows on the characteristics of the liquid flow, it can be seen that the steady state signal must also be a function of the liquid flow.

The dependence of the mass spectrometer oxygen signal on the volume flow rate produced by the peristaltic pump in Experiment 3 is thus explained.

Experiment 4

5ml samples of heparinised, human blood were tonometered at $37^{0}C$ with a series of known humidified gas mixtures and passed through the diffusion cell by the peristaltic pump. The mass spectrometer signals for oxygen, carbon dioxide, and nitrogen were recorded for each mixture. Within the limits of experimental error discussed in connection with 'Experiment 3' results such as those in Figure 15 showed that although output was linear with respect to PCO_2 and PN_2, it was non-linear with respect to PO_2, the non-linearity tending to lessen as blood flow increased.

As the degree of non-linearity altered with flow, it seemed possible that its cause was connected with the consumption of the mass spectrometer depleting the blood's oxygen - if the consumption was high enough for the plasma close to the membrane to give up sufficient oxygen to significantly shift the dynamic oxygen equilibrium between plasma and red cells, then oxygen molecules would diffuse out of the cells to the plasma until a new steady state was reached, and so the mass spectrometer signals could be responding to some extent to the oxygen content of the blood, rather than to its partial pressure. This might explain the non-linear response.

If this was so, the non-linearity would become more marked as consumption was increased, and it was decided to check this by repeating the tonometered blood experiment with a polyethylene membrane of half the thickness ie. of double the consumption. A comparison of Figures 15 and 16 shows that the degree of

FIGURE 15 : Flowing tonometered blood response

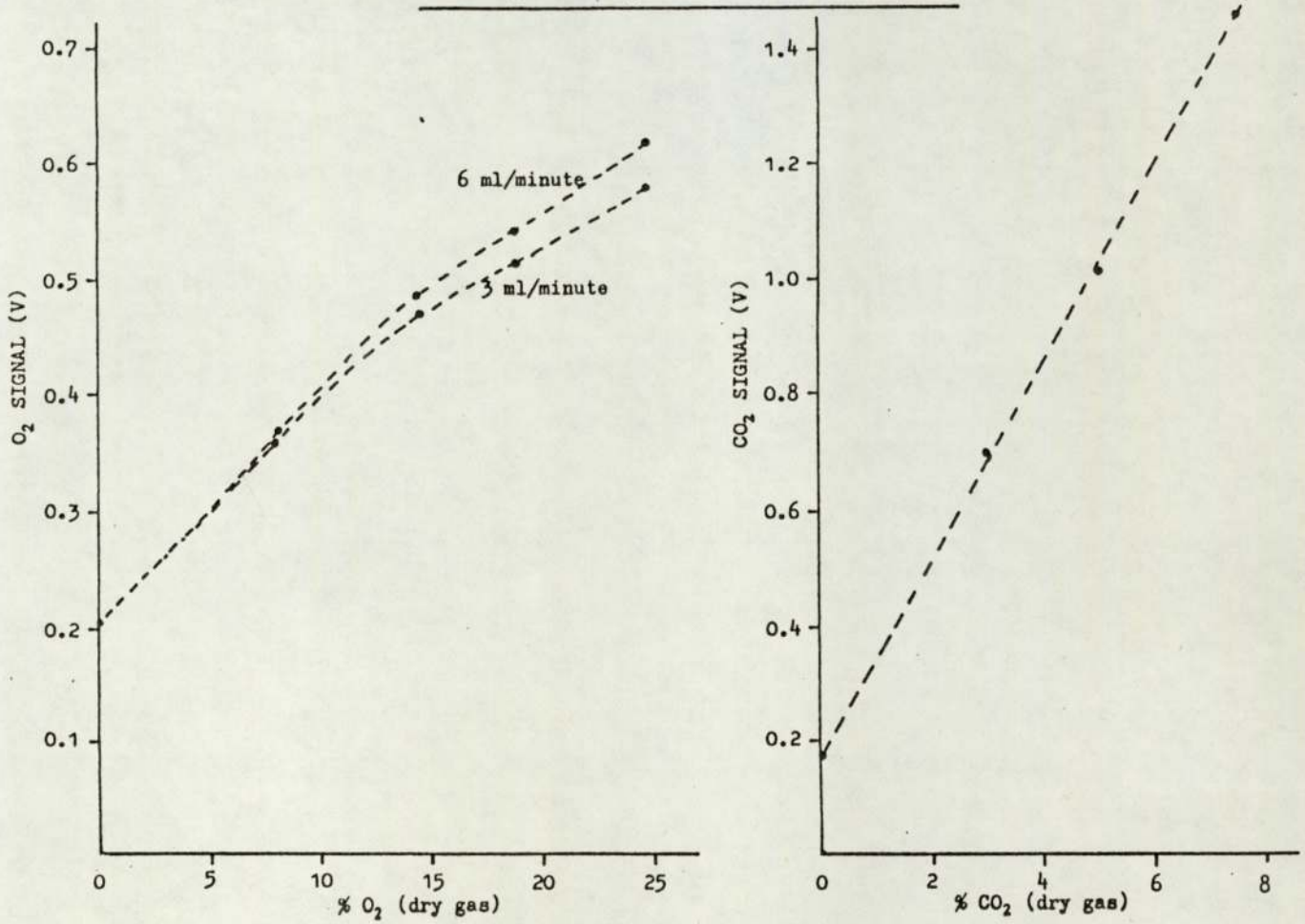
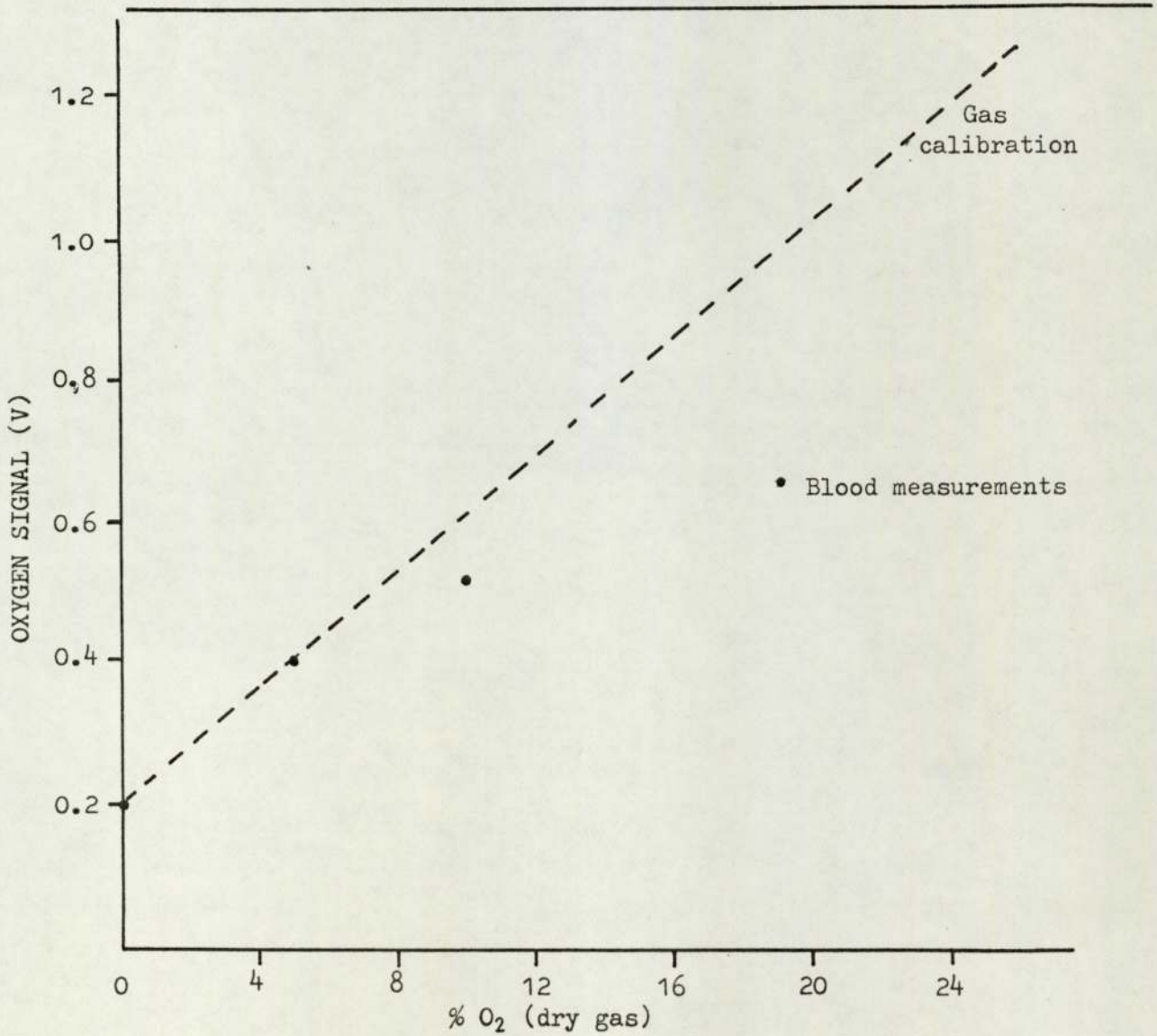


FIGURE 16 : Tonometered blood oxygen response with 12.5μm Polyethylene



non-linearity in the oxygen response was increased, but whether the increase could be considered significant was open to doubt, as the reproducibility of results from day to day was very poor. Some of the problems lay in faults in the mass spectrometer, but in addition it became clear that there was room for improvement in the experimental procedure as regarded damage done to the blood's red cells, the constancy of the blood flow through the cell, and the limited number of analysed cylinder gas mixtures available for use in tonometry - in general, when the PO_2 was altered from one blood sample to another, the PCO_2 was altered too.

With the experience and information obtained from these preliminary experiments, further work was planned to pinpoint the cause of the non-linearity of blood oxygen signals with respect to partial pressure as this would obviously complicate any possible calibration of the system before unknown oxygen partial pressures could be measured. It was noted that as the underlying process seemed to be linked to the dissociation of oxyhaemoglobin in the red cells, any factors that alter the oxygen binding curve, such as PCO_2 and pH, would have to be monitored and if possible, held constant at physiological values so that the effect of varying PO_2 in blood could be studied in isolation. The experiments that were devised for this purpose, and the results that followed, are described in detail in the next chapter.

Chapter 6

Study of Membranes for Blood Gas Analysis

6.1 Oxygen Linearity Experiments

The objectives of this set of experiments were to establish whether the non-linearity of blood oxygen signals would be observed under controlled conditions, to record the effects of changing the conditions, and if possible to draw conclusions from these data with regard to the processes underlying the observed phenomenon.

Summary of Experimental Procedure

A polyethylene membrane 12.5 μm thick was inserted into the test cell described in Chapter 5. The brass support for the membrane effectively exposed 14.3 mm^2 of polyethylene to the vacuum of the mass spectrometer's inlet line in the form of 56 circular areas separated by less than twice their diameter. The mass spectrometer with its polyethylene inlet system was calibrated with two humidified gas mixtures at 37°C.

For each 5 ml blood sample, the haemoglobin concentration was measured with a photometer - the Corning model 950 - and its haematocrit was recorded. The blood was tonometered for 15 minutes while pumping some of the tonometry gas through the mass spectrometer cell, the whole apparatus being kept at 37°C. The steady mass spectrometer oxygen signal was recorded and then the peristaltic pump was tested, changing its tubing if necessary, to achieve a liquid flow

rate of 9 ml/minute. The tonometered blood was then pumped through the cell and the oxygen signal recorded - usually the signal became steady within six minutes. 2 ml of blood was anaerobically withdrawn from the system and analysed by the Corning 165 for pH, PO_2 and PCO_2. In some cases, the oxygen content of this blood sample was measured too, utilising a galvanic cell system - the Lex-O_2-Con TL. From the remaining blood, 1 ml was centrifuged and the haemoglobin concentration in its plasma measured with the Corning 950. The tonometry circuit including the cell was well flushed with distilled water, and the next blood sample tonometered. This procedure was repeated for each blood sample, and at the end of each set of samples the cell was cleaned by flushing with sodium hypochlorite solution and then distilled water.

Discussion of Experimental Procedure

Blood. It was not possible to arrange for a regular supply of blood from either healthy or ill volunteers, and so the minimum volumes necessary were taken as and when required from blood set aside for transfusions but unused within its 'shelf life' of 21 days from donation. The anticoagulant used for such blood is sodium citrate solution and is particularly effective at preserving the red cells but does have the effect of lowering the blood pH considerably from its value in vivo. When such blood is used for transfusions it is added to the bloodstream either sufficiently slowly, or in combination with a bicarbonate solution, to ensure that the acid/base balance of the body is not seriously affected, but for the in vitro experiments to be

described, the blood pH was necessarily relatively low. To avoid reducing the pH still further, no carbon dioxide was included in the gas mixtures used to tonometer the citrated blood, although in turn blood of zero PCO_2 cannot be accepted unquestioningly as directly representing blood as it is in vivo.

Tonometry. Oxygen and nitrogen from cylinders of the compressed pure gases were fed separately through rotameters (British Oxygen Company, 0-15 l/minute flowmeters), then mixed and passed through a humidifier. This consisted of a sintered glass gas distributor immersed in distilled water and held at 37°C. The humidified gas mixture was then fed into the tonometry vessel, also held at 37°C. It was a 250 ml spherical-bottomed glass flask, used to tonometer 5 ml of blood at a time. The neck of the flask was fitted with a rubber bung with four ports. One allowed for gas entry via a thick walled plastic tube reaching down almost to the surface of the blood; another was simply a vent to let the gas out and keep the flask's interior at atmospheric pressure. Narrow stainless steel tubing passed through the other two holes - one short piece that just emerged through the rubber bung, and one long piece that could be pushed right down to below the surface of the pool of blood at the bottom of the flask. These were required firstly for obtaining a sample of the tonometry gas mixture to be analysed by the mass spectrometer cell, and secondly for withdrawing the tonometered blood anaerobically from the flask through the cell and back to the flask in a closed circuit.

The efficiency of the tonometry was checked by using the system to tonometer 5 ml of distilled water with 100% nitrogen. Analysis of

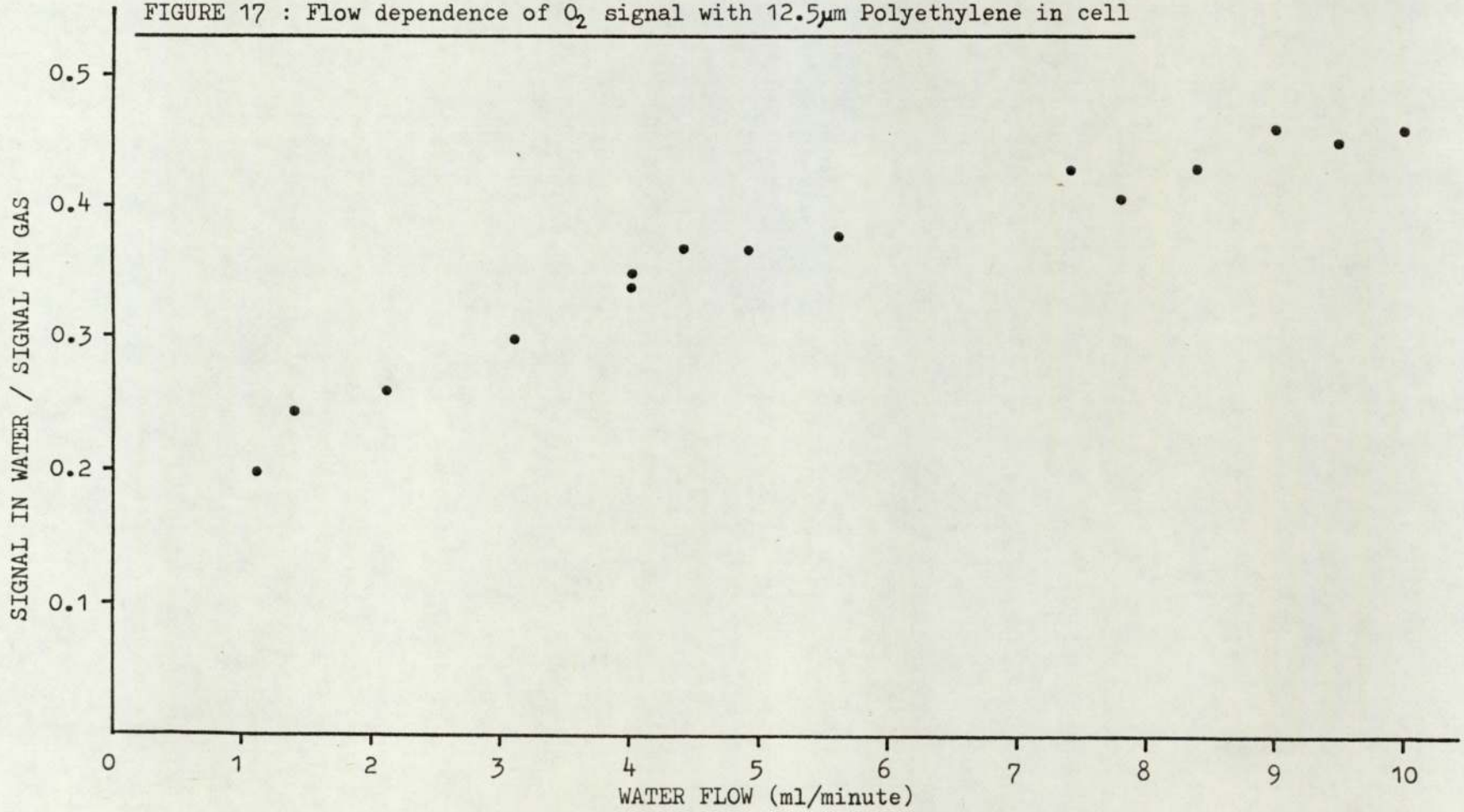
the water by the mass spectrometer after 12 minutes tonometry with a gas flow of 1 l/minute showed that there was less than 7 torr of oxygen present, and repeating this check with blood confirmed this as an upper limit to the inaccuracy of the tonometry system.

Flow. The flow dependence of the membrane inlet system was studied by pumping air-equilibrated water through the cell with the Watson-Marlow H.R. Flow Inducer and recording the steady state oxygen signal as a function of flow rate. From the results, shown in Table 4 and Figure 17, a flow rate of 9 ml/minute was chosen as suitable for the blood analysis to be described - at this rate, flow variations of $\pm$0.5 ml/minute would cause variations in the oxygen signal of about $\pm$2%. Extrapolating from Figure 17 and the results of work described in Appendix 1, it could be seen that an even higher flow rate would have been preferable in that variations in flow about the target value would have had less effect on the oxygen signal. However, it was found to be very difficult to maintain such a high flow rate with the pump system available, particularly because the vinyl tubing around the pump rollers tended to flatten quickly, so reducing the flow through it. It had been found necessary to replace the silicone rubber tubing normally used for peristaltic pumps with vinyl tubing, as the former had an unacceptably high permeability to oxygen. The latter while less resilient did nevertheless allow the pump to work and was found to have negligible permeability for oxygen - this was tested by pumping blood of zero PO_2 through lengths of tubing much longer than required in practice and analysing the oxygen in the blood with the mass

TABLE 4 Flow dependence with 12.5μm Polyethylene in cell

Water flow (ml/minute)	O_2 signal in water/O_2 signal in air
10.0	0.46
8.4	0.43
7.8	0.41
4.9	0.37
4.4	0.37
4.0	0.35
3.1	0.30
1.4	0.24
2.1	0.26
9.5	0.45
5.6	0.38
4.0	0.34
1.1	0.20
7.4	0.43
9.0	0.46

FIGURE 17 : Flow dependence of O_2 signal with 12.5μm Polyethylene in cell



spectometer cell, as described above for the tonometry checks.

Red Cell Damage. It was thought that some of the variability in preliminary experiments could be due to variation in the amount of damage inflicted in the course of the measurements on the red blood cells. Avoiding damage altogether was not possible. An early attempt to use dog blood was abandoned as dog red cells were found to be particularly fragile, but even the human transfusion blood chosen for these experiments was easily damaged, perhaps because of the aging of the cells. Factors that caused damage included any excess moisture in the humidified tonometry circuit, insufficient moisture in the tonometry gas causing the blood to dry out during the tonometry, and the mechanical action of the rollers of the peristaltic pump. Such damage could have an effect on the oxygen flux by altering properties of the blood such as its viscosity, its overall diffusivity for oxygen, or the distribution of oxyhaemoglobin next to the membrane. The damage inflicted on each sample was calculated from measurements of the haemoglobin density in the whole blood, the density of haemoglobin liberated into the plasma by lysed red cells, and the haematocrit. Only results obtained on samples suffering less than 7% damage were accepted as representing 'undamaged' blood.

Experiment 5

Tonometry and analysis were carried out as described above on samples of citrated blood. Results are summarised in Table 5 and illustrated in Figures 18 and 19. Figure 18 shows that the oxygen signal of the mass spectrometer in blood was obviously not directly proportional to the partial pressure of the oxygen in the tonometry gas mixture over the range of PO_2 studied. Between about 40 and 180 torr, the change in the oxygen signal for a given partial pressure difference was much lower than below 40 torr, where, in fact, the response seemed to be linear. Figure 19 shows that plotting the mass spectrometer oxygen signal in blood against the corresponding Corning PO_2 analysis gave the same pattern of non linearity, so this pattern cannot be explained in terms of peculiarities or errors in the tonometry of the blood.

TABLE 5 Whole citrated blood results

M.S. Gas PO_2 (torr)	M.S. Blood PO_2 (torr)	Corning Blood PO_2 (torr)	pH	O_2 Content (vol %)	Haemolysis (%)
105	68.4	97			3.0
109	69.8	108			2.2
139	81.2	134	6.75		6.4
56.1	58.3	56	6.97		6.7
16.6	20.5	18.5	6.80		2.7
148	86.2	138	6.77		2.5
82.6	57.3	76.0	6.73		5.3
74.4	60.6	75.1	6.73		5.6
114	71.2	112	6.75		3.7
167	91.5	148	6.73		3.6
7.8	7.8	8.1	6.85	1.5	6.2
24.2	31.2	24	6.75	9.5	
44.7	53.8	45	6.70	15.3	2.0
78.0	70.2	83.7	6.78		3.7
87.2	68.0	93.5	6.96		2.5
95.6	74.6	101	6.87		1.8
213	115	190	6.86		3.8
147	92.6	138	6.88		4.1

FIGURE 18 : Citrated blood with 14.3mm^2 of 12.5μm Polyethylene

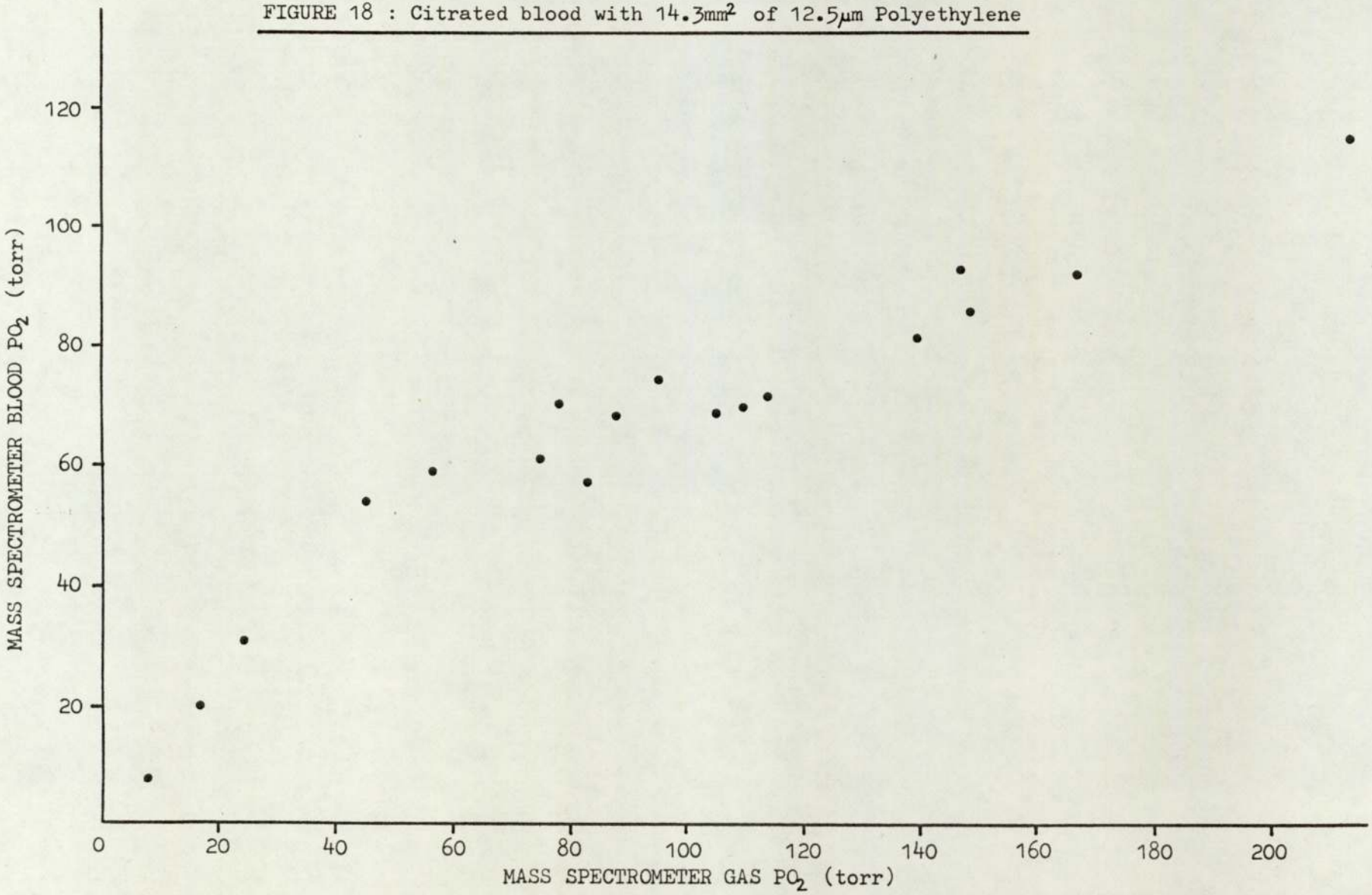
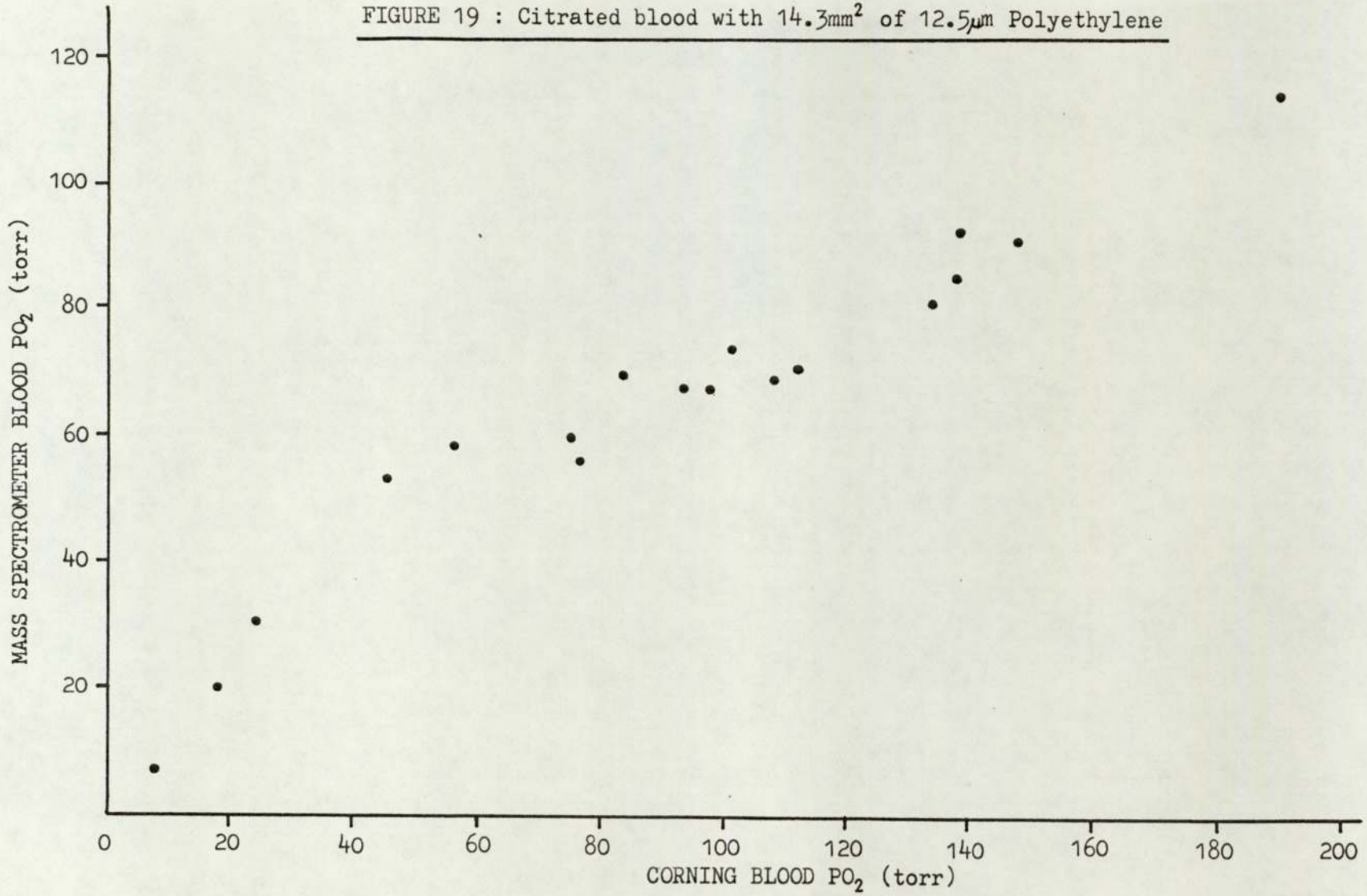


FIGURE 19 : Citrated blood with 14.3mm^2 of 12.5μm Polyethylene



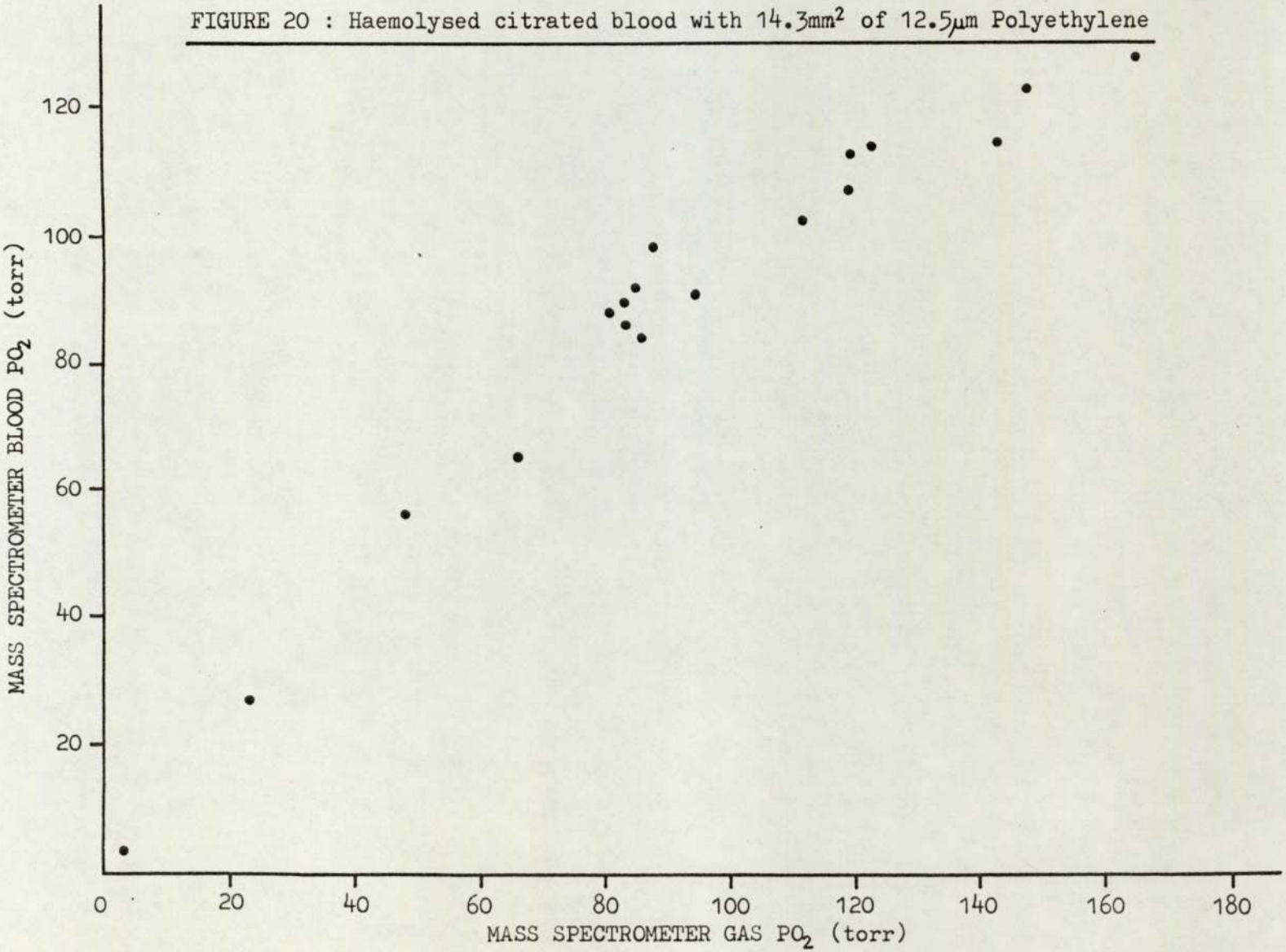
Experiment 6

Tonometry and analysis were carried out as before this time using blood that was completely haemolysed before tonometry by freezing and then rapid thawing. Results are given in Table 6 and Figure 20.They show that the oxygen flux through the polyethylene membrane to the mass spectometer was not linear ly related to the partial pressure of oxygen in the haemolysed blood. The response began with a linear slope of about the same magnitude as with undamaged blood, but then as PO_2 increased the deviation from linearity was much less marked.

TABLE 6 Haemolysed citrated blood results

M.S. Gas PO_2 (torr)	M.S. Blood PO_2 (torr)	Corning Blood PO_2 (torr)	pH
112	102	113	6.85
3.5	3.5	3.8	6.97
84.7	91.8	86.5	6.85
120	112	118	6.86
95.7	90.1	94.8	6.60
83.1	86.6	54.3	6.63
85.9	84.5	62.2	6.62
23.2	27.4	19.9	6.70
88.6	98.4	86.3	6.65
47.8	56.2	38.3	6.68
148	123	137	6.63
83.2	89.5	84.3	6.76
81.8	88.1	86.1	6.76
65.8	65.0	70.5	6.76
122	113	123	6.73
165	127	148	6.76
119	107	115	6.72
143	114	132	6.72

FIGURE 20 : Haemolysed citrated blood with 14.3mm^2 of 12.5μm Polyethylene



Experiment 7

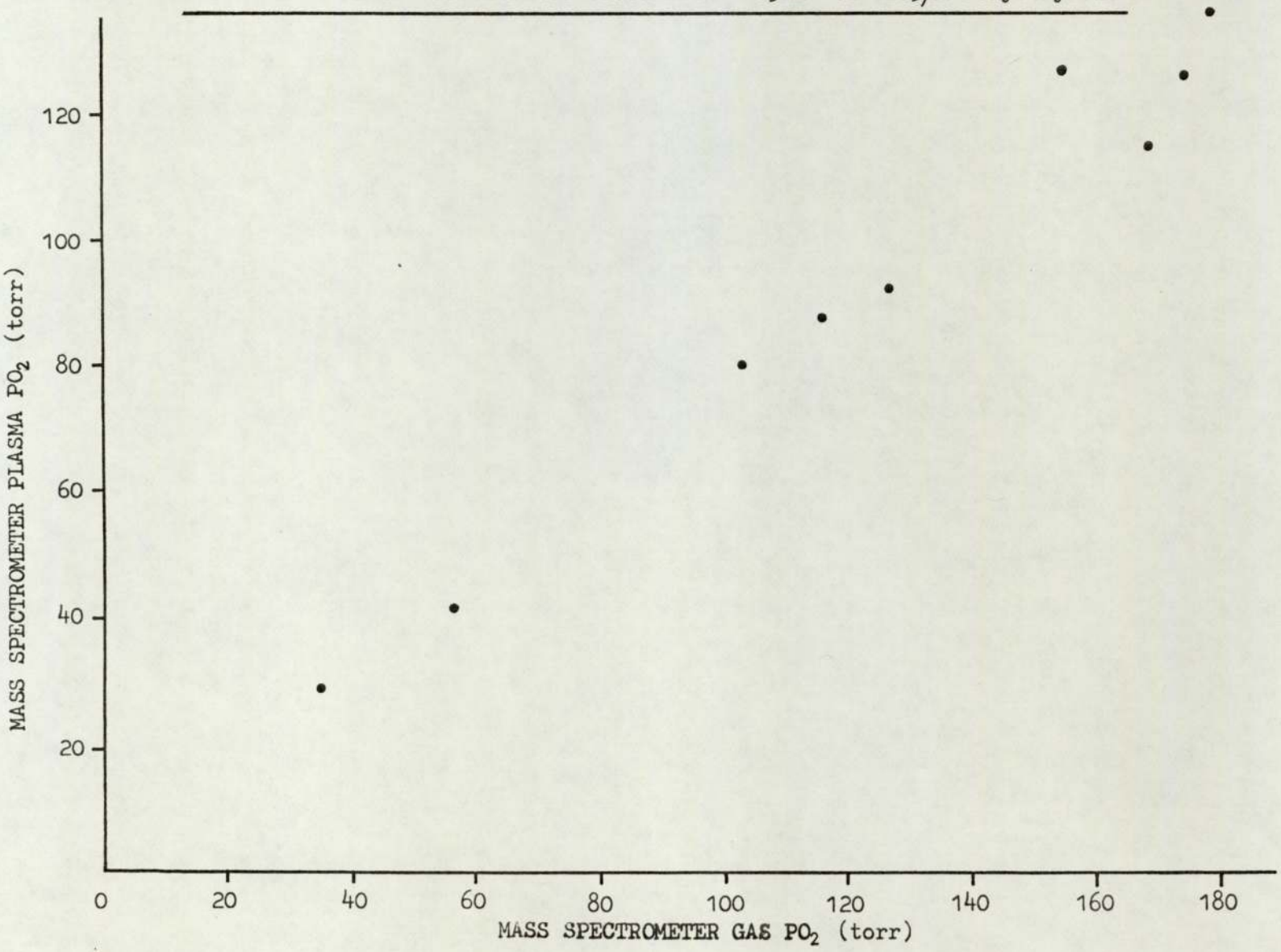
The significance of the presence of red cells to the phenomenon of non-linearity of oxygen flux with respect to partial pressure was investigated by repeating the measurements with the plasma of citrated transfusion blood. There were problems with contamination of the plasma with bacteria such as Klebsiella pneumoniae which could conceivably consume oxygen at such a high rate as to invalidate the tonometry. Such contamination presumably also occured occasionally with the previous experiments on whole blood, but the oxygen buffering properties of the red cells could have minimised its effects. The absence of such buffering in plasma also amplified the effects of any air leaks during tonometry or analysis.

The results, shown in Table 7 and Figure 21, are too few in number and too scattered to be completely convincing, but in the absence of further data the points were taken as representing a linear relationship between the mass spectrometer oxygen signal and the PO_2 of the plasma. The slope of the line was considerably less than the initial slope of the corresponding data in whole blood, so the effect of 'adding' red cells to plasma seems to be an increase in oxygen flux through the membrane at low PO_2 values.

TABLE 7 Citrated plasma results

M.S. Gas PO_2 (torr)	M.S. Plasma PO_2 (torr)	Corning Plasma PO_2 (torr)	pH
116	87.8	127	7.93
56.5	41.8	68	7.84
169	115	165	7.70
174	125	158	7.74
126	91.8	125	
35.4	29.3	42.3	8.03
103	80.0	96.4	8.06
154	126	131	8.04
178	135	155	8.11

FIGURE 21 : Plasma of citrated blood with 14.3mm^2 of 12.5μm Polyethylene



Experiment 8

One obvious drawback with the last three experiments described is that they were all carried out on out of date transfusion blood. The non-linearity found with respect to oxygen partial pressure could be connected with the non-linear dissociation curve of blood, and since this curve is significantly affected by pH, PCO_2, and ageing (which reduces 2,3-diphosphoglycerate concentration) the 'unphysiological' values of pH and PCO_2 and the age of the blood could be creating a situation in the experiments quite unlike that applicable to normal human blood in vivo. It was therefore arranged to obtain a limited number of fresh blood samples from patients.

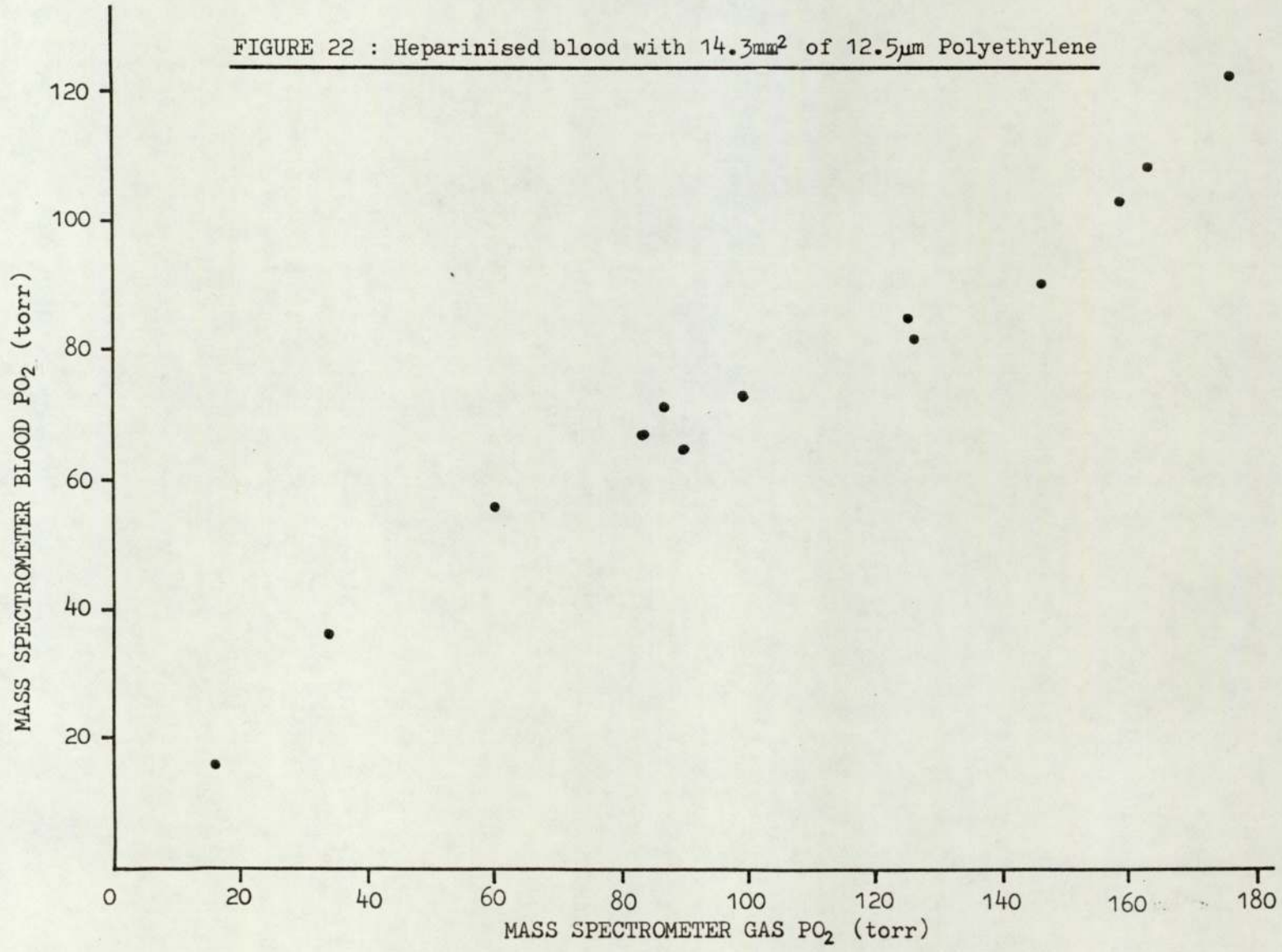
These samples were anticoagulated with heparin, which had no significant effect on the blood pH. In order to approach physiological conditions more closely, about 5% carbon dioxide was included in the tonometry gas mixture. With the available flowmeters, achieving a reasonably reproducible proportion of carbon dioxide in the mixture, or even just keeping the proportion constant throughout the tonometry time of 15 minutes was not possible as a matter of course, and the results of many of the samples tonometered had to be abandoned when analysis showed the blood PCO_2 to be outside the chosen range of 28 torr to 48 torr. The remainder of the experimental procedure was unchanged.

Results are given in Table 8. The oxygen flux to the mass spectrometer is seen in Figure 22 to again be non-linearly related to the PO_2 of the blood. In fact, a third order polynomial was found to

TABLE 8 Whole heparinised blood results

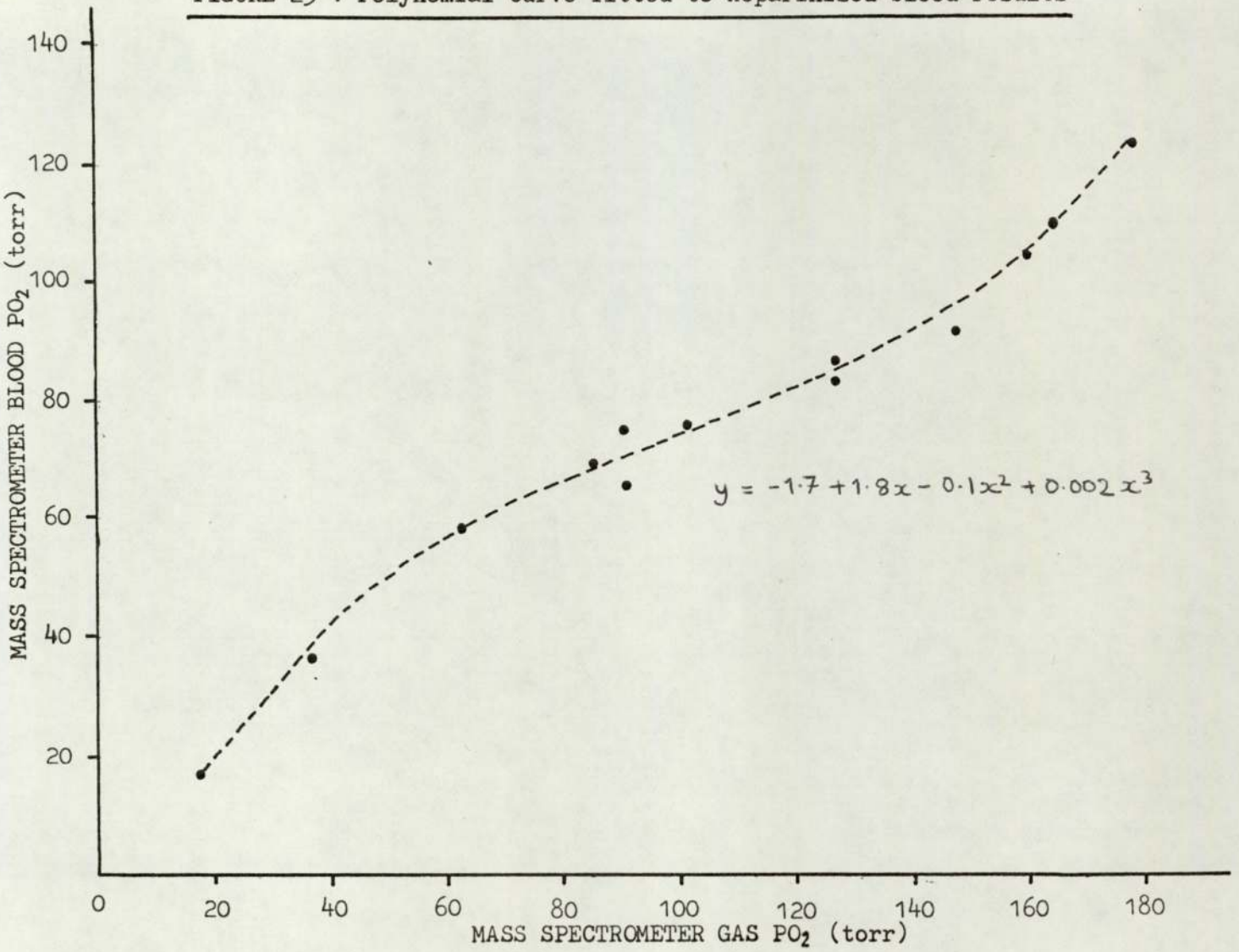
M.S. Gas PO_2 (torr)	M.S. Blood PO_2 (torr)	Corning Blood PO_2 (torr)	pH	Corning PCO_2 (torr)	Haemolysis %
84.5	67.6	81.9	7.36	31.5	4.5
125	85.2	106	7.34	37.0	2.6
99	73.3	98.8	7.21	39.7	4.5
59.6	55.4	64.3	7.41	28.4	1.4
87.0	71.3	86.3	7.36	37.6	2.7
34.5	36.6	34.6	7.38	43.1	4.5
16.6	15.6	19.7	7.36	45.6	
176	122	166	7.37	39.1	3.1
89.7	64.5	88	7.35	32.5	1.8
126	81.9		7.35	38.6	5.4
146	90.3	136	7.44	37.3	3.4
163	108	151	7.29	43.4	
159	103				3.0

FIGURE 22 : Heparinised blood with 14.3mm^2 of 12.5μm Polyethylene



give a good fit to the measured points, as shown in Figure 23. The details of the curve differed from those of whole citrated blood - the initial slope was lower (though not as low as that of citrated blood's plasma) and the curve seemed to restraighten at about 150 torr - but the general deviation from linearity was clear, and qualitatively similar to that found in the experiments with citrated blood.

FIGURE 23 : Polynomial curve fitted to heparinised blood results



Experiment 9

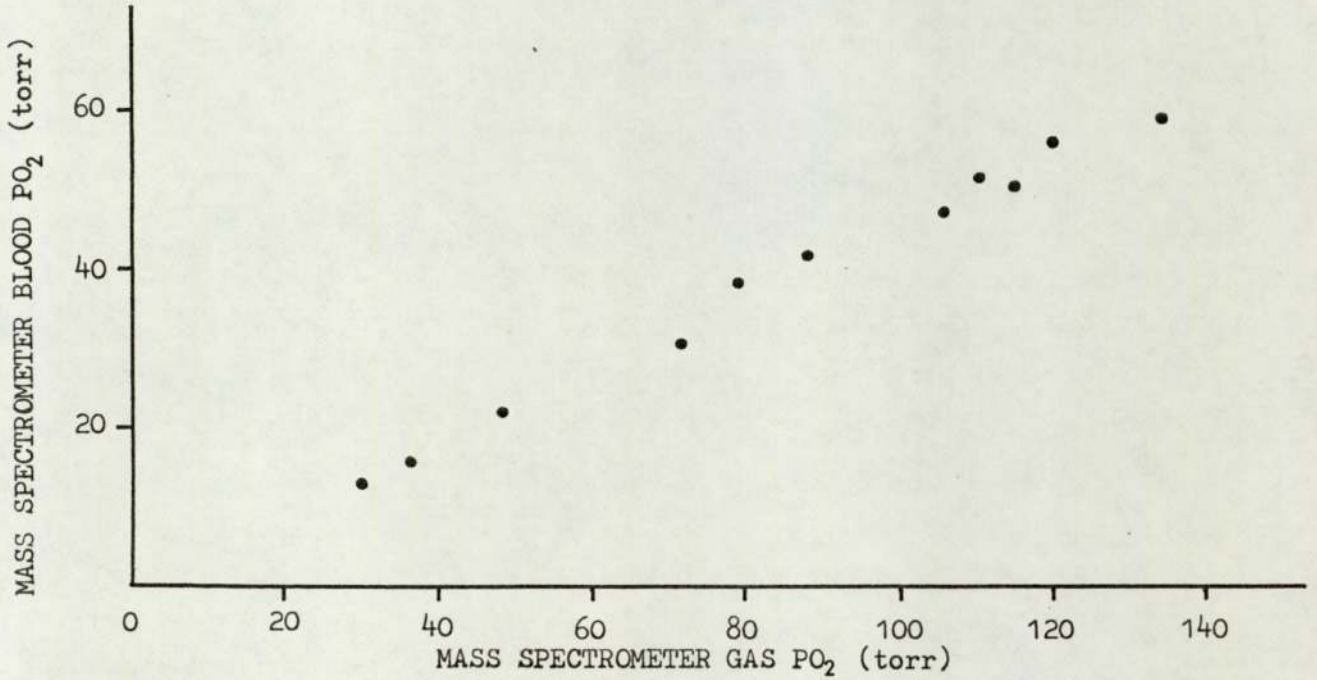
To establish whether the haemoglobin/oxygen reactions occurring within the blood red cells were responsible for the non-linearity of oxygen flux to the mass spectrometer, tonometry gas mixtures were made up with approximately 2% carbon monoxide so that most of the haemoglobin in the red cells would combine with the carbon monoxide in the mixture rather than with the oxygen. For example, at a PO_2 of 100 torr, about 30 times as much carboxyhaemoglobin would be present as oxyhaemoglobin. Citrated transfusion blood with zero PCO_2 was used for this experiment.

Results are given in Table 9. Figure 24 shows that the effect of saturating the majority of the haemoglobin in blood with carbon monoxide is to linearise the oxygen flux to the mass spectrometer. The low slope of the line shows that the presence of oxyhaemoglobin in 'normal' blood must cause the oxygen flux to the mass spectrometer to be greatly increased, at least at low to medium PO_2 values.

TABLE 9 CO-treated citrated blood results

M.S. Gas PO_2 (torr)	M.S. Blood PO_2 (torr)	Corning Blood PO_2 (torr)	pH	O_2 Content (vol %)	Haemolysis (%)
78.8	38.7	80	6.98	0.6	1.8
88.0	41.5	86	6.98	0.5	1.3
106	47.9	101	6.99	0.4	1.8
120	56.3	112	6.99	0.4	1.3
30.4	13.5	36	6.86	0.3	4.9
48.9	21.9	52	6.89	0.4	3.1
110	42.4	110	6.90	2.9	6.8
71.6	30.5	76	6.83	0.7	6.2
114	51.0	102	6.87	1.4	4.5
36.3	15.6	36	6.82	0.3	5.3
134	58.8	118	6.76	1.1	6.0

FIGURE 24 : CO-treated citrated blood with 14.3mm^2 of 12.5μm Polyethylene



Experiment 10

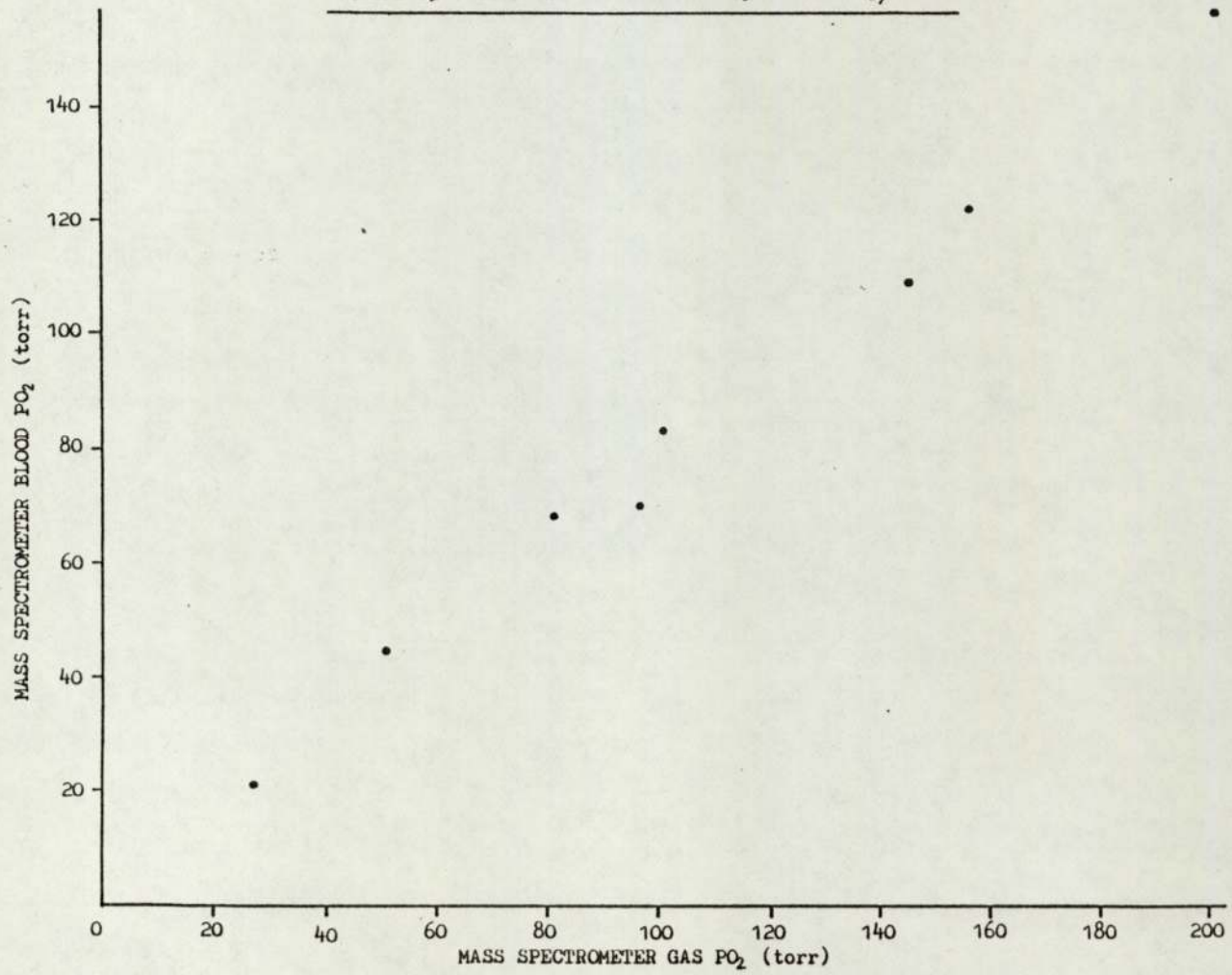
To establish quickly whether drastically reducing the oxygen consumption by the cell/mass spectrometer system would eliminate the oxygen signals' non-linearity, the polyethylene membrane in the cell was replaced with one of 12.5 μm polyvinyl chloride which was estimated to reduce oxygen consumption by a factor of 50. Citrated transfusion blood was tonometered and analysed according to the procedure already described. Because of the comparatively low steady state oxygen flux, the mass spectrometer signals were very small, with a signal to noise ratio of at best 15 to 1. Results are shown in Table 10 and Figure 25.

It can be seen that the oxygen flux to the mass spectrometer was directly proportional to the blood partial pressure. Reducing the cell's permeability to oxygen by changing from polyethylene to PVC thus seemed to remove the phenomenon of non-linearity of oxygen signals in blood.

TABLE 10 Whole citrated blood results with PVC

M.S. Gas PO_2 (torr)	M.S. Blood PO_2 (torr)	Corning Blood PO_2 (torr)	pH	Haemolysis %
81.1	68.9	88.4	6.83	5.4
96.5	70.4	96.4	6.84	3.8
101	83.1	102	6.83	3.8
50.7	44.6	69.6	6.80	5.0
202	157			6.7
156	122			5.6
26.4	21.1	24.7	6.87	3.7
145	109	138	6.87	1.9

FIGURE 25 : Citrated blood with 14.3mm² of 12.5μm PVC



6.2 Discussion of Oxygen Linearity Experiments

Linearity was found between mass spectrometer oxygen signals and PO_2 for plasma. This can be explained as follows - the mass spectrometer oxygen signal is directly proportional to the oxygen flux through its membrane inlet system, this flux is directly proportional to the oxygen concentration in the plasma at the surface of the membrane, and this oxygen concentration is directly proportional to the plasma PO_2, the constant of proportionality being the Henry's law solubility. One reason for the slope of the plasma oxygen line being less than unity ie. for the relatively low reading of the mass spectrometer in plasma compared to gas of the same PO_2 is that depletion of the plasma oxygen by the membrane/mass spectrometer system lowers the concentration immediately next to the membrane relative to that of the bulk of the plasma. This is in spite of diffusion and bulk plasma flow acting in a direction tending to redress the balance, as discussed in connection with Experiment 3 in Chapter 5. So depletion lowers the steady state oxygen flux to the mass spectrometer from that exactly representative of the bulk plasma oxygen concentration, but the <u>proportion</u> by which the PO_2 is lowered is independent of the actual bulk PO_2, and so linearity between oxygen flux and bulk PO_2 (ie. the PO_2 of the tonometry gas) is maintained.

Linearity between mass spectrometer oxygen signals and PO_2 was not found for whole blood. The oxygen concentration in blood is not directly proportional to PO_2, as the major proportion of the oxygen carried is present in reversible chemical combination with haemoglobin in the red blood cells, and the affinity of haemoglobin for oxygen

varies in a highly non-linear way with PO_2, as illustrated in a typical oxygen dissociation curve shown in Figure 1. Consideration of this curve together with the way that the mass spectrometer samples oxygen from the blood can explain the experimentally found non-linearity in qualitative terms, as follows.

In vivo, oxygen diffuses from the plasma into the respiring tissues, to be replaced by oxygen dissociating from the oxyhaemoglobin in the red cells and then diffusing out into the plasma. Similarly, in vitro, the oxygen diffusing across the polyethylene membrane to the mass spectrometer must originate from the plasma of the blood, with the red cells giving up oxygen in turn to keep a balance. Now consider the effect of depletion on blood of high, medium and low PO_2 in turn, with reference to Figure 1, keeping in mind that the depletion is only significant in a relatively narrow zone - of the order of 10^{-5}m - close to the membrane.

When the PO_2 at the membrane surface is high enough for the haemoglobin to be virtually 100% saturated, its affinity for oxygen is high , very little oxygen, if any, will be given up to the plasma to make up for losses by depletion, and the presence of haemoglobin can effectively be ignored. Depletion will therefore occur just as for plasma and linearity between oxygen flux to the mass spectrometer and PO_2 of the bulk blood can be expected.

If the PO_2 at the membrane is low enough for the haemoglobin to be significantly less than fully saturated, then as the plasma is depleted of oxygen, the haemoglobin will give up some of its bound oxygen to the plasma. This extra oxygen will diffuse through the membrane to the mass spectrometer and, assuming the blood is flowing

so that there is a continuous supply of red cells to the depleted zone, fresh oxygen can be continuously given up by the haemoglobin to the plasma. The steady state supply of oxygen to the membrane is thus augmented by the oxyhaemoglobin in the red cells of the blood.

At very low PO_2, the haemoglobin has little oxygen to give up, and its affinity for what it has is high, so augmentation of the plasma's oxygen is likely to be minimal.

The mass spectrometer oxygen signal as a function of blood PO_2 can be considered as the sum of the basic plasma supplied oxygen, which varies linearly with PO_2, and the extra oxygen given up by haemoglobin, which varies non-linearly with PO_2. Figure 26 shows diagramatically how this hypothesis can predict the general shape of the oxygen response by the mass spectrometer as found experimentally. As the PO_2 at the membrane surface is depleted in proportion to the bulk PO_2, the difference between bulk PO_2 and the plasma depleted PO_2 depends linearly on the bulk PO_2. So considering two 'states' on the dissociation curve, one representing bulk PO_2 and the other the depleted PO_2, the horizontal separation of these states ie. the PO_2 difference is greater the greater the value of the bulk PO_2. The 'willingness' of the haemoglobin to donate oxygen depends on the vertical separation of bulk and depleted states. So this 'willingness' is not simply proportional to the slope of the dissociation curve at a point, but to the difference in saturation between two points on the curve, which varies with slope and position. This sort of model for the blood/membrane/vacuum system can lead to quantitative predictions for the oxygen flux as a function of blood PO_2, as follows.

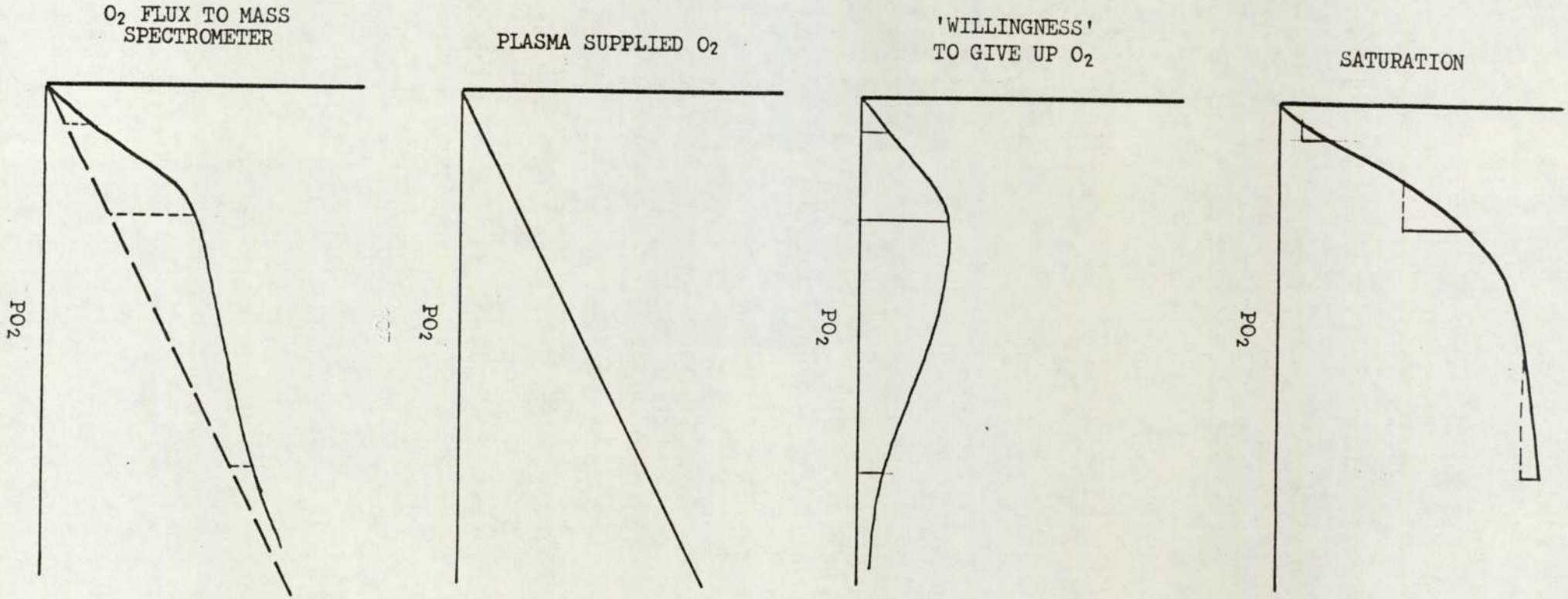


FIGURE 26 : Graphical models for PO_2 non-linearity

Consider tonometered blood at a certain PO_2. Let the oxygen concentration in the plasma be cp_1 and the oxygen concentration in the red cells be cr_1. Then the ratio cr_1/cp_1, R_1 say, is determined by the 'position' of the blood on its oxygen dissociation curve ie. R_1 is a function of cp_1. Now consider the processes leading to a steady state oxygen flow from blood through the membrane as if they occurred in two stages.

Close to the membrane, as oxygen diffuses into the evacuated inlet system of the mass spectrometer, the oxygen concentration in the plasma falls. Suppose the concentration were to fall to $cp_2 = f\ cp_1$ where f is some fraction, constant for a given membrane and blood flow system. Then the corresponding oxygen concentration in the red cells would be $cr_2 = R_2\ cp_2$ where R_2 is the ratio of oxygen concentrations that would correspond to the new plasma concentration, via the dissociation curve. However, the actual concentration in the cells is cr_1, which is greater than cr_2, so some oxygen will be transfered from the cells to the plasma until equilibrium is regained.

At equilibrium, let the concentration of oxygen that has been transfered from cells to plasma be x. Then the final plasma oxygen concentration cp_3 and red cell oxygen concentration cr_3 are given by

$$cp_3 = cp_2 + x = f\ cp_1 + x$$
$$cr_3 = cr_1 - x$$

Now assume that the oxygen transfered from the red cells is proportional to the difference between cr_1 and cr_2 ie. the difference between the cells' original oxygen concentration, and that

concentration that would have corresponded to the initially depleted plasma oxygen concentration.

$$x = g(cr_1 - cr_2) \text{ say, where } g < 1$$

Then the final equilibrium plasma oxygen concentration is given by

$$\begin{aligned} cp_3 &= f\, cp_1 + g(cr_1 - cr_2) \\ &= f\, cp_1 + g(R_1 cp_1 - R_2 f\, cp_1) \\ &= (f + gR_1 - gf\, R_2) cp_1 \end{aligned}$$

Now the observed mass spectrometer oxygen signal Sobs will be proportional to this plasma concentration cp_3, rather than to the expected, tonometered concentration cp_1.

$$S_{obs} = (f + gR_1 - gf\, R_2)\, S_{exp}$$

To test this model, vales of f and g were taken as parameters and values of R_1 and R_2 calculated from standard oxygen dissociation curve data to give curves like those shown in Figures 27 and 28. It was found that taking f = 0.65 and g = 0.01 gave the curve shown dotted in Figure 29 with the experimentally obtained data from Experiment 8. The fit is seen to be reasonably good, so the proposed model - of oxygen depletion partially compensated by oxyhaemoglobin dissociation - could be valid.

The difference between the results for Experiments 5 and 8,

FIGURE 27 : Predicted response to blood PO_2; g constant, 4 values of f

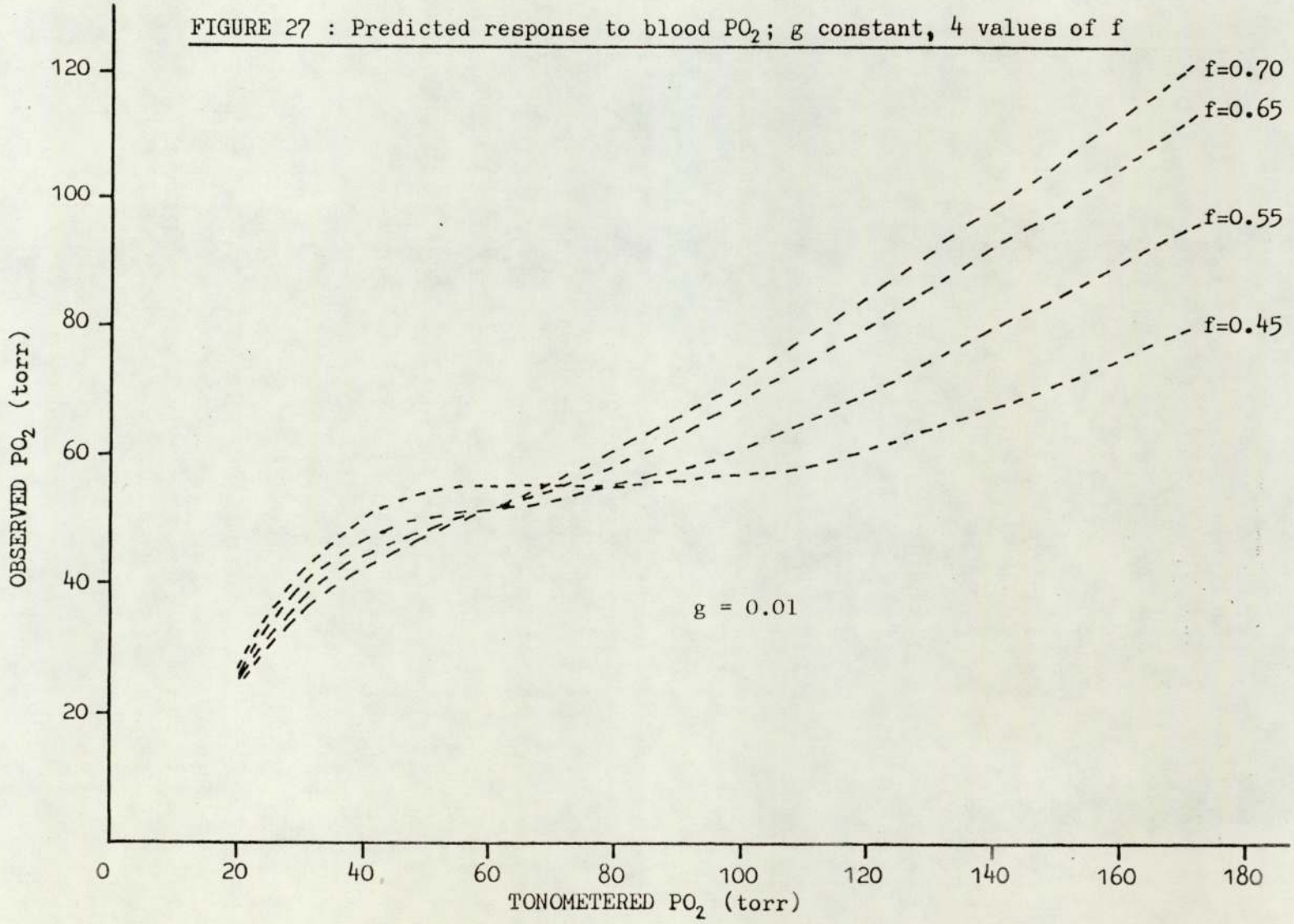


FIGURE 28 : Predicted response to blood PO_2; f constant, 4 values of g

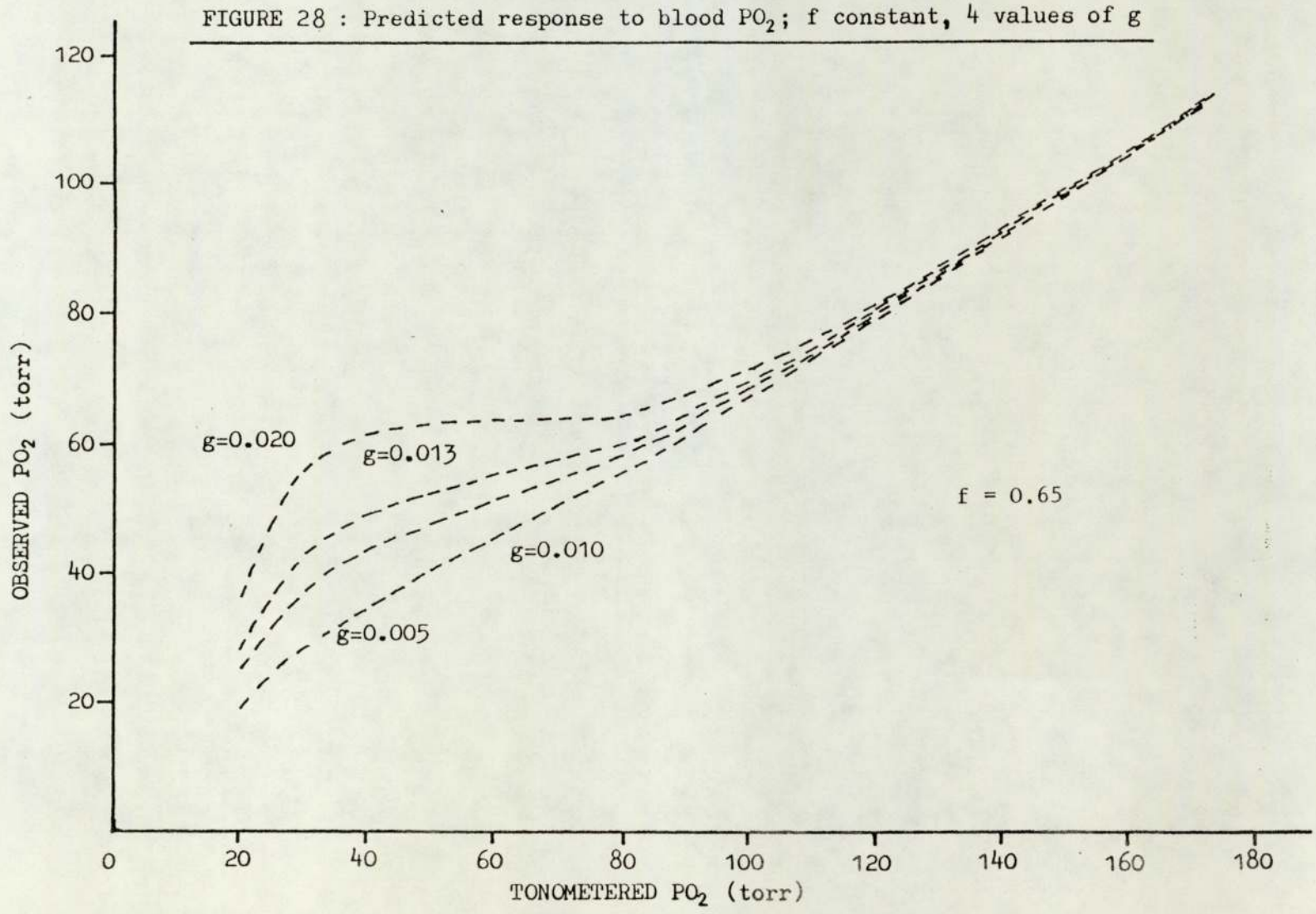
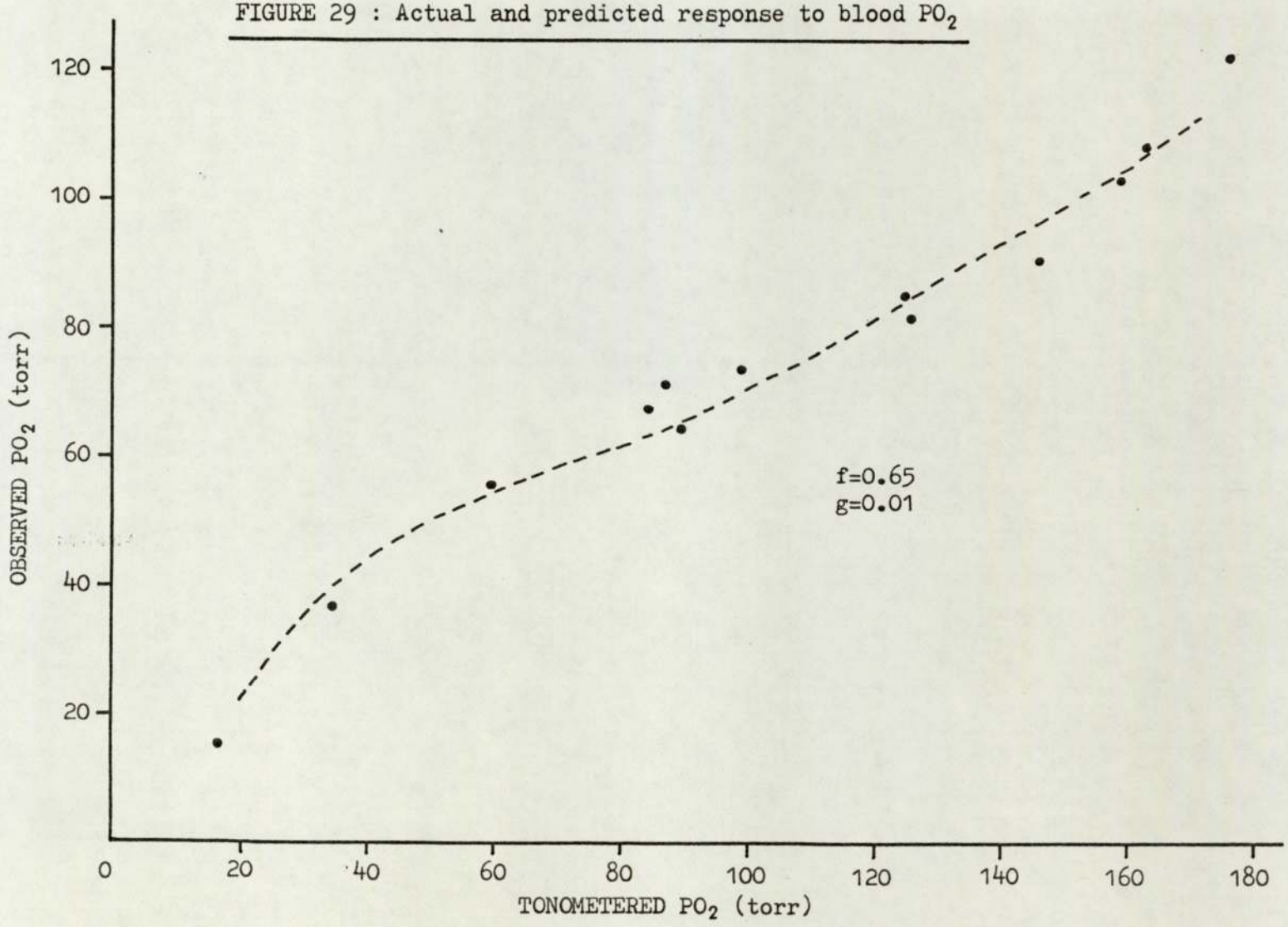


FIGURE 29 : Actual and predicted response to blood PO_2



citrated and heparinised blood, could be due simply to the effects of pH, PCO_2 and ageing in shifting the dissociation curve, and so the 'willingness' of the haemoglobin to part with oxygen as a function of PO_2.

The reason for the difference in response betwen haemolysed and unhaemolysed citrated blood is not clear. It could be linked to a change in oxygen diffusivity in the blood, or possibly a more effective augmentation of the plasma oxygen by haemoglobin, as it is spread out more homogeneously through the plasma than when confined within red cells.

Linearity was found between mass spectrometer oxygen signals and PO_2 for blood whose haemoglobin was to a large extent bound with carbon monoxide rather than oxygen. This supports the idea that augmentation of the plasma oxygen from oxyhaemoglobin is the cause of non-linearity in 'normal' blood, as obviously the concentration of oxyhaemoglobin available to give up any oxygen to the plasma is considerably reduced. The fact that linearity was found between mass spectrometer signals and blood PO_2 in Experiment 10 is understandable, as using PVC rather than polyethylene drastically reduced the permeability of the sampling system to oxygen. This meant that depletion of the plasma oxygen must also have been much reduced, so creating little drive for the oxyhaemoglobin to dissociate and provide extra oxygen.

In summary, the non-linear response of the mass spectrometer polyethylene inlet system to the PO_2 of whole blood was thought to be due to the dissociation of oxyhaemoglobin augmenting the plasma oxygen, that had been depleted by the consumption of the inlet system.

However, two major criticisms could be levelled at the set of membrane experiments just described. One is that the use of citrated transfusion blood produced conditions quite unlike those in vivo; the pH in particular was very different. Although one experiment was carried out on heparinised blood and non-linearity was found, the results were rather different from those of citrated blood, and so it was decided that the other experiments - on plasma, haemolysed blood, and so on - should all be repeated with fresh heparinised blood. Since the haemoglobin/oxygen reactions seemed to affect the oxygen flux through the membrane to the mass spectrometer, the haemoglobin concentration of the blood could be significant, and so it was considered desirable to conduct each experiment with blood taken on one occasion from one person, so that the blood used for each tonometered sample analysed during a single experiment would have the same haemoglobin concentration. To avoid having to take large amounts of blood from each volunteer - about 50 ml would be required for each set of results using the existing protocol - it was proposed that after a steady mass spectrometer oxygen signal had been recorded from each blood sample, and the minimum volume necessary for electrochemical and haemolysis analysis had been withdrawn, the remaining blood should just be topped up to the required volume and retonometered for the next PO_2 point. The objection to this was that the haemolysis caused in the first run would be increased with each successive run and quickly reach an unacceptably high value. To minimise haemolysis it was decided to abandon the tonometry system constructed for the previous experiments, and use a commercial

manufactured tonometer which had not been available to me before - the IL model 237. This instrument used a slightly different method of tonometry - a cylindrical vessel with a hemispherical bottom was repeatedly spun then stopped, with a period of a few seconds so that in each cycle the blood within was forced into a thin film around the walls and then drained back into a pool at the bottom, the vessel being continuously washed through by the humidified tonometry gas. This tonometer itself probably caused less damage to the red cells than the other had, and in addition it was decided to alter the experimental procedure by flushing the tonometry circuit with isotonic saline immediately before pumping blood through it, to remove any water that had condensed out of the humidified gas mixture. Trials showed that together, these two measures reduced haemolysis so that the topping up prodedure was feasible, and so, in turn, was the use of fresh heparinised blood from volunteers, one donor for each experiment.

The second major criticism applicable to the experiments so far is that the system of gas mixing for the tonometry gas mixture was not good enough to maintain a mixture of constant proportions throughout the tonometry and analysis time of about 25 minutes. Obviously if the partial pressures in the gas phase altered significantly during this time, a steady state could not be assumed between gas and blood phases at the end of the tonometry period or during the analysis by the spectrometer. The situation would become even less acceptable when heparinised blood was used, as it would be necessary to introduce carbon dioxide to the mixture, and keep its partial pressure constant at about 40 torr to correspond to its normal value in vivo. A new set

of rotameters calibrated in the required ranges for nitrogen, oxygen and carbon dioxide respectively was therefore obtained. With these flowmeters ('MeTeRaTe' flowmeter tubes, Jencons Scientific Limited) and a corresponding set of needle valves it was possible to both make up mixtures of the required proportions, and to keep these proportions constant during the time necessary for tonometry and analysis. A measure of the accuracy of this gas mixing system can be gauged from the fact that in the experiments to be described the PCO_2 of the tonometered blood varied by less than $\pm$ 7 torr from its target value of 40 torr (an error of less than $\pm$1% of the total gas pressure) in 80 consecutive samples over a period of 35 days.

Having revised a large part of the protocol for the oxygen linearity experiments on blood, it was thought appropriate to consider any other areas of improvement so that all of the alterations necesary could be inaugurated at the same time, and the possibility of requiring further repetition of experiments could be minimised. The main area in which improvement was both desirable and possible was in the accuracy of the reference blood samples by the Corning electrochemical blood gas analyser. Previously, this analyser had been situated in a laboratory some distance from that containing the mass spectrometer and the rest of the apparatus, and the time that necesarily elapsed between the mass spectrometer analysis and the Corning analysis, together with the necessity to calibrate the Corning before each measurement, and the inconvenience of doing this in advance, must in general have resulted in inaccurate measurements. However, the mass spectrometer, the cell, and the attendant apparatus had recently been moved into the laboratory housing the Corning, and

so the possibility of setting up the analyser specifically for the tonometered blood measurements with the minimum delay existed. To achieve much more accurate reference measurements, especially of oxygen, was now a reasonable aim, so an investigation into the operation of the Corning blood gas analyser was carried out and is described below.

6.3 Blood Gas Measurements with the Corning 165

Four 2ml samples of 1 day old heparinised dog blood, anaerobically stored in glass syringes, were obtained. Two samples (numbers 1 and 2) were kept at room temperature and the others (numbers 3 and 4) were brought to 37°C in a water bath. Each sample in turn was well mixed in its syringe and then introduced to the measuring cuvette of the Corning, in most cases in successive aliquots of 0.25 ml. The outputs of the PO_2 and PO_2 electrodes were recorded after each aliquot was introduced and the steady or peak readings were taken. The results are summarised in Table 11.

They show that successive aliquots of blood gave consistently lower PO_2 readings, especially for higher initial PO_2's - or possibly especially when the blood sample is initially at a higher temperaure. Even at a lower PO_2, as for sample (1), 0.5 ml was required to maintain a reasonable plateau for the PO_2 signal, and even this plateau shifted downwards for the next few aliquots. The question remained - was the PO_2 reading after the first aliquot 'right' because the blood itself had had the minimum time to consume its own oxygen, and so its PO_2 was the closest to its initial tonometered or 'true' value? Or was the second or one of the later readings 'right' because only then was the cuvette sufficiently well flushed through with the blood to be negligibly affected by 'memory'?

Further samples of the same type were obtained, this time of 1 ml volume. The first of these was introduced, in 0.25 ml aliquots as before, immediately after filling the Corning cuvette with one of the gas mixtures used to calibrate it, with a PO_2 of about 140 torr and a

TABLE 11 Initial results with the Corning 165

Sample	PO_2 (torr)	PCO_2 (torr)
0.25 ml into Corning	69.6 (Peak)	22.3
+0.25 ml "	69.3 (Plateau)	24.8
+0,25 ml "	64.9 "	25.2
+0.25 ml "	64.8 "	25.3
+0.25 ml "	63.4 "	25.3
+0.25 ml "	64.0 "	25.4
+0.25 ml "	63.6 "	25.4
0.25 ml into Corning	91.4 (Peak)	16.8
+0.25 ml "	85.3 (Plateau)	20.7
+0.25 ml "	85.2 "	21.1
+0.25 ml "	85.3 "	21.1
0.25 ml into Corning	139 (Peak)	6.6
+0.25 ml "	133 "	7.0
+0.25 ml "	129 "	7.0
+0.25 ml "	126 "	7.0
+0.25 ml "	123 (Plateau)	6.9
+0.25 ml "	124 "	6.8
+0.25 ml "	121 "	6.9
0.25 ml into Corning	142 (Peak)	2.3
+0.50 ml "	133 "	2.1
+0.25 ml "	129 "	2.0
+0.50 ml "	127 "	1.9
+.50 ml "	125	1.9

PCO_2 of about 36 torr. A second sample was introduced straight after filling the cuvette with a second calibration gas mixture of zero PO_2 and a PCO_2 of about 75 torr. Results are given in Table 12. They show that filling the Corning cuvette with a gas mixture of low PO_2 and high PCO_2 did improve the precision of the measurements on blood of low PO_2 and high PCO_2 - the readings from the first 0.25 ml were closer to those of the second aliquot, and these readings were more reproducible. In fact, when primed in this way the Corning readings from the <u>first</u> 0.25 ml were exact for PO_2 and only about 2% too low for PCO_2.

Measurements were then made on 1 ml samples of blood of higher PO_2 and lower PCO_2, introducing each sample after one of the two calibration gases. The results, given in Table 13, show that two effects seemed to occur with blood of high PO_2 - there was a continuous fall in PO_2 with time, and also, when the cuvette was primed with a gas of low PO_2, it took about 0.75 ml of blood to flush away the 'memory' of the gas. Two more sets of measurements were made, as given in Table 13, and they showed that these effects were reproducible.

It was therefore concluded that when using the Corning as the reference blood gas analyser in tonometered blood experiments, the cuvette should be filled, after calibration, with the tonometry gas mixture immediately before introducing the blood. The PO_2 of the blood should then be read after the first 0.25 ml aliquot of blood, and the PCO_2 read after the final aliquot of blood.

TABLE 12 Further results with the Corning 165

Sample	PO_2 (torr)	PCO_2 (torr)
After high PO_2 low PCO_2 gas		
0.25 ml into Corning	44	68.0
+0.25 ml "	42.4 (plateau)	72.7
+0.25 ml "	42.0	73.5
+0.25 ml "	41.9	73.7
After low PO_2 high PCO_2 gas		
0.25 ml into Corning	41.5	72.9
+0.25 ml "	41.5 (plateau)	74.4
+0.25 ml "	41.3	74.4
+0.25 ml "	41.4	74.6

TABLE 13 Final results with the Corning 165

Sample	PO_2 (torr)	PCO_2 (torr)
1 After low PO_2 high PCO_2 gas		
0.25 ml into Corning	163 (peak)	17.0
+0.25 ml "	165 (plateau)	14.7
+0.25 ml "	167 "	14.2
+0.25 ml "	163	14.2
2 After high PO_2 low PCO_2 gas		
0.25 ml into Corning	163 (plateau)	15.3
+0.25 ml "	158 "	14.8
+0.25 ml "	156 "	14.5
+0.25 ml "	152 "	14.5
3 After high PO_2 low PCO_2 gas		
0.25 ml into Corning	145 (peak)	20.5
+0.25 ml "	139 (plateau)	20.3
+0.25 ml "	136 "	20.3
+0.25 ml "	131 "	20.3
4 After low PO_2 high PCO_2 gas		
0.25 ml into Corning	123 (peak)	23.7
+0.25 ml "	123 (plateau)	21.4
+0.25 ml "	119 "	20.9
+0.25 ml "	118 "	21.0

6.4 Revised Oxygen Linearity Experiments

Summary of revised experimental procedure

A polyethylene membrane 12.5 μm thick was inserted into the test cell previously described. 14.3mm^2 of this membrane was exposed to the vacuum of the mass spectrometer's inlet line in the form of 56 circular areas separated by less than twice their diameter. The mass spectrometer with its polyethylene inlet system was calibrated with two humidified gas mixtures at 37°C.

About 25ml of fresh heparinised venous blood was obtained from healthy volunteers . Its haemoglobin concentration was measured with a Hilger-Watts Unichem spectrophotometer model H1620 using a BCL haemoglobin kit. The haematocrit was recorded too, and then a 3ml sample of the blood was tonometered in the IL 237 with a gas flow of 500 ml/minute for 10 minutes while pumping some of the tonometry gas through the mass spectrometer cell. The whole apparatus was kept at 37°C. The steady mass spectrometer oxygen signal was recorded and then the tonometry circuit was flushed with isotonic saline. The liquid flow rate produced by the peristaltic pump was adjusted to 9ml/minute, and then the tonometered blood was pumped through the cell.

The mass spectrometer oxygen signal was recorded over the next 5 or 6 minutes. During this time the Corning 165 was calibrated and then used to analyse a sample of the tonometry gas mixture. Once a steady state mass spectrometer oxygen signal was recorded, 1ml of blood was anaerobically withdrawn from the tonometry circuit and analysed by the Corning 165 for PO_2, PCO_2, and pH. In some cases, the oxygen content

was measured too, by the Lex-O_2-Con TL, and 0.5ml of the blood was spun down and the haemoglobin concentration of the plasma was measured, so that the red cell damage could be calculated. Any blood remaining after analysis was returned to the pool within the tonometry vessel, and the pool was topped up to 3ml. The tonometry circuit including the cell was flushed with saline while the flowmeter needle valves were adjusted to give a new tonometry gas mixture, and the next tonometry was begun.

At the end of the set of samples the cell and the tonometry circuit were flushed with sodium hypochlorite solution and then distilled water.

Experiment 11

Tonometry and analysis were carried out as described above on samples of fresh heparinised whole blood. Two complete sets of analyses were carried out, using blood from two different volunteers. The results are summarised in Table 14 and illustrated in Figure 30. The graph shows that the blood oxygen signal of the mass spectrometer departed from a linear response with respect to PO_2 at about 50 torr. There was little evidence for a return to a linear relationship at high PO_2 but otherwise the general pattern of the response was similar to that obtained in Experiments 5 and 8. The blood readings were not linearly related to the oxygen content, but were possibly slightly dependent on the haemoglobin concentration.

Tonometry and analysis were carried out on two sets of heparinised whole blood, freshly haemolysed by freezing and thawing. The results are summarised in Table 15 and illustrated in Figure 31. The graph shows that the oxygen flux from blood through the membrane to the mass spectrometer depended non-linearly on the partial pressure of oxygen, and in fact the measured points lay on roughly the same curve as those for non-haemolysed blood.

Next, the plasma of fresh heparinised blood was tonometered and analysed. The results are given in the first part of Table 16. Large discrepancies were seen to exist between the Corning measurements of plasma PO_2 and the mass spectrometer and the Corning measurements of the gas PO_2. The discrepancies were most marked at low PO_2, so it seemed likely that air was contaminating the plasma due to inefficient tonometry, leaks in the tonometer/mass spectrometer cell circuit, or

TABLE 14 Blood with 14.3mm^2 of 12.5μm Polyethylene

M.S. Gas PO_2 (torr)	M.S. Blood PO_2 (torr)	Corning Blood PO_2 (torr)	Corning Blood PCO_2 (torr)	pH	O_2 Content (vol %)	Red Cell Damage (%)
165	107	158	41.1	7.31	19.3	1.6
50.0	51.4	52.2	42.4	7.30	17.1	
87.3	72.7	95.0	40.4	7.29	19.4	1.2
28.7	30.9	31.1	39.2	7.30	10.6	2.7
134	92.1	136	37.3	7.29		4.0
75.5	68.3	83	42.9	7.29		1.8
38.7	39.3	38.8	39.2	7.26		2.7
50.5	53.3	55.8	41.5	7.20		5.8
68.4	53.6	75.1	43.4	7.34	16.0	2.0
81.9	66.8	79.7	37.4	7.33	15.9	
44.0	45.7	54.3	34.3	7.36		3.1
84.0	68.8	83.4	39.4	7.33		2.4
96.1	73.9	95.1	39.5	7.33	15.4	3.2
61.9	52.9	57.4	41.4	7.33	13.4	4.5
104	74.7	105	37.6	7.35	15.7	2.0

FIGURE 30 : Blood with 14.3mm² of 12.5μm Polyethylene

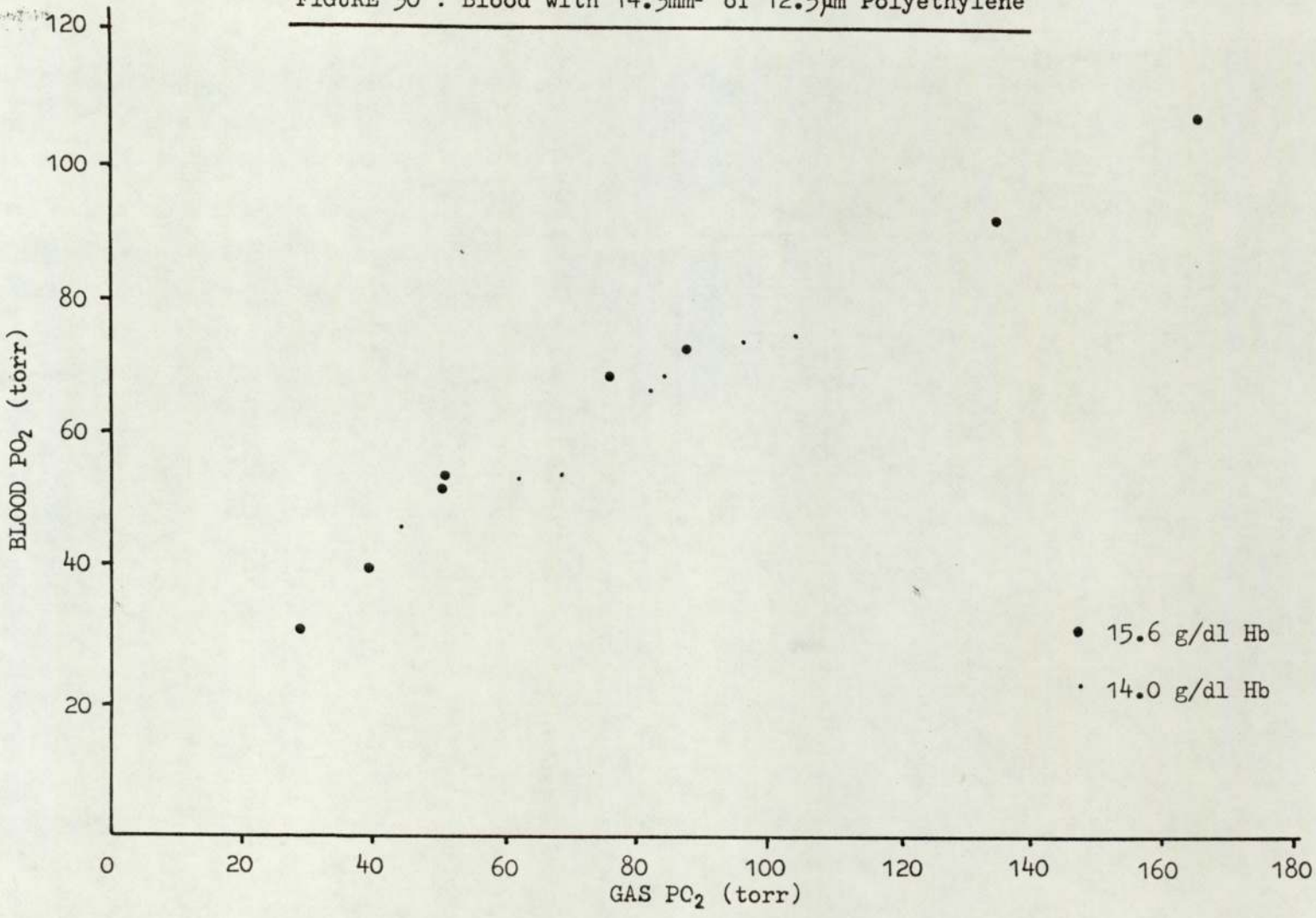


TABLE 15 Haemolysed blood with $14.3mm^2$ of 12.5μm Polyethylene

M.S. Gas PO_2 (torr)	M.S. Blood PO_2 (torr)	Corning Blood PO_2 (torr)	Corning Blood PCO_2 (torr)	pH
95.2	72.9	99.2	39.6	7.29
39.3	43.8	44.9	41.5	7.27
112	71.8	118	34.0	7.31
33.5	36.5	34.9	41.9	7.25
62.3	53.8	68.6	40.4	7.27
67.7	52.8	77.0	39.8	7.25
156	91.3	165	38.5	7.25
64.5	55.1	73.0	44.7	7.22
127	78.9	132	37.9	7.28
69.2	66.3	64.7		7.24
94.8	75.6	93.3		7.17
126	74.9	127		7.22
176	89.2	175		7.18
49.6	44.7	53.7		7.11
103	69.8	112	44.8	7.10
118	77.0	126	37.1	7.16
58.6	53.6			
86.2	63.6	92.0	47.2	7.05

FIGURE 31 : Haemolysed blood with 14.3mm^2 of 12.5μm Polyethylene

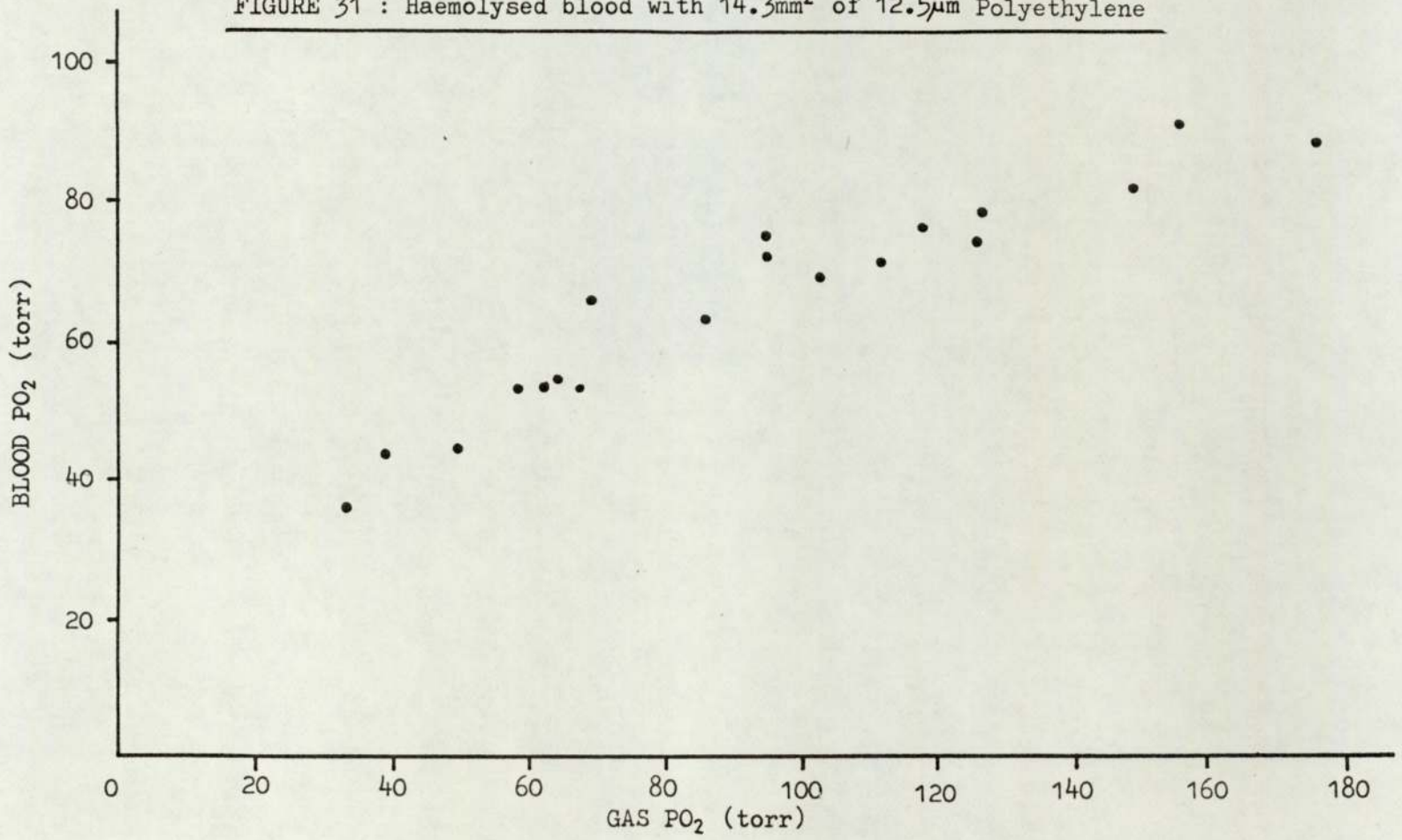


TABLE 16 Plasma with 14.3mm^2 of 12.5μm Polyethylene

M.S. Gas PO_2 (torr)	M.S. Plasma PO_2 (torr)	Corning Gas PO_2 (torr)	Corning Plasma PO_2 (torr)	Corning Plasma PCO_2 (torr)
49.5	35.2	54.4	78.1	40.0
146	72.4	146	143	32.9
73.1	41.2	69.5	86	37.0
62.6	40.7	66.7	83.3	37.0
84.7	50.6	85.0	100	36.7
30.1	22.5	31.4	59.3	39.4
140	70.8	127	131	43.7
85.6	43.4	83.4	83.0	43.2
111	57.5	105	100	37.7
26.6	14.7	26.3	31.0	39.0
53.8	30.1	55.4	57.4	43.8
93.0	47.1	100	93.8	41.0
85.6	42.2	84.2	85.6	41.4
99.2	50.2	97.0	95.2	39.1
24.9	14.4	29.2	44.8	47.0
46.5	23.8	51.0		
63.5	30.3	68.8	72.4	35.9
116	57.2	122	112	45.4

errors in sampling and analysis by the Corning. Tests on the tonometer and the pumping were carried out and they showed that much of the air contamination could be avoided by replacing as much of the vinyl tubing as possible with stainless steel tubing. It was also found that for low PO_2 plasma samples a more definitely determined PO_2 reading could be achieved by filling the Corning cuvette with a zero oxygen gas mixture immediately before analysis, although the actual reading taken was not significantly different from that obtained by filling the cuvette with tonometry gas before analysis.

These changes were effected and the experiment repeated on a fresh stock of plasma. The results are given in Table 16 and plasma PO_2 v. gas PO_2, as measured by the mass spectrometer, is plotted in Figure 32. The flux of oxygen to the mass spectrometer is seen to depend linearly on PO_2.

Tonometry and analysis were then carried out on fresh heparinised whole blood, adding about 2% carbon monoxide to each tonometry gas mixture, so that most of the blood's haemoglobin would combine with carbon monoxide rather than oxygen. The results are given in Table 17 and the oxygen response shown in Figure 33. The relationship between the oxygen signal from the mass spectrometer and the blood PO_2 is seen to be approximately linear.

Computer programs that could fit straight lines or 3rd order polynomial curves to sets of points were run on the experimental results. The standard deviation of each set of points from the calculated line or curve was taken as a measure of the goodness of fit, and is recorded in the first part of Table 18. It can be seen that fitting 3rd order polynomials to the whole blood data gave

FIGURE 32 : Plasma with 14.3mm^2 of 12.5μm Polyethylene

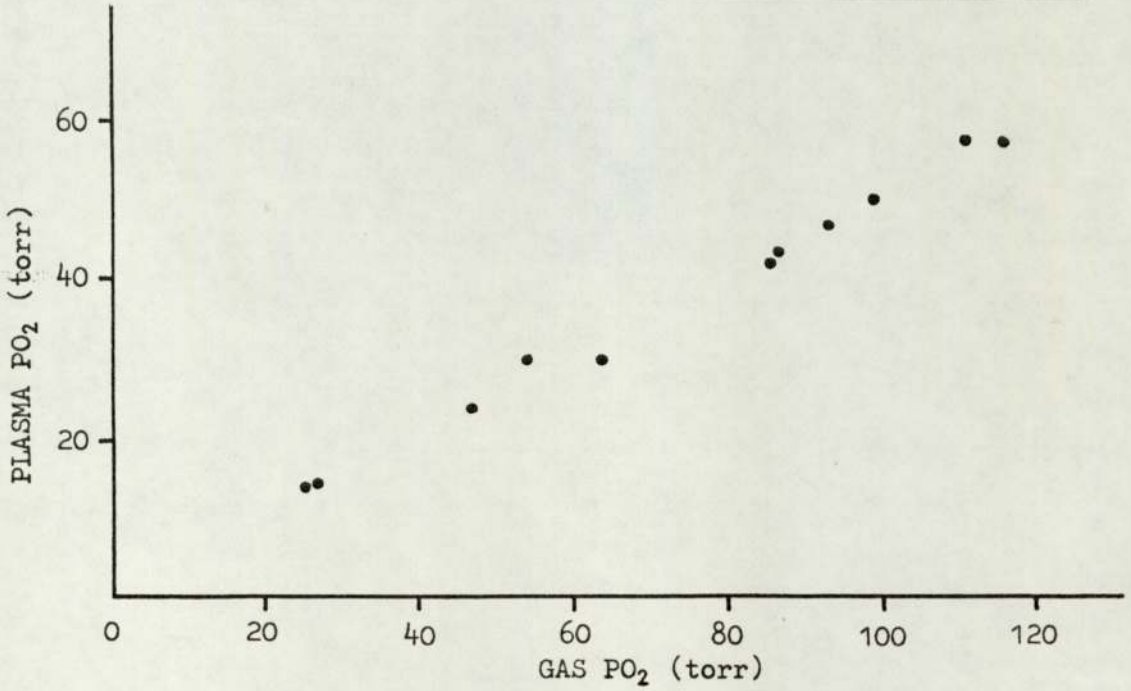


TABLE 17 CO-treated blood with 14.3mm^2 of 12.5μm Polyethylene

M.S. Gas PO_2 (torr)	M.S. Blood PO_2 (torr)	Corning Blood PO_2 (torr)	Corning Blood PCO_2 (torr)	pH	O_2 Content (vol %)	Red Cell damage (%)
39.8	19.6	37.5	49.6	7.19	0.1	2.9
93.3	45.2	98.8	44.0	7.16	0.5	4.0
65.7	28.8	66.0	35.8	7.19	0.5	
120	49.9	117	39.1	7.16	1.0	
128	57.7	122	43.9	7.25	1.6	5.5
79.7	34.7	81.4	46.4	7.23	0.5	6.0
101	48.4	101	46.6	7.25	2.3	
95.8	39.6	103	46.3	7.26	0.8	

FIGURE 33 : CO-treated blood with 14.3mm^2 of 12.5μm Polyethylene

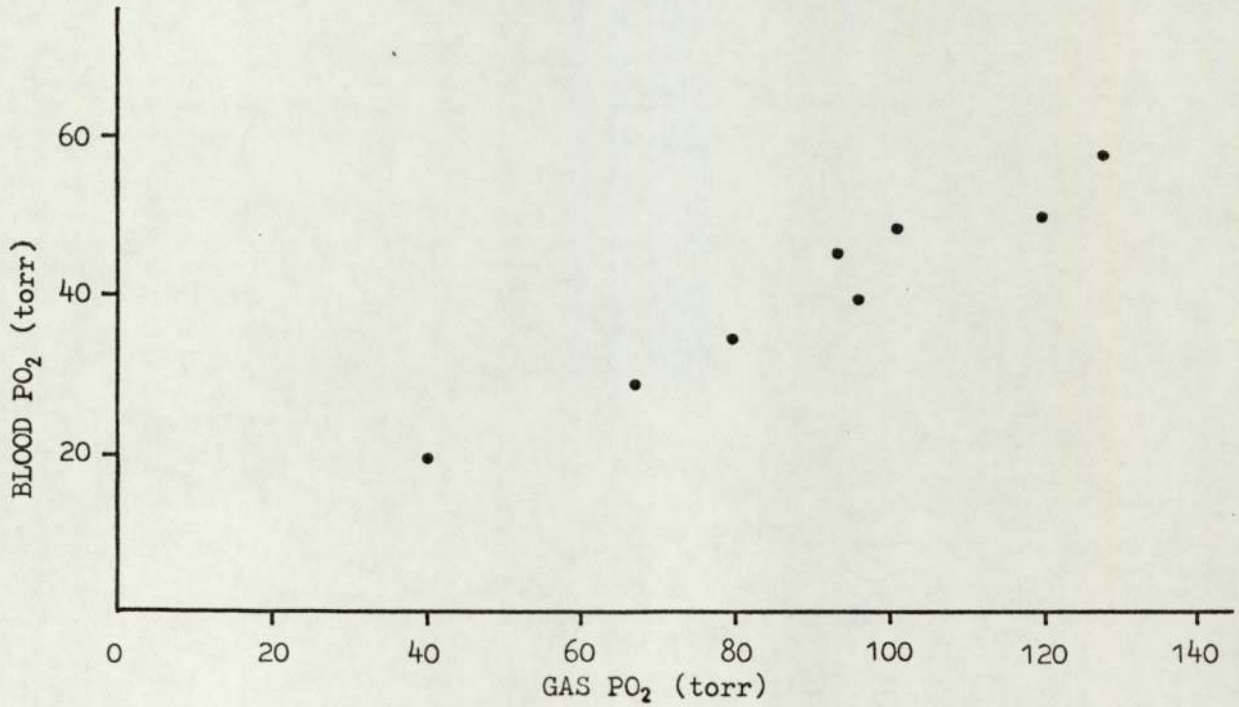


TABLE 18 Results of line and curve fitting programs on experimental data

Membrane		Fluid	Standard Deviation for 3rd Order Polynomial (torr)	Standard Deviation for straight line (torr)
14.3 mm^2 Polyethylene	★	Blood	1.44	8.32
"	★	Blood	2.52	5.12
"	★	Haemolysed Blood	3.56	7.96
"		Plasma	1.21	1.35
"		CO treated Blood	2.48	2.69
14.3 mm^2 of PVC		Blood	4.42	4.87
"		Blood	3.89	4.31
14.3 mm^2 of Polypropylene	★	Blood	2.94	6.48
"		Plasma	2.05	2.28
3.3 mm^2 of Polyethylene	★	Blood	2.39	7.35
3.0 mm^2 of Polyethylene	★	Blood	2.24	7.06
3.0 mm^2 of Polypropylene		Blood	3.90	7.36

standard deviations between 2 and 6 times smaller than those resulting from fitting straight lines, so this data could reasonably be taken as representing a non-linear dependency of oxygen flux from the blood on PO_2. For the plasma and carbon monoxide data however, the standard deviations were virtually unchanged, so the data could reasonably be taken as representing a linear relationship between oxygen flux and PO_2.

The results of Experiment 11 can be explained in terms of the depletion model described in the Discussion of the previous set of oxygen linearity experiments. In brief, the oxygen flux from plasma is linearly related to PO_2 because the concentration of oxygen in the plasma is directly proportional to PO_2 and depletion close to the membrane merely lowers the absolute size of the oxygen flux relative to that corresponding to the bulk oxygen concentration; the oxygen flux from blood is non-linearly related to PO_2 because the basic contribution from plasma-dissolved oxygen is augmented by a partial dissociation of oxyhaemoglobin in the depleted zone, and this augmentation is determined by the non-linear oxygen dissociation curve for blood; the oxygen flux from carbon monoxide treated blood is linearly related to PO_2 because there is too little oxyhaemoglobin present to effectively augment the steady state flux.

This hypothesis would suggest that the non-linearity found for blood could be virtually elimin ated if the permeability of the membrane system were reduced to a great enough extent for the depletion of oxygen in the plasma to be too slight to significantly shift the oxygen equilibrium between red cells and plasma.

The permeability of the system as it stood (ie. with 14.3 mm^2 of

12.5 μm polyethylene) was measured by attaching a glass capillary tube (of capacity 1 μl per mm length) to one outlet of the cell, sealing off the other outlet, and timing the passage of a small bolus of ink along the tube towards the membrane. From this, the rate at which air was sampled by the membrane inlet system was found to be $9 \pm 1 \times 10^{-6}$ ml/s, corresponding to an oxygen flux of approximately 10^{-8} ml/s per torr O_2 partial pressure difference.

The polyethylene membrane was then replaced by one of 12.5 μm thick PVC. Its oxygen sampling rate was measured in terms of that of the polyethylene membrane by recording the oxygen signal output for a given partial pressure input for each system in turn while holding the mass spectrometer sensitivity constant. In this way, the specific gas flux through the PVC was found to be about 5% of that through the polyethylene membrane, ie. 5×10^{-10} ml/s per torr O_2 partial pressure difference.

Experiment 12

Tonometry and analysis were carried out as before but with the PVC membrane in the cell instead of polyethylene. The results are given in Table 19. The oxygen signals were very small - of the order of tens of millivolts - and correspondingly noisy. The signal to noise ratio when sampling 25% oxygen in the gas was typically 12:1. The resolution of the mass spectrometer was reduced to the extent that there was a 10% valley between oxygen and nitrogen peaks in Spectrum mode, so that the sensitivity was increased. In addition, the oxygen output signal was smoothed before it was recorded, using a filter of 100 kΩ resistance and 10 μF capacitance. With these alterations tonometry and analysis were carried out on another set of heparinised blood samples. The results are given in Table 19 and Figure 34 shows both sets of measured points. The oxygen response of this membrane inlet system is seen to be linear with respect to PO_2.

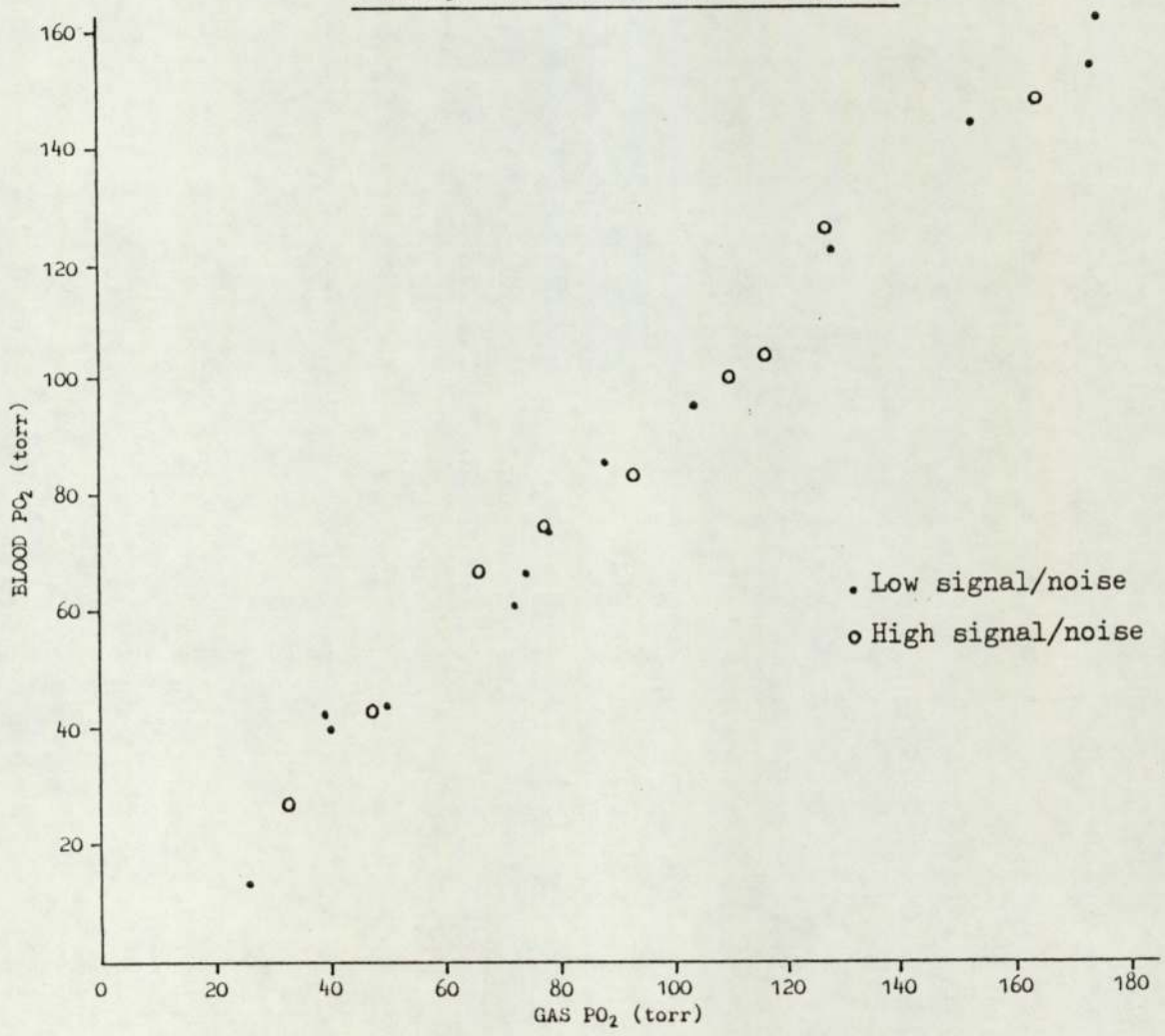
Curve fitting programs run on this data showed that straight lines fitted the oxygen response quite well, and that the standard deviation of the data from the line was slightly lower for the second set of points than the first - see Table 18.

It could be concluded then that the PVC inlet system that drew an oxygen flux from air of about 5×10^{-10} ml/s per torr O_2 partial pressure difference was of low enough permeability to avoid the excessive depletion of blood oxygen that would cause non-linearity of the oxygen signal with respect to PO_2.

TABLE 19 Blood with 14.3mm^2 of 12.5μm PVC

M.S. Gas PO_2 (torr)	M.S. Blood PO_2 (torr)	Corning Blood PO_2 (torr)	Corning Blood PCO_2 (torr)	pH	Red Cell Damage (%)
128	122	127	42.5	7.36	1.5
175	162	165	36.4	7.30	3.8
74.5	66.2	79.6	43.4	7.24	2.9
104	94.9	121	37.0	7.26	4.9
25.6	12.8	32.8	33.8	7.30	4.6
49.8	44.0	45.2	38.8	7.26	3.5
87.9	85.0				3.3
174	154	162	41.6	7.23	3.3
39.2	42.0	49.7	40.4	7.20	
78.4	72.8	97.6	22.8	7.34	4.8
153	144	154	40.0	7.14	
72.2	60.5	65.8	44.6	7.10	
39.9	39.9	37.7	31.5	7.08	
77.3	73.5	79.7	40	7.39	1.8
110	99.9	117	34.5	7.38	
32.4	27.1	40.2	41.3	7.34	2.4
93.2	83.0	101	40.9	7.31	2.9
66.2	66.2	68.4	49.7	7.26	1.6
127	126	137	37.9	7.28	3.4
164	148	154	37.6	7.29	0.9
116	104	118	42.2	7.23	1.4
47.5	42.9	48.1	43.4	7.20	3.1

FIGURE 34 : Blood with 14.3mm^2 of 12.5μm PVC



Experiment 13

The PVC membrane was replaced by one of polypropylene 25 μm thick. The oxygen consumption of the mass spectrometer with this modified inlet system was found to be about 15% of that with 12.5 μm polyethylene, ie. approximately 2×10^{-9} ml/s per torr O_2 partial pressure difference.

The flow dependence of the oxygen signal for air-equilibrated distilled water was recorded, and shown in Figure 35.

Tonometry and analysis were carried out on samples of fresh whole blood. The results are given in Table 20 and Figure 36. The experiment was repeated on the plasma of fresh heparinised blood, and the results are given in Table 21 and Figure 37. It can be seen that the oxygen response of this polypropylene inlet system was non-linear in blood and linear in plasma, just as for the polyethylene experiments. Standard deviation data is given in Table 18.

The results of Experiments 11, 12, and 13 suggested that while the depletion caused by membrane inlet systems allowing a specific gas flux of 10^{-8} ml/s/torr or even 2×10^{-9} ml/s/torr was so large as to cause non-linearity between the oxygen flux to the mass spectrometer and blood PO_2, the depletion caused by a membrane system of 5×10^{-10} ml/s/torr was small enough to avoid such non-linearity. Further attention was thus given to the low permeability PVC membrane inlet system, with a view to its potential use for blood gas analysis.

The mass spectrometer output signals were small with this system, and various measures were taken to try to improve the signal to noise ratio of the oxygen signal in particular. In the process, a related complication became apparent - large, low frequency changes of the

FIGURE 35 : Flow dependence of O_2 signal

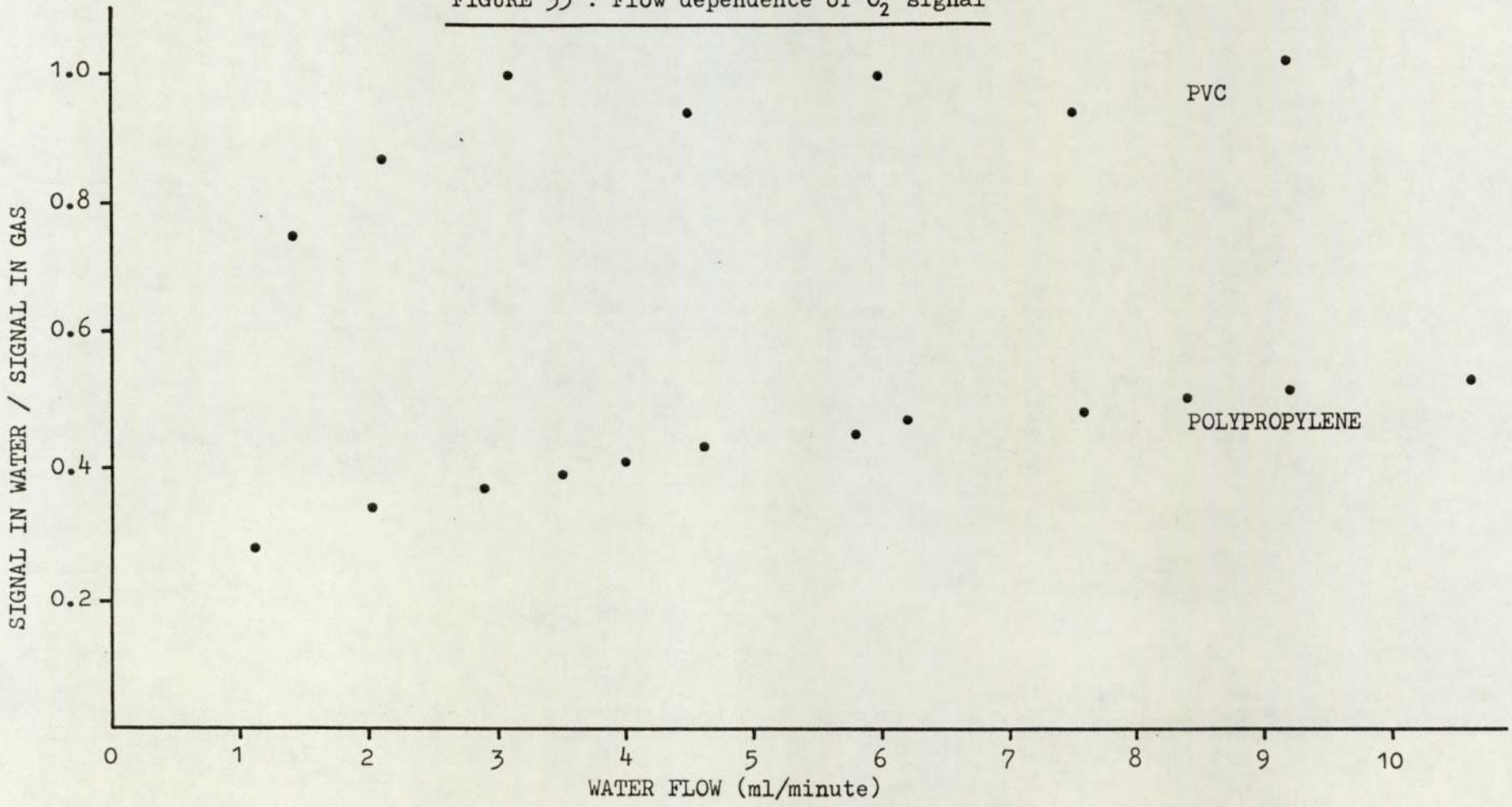


TABLE 20 Blood with 14.3mm^2 of 25.0μm Polypropylene

M.S. Gas PO_2 (torr)	M.S. Blood PO_2 (torr)	Corning Blood PO_2 (torr)	Corning Blood PCO_2 (torr)	pH	O_2 Content (vol %)	Red Cell Damage (%)
50.0	51.5	51.3	43.5	7.30		1.2
92.9	66.7	96.6	41.0	7.25	16.6	4.4
109	83.0	113	44.2	7.24	18.1	1.5
156	104	154	38.9	7.27	18.3	
130	84.6	139	43.3	7.22		2.3
60.6	53.6	60.8	43.3	7.20	15.3	2.7
81.8	62.6	84.6	40.6	7.33		1.3
36.4	37.3	37.1	36.9	7.35		2.0
25.5	26.4	25.0	35.3	7.34		2.7

FIGURE 36 : Blood with 14.3mm^2 of 12.5μm Polypropylene

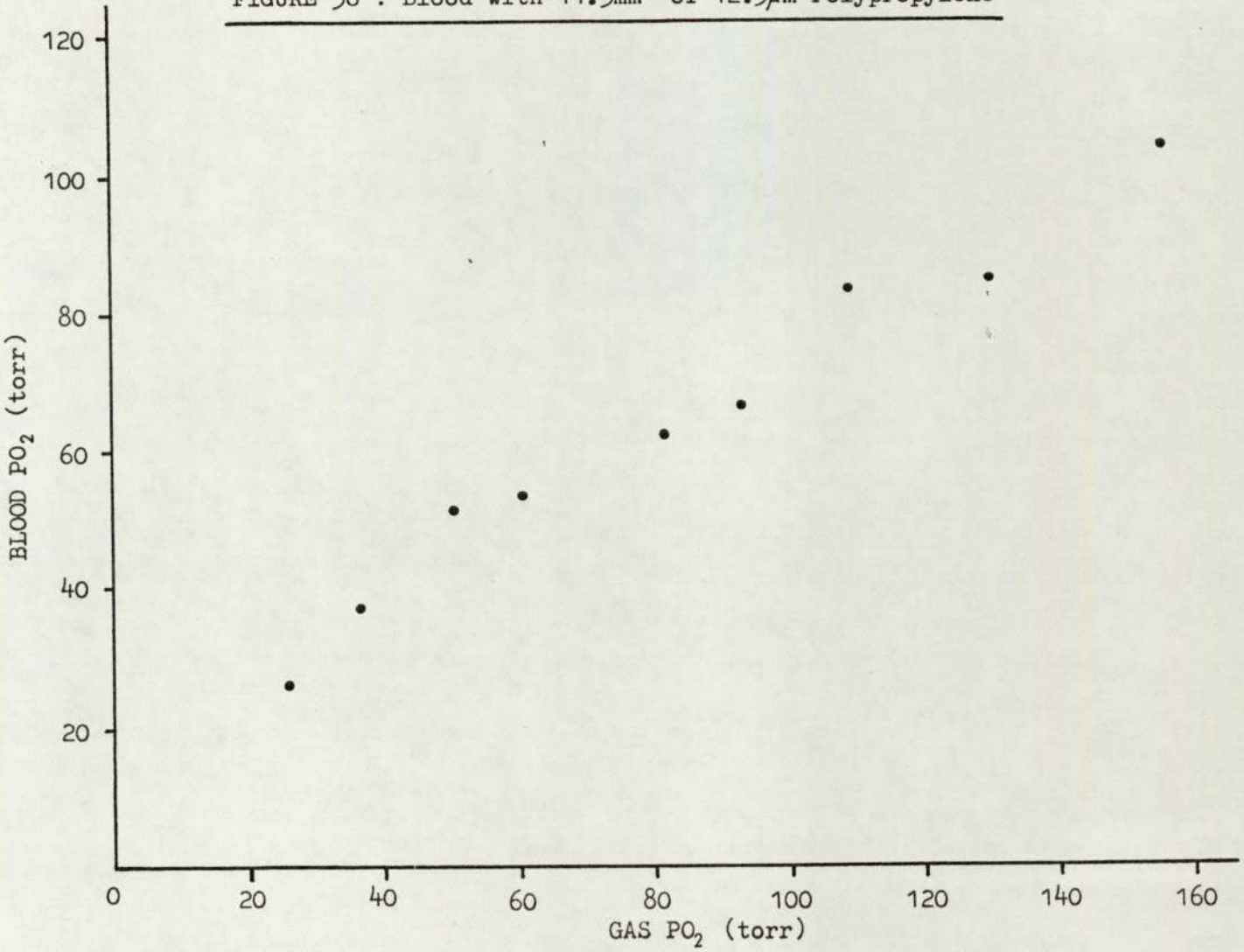
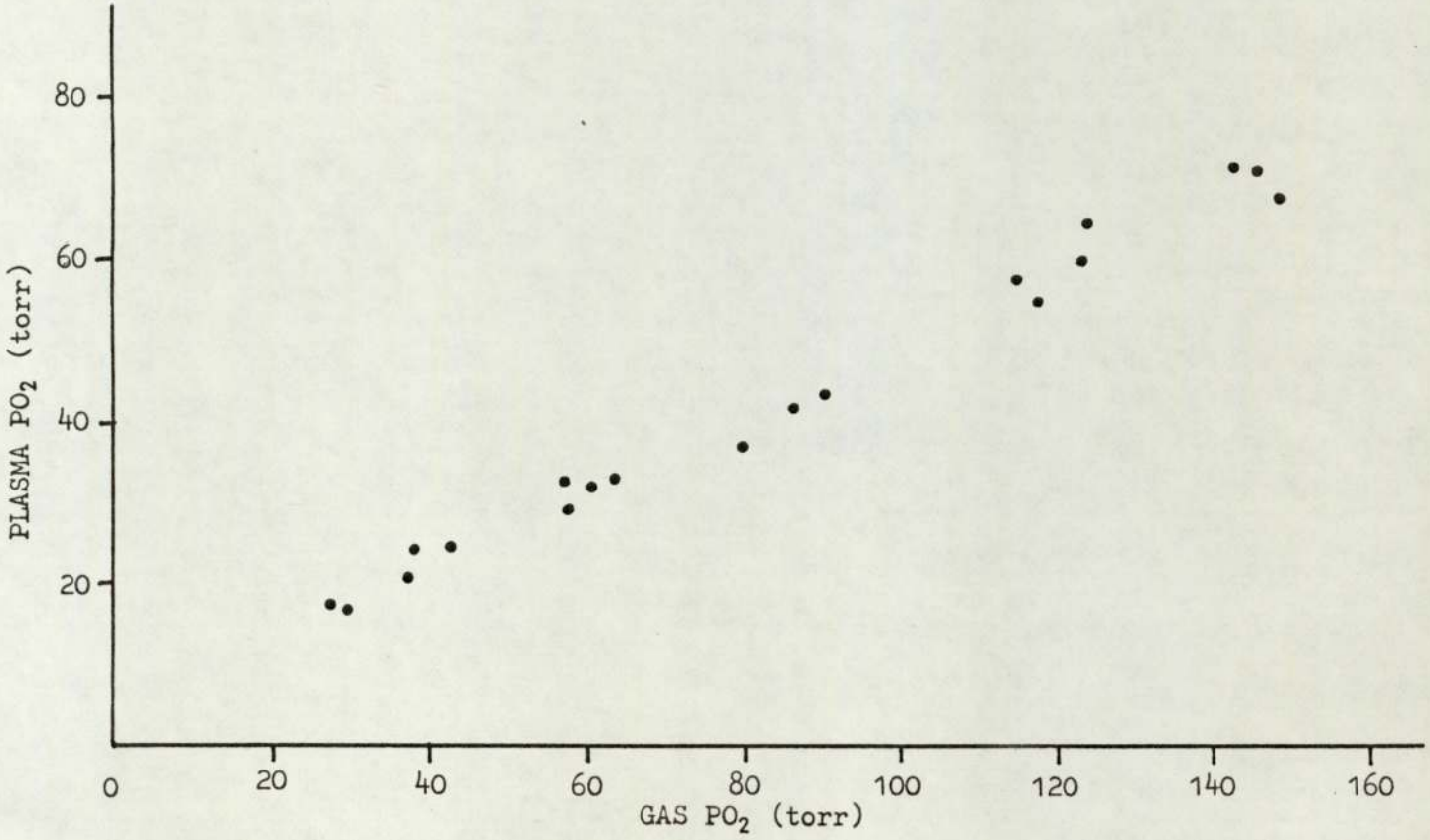


TABLE 21 Plasma with 14.3mm^2 of 25μm Polypropylene

M.S. Gas PO_2 (torr)	M.S. Plasma PO_2 (torr)	Corning Plasma PO_2 (torr)	Corning Plasma PCO_2 (torr)	pH
56.8	32.7	58.6	41.4	7.34
146	71.3	139	38.8	7.37
79.8	37.7	90.8	34.9	7.41
90.6	44.0	100	39.6	7.34
37.7	21.5	50.8	35.9	7.40
118	55.6	130	45.1	7.31
42.8	24.9	57.2	38.5	7.44
60.3	32.7	73.2	38.9	7.38
57.4	29.8			
149	68.2	146	41.9	7.42
63.0	33.5	70.4	38.8	7.42
124	64.8	124	40.6	7.43
115	58.1	115	46.5	7.34
38.1	24.6	46.9	45.6	7.34
27.6	17.3	42.7	43.0	7.36
28.8	16.7	39.3	43.9	7.39
86.4	42.4			
123	60.4			
143	71.4			

FIGURE 37 : Plasma with 14.3mm^2 of 25μm Polypropylene



oxygen and carbon dioxide signals linked not to any changes in the applied sample partial pressures but to the wetness of the PVC membrane. For example, when dry gas was sampled, the peak-to-peak oxygen signal variation was about 4% of the signal, when wet gas was sampled, the amplitude of the variation was about 5% of the signal, and after water was pumped through the cell variations of up to 24% of the mean signal size were found. Changing the membrane to a fresh, dry one reduced the 'noise' to about 5%, as did allowing a very wet membrane to dry out over several hours. The signal variations, of mean period of the order of 45s, were not found to be linked to any changes in emission current or analyser pressure, but the oxygen and carbon dioxide signals definitely changed together, increasing and decreasing in synchrony. The water signal itself did not show corresponding variations.

The basic cause of these output signal changes was not discovered - possible explanations include the existence of pores in the PVC membrane blocking and unblocking with droplets of water, a variable degree of wetting over the surface of the PVC membrane, 'microbubbles' of gas in a surface film of water, and stretching and relaxing of the membrane over the holes in the cell's metal support causing changes in its thickness and so its permeability.

Accepting these signal variations, filtering across the input to the chart recorder was tried. With a resistance of 100 kΩ and a capacitance of 10 μF the oxygen signal to noise ratio was virtually unchanged from its initial value of 10:1, taking the noise as the peak to peak amplitude of the signal variations. Increasing the capacitance to 100 μF, and so the time constant to 10s, improved the signal to

noise ratio slightly to 13:1 but the main problem was present as relatively low frequency changes of the signal rather than high frequency noise that could be easily filtered out.

The mass spectrometer was then switched into Spectrum mode and the scan width was gradually reduced to zero while keeping the scan centered on the oxygen peak. By this means the output signal from the mass spectrometer represented oxygen alone and corresponded to the number of oxygen ions collected over time periods long compared to those employed in Peak Select mode. The signal to noise ratio was indeed improved, to typically 20:1, but was far from ideal.

To avoid the problems inherent with such a low permeability inlet system, an alternative method of reducing depletion was sought. Following suggestions by, among others, Schuler et al [Reference 19] it was decided to change the distribution of holes of the membrane support in the cell - separating the holes (through which gas diffused from the blood through the membrane into the evacuated inlet line of the mass spectrometer) by six or seven times their diameter rather than twice as at present, would, it was hoped, ensure that each depleted blood zone (corresponding to each hole) would act independently so that the overall effect of depletion would be no greater than that due to any one hole, but the total gas flux withdrawn, and so the output signal size, would correspond to the sum of the areas of the holes. In this way it was hoped to reduce depletion but maintain sensitivity.

A suitable support material - nickel sheet photo-etched with 20 μm holes 136 μm apart - was obtained. Inserting this into the cell already constructed would, however, not only change the distribution

of the areas through which the gas was sampled, but would also reduce the total area from 14.3 mm^3 to 3.0 mm^2. In order to be sure that any change observed was not simply due to the reduction in area, before the new support material was tried the original support was modified, filling in 43 of the 56 holes with solder so that the area was reduced to 3.3 mm^2. The pattern of holes was filled in from the periphery inwards, leaving a central group untouched so that the average separation between these remaining holes was unchanged. A 12.5 μm polyethylene membrane was fitted and a set of fresh heparinised blood samples was tonometered and analysed, just as in the previous experiments. The results are given in Table 22, and Figure 38 shows that non linearity between oxygen flux and blood PO_2 was found, as before.

The cell was then altered to accommodate the new support material and a tonometered blood experiment was carried out with a 12.5 μm polyethylene membrane. The specific gas flux through this system was calculated to be approximately 2×10^{-9} ml/s per torr O_2 partial pressure difference. The results are given in Table 23 and Figure 39. The PO_2 response was indistinguishable, within the limits of experimental error, from that of the original system. This suggested that, in fact, depletion had <u>not</u> been reduced by changing the distribution of areas through which gas was sampled from the blood. The flow dependence of the system was recorded using air equilibrated water and the results, given in Table 24, were found to be very similar to those for the original cell with either the same polyethylene membrane (see Table 4) or polypropylene. The flow dependence of the cell with PVC, the only linear system found so far,

TABLE 22 Blood with $3.3mm^2$ of 12.5μm Polyethylene

M.S. Gas PO_2 (torr)	M.S. Blood PO_2 (torr)	Corning Blood PO_2 (torr)	Corning Blood PCO_2 (torr)	pH	Red Cell Damage (%)
64.9	61.4	67.2	44.1	7.28	1.4
150	91.0	146	41.0	7.22	2.4
86.8	73.4	93.4	44.0		2.9
36.7	37.2	44.8	42.5		2.8
36.8	40.1	45.3	40.2	7.11	
128	90.2	119	39.5	7.16	2.9
23.5	27.3	28.5	41.4	7.15	4.4
132	92.9	134	38.5	7.13	5.4
95.7	72.2	97.8	38.3	7.13	5.4
63.2	58.3	70.2	38.7	7.13	5.3
27.2	33.9				
93.2	79.4				
107	82.1	106	42.9	7.13	7.3

FIGURE 38 : Blood with $3.3mm^2$ of $12.5\mu m$ Polyethylene

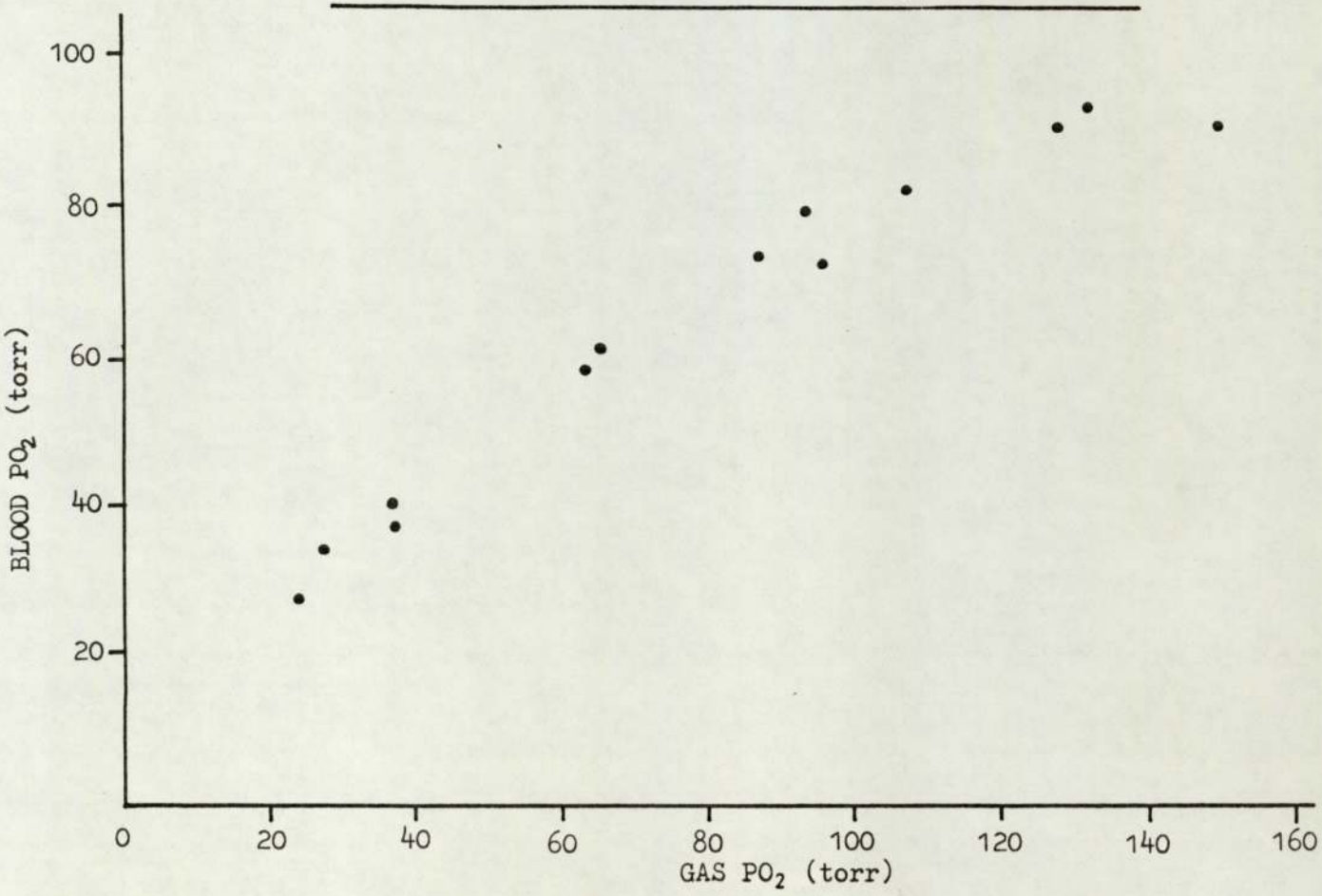


TABLE 23 Blood with 3.0mm^2 of 12.5μm Polyethylene

MS Gas PO_2 (torr)	MS Blood PO_2 (torr)	Corning Blood PO_2 (torr)	Corning Blood PCO_2 (torr)	pH	Red Cell Damage (%)
51.1	53.3	52.1	37.7	7.19	4.0
94.7	67.3	90.4	35.7	7.17	5.5
37.7	40.7	38.1	36.0	7.18	5.3
112	74.4	112	35.7	7.35	
61.4	51.6	65.2	33.0	7.33	
107	70.0	111	38.8	7.27	
59.5	54.5	56.0	37.5	7.26	
142	92.5	142	36.7	7.24	
82.2	57.2	88.1	37.3	7.11	100
24.3	32.2	28.3	37.2	7.10	100

FIGURE 39 : Blood with 3.0mm^2 of 12.5μm Polyethylene

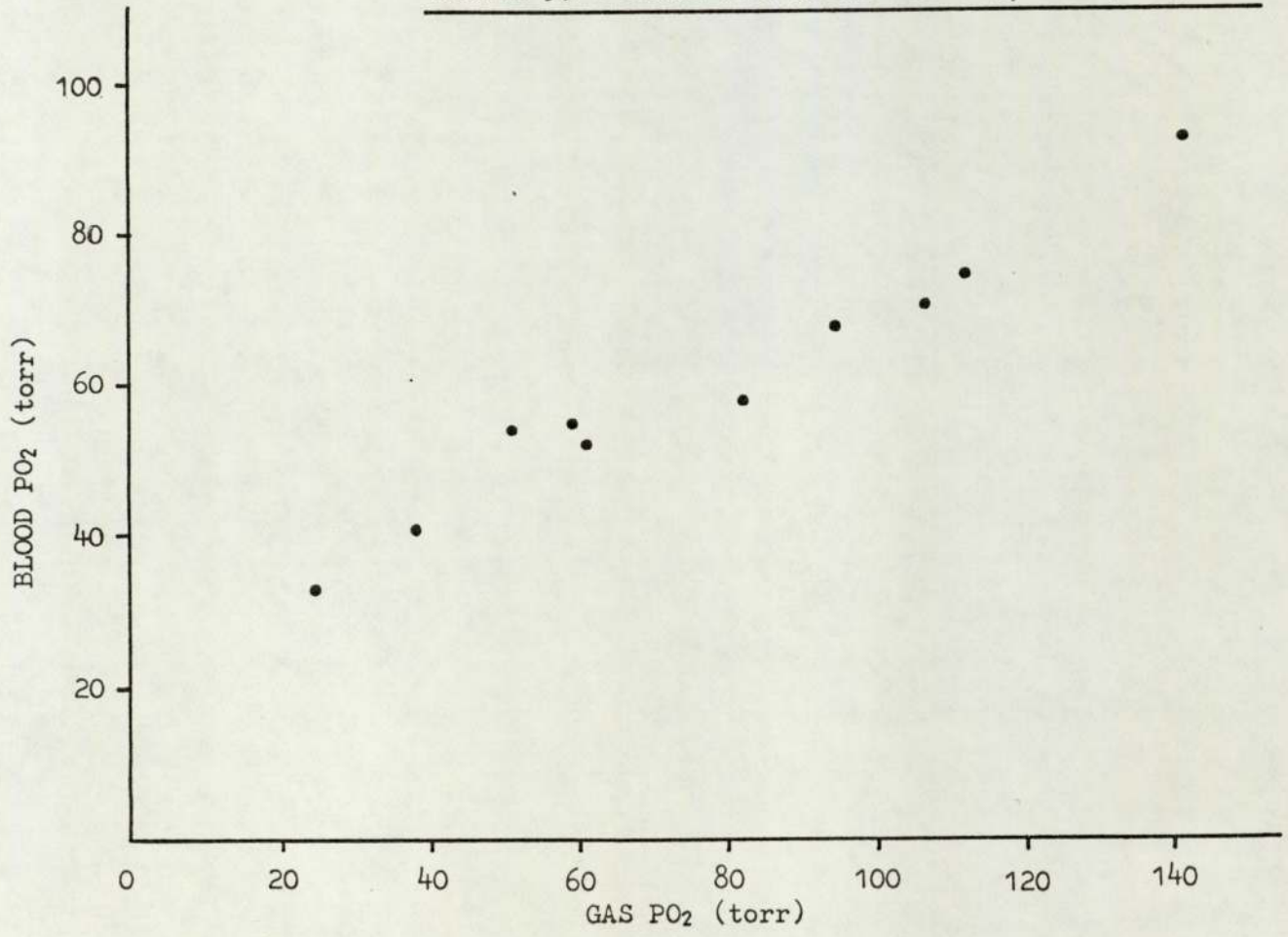


TABLE 24 Flow dependence with 3.0mm^2 of 12.5μm Polyethylene

Water Flow (ml/minute)	8.1	5.4	2.9	7.5	5.4	6.2	4.0	2.0	0.9	9.5
Oxygen $^{SL}/_{SG}$	0.46	0.42	0.33	0.45	0.43	0.44	0.41	0.31	0.22	0.46

was quite different, as Figure 35 shows. Taking the flow dependence as an index of the relative depletion caused, either of two possible conclusions could be drawn from these last two sets of results. One is that even separating the sampling areas by 6.8 times their diameter was insufficient to ensure that each acted effectively independently. The alternative interpretation is that a separation of only twice their diameter, as in the original cell construction , was itself sufficient for this independence but that, even so, depletion occurred to the extent of causing significant dissociation of oxyhaemoglobin, and so non-linearity of measured oxygen flux with respect to PO_2.

To check whether reducing permeability further with the new cell would result in a linear oxygen response, a 25 μm polypropylene membrane was fitted into the cell, and a tonometered blood experiment were carried out. This system, of specific gas flux 6×10^{-10} ml/s/torr O_2, was found to have a linear oxygen response, as shown in Table 25 and Figure 40.

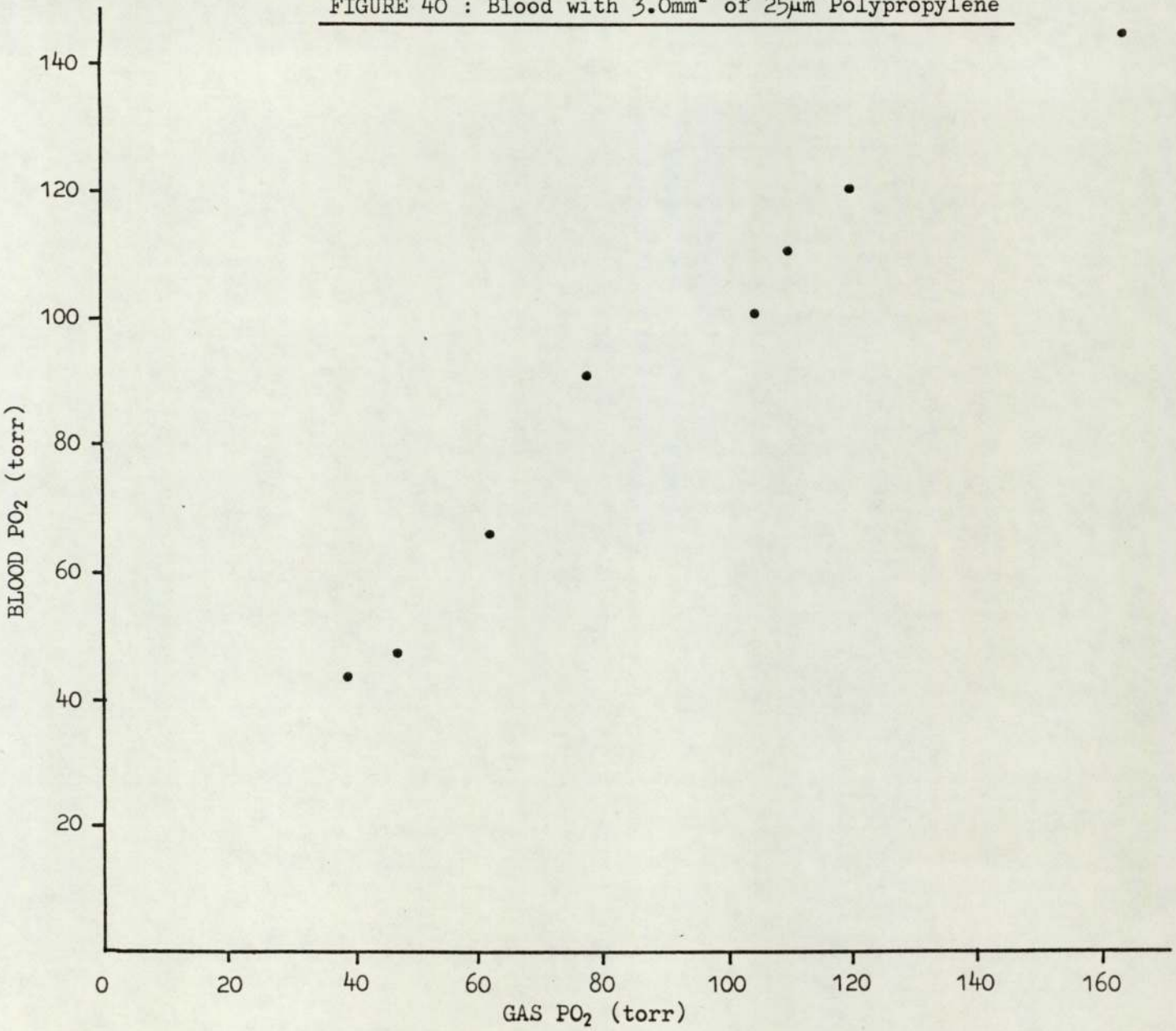
The results of fitting curves to each set of experimental data from Experiment 11 onwards are summarised in Table 18. The results indicated by asterisks (for blood analysed by the more permeable membrane systems) can be seen to be far better described by 3rd order polynomial curves than by straight lines. For all the other results, however, (for blood analysed by the less permeable membrane systems, for CO-treated blood, and for plasma) the differences between the standard deviation values were relatively small, so a linear response could be taken to exist in these cases.

Trying just a few blood PO_2 analyses with a 25 μm polyethylene membrane (giving an oxygen sampling rate of approximately 10^{-9}

TABLE 25 Blood with 3.0mm^2 of 25μm Polypropylene

MS Gas PO_2 (torr)	MS Blood PO_2 (torr)	Corning Blood PO_2 (torr)	Corning Blood PCO_2 (torr)	pH
62.2	65.5	58.6	44.8	7.26
164	144	153	40.4	7.27
46.9	46.9	43.9	44.2	7.26
39.3	42.8	40.4	38.7	7.26
78.2	90.2			
120	120	123	41.6	7.10
110	110	112	36.0	7.12
105	100	110	36.5	7.05

FIGURE 40 : Blood with $3.0mm^2$ of 25μm Polypropylene



ml/s/torr O_2) showed evidence of non-linearity; the data is shown in Figure 41 together with all the other non-linear oxygen data from Experiment 11 onwards (ie. since the experimental procedure was thoroughly revised). The Figure also shows the curve predicted by the model described in Section 6.2, putting f=0.65 and g=0.01 in Equation (2) and calculating the required intermediate values as given in Table 26.

The experimental work was concluded at this point.

FIGURE 41 : Accumulated blood data and predicted curve

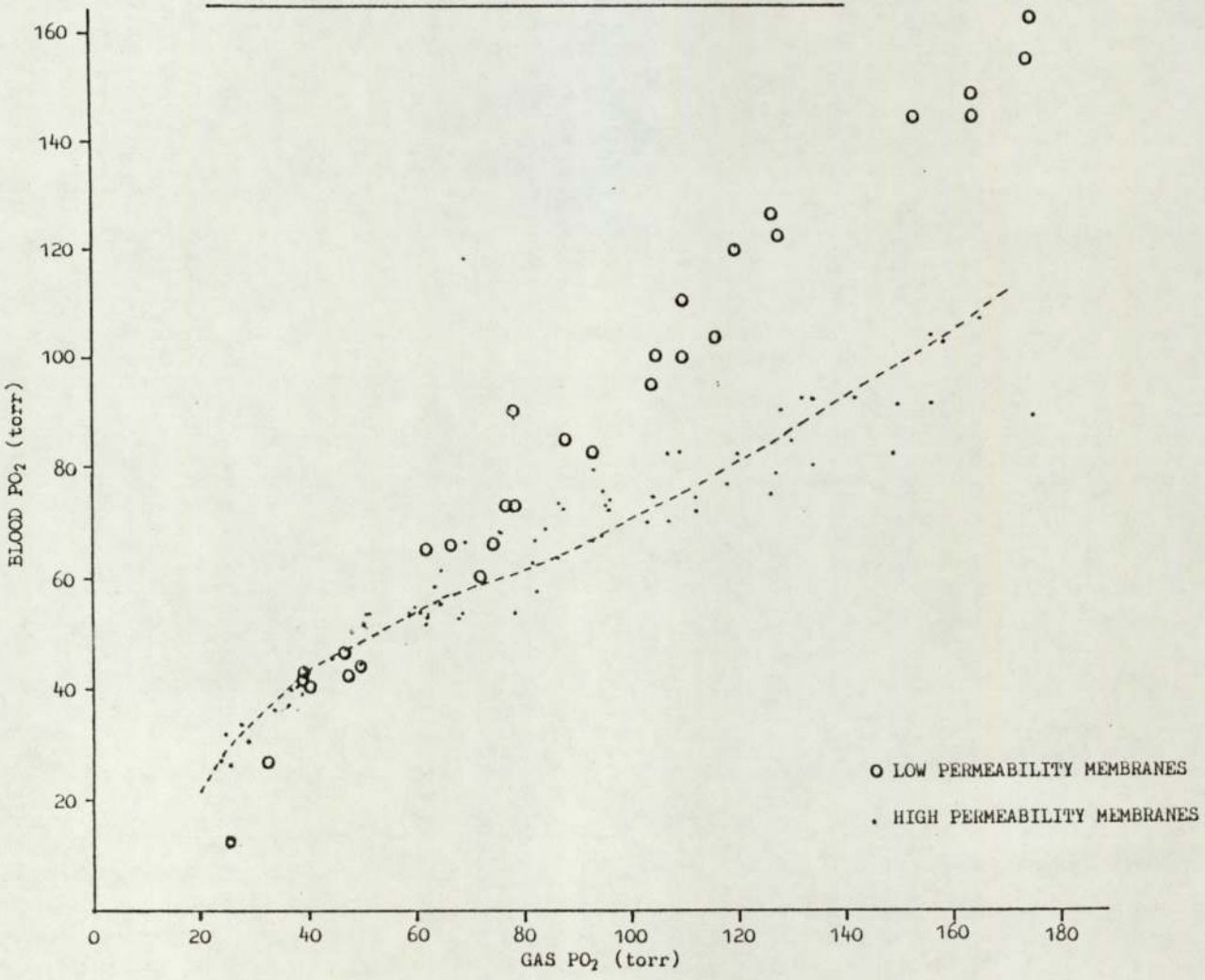


TABLE 26 Predicted response of membrane sampling systems

Tonometered PO_2 (torr)	Saturation (%)	fPO_2 (torr)	Saturation for fPO_2 (%)	R_1	R_2	Predicted PO_2 (torr)
20	24	13	11	82.0	57.8	21.9
30	46.3	19.5	23	105	80.6	35.3
40	64.5	26	37.5	110	98.5	44.4
50	76.8	32.5	51.7	105	110	49.3
60	85.2	39	63	97.0	110	54.3
70	90.5	45.5	72	88.3	108	58.2
80	93.4	52	78.8	79.7	104	61.7
90	95.1	58.5	84.2	72.2	98.3	66.0
100	96.3	65	88.5	65.8	93.0	70.4
110	97.0	71.5	91.0	60.2	86.9	75.6
120	97.5	78	92.9	55.5	81.3	81.2
130	98.0	84.5	94.3	51.5	76.2	87.1
140	98.2	91	95.3	47.9	71.5	93.0
150	98.4	97.5	96.1	44.8	67.3	99.1
160	98.6	104	96.6	42.1	63.4	105
170	98.8	111	97.1	39.7	59.7	112

Temperature = 37°C pH = 7.24 f = 0.65 g = 0.01

Chapter 7 - Conclusions

The work described in Chapter 5, Section 1 showed that the Spectra-cath catheter, used in conjuction with the 200 MGA mass spectrometer, was not satisfactory for the measurement of the respiratory gases in blood. The grounds for this assertion are basically that after calibration in accordance with the manufacturers' instructions, agreement was not found between blood PO_2 measured using the catheter and using a standard Clark-type electrode system, in vitro and in vivo. These findings conflict with published work by Brantigan et al [Reference 60] and one likely explanation for this is that during the relatively large-scale manufacture of these catheters it may not have been possible to keep the physical properties of the teflon membranes sufficiently uniform either over each individual catheter or as regards one catheter compared with another. This would account for the inconsistency between results, and in fact since this work was carried out, the Spectra-cath catheter has been withdrawn from the market while some design modifications are developed. This catheter had seemed by far the most promising of those available, so it was concluded that there was little or no prospect of using such ready-made catheters for blood gas analysis in the near future.

The work described in Chapter 5, Section 2 showed that a very simple inlet system - a thin polyethylene membrane over a perforated metal plate pumped by the mass spectrometer - was reasonably satisfactory for the measurement of nitrogen, carbon dioxide and oxygen in water, and for the measurement of nitrogen and carbon

dioxide in blood, but that the measurement of blood PO_2 was complicated by an apparent non-linearity between the measured oxygen signal and the true PO_2. Non-linearity in the measurement of blood PO_2 had been reported previously, and some tentative explanations put forward, for example by Key [Reference 68] in connection with electrochemical analysis, but the effect had generally been small enough to ignore in comparison with other errors. In these initial mass spectrometer experiments however, the effect was large, and as the measurement of blood PO_2 is of particular clinical importance, it was decided to investigate the phenomenon experimentally in more detail than had previously been done, and to attempt to formulate a theoretical explanation.

The results of Experiments 5 to 9, described in Chapter 6, showed that the non-linearity occurred for whole blood, whether citrated or heparinised, and haemolysed blood, but not for plasma or blood tonometered with carbon monoxide - this suggested a link with the presence of oxyhaemoglobin. The importance of depletion as a causative factor was suggested by Experiment 10, in which the oxygen flux sampled was cut by a factor of 20 and non-linearity eliminated.

The following hypothesis was then put forward. It is well established that with any membrane system sampling a gas from a liquid, a gas depleted zone is formed in the liquid adjacent to the membrane. It is now proposed that when this phenomenon occurs in blood, the depletion of oxygen may be offset to a considerable extent by the oxyhaemoglobin within this zone. As this dissociation is non-linearly related to the partial pressure of oxygen (for important physiological reasons) the effect it has in augmenting the oxygen flux

from blood through the membrane to be measured is correspondingly non-linearly related to partial pressure. This process must occur with any membrane system with a sampling rate high enough to cause significant depletion; the experiments indicated that a sampling rate of 10^{-8} ml O_2/s/torr was high enough for this to occur while $5x10^{-10}$ ml O_2/s/torr was not.

The practical importance of this hypothesis lies in the fact that currently available medical mass spectrometers require oxygen sampling rates of the order of the former rather than the latter value for an acceptably low output signal to noise ratio, and so the predicted non-linearity is likely to be a real problem in the use of such instruments for the measurement of blood PO_2.

A mathematical treatment of this depletion/dissociation model, set out in Chapter 6, Section 2, was found to yield quantitative data in reasonable agreement with experimental results. There was however much room for improvement in the practical details of the experiments carried out so far, so the confidence that could be placed on the agreement between theory and experiment was limited. The technical details of the experiments were accordingly modified and the experiments repeated. The results were not essentially different, and extra experiments were carried out, checking for PO_2 linearity with a selection of membrane sampling systems.

It was found that with all those systems that sampled oxygen at rates in the range of approximately $2x10^{-9}$ ml/s/torr up to 10^{-8} ml/s/torr the same non-linear relationship between measured oxygen flux and blood PO_2 existed. This included polyethylene and polypropylene membranes of varying thickness, area, and pattern of

sampling. Predictions yielded by the proposed model were found to fit these results quite well, as shown in Figure 41. The two systems that sampled at the much lower rate of about 5×10^{-10} ml/s/torr (one of PVC and one of polypropylene) did not exhibit this non-linearity.

These facts are all compatible with the idea of oxyhaemoglobin dissociation, induced whenever the depletion inherent with the membrane diffusion sampling technique is sufficiently great, contributing to the steady state oxygen flux analysed.

These conclusions have serious implications for the use of mass spectrometry in blood gas analysis. If the membrane diffusion technique is employed, and other methods are of very limited application, the possibility of non-linearity in the oxygen analysis has to be taken into account. It will occur with all membrane systems of high oxygen sampling rates (with the possible exception of systems with very widely separated, small sampling areas) and such systems are otherwise generally desirable, as they can make high sensitivity and short response times possible. If such systems are used then, either it must be for blood gases <u>other</u> than oxygen, or, if enough data is collected to make up an acceptably well defined oxygen calibration curve, oxygen analysis could still be feasible. One possible problem with the latter course is that the curve flattens in the region of normal arterial partial pressures - 60 to 100 torr - so accuracy would be low in this range.

An obvious alternative is to use membrane systems that sample at much lower rates and so keep depletion so low that the processes resulting in non-linearity of oxygen flux with repect to PO_2 are not called into being. This could well be the direction in which future

work in this field will progress, but its success will depend on improving the quality of the mass spectrometer analysis if acceptably high signal to noise ratios, and hence good precision, are to be achieved. Possible approaches to this could include the use of a low noise electrometer amplifier rather than an electron multiplier as the ion detector, slowing the voltage scan speed of the quadrupole filter, improving the vacuum system, and the discriminate use of electronic signal smoothing.

Appendix 1

Flow Dependence of Chemitron Silastic Catheters

A detailed investigation of the flow dependence of these commercially manufactured catheters was carried out, partly just to find out how much in error measurements of blood gas partial pressures in vivo would be, due to blood flow changes, and partly with a view to using this flow dependence to actually measure blood flow in vivo by monitoring variations in the signal of a reference gas, of constant partial pressure. This work was carried out in collaboration with J.S. Lundsgaard and J.Groenlund of Odense University, Denmark.

Current methods for measuring blood flow can be categorised according to the type of interaction or energy transfer between the transducer and the blood. One group of methods is electromagnetic - these depend on measuring the potential difference induced between two electrodes placed at right angles to the blood flow in the presence of a transverse magnetic field. Interactions between the propagation of ultrasonic waves and flowing blood give rise to a second group of measuring techniques. Thermal methods depend on measuring the rate of heat loss from a probe held in blood flowing past it at a slightly lower temperature. There are also several mechanical methods of measuring flow, involving the measurement of viscous drag, pressure at chosen points around obstructions to flow, or simply the deflection of a suspended 'pointer'.

The method now proposed for blood flow measurement is based on

the effect of bulk liquid flow in limiting the diffusive gas flow through a membrane in the liquid to the mass spectrometer. Flow variations alter the effective thickness of the liquid 'barrier' to gas flow, as described here; the situation may be quantitatively analysed as follows.

From the Nernst theory [Reference 58] the thickness Y of the 'static' layer next to a moving fluid is given by

$$Y = k\left[\frac{\mu}{D_0}\right]^{1/6}\sqrt{\frac{D_0 d}{v}}$$

$$= \frac{c}{\sqrt{v}} \quad \text{say} \qquad (1)$$

where c is a constant for a given gas, liquid and membrane, and v is the 'streaming velocity' of the bulk of the liquid.

As shown by Lunsdgaard et al [Reference 58] the mass spectrometer signals in a gas of constant partial pressure and a liquid equilibrated with a gas are related to the 'static layer' thickness by

$$\frac{S_g}{S_l} = A\left(Y + 1/A\right) \qquad (2)$$

where S_g is the mass spectrometer signal for the gas phase

S_l is the mass spectrometer signal for the liquid phase

and A is a constant for a given gas, liquid and membrane

Substitute for Y from equation 1 into equation 2

$$\frac{S_g}{S_l} = A\left[\frac{c}{\sqrt{v}} + \frac{1}{A}\right]$$

$$\frac{S_g}{S_l} = \frac{B}{\sqrt{v}} + 1$$

where B is a constant for a given gas, liquid, and membrane.

So a plot of S_g/S_l against $1/\sqrt{v}$ should be a straight line.

In the experiments carried out to check this, using a catheter in a tube of flowing liquid to represent its use in vivo, there was no obvious way of interpreting 'bulk streaming velocity' v, and in any case the quantity it was desired to measure was flow Q, of dimensions volume per unit time. So it was necessary to calculate how this flow Q was related to some measure of liquid velocity.

Assuming laminar flow in a tube of radius a and length l where $l \gg a$, the velocity u as a function of radius r is given by

$$u = \frac{\Delta p}{4\eta l}\left(a^2 - r^2\right) \qquad (3)$$

where Δp is the pressure difference along the tube and η is the viscosity.

The presence of a catheter along the axis of the tube must, however, disturb the parabolic velocity profile suggested by equation 3.

Consider the general solution for laminar flow in a tube

$$u = -\frac{\Delta p}{4\eta l} r^2 + G \ln r + H \qquad ④$$

where G and H are constants to be determined.

Let the catheter have radius R_2 in a tube of radius R_1.

Then the boundary conditions are

$$\text{when } r = R_1 \text{ , } u = 0$$

$$\text{and when } r = R_2 \text{ , } u = 0$$

Using the first boundary condition, equation 4 becomes

$$0 = -\frac{\Delta p \, R_1^2}{4\eta l} + G \ln R_1 + H$$

$$\longrightarrow \quad H = \frac{\Delta p \, R_1^2}{4\eta l} - G \ln R_1$$

Substitute in ④

$$u = \frac{\Delta p}{4\eta l}\left(R_1^2 - r^2\right) + G \ln\left(\frac{r}{R_1}\right) \qquad ⑤$$

Using the second boundary condition, equation 5 becomes

$$0 = \frac{\Delta p}{4\eta l}\left(R_1^2 - R_2^2\right) + G \ln\left(\frac{R_2}{R_1}\right)$$

$$\longrightarrow \quad G = -\Delta p \, \frac{\left(R_1^2 - R_2^2\right)}{4\eta l \ln\left(\frac{R_2}{R_1}\right)}$$

Substitute in ⑤

$$u = \frac{\Delta p}{4\eta l}\left(R_1^2 - r^2 - \left[\frac{R_1^2 - R_2^2}{\ln\frac{R_2}{R_1}}\right]\ln\frac{r}{R_1}\right) \qquad ⑥$$

Now take the maximum velocity u_m to be a reasonable 'measure' of the velocity distribution, corresponding to bulk streaming velocity in a non-cylindrical geometry. u_m is found by putting

$$\frac{du}{dr} = 0$$

$$\rightarrow \quad \frac{\Delta p}{4\eta l}\left[-2r - \frac{1}{r}\left[\frac{R_1^2 - R_2^2}{\ln\frac{R_2}{R_1}}\right]\right] = 0$$

$$\rightarrow \quad 2r^2 = \frac{R_2^2 - R_1^2}{\ln\frac{R_2}{R_1}}$$

$$\rightarrow \quad r = \sqrt{\frac{R_2^2 - R_1^2}{2\ln\frac{R_2}{R_1}}} = X \text{ say}$$

$$\text{So } u_m = \frac{\Delta p}{4\eta l}\left\{R_1^2 - X^2 + \left[\frac{R_2^2 - R_1^2}{\ln\frac{R_2}{R_1}}\right]\ln\left(\frac{X}{R_1}\right)\right\} \qquad ⑦$$

$$\text{Now, flow } Q = \int_{R_2}^{R_1} (u 2\pi r)\, dr$$

$$\text{From ⑥} \quad Q = \frac{\pi \Delta p}{2\eta l} \int_{R_2}^{R_1} \left(rR_1^2 - r^3 - \frac{r(R_1^2 - R_2^2)\ln \frac{r}{R_1}}{\ln \frac{R_2}{R_1}} \right) dr$$

This reduces to

$$Q = \frac{\pi \Delta p}{4\eta l} \left(\frac{R_1^4 - R_2^4}{2} + \frac{R_1^2 - R_2^2}{\ln \frac{R_2}{R_1}} \right) \qquad ⑧$$

For a given tube and catheter (R_1,R_2 constant) equations 7 and 8 show that the flow Q is proportional to the maximum velocity u_m, so if u_m can be considered as corresponding to 'bulk streaming velocity' v in equation 1, to say a plot of S_g/S_1 against $1/\sqrt{v}$ should be a straight line is equivalent to saying that a plot of S_g/S_1 against $1/\sqrt{Q}$ should be a straight line in the cylindrical geometry of the experiments to be described.

The catheters used in these experiments had diffusion membranes of silastic rubber suported on slotted stainless steel tubing.

Initially, one of these catheters, attached to the blood gas inlet of the mass spectrometer, was positioned along the axis of a 9 mm diameter tube in the circuit shown in Figure 42. Air equilibrated water was continuously pumped past the catheter, and the steady state mass spectrometer oxygen and nitrogen signals were recorded at each of a series of flow rates. The signals from the catheter in humidified air were then recorded, and the results in water expressed as fractions of the gas values, as given in Table 27. Figure 43 shows the

FIGURE 42 : Apparatus for flow dependence experiments

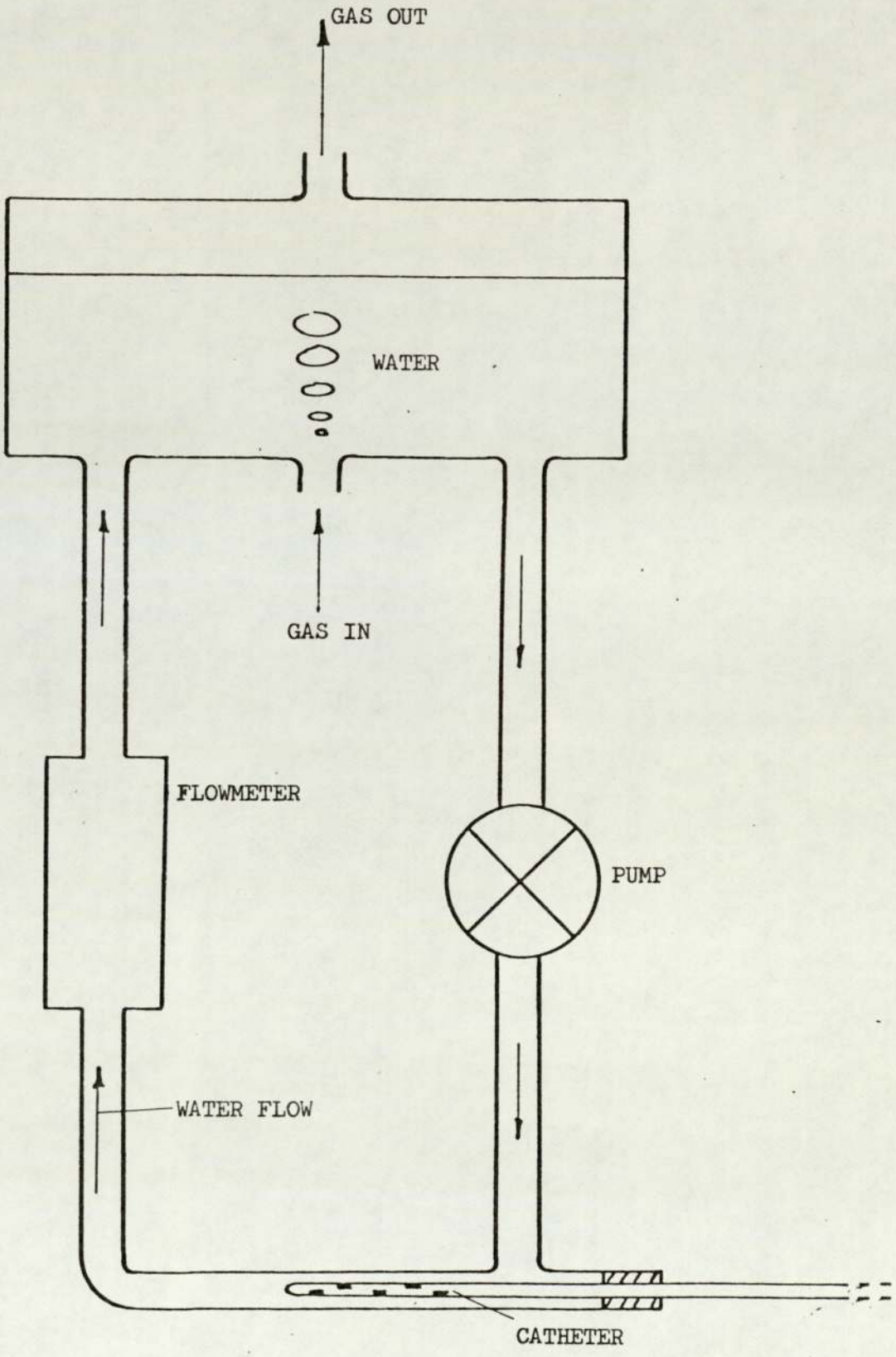
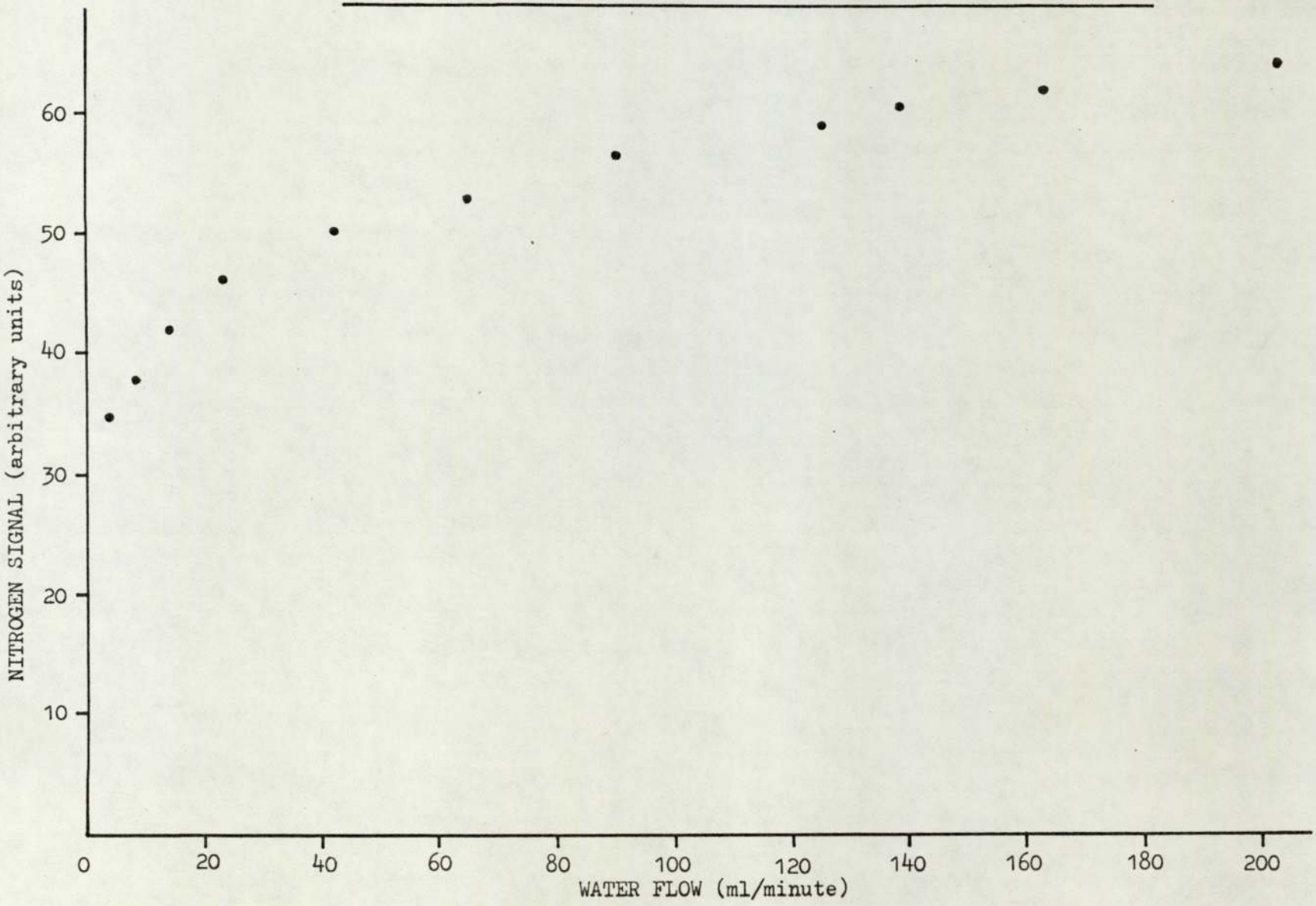


TABLE 27 Flow dependence results - see Figures 43 and 44

N_2 signal in water	N_2 $^{SG}/_{SL}$	Q (ml/minute)	$^{1}/\sqrt{Q}$	O_2 $^{SG}/_{SL}$	$^{1}/\sqrt{Q}$
64	1.45	203	0.070	1.38	0.070
62	1.50	164	0.078	1.43	0.075
60.5	1.54	139	0.085	1.52	0.08
59	1.58	125	0.090	1.53	0.09
56.5	1.65	90	0.11	1.56	0.10
53	1.75	65	0.13	1.63	0.11
50	1.86	43	0.15	1.67	0.14
46.5	2.00	23	0.21	1.83	0.17
42.5	2.19	14	0.27	1.88	0.21
38.5	2.42	7.5	0.37	2.01	0.27
35	2.66	3.0	0.58	2.56	0.58

FIGURE 43 : Flow dependence of N_2 signal with Chemitron catheter



flow dependence of the nitrogen signal; the oxygen flow dependence was almost identical. Figure 44 shows S_g/S_1 plotted against $1/\sqrt{Q}$ for each gas. The results did not agree with theoretical predictions in that the relationship was not a linear one.

A calculation was carried out to find whether laminar flow conditions were likely to apply to the part of the circuit in which the catheter was positioned using the condition that Re, the Reynold's number for the flow, D the diameter of the tube, and 1 the distance of the catheter tip from the nearest bifurcation in the circuit should be linked by

$$\frac{1}{D} \geqslant 0.058\ R_e$$

In the experiment $1 = 3\times10^{-2}$m

$D = 10^{-2}$m

$R_e = 10^4 v$

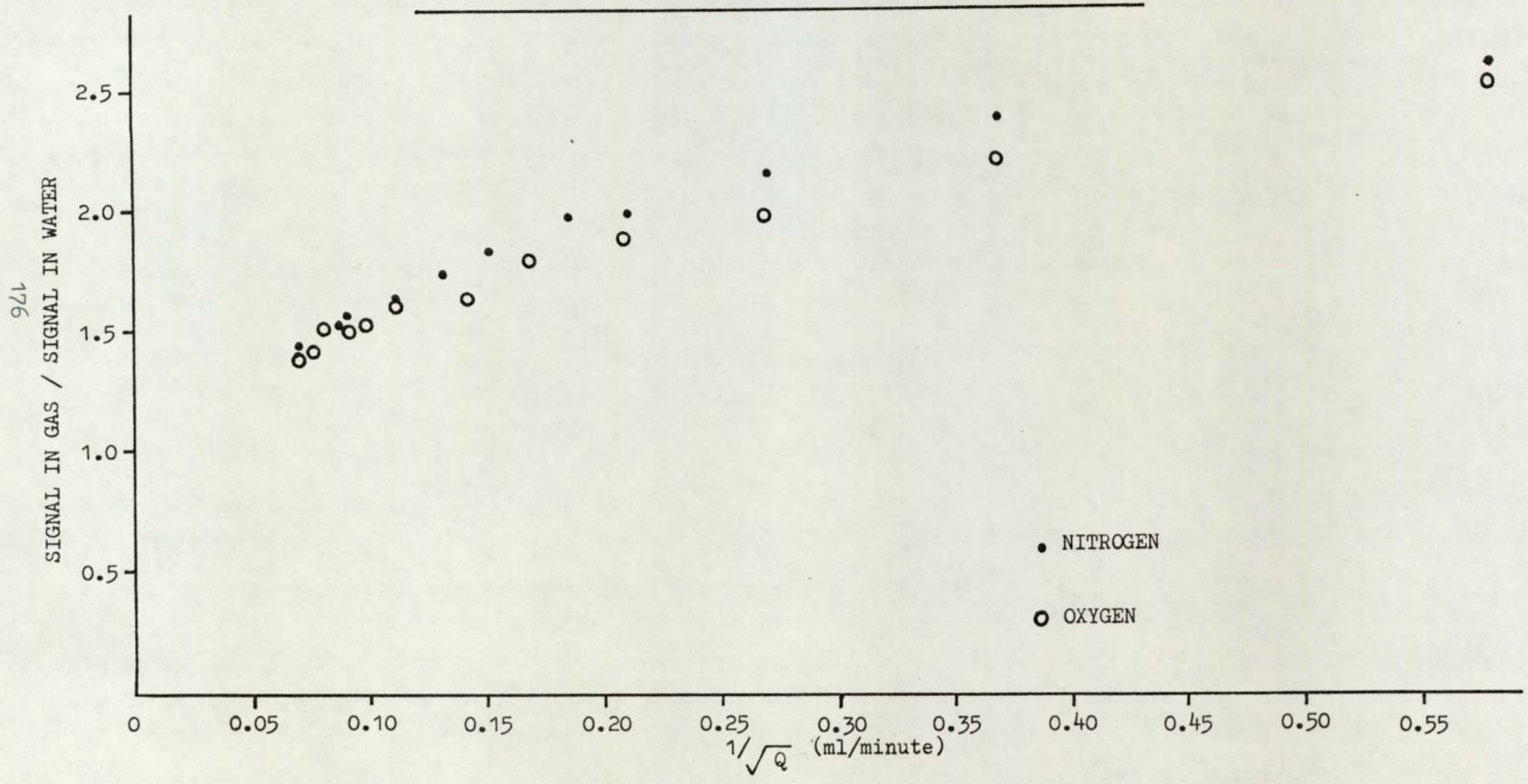
$\longrightarrow$ $v \leqslant 0.5$ cm/s

$Q \leqslant 25$ ml/minute

This condition for laminar flow was only satisfied by the four points on the right hand extremes of the graph for each gas, and these points could be taken to be on straight lines.

The experimental circuit was altered so that the membrane of the catheter was far enough from any bifurcations for laminar flow conditions to be approached. The catheter was positioned along the axis of a tube of 9 mm diameter filled with distilled water. The air-equilibrated water was pumped along the tube by a Watson-Marlow

FIGURE 44 : 'Derived' flow dependence of Chemitron catheter



Flow Inducer. The steady oxygen signal from the mass spectrometer was recorded at each of a series of flow rates, the whole apparatus being kept at room temperature. The catheter was calibrated before and after the experiment in humidified gas mixtures. Results are given in Table 28. Figure 45 shows the oxygen signal as a function of flow, and Figure 46 shows the reciprocal of the oxygen signal as a function of the reciprocal of the square root of flow. The latter is seen to approximate to a straight line as theory predicted.

TABLE 28 Flow dependence results - see Figures 45 and 46

Flow (ml/min)	O_2 signal (V)	$^1/_{O_2}$ signal (V^{-1})	$(^1/\text{flow})^{\frac{1}{2}}$
160	1.26	0.79	0.079
140	1.23	0.81	0.085
120	1.21	0.83	0.09
100	1.18	0.85	0.10
80	1.15	0.87	0.11
60	1.11	0.90	0.13
50	1.09	0.92	0.14
40	1.06	0.94	0.16
30	0.99	1.01	0.18
120	1.22	0.82	0.09
20	0.95	1.05	0.22

FIGURE 45 : Flow dependence of O_2 signal with Chemitron catheter (laminar flow)

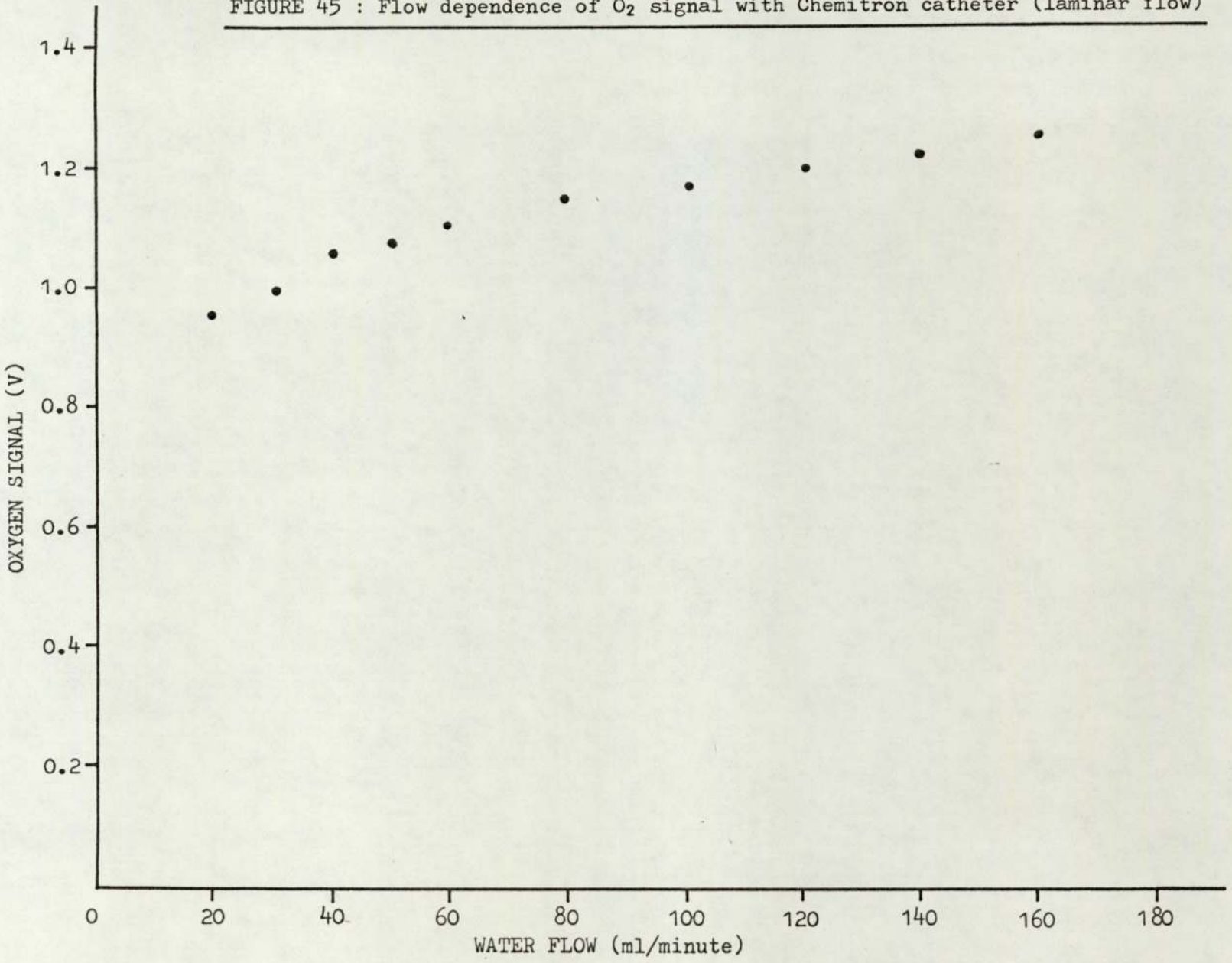
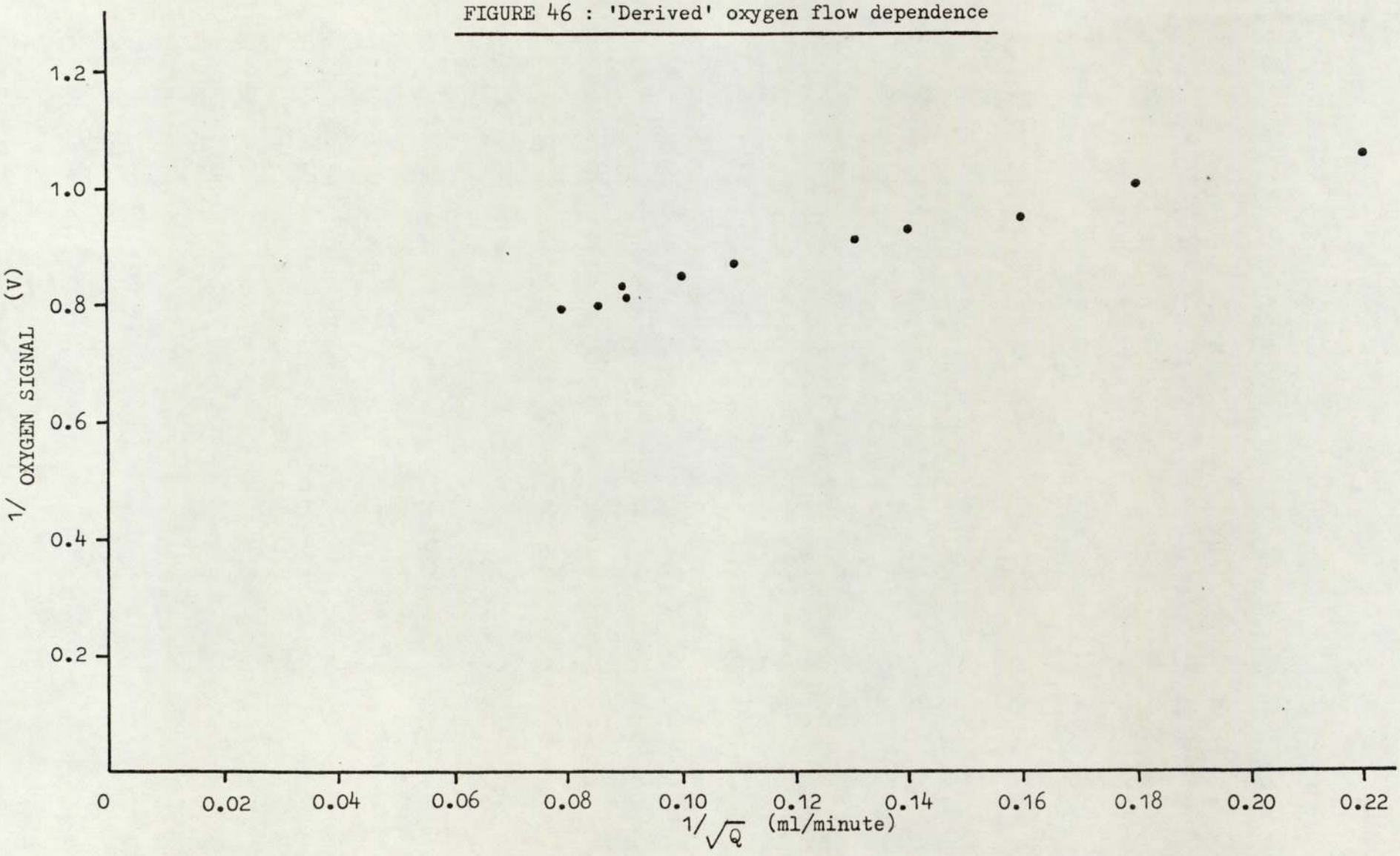


FIGURE 46 : 'Derived' oxygen flow dependence



The catheter was positioned first along the axis of a 9 mm diameter tube, then close to the walls of the tube, and finally in an intermediate position. At each position, the flow rate of the water in the tube was altered through a series of values and the corresponding mass spectrometer signals for oxygen and nitrogen were recorded. Results are given in Table 29. Figures 47 and 48 show that although the flow dependence followed the same general shape for each position the actual values of the signals were significantly affected by position - a lateral shift of the catheter within the tube caused variations of up to 20% depending on the value of the flow itself and the extent of the shift.

TABLE 29 Effect of catheter position on O_2 and N_2 flow dependence (pulsatile flow)

Flow (ml/min)	Catheter axial		Catheter peripheral		Intermediate position	
	O_2OP(V)	N_2OP(V)	O_2OP(V)	N_2OP(V)	O_2 OP(V)	N_2 OP(V)
160	0.29	0.81	0.26	0.73		
140	0.29	0.80	0.25	0.71		
120	0.28	0.79	0.24	0.68		
100	0.28	0.77	0.24	0.66	0.26	0.72
80	0.27	0.76	0.23	0.63	0.26	0.70
60	0.26	0.74	0.22	0.60	0.25	0.67
40	0.24	0.68	0.20	0.54	0.23	0.61
30	0.22	0.64	0.18	0.49	0.21	0.58

FIGURE 47 : Effect of catheter position on O_2 flow dependence

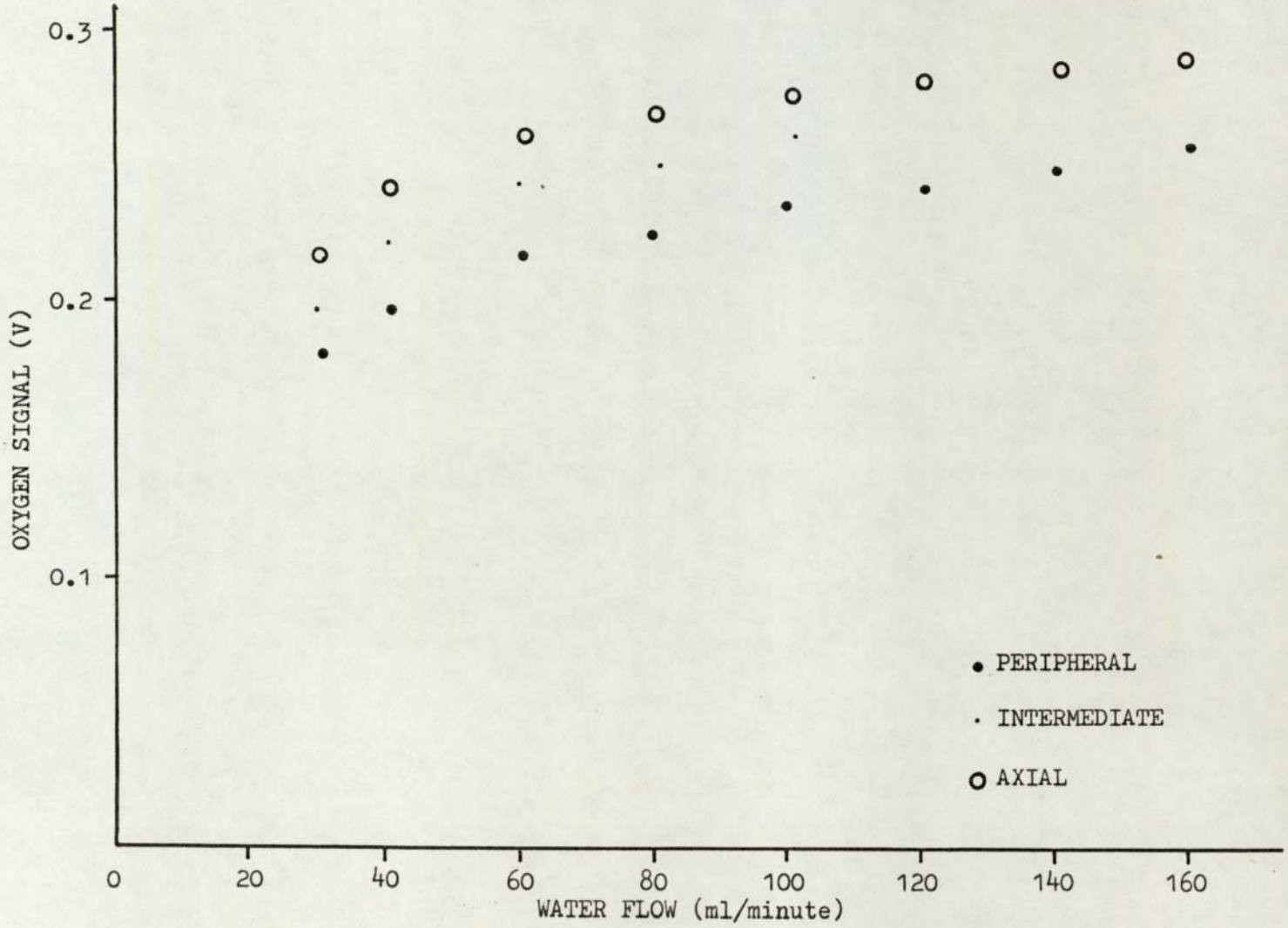
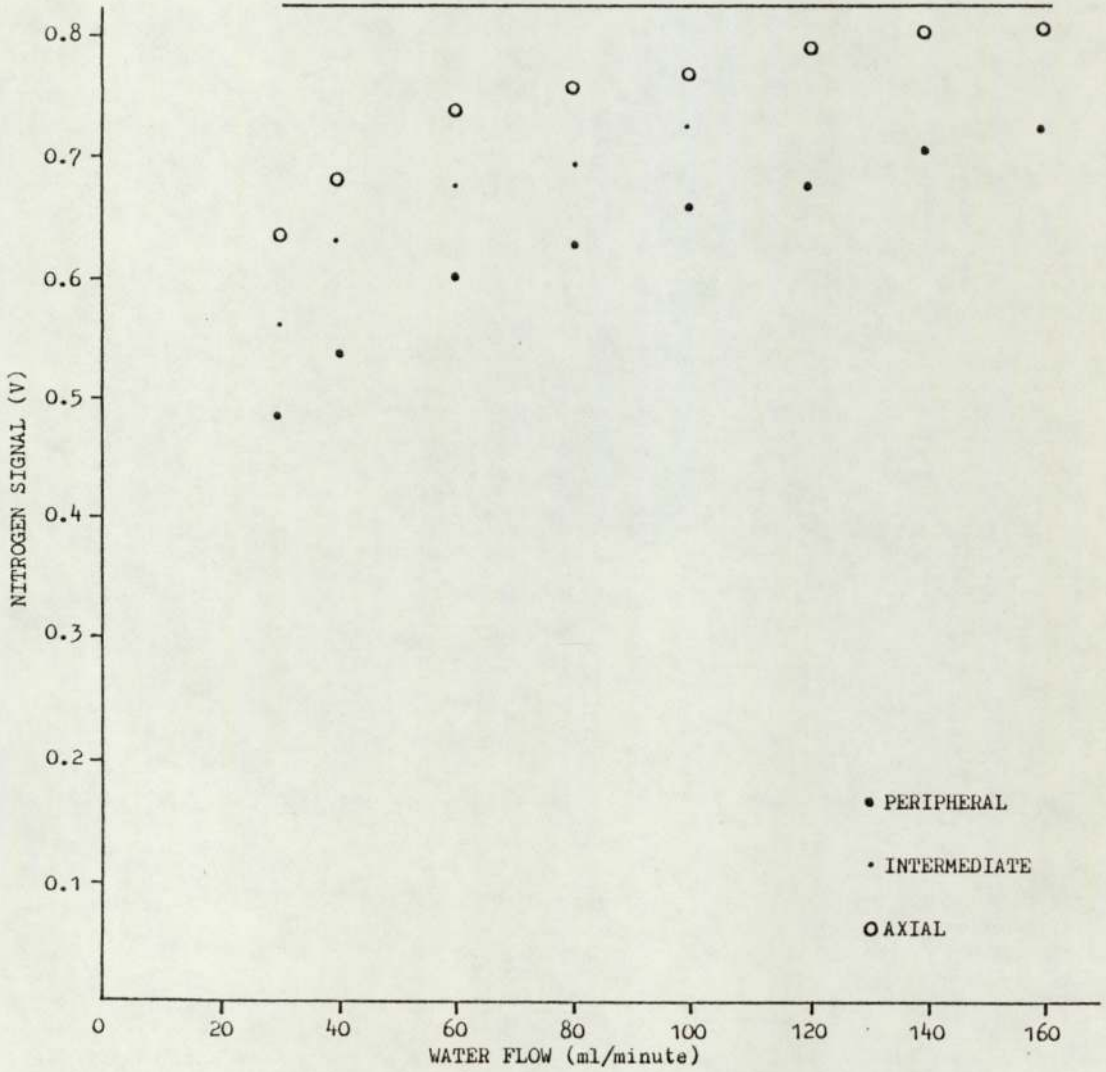


FIGURE 48 : Effect of catheter position on N_2 flow dependence

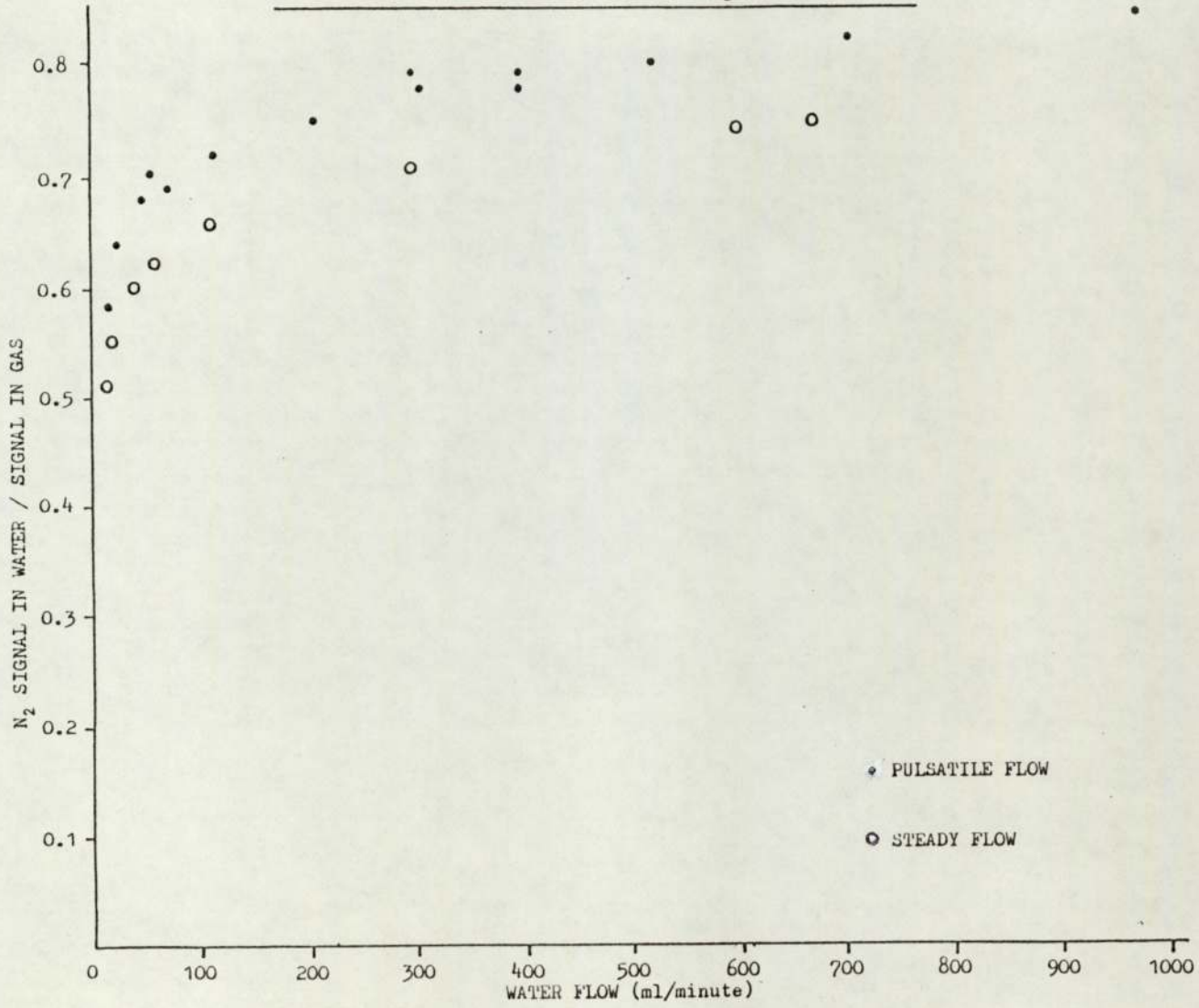


The catheter was positioned along the axis of a 9 mm diameter tube. The nitrogen signal was recorded as a function of water flow rate, firstly using the peristaltic pump to propel the water, and secondly letting the water flow through under gravity. Results are given in Table 30. Figure 49 shows that in the second case, for which the water flow was more likely to be laminar, the signals were generally lower than in the first case, in which the flow was pulsatile.

TABLE 30 Effect of type of flow on N_2 flow dependence

Steady Flow		Pulsatile Flow	
Flow (ml/min)	N_2 signal in water/air	Flow (ml/min)	N_2 signal in water/air
667	0.75	300	0.79
600	0.74	400	0.79
300	0.71	522	0.80
107	0.66	706	0.82
59	0.62	973	0.84
41	0.60	308	0.78
20	0.55	207	0.75
9	0.51	115	0.72
2	0.43	68	0.69
		55	0.70
		45	0.68
		24	0.64
		15.5	0.58

FIGURE 49: Effect of type of flow on N_2 flow dependence

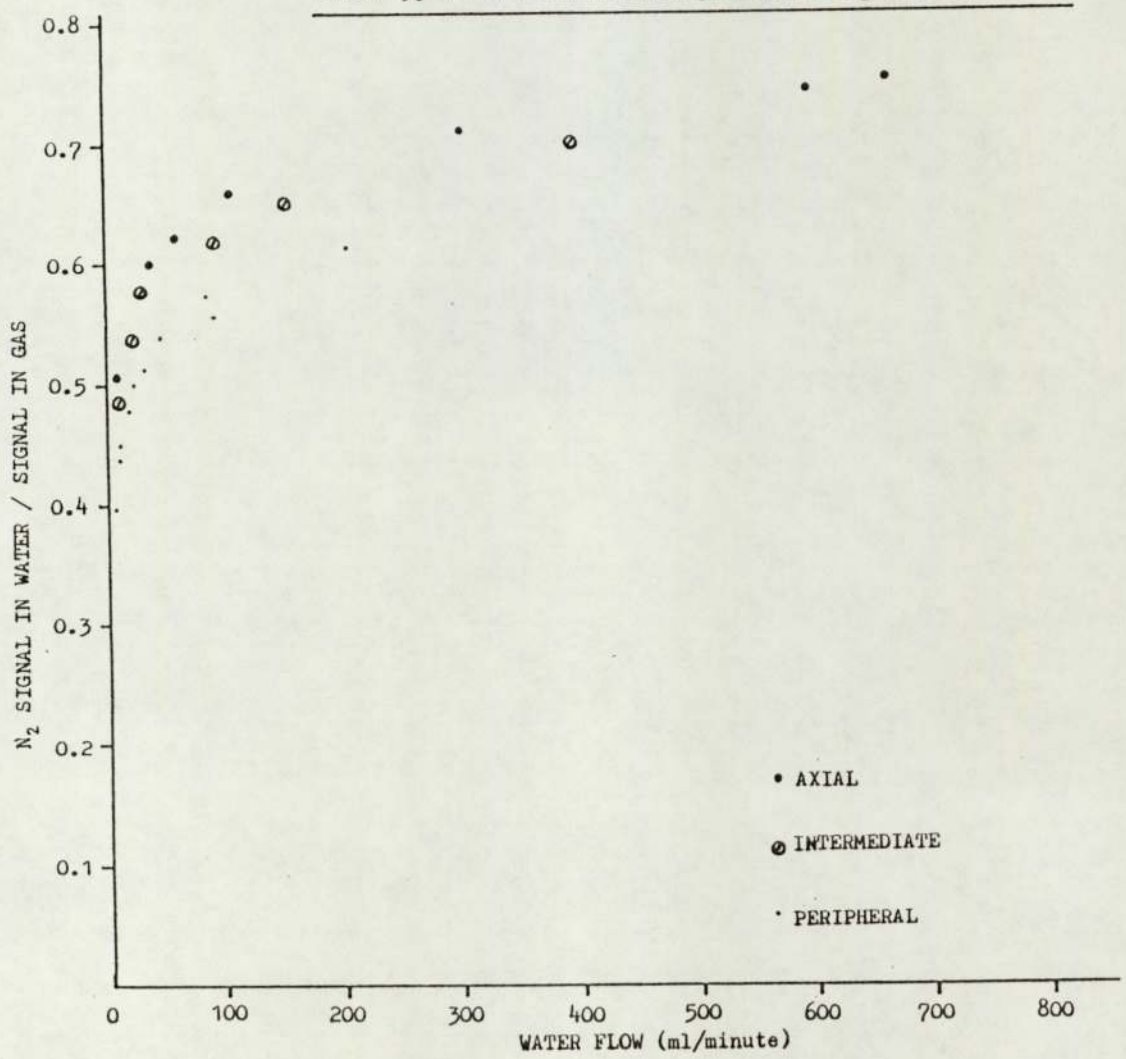


The catheter was positioned axially, then peripherally and finally in an intermediate position along a 9 mm diameter tube. The mass spectrometer nitrogen signal was recorded as a function of flow rate at each of the 3 positions, the water flowing in each case under gravity rather than by pumping. The results are given in Table 31. Figure 50 shows how the flow dependence is shifted according to the catheter's position.

TABLE 31 Effect of catheter position on N_2 flow dependenne (steady flow)

Flow (ml/min)	Signal in water/Signal in gas	
3	0.398	Catheter in peripheral position
8	0.44	
15	0.48	
26	0.50	
45	0.54	
86	0.56	
207	0.61	
85	0.57	
45	0.54	
28	0.51	
11	0.45	
4	0.40	
667	0.75	Catheter in axial position
600	0.74	
300	0.71	
107	0.66	
59	0.62	
41	0.60	
20	0.55	
9	0.51	
2	0.43	
2	0.43	Catheter in intermediate position
9	0.49	
20	0.54	
30	0.58	
93	0.62	
154	0.65	
400	0.70	
1017	0.80	

FIGURE 50 : Effect of catheter position on N_2 flow dependence

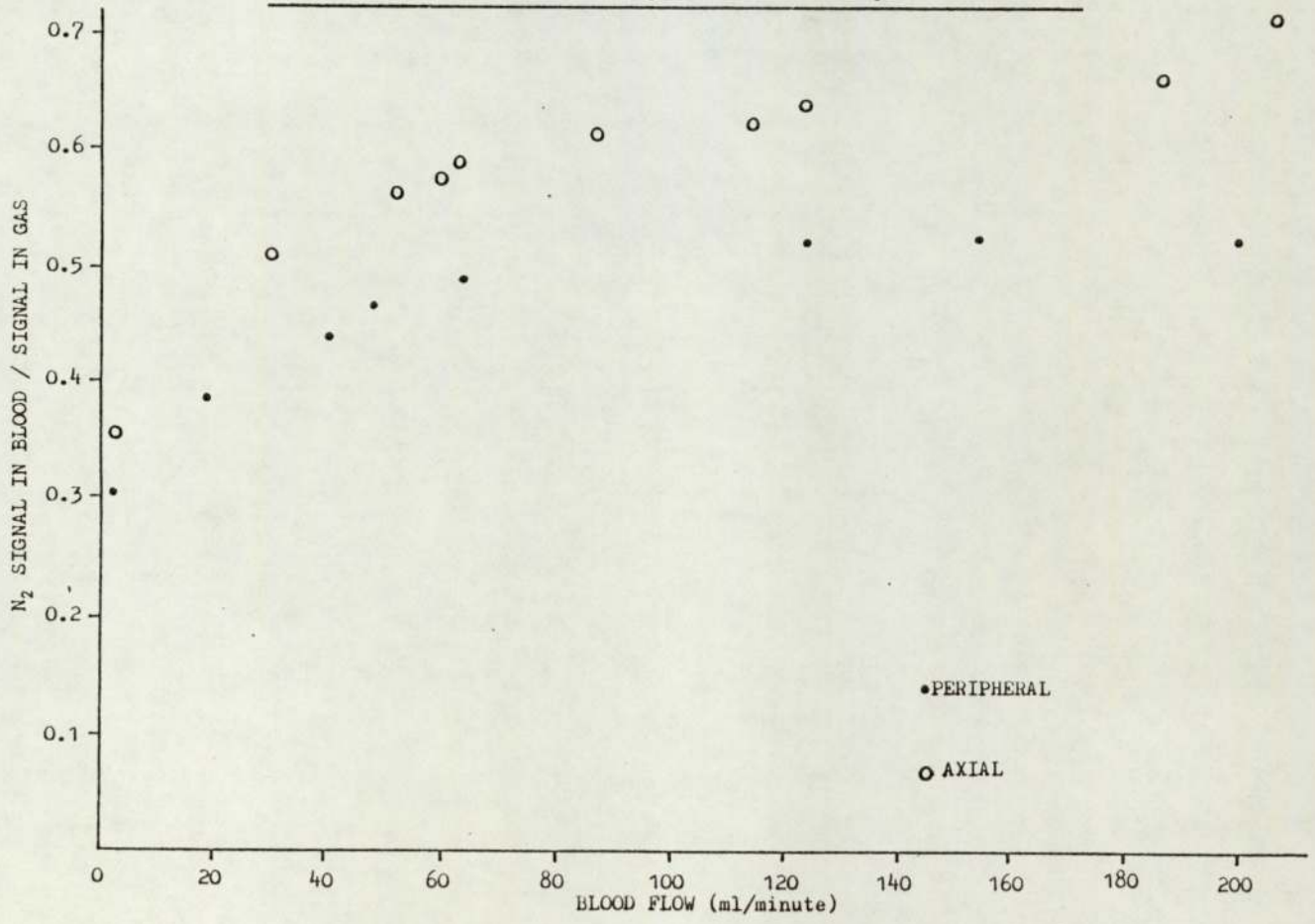


The catheter was positioned along the axis of a 9 mm diameter tube filled with citrated air-equilibrated blood. The blood flowed along the tube under gravity, and the steady mass spectrometer nitrogen signal was recorded at each of a series of flow rates, achieved by altering the blood pressure head. The procedure was repeated with the catheter positioned close to the wall of the tube. Results are given in Table 32. Figure 51 shows that the flow dependence followed the same general form in blood as in water, and that the position of the catheter across the tube was of importance.

TABLE 32 Effect of catheter position on flow dependence in blood

Flow (ml/minute)	N_2 signal in water/air	
188	0.67	
63.8	0.59	
125	0.64	
28	0.52	Catheter
53.3	0.57	
115	0.62	axial
60	0.58	
88	0.62	
3	0.35	
1	0.30	
19	0.38	
40	0.44	
48	0.47	Catheter
64	0.49	peripheral
124	0.52	
154	0.53	
200	0.52	

FIGURE 51 : Effect of catheter position on flow dependence in blood



Discussion of Flow Dependence Experiments

The flow dependence of the mass spectrometer oxygen and nitrogen signals using Chemitron silastic catheters was found to be large at low flows and to level off at high flows, in a form fitting the predictions of the 'static layer' theory. Typically, flow variations of $\pm$ 10% about a value of 900 ml/minute, corresponding to an average velocity of about 6 cm/s, caused the nitrogen signal to vary by $\pm$ 1.2%. In general, however, arterial flow velocities are much higher than this, in a range for which signal variations would be much lower. In fact the range in which these mass spectrometer measurements could provide a qualitatively useful index of liquid flow was found to be up to about 0.6 cm/s and therefore too low to be of physiological interest.

The flow dependent gas signals were also found to be significantly affected by the position of the catheter relative to the velocity profile of the flowing liquid, by the nature of the flow (pulsatile flow gave different results to steady flow), and by the nature of the fluid (blood gave different results to water). All these factors would cause problems in the calibration and operation of these catheters as flow monitoring instruments. It was concluded that these particular catheters could not easily be applied to blood flow measurements in vivo, although the principle of monitoring diffusive gas flow through a membrane as a measure of liquid flow remains valid and could be of practical use with catheters of higher gas permeability.

Appendix 2

In Vitro Measurement of Blood Gas Contents

It was required to measure low concentrations - of the order of 10 parts per million - of gases such as ethane, acetylene, halothane and helium, introduced to the bloodstream in studies of pulmonary ventilation and perfusion.

For a preliminary study of the problem the apparatus shown in Figure 52 was constructed. The control logic of the mass spectrometer was altered so that access either to the analyser section of the instrument or to the roughing rotary pump could be manually gained for the respiratory inlet. The experimental procedure was as follows.

5 ml of blood was tonometered with a mixture of 5% sulphur hexafluoride, 5% cyclopropane and 15% ethane in nitrogen. A sample of the tonometry gas was taken into a 1 ml glass syringe which was then fitted into the inlet apparatus at point A. The dead space of the glass tap T_2 was flushed with the gas and then the tap was closed. The apparatus was connected to the respiratory inlet system of the mass spectrometer at point B with tap T_1 closed. In a short time the inlet warning light of the mass spectrometer lit up indicating that the system was evacuated up to tap T_1. The now manually operatable valve to the analyser was briefly shut off while opening T_1 to protect the analyser from a sudden surge of gas. When the inlet light came on

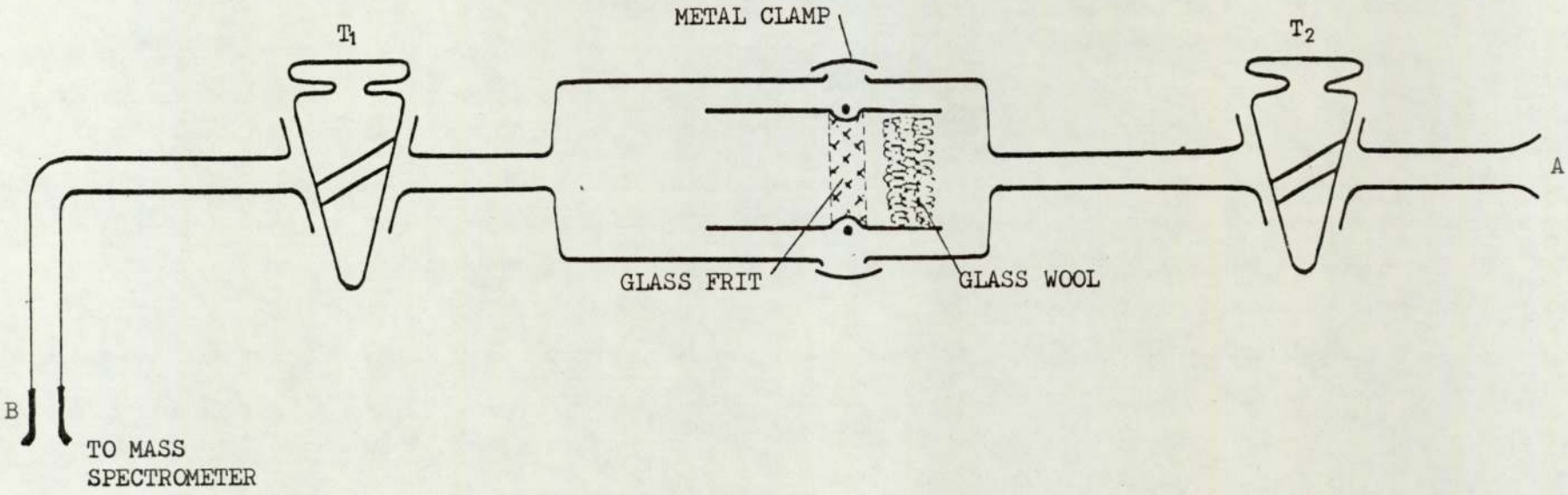


FIGURE 52 : First inlet system for content measurements

again, T_1 was closed and T_2 opened so that the contents of the syringe were sucked into the central glass chamber. T_2 was closed and access to the roughing pump shut off so that the gas sample would be pumped through the molecular leak to the diffusion pump via the analyser. T_1 was then opened and the steady state mass spectrometer signals recorded. The whole procedure was then repeated with a sample of the tonometered blood.

Only one complete set of measurements was successfully made. The results are given in Table 33 and are seen not to correspond very well with the known solubilities of the three gases in blood. Typical problems with this system were leaks into the inlet, loss of sample when flushing T_2, and noisy, unstable mass spectrometer signals when the blood gases were finally analysed.

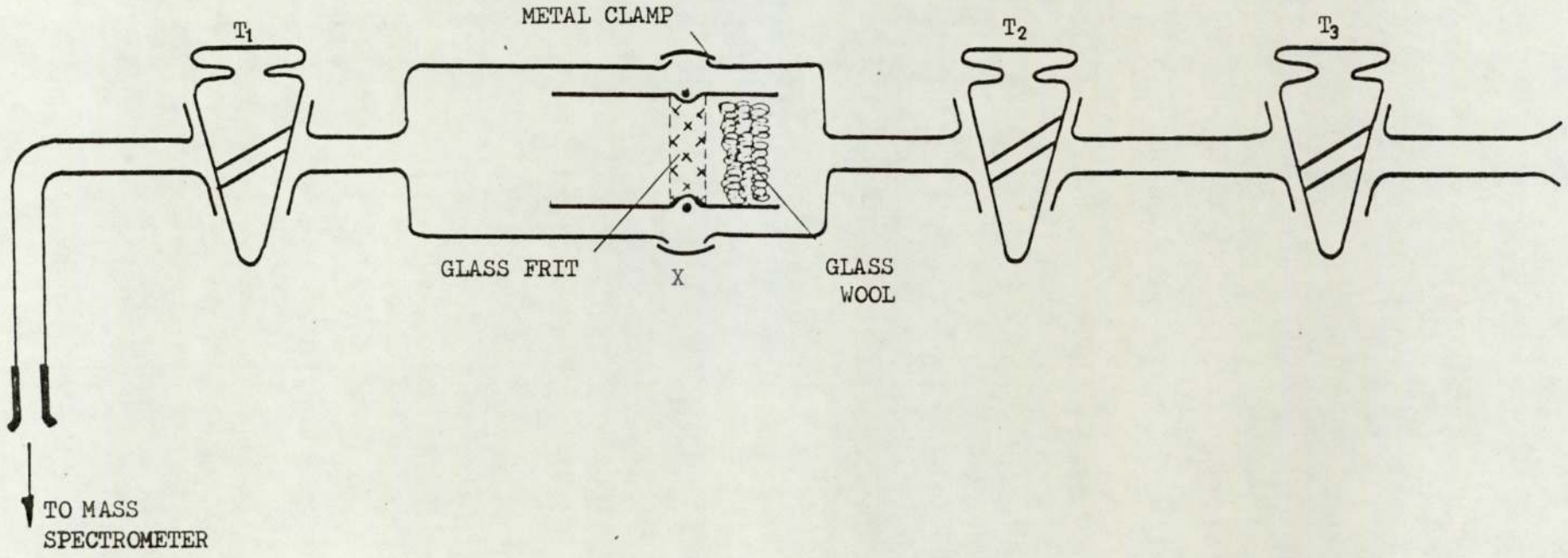
The sensitivity of the mass spectrometer was increased by adding a photon counter - Model 1120 of SSR Instruments Co. - after the electron multiplier in the analyser of the instrument. Instead of a simple on/off valve a manually adjustable needle valve was inserted between the inlet and the analyser. An extra two-way tap T_3 was joined to the inlet apparatus, as shown in Figure 53. The experimental procedure was accordingly revised. Blood samples were made up with known concentrations of halothane, diethyl ether, and acetone, in three batches - one of 50 ppm of each gas, one of 10 ppm, and the reference batch of 0 ppm.

The glass inlet apparatus was disconnected at X, held vertically and filled up to tap T_2 with blood. Taps T_2 and T_3 were then closed and the apparatus was reconnected with the needle valve to the analyser closed off, and the valve to the roughing pump open. The

TABLE 33 Results with inlet system of Figure 52

Gas	Solubility (ml/100ml/torr)	Output for gas (V)	Output for blood (V)	Calculated Partition Coefficient
5%SF_6	0.00084	1.55	0.024	0.015
5%C_3H_6	0.0762	1.38	1.59	1.15
15%C_2H_6	0.00059	2.42	0.45	0.19

FIGURE 53 : Second inlet system for content measurements



needle valve was gradually opened fully and left until the pressure in the analyser, as recorded on a Penning gauge, dropped below 10^{-5} torr. Then Tap T_1 was closed, the needle valve was closed, and tap T_2 opened so that the blood was sucked into the glass chamber X and degassed. The valve to the rotary pump was closed off and tap T_1 opened. The needle valve was opened until the pressure in the analyser was exactly 10^{-5} torr. The output of the photon counter was recorded as each mass of interest was manually tuned in turn. Mass numbers 57,58,59,74 and 117 were used for the gas analysis and mass 40 for reference. After each set of results, the apparatus was disconnected at X, washed and thoroughly dried and the glass wool, present to encourage degassing of the blood sample, was renewed.

The results, given in Table 34, were unsatisfactory in that the mass spectrometer output signals were not found to be proportional to the concentration. The argon readings, on mass 40, were seen to vary considerably too. The main problem was thought to be that the rate of gas leakage into the analyser through the needle valve could not be repeatedly reset with precision nor was the pressure determination by the Penning gauge very accurate. Leaks also occurred around the clamp connection at X.

The glass inlet apparatus was altered, as shown in Figure 54. The needle valve was replaced by the original on/off valve, without manual control. Batches of saline samples were made up with known concentrations of halothane, diethyl ether and acetone. Tap T_1 was kept closed while the glass inlet apparatus was disconnected at joint J, held vertically and filled with saline up to tap T_2. Taps T_2 and T_3 were closed enclosing a volume of saline of just under 2 ml. The

TABLE 34 Results with inlet system of Figure 53

Mass Number	OP at 50 ppm (s^{-1})	OP at 10 ppm (s^{-1})	OP at 0 ppm (s^{-1})
57	72	51	38
58	435	105	54
59	2050	230	38
74	1100	110	35
117	90	47	11
40	10^7 antilog 0.49	10^7 antilog 0.565	10^7 antilog 0.29

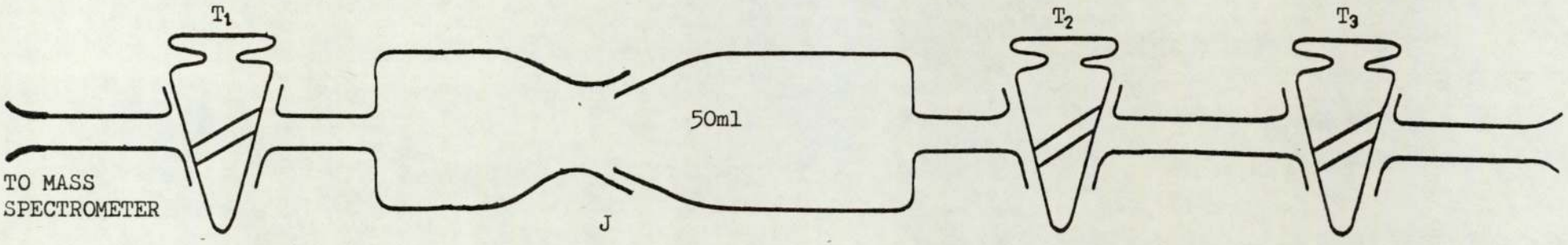


FIGURE 54 : Third inlet system for content measurements

apparatus was reconnected at joint J, and tap T_1 opened. After the inlet warning light came on and the pressure in the inlet system dropped to about 5×10^{-3} torr, tap T_1 was closed and tap T_2 opened so that the saline was drawn into the central chamber. After about 2 minutes, when all vigorous bubbling had ceased, the valve to the roughing pump was closed and tap T_1 opened. The signals from the photon counter were read for each mass number of interest manually tuned in sequence.

It was found that the signals of interest peaked almost immediately and then decayed fast, so the time taken for manual mass tuning was an important limitation on the accuracy of the measurements. The results obtained with saline samples are shown in Table 35.

Inaccuracies in making up the saline samples, ie. in pipetting and diluting, were probably the cause of the discrepancies found between different batches of samples, but within each batch reasonable reproducibility was found, even at levels of 10ppm. The results were encouraging enough for a further improved inlet system to be proposed - one with all metal taps and connections rather than glass to reduce the leaks which were a major problem with all the systems tried to date. The inlet system was not constructed in time for it to be tried on the 200 MGA, modified by the addition of the photon counter, with more accurately made up samples of saline and blood, but remains a possibility for the future.

TABLE 35 Results with inlet system of Figure 54

	Mass Number	Output of Photon Counter (s^{-1})		
2 batches	58	[1] 5400	[2] 5600	
at 70 ppm	74	2600	2600	
	117	1600	1300	
1 batch at	58	[1] 10,000	[2] 1800	[3] 1700
50 ppm, next 2	74	1200	200	210
diluted to 10 ppm	117	8000	1400	1200
3 batches	58	[1] 2000	[2] 2100	[3] 2200
at 10 ppm	74	300	320	320
	117	840	900	900

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