The Analyst

Tutorial Review

Electrochemical analysis of clinical blood-gases, gases and vapours

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This tutorial review charts the development of electrochemical sensors for the analysis of blood-gases, gases and vapours in clinical medicine over the past four decades. The development of each sensor is set in its historical and clinical context, and the first part of the review concentrates on aqueous electrolyte electrochemistry and on those sensors which have made a major impact on the clinical measurement of the partial pressures of oxygen and carbon dioxide in the blood. The electrochemical interference effects of anaesthetic agents on these measurements are also described. Those electrochemical sensors which have failed, in the past, to make a clear impact in this area are not considered, but the few attempts to devise aqueous electrolyte electrochemical sensors for anaesthetic agent measurement are reviewed. The second part of the review describes the chequered history of the development of non-aqueous solvent electrochemical sensors to measure the partial pressures of oxygen and carbon dioxide, in both the presence and absence of each other, in the gas phase. The last part of the review examines various attempts, using non-aqueous solvent electrochemistry, to measure the concentration of inhalational anaesthetic vapours in the gas phase. These sensors have yet to make an impact on clinical practice. Throughout this tutorial review, theoretical models of membrane-covered electrochemical sensors are described where appropriate. This review represents a personal view of the development of electrochemical sensors for clinical measurement, and it is therefore necessarily selective in its approach and emphasis.

Keywords: *Review; clinical medicine; blood-gases; gases; vapours; electrochemical sensors*

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Introduction

The massive contribution made by chemists to the development and understanding of blood-gas and acid-base balance over the past centuries is chronicalled in the book *The History of Blood-Gases, Acids and Bases* by Poul Astrup and John Severinghaus[1] and in their historical essays on blood-gas analysis.[2–6] The list is long and very distinguished but, right up to the 1950s, much of this basic research and development work remained confined to chemistry, biochemistry and physiology laboratories. Furthermore, despite the fact that dissolved gases had been extracted from blood by vacuum techniques for more than 300 years, with their identity known for 200 years and their contents having been measured for more than 150 years, these blood-gas content measurements had contributed relatively little to patient care over this period of time.[5]

However, a succession of external 'forcing' factors have contributed, over the past 60 years, to the increasing utilisation of blood-gas measurements in the treatment and care of patients and to the concomitant development of modern blood-gas electrochemical analysis techniques. First, physiological research into high altitude acclimatisation, deep sea diving and aviation medicine began to burgeon during World War II, and the limitations of the classical volumetric analysis of gases dissolved in blood (*i.e.*, the blood-gases) became so apparent that respiratory physiologists and clinicians began to seek other ways of making these measurements. (The chemical analyses of anaesthetic gases and vapours were to follow some decades later.) Following this initial war interest, two unconnected events, both in the 1950s, led to the rapid development of modern electrochemical sensors for measuring blood-gases and, amazingly, these early electrochemical devices were so good that they have remained relatively unchanged over the succeeding four decades.

The first event, the massive poliomyelitis (polio) epidemic that struck both Copenhagen and the USA in 1952, focused the minds of chemists, physiologists and clinicians very sharply. Polio was a very important disease which consumed the resources of many hospital departments, and this disease led directly to the development of intensive care units, modern

artificial ventilation and the potentiometric electrochemical analysis of the partial pressure of carbon dioxide (P_{CO_2}) dissolved in blood. The inventor of this electrochemical technique, Richard Stow, was a scientist working in the Physical Medicine Department at Ohio State University, where there were desperate attempts to measure the blood-gases of polio patients who were receiving artificial ventilation. His pioneering work was developed further by John Severinghaus, an anaesthetist in San Francisco, who successfully developed the first practical electrochemical P_{CO_2} sensor (or 'electrode') for clinical use.[4]

The second event was the development, in the 1950s, of blood bubble oxygenators for human use. It was quickly realised, at that time, that there was a concurrent necessity to measure the partial pressure of oxygen (P_{O_2}) dissolved in blood, in order to check that the oxygenators themselves were working efficiently and properly.[5] A biochemist and physiologist, Leland Clark, not only developed some of the first of these new oxygenators, but also invented the first practical electrochemical sensor for measuring the P_{O_2} of whole blood.[5,6]

So, the first two practical electrochemical medical devices were developed out of acute clinical necessity by clinical scientists and clinicians working in hospital laboratories, but these workers had built upon an experimental framework which had already been firmly established by the electrochemist in the chemistry laboratory.[1]

From this point on, major contributions to the development of practical electrochemical blood-gas, gas and anaesthetic vapour sensors were made by chemists, physiologists, bioengineers and clinicians alike. Unfortunately, it is, and has been, generally true that clinicians and electrochemists rarely seem to read each other's research papers, or appreciate the real value of each other's contribution to the field, and so there is still today a great communication gap between the clinician and the chemist. The author of this tutorial review hopes to bridge this gap somewhat and (i) to introduce the chemist to some of the very real problems facing the use and development of blood-gas sensors in clinical practice and (ii) to correct some misconceptions concerning the measurement of gas and vapour concentrations in the blood.

Physiological background

Before considering the electrochemical techniques themselves, the whole subject matter needs to be placed within its relevant physiological and clinical context, since this is the *raison d'être* for the development of the electrochemical gas and blood-gas measurement techniques themselves.

Cardiopulmonary homeostasis

The primary respiratory functions of blood are to transport oxygen from the lungs to body tissues, carbon dioxide from the tissues to the lungs and hydrogen ions from the tissues to the kidneys. The function of the lung is to facilitate the exchange of these gases between the blood and the outside atmosphere. The determination of blood acid–base status (*i.e.*, clinical chemistry) is extremely important in the critically ill patient, but that subject, including the measurement of hydrogen ion concentration, is outside the brief of this tutorial review. This review is concerned solely with the determination of the blood-gases and the inspired or expired gases and vapours. Here, the blood-gases referred to are the partial pressures of oxygen or carbon dioxide in blood (P_{O_2} and P_{CO_2} respectively) and this tutorial review will exclude the measurement of the partial pressures of anaesthetic gases dissolved in blood (for reasons given below). The respiratory blood gases themselves reflect cardiopulmonary homeostasis. This is the ability of the cardiopulmonary system to maintain a constant relationship between respiration in the cells of the body, the supply of oxygen from the lungs to the blood and the elimination of carbon dioxide by the lungs. During inspiration, fresh gas in drawn into the lungs and gas exchange takes place between the gas in the lung and the blood flow entering and leaving the lung. The blood which leaves the lung is freshly oxygenated and is pumped, by the left side of the heart, through a system of arteries to the main body organs and the body tissues. In these body tissues, internal respiration takes place and gas exchange also occurs. The respiratory product of this internal respiratory system, carbon dioxide, is transported back to the right side of the heart, and is pumped back through the lungs (as mixed-venous blood) by the right ventricle. When this venous blood enters the lung, carbon dioxide is offloaded and is expelled in expired air. For a normal human being, about 350 l of carbon dioxide are expired every day!

Thus, the body constantly consumes oxygen and produces carbon dioxide, and there is a 'normal' balance between the absolute contents and the partial pressures of these two gases in the blood. Cardiopulmonary function acts as a complex feedback system to keep the blood-gas partial pressures of O_2 and CO_2 at their 'normal' physiological values. This balance can be upset by many physiological or clinical factors or problems, but cardiopulmonary function has a remarkable ability to adapt to compensate for these changes, especially to keep the blood-gases at their 'normal' values.

Carbon dioxide homeostasis

Broadly, the P_{CO_2} of arterial blood (*i.e.*, oxygenated blood which is leaving the lung) reflects the adequacy of the ventilation of gas within the lung. Arterial blood normally has a P_{CO_2} of 5.3 kPa and mixed-venous blood has a P_{CO_2} of 6.1 kPa for a man breathing room air. These P_{CO_2} values are kept constant by the lung ventilation rate, which is about 7 $dm^3\ min^{-1}$. Carbon dioxide homeostasis is concerned with balancing the metabolic rate of production of CO_2 in the body against the effectiveness of lung ventilation. If metabolic CO_2 production increases, lung ventilation must be increased to excrete the CO_2 produced by the body, and so keep arterial P_{CO_2} constant. There is an inverse law relationship between lung ventilation rate and arterial P_{CO_2}, at a constant metabolic CO_2 production rate. In fact, very little CO_2 is carried in blood as a dissolved gas, since by far the majority is carried as hydrogencarbonate or by combination with haemoglobin in the red blood cells. This chemically stored CO_2 is released in the lung, in gaseous form, through the action of carbonic anhydrase, and this CO_2 is expelled in expired air. The actual total CO_2 content (*i.e.*, chemically bound and dissolved) in blood is related to the P_{CO_2} through well defined but complex biochemical relationships, and this is a key part of blood acid-base chemistry. As stated previously, this is outside the remit of this teaching review. It is sufficient to note here that the magnitude of the arterial blood P_{CO_2} is the key clinical measure of the effectiveness of the lungs to expel CO_2.

Oxygen homeostasis

As with CO_2, there is also a very well defined physiological relationship between P_{O_2} and oxygen content in the blood. This well known relationship is illustrated in the shape of the oxyhaemoglobin dissociation curve (the blood oxygen content–P_{O_2} relationship) shown in Fig. 1 for both foetal and adult haemoglobin. The sigmoid shape is due to the way in which oxygen combines reversibly with haemoglobin in the red blood cells, and the chemistry of this relationship has exercised the minds of chemists and physiologists for decades.[1] The ways in which this oxygen content–P_{O_2} relationship can be compromised are legion, and the interested reader can be referred to a readable applied respiratory physiology textbook for this

purpose.[7] For the purpose of this review, it is sufficient to state that the measure of the P_{O_2} in arterial blood is one important indication of how efficient the lungs are in oxygenating the blood, but the measurement of arterial blood P_{O_2} is not, in itself, an indication of adequate tissue oxygenation. The composition of the inspired gases, the affinity of haemoglobin for oxygen, the amount of haemoglobin present in the blood and the heart cardiac output all play major roles in the adequate oxygenation of the body tissues.[1,7]

Respiratory support

Respiratory support, or artificial ventilation, is applied to patients when their lungs begin to fail, because of clinical reasons such as neuromuscular weakness, a decrease in the respiratory drive signals emanating from the brain, an increase in the impedance to respiration or acute lung disease in the severely sick.[7,8] All these conditions lead to a failure of the lung to clear the body's CO_2 production, together with a decrease in the arterial blood P_{O_2} when the patient is breathing air. A mechanical device, commonly called a ventilator, is therefore connected to the patient's airway to inflate the lungs and to expel the CO_2. Supplementary oxygen is added to the input of the ventilator, so that the patient is oxygenated to the desired level. The efficiency of this artificial ventilation respiratory support is ascertained by obtaining arterial O_2 and CO_2 blood-gas samples, measuring their partial pressures and then comparing these with the values expected for efficient gas exchange in the lung.

As stated previously, the polio epidemic in the 1950s led directly to the development of mechanical ventilators and modern intensive care units. There is now a galaxy of mechanical and electromechanical high-technology ventilators in current use, and the principles and practice of these devices have been very well described by Sir Keith Sykes in his book *Principles and Practice of Respiratory Support*.[8] Intensive care units for sick babies, especially prematurely born infants, have also developed rapidly over the past three decades. These units, too, have placed growing demands on the technologist (including the chemist) to make urgent blood-gas and acid–base analyses with minute blood samples, typically less than 50 μl. The frightening speed at which respiratory disease in both adult and neonatal patients can degenerate has also led to a perceived need to measure arterial P_{O_2} and P_{CO_2} *in vivo* with intravascular electrochemical transducers, because it is felt that the patient might deteriorate within the analysis time taken by serial discrete blood samples. Whether this need is true or not is a matter of clinical judgement,[9,10] but this need has placed an even greater burden on the technologist because it is no easy matter to make on-line electrochemical measurements in such a hostile environment as flowing blood in a very sick patient.

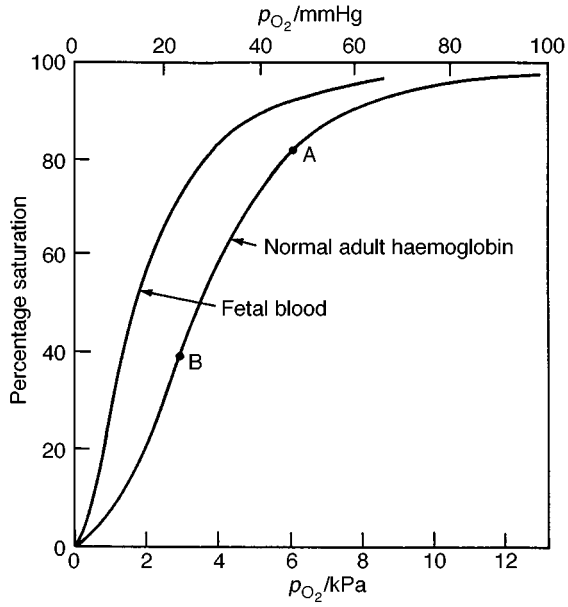

Fig. 1 Oxyhaemoglobin dissociation curves for normal adult haemoglobin compared with foetal haemoglobin. After birth, foetal haemoglobin is progressively replaced with adult haemoglobin and the foetal blood dissociation curve gradually moves across to the right to form the adult curve. Point A represents serious hypoxia which requires urgent treatment in an adult patient with a normal haemoglobin concentration and normal circulation. Point B shows the arterial P_{O_2} which corresponds to the threshold of loss of consciousness from hypoxia. (Taken from ref. 7.)

Hence the demand for blood-gas analysis, whether using discrete samples or on-line, is of paramount importance for both adult and the neonatal intensive care, and the needs of these two different clinical units can tax the ingenuity of even the cleverest developer of blood-gas sensors. Some perceived clinical demands may just be wishful thinking, as alluded to above, and may be totally impossible to meet with current electrochemical techniques. Before embarking upon a fruitless development exercise, the chemist needs to think long and hard before he or she sets off on a journey to devise the perfect sensor–because it may not, in reality, be needed and might also never be used in clinical practice[9,10] (*i.e.*, outside the experimental laboratory).

Inhalational anaesthesia

Patients undergoing major surgery are still mostly anaesthetised by *inhalational* anaesthetic agents, delivered by an anaesthetic machine. Inhalational anaesthesia has had a long history, lasting over 150 years now, and the ideal modern anaesthetic agent should induce anaesthesia rapidly, maintain it with the minimum of unwanted side effects for the duration of the operation and should present both the patient and the anaesthetist with the minimum of complications at the end of the operation. It is, of course, impossible to achieve all these *ideals* since the agent is a drug and, of necessity, this means that it has the potential to harm the patient if administered in too high a concentration or if the equipment delivering the drug to the patient fails to work properly. Because there are so many interconnections between an anaesthetic machine, the ventilator and the patient, and because the agent is delivered by a vaporiser which contains mechanical and/or electronic components, the possibility of mishap is always present. The fact that anaesthesia is a very safe process is a testimony to the reliability of modern instrumentation and to the skill of the clinical anaesthetist, but anaesthetic agent measurement devices *are* needed to monitor not just the patient, but also the anaesthetic machine itself, if the risk of mishap is to be minimised.

It is important, at this point, to correct a misconception which has grown concerning a perceived necessity to measure the partial pressure of inhalational anaesthetic agents in the blood.[11] Although the direct molecular mechanism whereby the anaesthetic agent induces a deep state of hypnosis is still not yet fully understood, it has been clear for decades now that it is the partial pressure of a given anaesthetic agent which determines the depth of hypnosis in a given patient. This anaesthetic partial pressure in the blood determines the quantity of anaesthetic which passes across the blood–brain barrier, and the clinical effect of the anaesthetic is titrated against this partial pressure by the anaesthetist using clinical judgement. The important point to grasp here is that, because the agent is breathed in and out of the lungs by spontaneous or artificial respiration, the partial pressure of an inhalational agent in the blood cannot exceed that of the inspired partial pressure (*i.e.*, that partial pressure delivered by the anaesthetic machine). The patient

therefore eventually comes into partial pressure equilibration with the setting delivered by the anaesthetic machine *via* an exponential time process, and neither the patient's expired anaesthetic gas nor the anaesthetic blood partial pressures can rise above the partial pressure set by the anaesthetic machine, because of the law of mass balance. The actual *concentration* of the agent in the patient's lungs and body organs is governed, of course, by the tissue–gas partition coefficient of that particular agent in the body tissue—and either much, or little, of the anaesthetic agent is stored in the body tissues, according to the individual solubility of the anaesthetic agent in the body tissues. An agent with a low tissue–gas solubility coefficient provides a fast induction for, and recovery from, an inhalational anaesthetic, and this is another ideal aim for the chemist and pharmacologist designing new anaesthetic drugs.

The point at issue here is that, unlike oxygen and carbon dioxide (which are chemically bound to haemoglobin and whose partial pressures in expired gas, and in arterial and venous blood, are governed by complex physiological relationships), the inhalational agent will (theoretically) eventually come into partial pressure equilibrium throughout the whole body (*i.e.*, in both inspired and expired gas, and in arterial and venous blood). This does not mean that the uptake and excretion of inhalational agents are not governed by complex physiological/pharmacological relationships, but it is assumed for pharmacokinetic modelling purposes that they are 'inert'. Therefore, measurement of the partial pressure of the agent in inspired and expired gas will provide the anaesthetist with the information which is required concerning patient safety. Since these measurements confirm the vaporiser setting, it is unnecessary to measure the blood-gas partial pressure of an inhalational anaesthetic. Such measurements are only required for research purposes, in order to develop new, or confirm old, models of anaesthetic uptake and elimination by human beings and animals.

Even in the case of low-flow, or semi-closed, anaesthesia, where it is possible to accumulate an anaesthetic agent through the inappropriate use of certain vaporisers, the present day recommended practice is to measure the inspired and expired agent gas concentrations at the patient's mouth to ensure that a dangerous (or too low, in some circumstances) level of anaesthetic is not delivered. Although it might be argued by some practitioners that monitoring the partial pressure of the volatile agent in the patient's blood on a frequent basis might aid the anaesthetist, the clinical demand is simply not there at the moment.

The above arguments do not apply when a *non-inhalational anaesthetic drug* is delivered intravenously, since a drug delivered in this fashion can build up inexorably within the body, if the body metabolism does not break the drug down into safe by-products in sufficient time, *i.e.*, the patient can be 'overdosed'. Great care must therefore be taken concerning the *infusion* of anaesthetic agents—but the topic of total intravenous anaesthesia is outside the brief of this tutorial review.

Measurement summary

In summary, therefore, it is clinically important to measure the *inspired* and *expired partial pressures* of oxygen and carbon dioxide in the critically ill adult and infant patient. It is equally important to measure the P_{O_2} and P_{CO_2} of arterial blood, but here a choice has to be made whether blood samples are taken and analysed by a static laboratory bench analyser, or whether it is possible (or economically viable) to make these measurements *in vivo*. Sometimes, it will be necessary to measure the P_{O_2} and P_{CO_2} of mixed-venous blood (that is, the blood which is returning directly to the lungs). Sometimes it may be possible to deduce the blood partial pressures by making *in situ* measurements on the skin of a patient (so-called transcutaneous measurements, described in a later section). Lastly, it will be important to measure the inspired and expired concentrations of oxygen, carbon dioxide and any present inhalational anaesthetic agent when an anaesthetic machine and/or a ventilator is connected to the patient and is thus taking on a *life support* function.

It is important to note that some of these measurements can be made relatively slowly, *e.g.*, the inspired oxygen and inhalational anaesthetic agent concentrations delivered by a machine, and static blood-gas analysis in a bench analyser. In other instances, very rapid response times of the order of 100 ms are required, such as for breath-by-breath inspired and expired gas analysis. Similarly, *in vivo* blood-gas sensors should respond reasonably rapidly, preferably with a response time of the order of seconds, if the measurements are to have any significant physiological meaning.

Before turning to the electrochemical analysis of the respiratory and anaesthetic gases, it must be pointed out that electrochemical techniques have serious rivals in the particular case of respiratory gas-phase measurements. Breath-by-breath O_2 gas analysis is now routinely performed by fast paramagnetic and magnetoacoustic O_2 analysers, and these analysers also incorporate infrared and photoacoustic gas analysis for CO_2 and the inhalational anaesthetic agents.[12] As matters now stand, these analysers will not be superseded by present-day electrochemical techniques. On the other hand, 'static' or slow response O_2, CO_2 and anaesthetic agent gas analysis could be performed electrochemically, and this could offer a cheap and viable alternative for monitoring the gas output concentration of the anaesthetic machine.

One final comment in this measurement summary section is on the question 'Why devise new sensors to make "old" measurements?'. Every reviewer will have his/her own opinion on this matter. By the time the reader has finished reading this review, it will be clear that all electrochemical sensors have their own drawbacks (some more major than others) and it is therefore perfectly legitimate for the chemist to explore other electrochemical avenues, or techniques, to make the same measurements. This is how research and development progresses in any science. If the new technique works better, is more reliable, is less invasive and is much better or cheaper, then the old technique will be abandoned and relegated to the electrochemical museum. If the new technique is no better than the old, then at least this avenue has been explored and tested. For this reason alone, clinicians and electrochemists alike have continued over the past four decades their relentless pursuit of developing the ideal chemical sensor for the respiratory and anaesthetic gases.

So, the clinical demands have already been outlined and have remained essentially unchanged over the past four decades. Many more chemical sensors have been developed over the years for P_{O_2} and P_{CO_2} measurements than can be mentioned in this tutorial review. They have all had their moments of glory and some examples include quinhydrone, conductometric, potentiometric, and CHEMFET sensors. Many of these have been described in past reviews[13–15] and in the historical series by Severinghaus and Astrup.[1–6] Also, the past two decades has witnessed a burgeoning interest in the measurement of O_2 and CO_2 using optical techniques, especially in the field of *in vivo* 'intravascular' blood-gas analysis, and a fibre-optic *in vivo* blood-gas monitor is now available for hospital use.[12] This particular topic is, again, outside the brief of this tutorial review and so will not be considered here.

Space constraints permit only a selection of electrochemical sensors to be described, and this reviewer has made his choice selectively, concentrating on those devices which have made the greatest impact or have shown the most promise. Electrochemical techniques still remain supreme in the laboratory

bench blood-gas analyser, and they are likely to do so for the foreseeable future.

Analytical techniques

Gas phase

Because steady state gas-phase measurements require only a relatively slow response time, electrochemical gas sensors are usually placed at the gas output of the gas delivery system (*i.e.*, the anaesthetic machine or the ventilator). The actual position of the gas sampling site can be important as far as patient safety is concerned. It is possible to sample at a given point in the patient's gas delivery system, in the belief that this measurement point is the correct one for monitoring the safety of the patient, and yet place the patient in danger—because an undetected gas delivery disconnection can occur between the patient and the point of gas measurement. These issues and potential mistakes are subtle in clinical practice, and have been discussed and illustrated by Sykes.[16] The ideal measurement point is at the patient's mouth, but this is impractical for electrochemical sensors, because of their size and the constant risk of infection—clinical electrochemical gas-phase sensors are not amenable to sterilisation. Furthermore, gas measurement at the mouth must be made with a very fast time response device, since the measurements must be able to follow both the inspiratory and the expiratory gas concentration patterns. A slow time response sensor will simply measure a running average of the inspiratory and expiratory gases, rendering the measurement useless for respiratory analysis. Thus, in the absence of very fast time response electrochemical sensors, measurements at the mouth are out of the question (even with side-stream remote analysis), and so electrochemical gas measurements must be confined to the parts of the gas delivery system which are distal to the patient's airway.

Blood phase

Here, three techniques are commonly used. The major technique, and that most used, is *in vitro* analysis of the blood, using electrochemical sensors installed in a blood-gas analyser situated either close to, or some distance away from, the patient. A blood sample is taken from the patient's artery in a gas-tight syringe, and this sample is then introduced to three electrochemical sensors which determine P_{O_2}, P_{CO_2} and hydrogen ion concentration separately. Less commonly used are *in vivo* techniques, which can be sub-divided into two approaches. The first approach (which is rarely used in the adult patient) is to insert an electrochemical sensor into the patient's artery so that blood-gas measurements can be made *in vivo*. The alternative approach is a non-invasive (transcutaneous) method of measuring blood-gas partial pressures, where the electrochemical sensor is placed on the patient's skin, and the measurement relies on O_2 and CO_2 diffusing through the skin to the electrochemical sensor.

Each different measurement approach has certain advantages and disadvantages, but all methods employ the same electrochemical principles to determine the gases in question. Almost without exception, separate electrochemical sensors are used to measure P_{O_2} and P_{CO_2}, and these sensors invariably use a two-electrode cell, with a working electrode to measure the variable and a reference electrode to standardise the measurement. A three-electrode cell is rarely employed, and this tutorial review will concentrate on the working electrode, at which the O_2, CO_2 or anaesthetic agent measurement is made. The reference electrodes (which are standard Ag/AgCl or calomel reference electrodes) will not be considered any further.

Aqueous electrolyte solution electrochemistry:carbon dioxide determination

Since the history and development of the electrochemical determination of CO_2 is less varied than that of O_2 determination, this topic will be considered first. Apart from very recent developments (to be described later), CO_2 determination in the blood has always been performed by potentiometric means using aqueous electrolytes. The P_{CO_2} sensor, first described by Stow *et al.*[17] in 1957 and then by Gertz and Loeschcke[18] in 1958, was developed into its present form by Severinghaus and Bradley[19] in 1958. This CO_2 sensor is commonly referred to as the Stow–Severinghaus electrode.

The Stow–Severinghaus sensor

This sensor is, in effect, a glass pH electrode housed behind a thin membrane which is permeable to CO_2, with a thin hydrogencarbonate layer placed between the pH electrode and the membrane. The working principle of this sensor can be illustrated by reference to Figs. 2 and 3. Fig. 2 shows a cross-

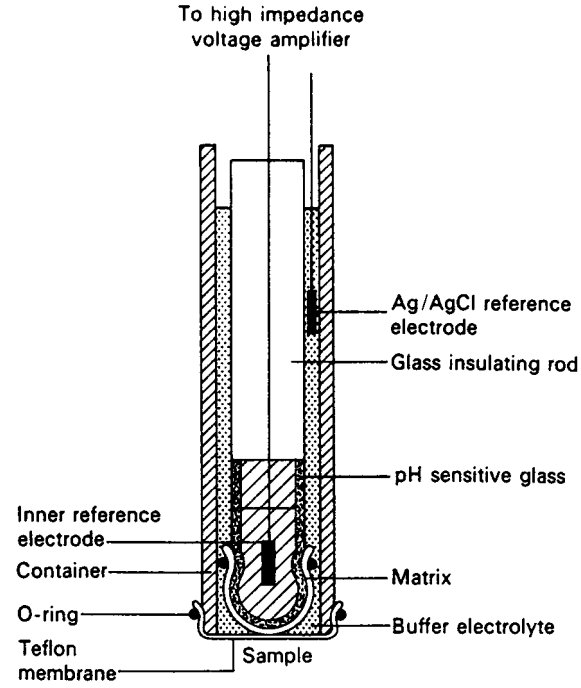

Fig. 2 A schematic outline of the Stow–Severinghaus P_{CO_2} sensor, suitable for *in vitro* blood-gas measurement.

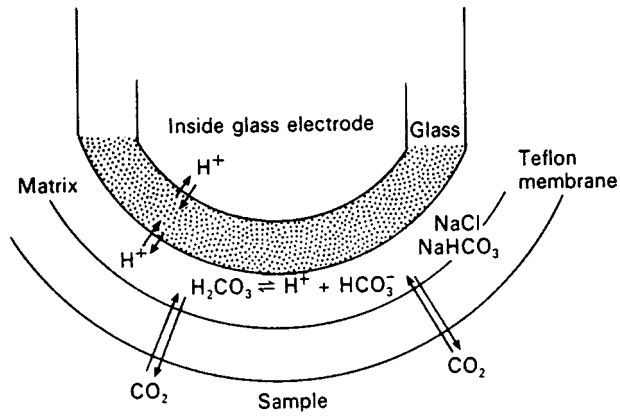

Fig. 3 Schematic diagram of the tip of the Stow-Severinghaus P_{CO_2} sensor showing the membrane, buffer electrolyte layer and the pH-sensitive glass of the pH electrode. The equilibria equations for CO_2 transport across the membrane are illustrated.

section of a practical P_{CO_2} sensor. The end of the pH glass electrode is covered by a matrix which holds a thin layer of solution containing $NaHCO_3$ with some NaCl, and sometimes AgCl, also added. An Ag/AgCl reference electrode, in physical contact with the hydrogencarbonate solution, completes the electrical circuit to the pH working electrode. The potential difference between the working electrode and the reference electrode is measured by a standard high-impedance voltage amplifier and display system.

Working principle

The pH sensor is separated from the blood, which is to be analysed, by a membrane (typically 20 μm thick Teflon) which is highly permeable to CO_2. When in practical use, the P_{CO_2} of the blood comes into equilibrium with the P_{CO_2} of the hydrogencarbonate solution in the matrix immediately adjacent to the H^+ ion-sensitive glass. This process is shown diagrammatically in Fig. 3. The equilibria now existing in this thin hydrogencarbonate layer are

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

$$HCO_3^- \rightleftharpoons H^+ + CO_3^{2-}$$

$$NaHCO_3 \rightleftharpoons Na^+ + HCO_3^-$$

By writing the dissociation constants K, K_1 and K_2 in terms of the chemical activity of each species present, and noting that the ionic products for the dissociation of water are given by

$$K_W = a_{H^+}a_{OH^-}$$

it can be shown that the CO_2 partial pressure, P_{CO_2}, in this thin film is given by

$$P_{CO_2} = \frac{a_{H^+}{}^2 + a_{Na^+}a_{H^+} - K_w}{\alpha K_1\left(1 + 2K_2 / a_{H^+}\right)} \quad (1)$$

where α is the solubility coefficient of CO_2 in the thin film of hydrogencarbonate, and it is assumed that Henry's solubility law holds in this thin film.

This principle was first determined by Stow in 1952, although his work was not published until 1957.[17] However, Stow's sensor utilised a thin layer of water between the membrane and the pH working electrode, and the sensor did not work well—it was unstable in practice, presumably because the pH of the distilled-water electrolyte changed very easily with slight contamination. Severinghaus's key contribution to the development of the sensor was to add sodium hydrogencarbonate to the water in order to stabilise the pH and to increase the sensor sensitivity. Accordingly, Severinghaus and Bradley[19] demonstrated that the addition of a 5–20 mM hydrogencarbonate concentration greatly increased the sensitivity of the sensor, and under these conditions, eqn. (1) simplified to

$$P_{CO_2} = \frac{a_{Na^+}a_{H^+}}{\alpha K_1} \quad (2)$$

Severinghaus was able to demonstrate that when the P_{CO_2} in the hydrogencarbonate layer changed to a new value, then [by taking logarithms of both sides of eqn. (2) and noting that both K_1 and a_{Na^+} are constants, and therefore cancel out when two a_{H^+} values are inserted], the relationship between the changes in the P_{CO_2} and pH in the bicarbonate layer was given by

$$\Delta \log P_{CO_2} = -\Delta pH \quad (3)$$

where the Δ terms represent the change in the logarithm of the P_{CO_2} and the change in pH. It must be noted that $-\Delta \log a_{H^+} = \Delta pH$, by definition, in the hydrogencarbonate layer.

These authors also defined the ratio $\Delta \log P_{CO_2}/\Delta pH$ as the sensitivity, S, of the sensor. Thus, if S is −1.00, then each 10-fold change in P_{CO_2} will theoretically induce a one unit change in the pH of the hydrogencarbonate solution. At a steady temperature of 37 °C, this would be registered as a voltage change of 61.5 mV, as predicted by the Nernst equation. Severinghaus and Bradley found that the sensor sensitivity was slightly less than this predicted change, and that S could typically be −0.97. It is clear from eqn. (3) that since the relationship between pH and potential difference is linear, and the relationship between P_{CO_2} and pH is logarithmic, then the carbon dioxide sensor produces a logarithmic relationship between P_{CO_2} and output voltage. All blood-gas analysers therefore employ an algorithm to linearise this relationship, so that P_{CO_2} output is displayed linearly.

Stability

It is important to note that the bulk of the electrolyte, shown in Fig. 2, plays no part in the equilibration process and merely serves as a reservoir and as electrical conductor. All active processes take place only in the thin hydrogencarbonate layer trapped adjacent to the glass electrode. It is therefore important to keep a stable calibrating gas, or liquid, solution in the sample chamber between readings, so that the sensor always works from a stable pH reference point in the hydrogencarbonate layer and returns to this same point after sampling. Sensor instability or drift will result if this regime is not adhered to, and modern blood-gas analysers pass through a sample analysis/wash/reference calibration point regime when used in clinical practice. If a sample is retained too long in the analysing chamber adjacent to the sensor membrane, the bulk of the sensor electrolyte solution will begin to come into equilibrium with the P_{CO_2} of the sample, and this will destroy the stability of the sensor.

Liquid-gas difference

Because all the electrode reactions described above are reversible, and since pH measurement itself is potentiometric, the overall electrochemical CO_2 process does not consume the analyte. Since the P_{CO_2} sensor does not consume CO_2 from the sample, it follows that, in principle, any liquid or gas sample which has the same CO_2 partial pressure will register the same output when analysed with the same sensor. There is therefore no so-called 'liquid–gas measurement difference' for the potentiometric Stow–Severinghaus P_{CO_2} sensor, and this is a major advantage over new rival amperometric techniques.

Calibration

The absence of a liquid–gas difference for the potentiometric CO_2 sensor means that the sensor can be calibrated with either a gas or liquid before it is used to measure the P_{CO_2} of a blood sample. Since the P_{CO_2} sensor output is logarithmically related to P_{CO_2}, a zero P_{CO_2} calibration point cannot be used. It is usually convenient to calibrate the sensor with two gases, or two liquids equilibrated with these gases, which have CO_2 contents which span the expected range of the blood P_{CO_2} values. Since the clinical range for patient blood P_{CO_2} values is expected to be between 2.7 and 8.0 kPa (or above in extreme cases), the two P_{CO_2} calibration values will normally lie between 3 and 10 kPa.

Uses and limitations

The Stow–Severinghaus CO_2 sensor, as described above, has a typical outside body diameter of 10 mm and an overall sensor length of 35 mm. It is very difficult to reduce these overall dimensions because of the very nature of the construction of pH glass electrodes. However, Parker *et al.*,[20] in 1978, made a

heroic attempt to develop an intravascular dual O_2/CO_2 sensor, and they managed to reduce the overall diameter of the sensor to 1.6 mm. However, this sensor was obviously very difficult to construct and must have been extremely fragile, and it does not appear to have been developed any further. Thus, the standard P_{CO_2} sensor is used solely for *in vitro* or transcutaneous (see later) CO_2 measurements in clinical practice, and it cannot be used for breath-by-breath CO_2 gas analysis because of its slow response time.

Nonetheless, this sensor has stood the severe test of time, and has been used (outside blood-gas measurement in medicine) for ambient gas monitoring, tissue studies, industrial fermentation control and even for satellite atmosphere monitoring.[4] As highlighted by Severinghaus and Astrup, Stow's development of the CO_2 sensor was a result of logical thinking and did not depend on a long series of prior discoveries, apart from the previous development of the glass pH electrode. The sensor was not patented, was not disputed and the inventor did not profit from his discovery[4]—an unusual occurrence in today's society.

Determination of oxygen

Danneel,[21] in 1897, reported that oxygen in aqueous solution reacted with negatively charged inert metals, and he demonstrated an approximately linear relationship between oxygen partial pressure and current when using two large polarised platinum electrodes. He, and others, attempted to use platinum electrodes for oxygen measurement in biological media, but found that the electrodes were rapidly poisoned, or else were coated with protein, causing decrease in the oxygen reduction current. Because the measurements were not dependable, Danneel's discovery fell into disuse. Much later, the discovery of the dropping-mercury electrode, and the subsequent measurement of dissolved oxygen with this electrode, was the prelude to a burgeoning interest in the measurement of dissolved oxygen. According to Severinghaus and Astrup,[6] it was work by Blinks and Skow[22] (in which they used platinum or gold instead of mercury as the working electrode) which led to the use of solid metal working electrodes for the measurement of dissolved oxygen, and thus the subsequent measurement of an ever-broadening range of other gases. Although Heyrovsky (the discoverer of the dropping-mercury electrode and the founder of polarography) had strongly objected to the use of solid metal working electrodes, because of their instability due to the 'poisoning' of the cathode surface,[5] Blinks and Skow persevered with their investigations of the use of platinum electrodes, and they demonstrated that they could produce results which were identical with those obtained with the dropping-mercury electrode. In fact they found that good oxygen reduction plateaux could be obtained in stirred solutions, with good current linearity over a range of oxygen concentration from 0 to 99.5% v/v. They further reported that oxygen was reduced to hydrogen peroxide during their investigations into oxygen evolution and consumption in plant cells, and they demonstrated that plant catalyse immediately broke down the hydrogen peroxide. Thus, Blinks and Skow appear to be the first workers to initiate the use of platinum working electrodes, at fixed polarising potentials, for the amperometric measurement of oxygen partial pressure in biological solutions, and they were also the first to demonstrate the linearity of the oxygen reduction current with P_{O_2}. Unfortunately, these workers appear to have been frequently overlooked when the history of the amperometric oxygen sensor has been reported in the past.

Thereafter, a whole succession of physiologists and chemists attempted to use solid wire electrodes to measure P_{O_2}, with little consistent success in biological media, and the solution to the problem of the measurement of P_{O_2} in liquids and biological solutions was finally proposed by Leland Clark.[23] Clark's great contribution to this field was the imposition of a gas-permeable membrane between the liquid sample and the working electrode, with the reference electrode also housed behind the same membrane. This innovative, but simple, step solved the problem of contamination or poisoning of the working electrode in one stroke.

This single change in design was a historical turning point for respiratory physiology, and led rapidly to the development of the modern blood-gas analysis machine. There followed an unparalleled explosion in the use of the oxygen electrode in clinical medicine, and it was rapidly used to measure gaseous oxygen, blood P_{O_2} and blood oxygen content and in, the study of the oxyhaemoglobin dissociation curve, and its use was also extended to the food, alcohol, aircraft and the space industries, to soil chemistry and to waste water and sewerage management.[5,6,13,24–27]

The development of this membrane-covered electrode also led to a variety of electrochemical control techniques being applied to the working electrode, in order to obviate, or overcome, some of the inevitable practical difficulties which would be encountered when the sensor was applied to practical clinical problems. It has also led to a research interest in the development of mathematical and computer sensor simulation models, in order to describe the reaction–diffusion processes taking place in the sensor and at the working electrode surface.

The Clark sensor

The fundamental principles of the Clark amperometric sensor are illustrated in Fig. 4. As with the Stow–Severinghaus CO_2 sensor, the Clark sensor comprises a container which houses the electrolyte, the working electrode (cathode) and the reference electrode. The external electrical circuit consists of a fixed voltage source and a current-to-voltage converter to measure the oxygen reduction current. A gas-permeable membrane separates the electrolyte from the oxygen sample and the working electrode is polarised at a steady negative voltage with respect to the reference electrode (which is almost invariably an Ag/AgCl electrode). The electrolyte solution is, typically, an aqueous buffer solution with the addition of Cl^- ions, and a pH is generally chosen to be in the region of 7. When used in a blood-gas analyser, the complete system is thermostatically

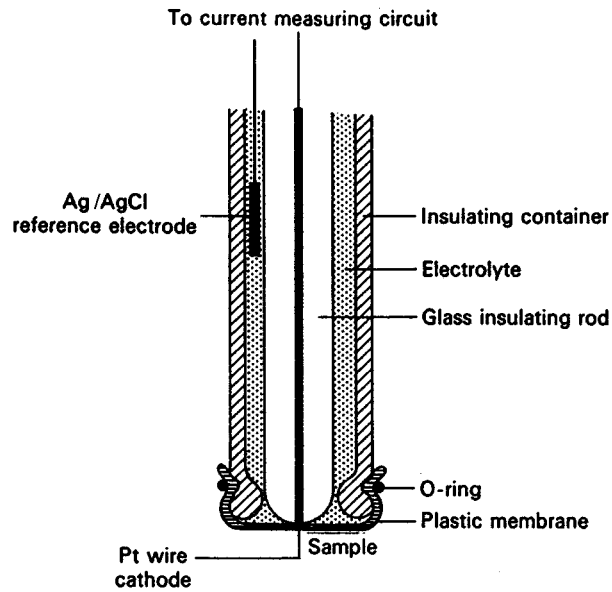

Fig. 4 Schematic outline of the Clark P_{O_2} sensor.

controlled at 37 °C, so that the blood-gas P_{O_2} measurements are made at normal body temperature.[28]

Electrode reactions

There has always been discussion and disagreement about the exact nature of the electrochemical reactions taking place at the electrode surface in the aqueous electrolyte solutions used in the Clark sensor.[14,27,28] Perhaps there will never be agreement, because the electrode reactions (and any combination of them) appear to depend upon poorly reproducible or uncontrollable conditions such as the past history of the cathode surface, the pH of the electrolyte, the metal used for the cathode and even the geometry of the cathodic compartment.[27] Furthermore, it could well be true that the combination of the reactions taking place at micro-cathodes (diameters less than 20 μm) might not be the same as those taking place at the conventional macro-cathodes (diameters greater than 200 μm) which are conventionally used for monitoring industrial processes. Various schemes have been proposed in the past,[14] with varying degrees of complexity, but it seems clear that the two products for the reduction of O_2 at noble metal surfaces, in alkaline media, are either the hydrogen peroxide ion, HO_2^-, or the hydroxyl ion, OH^-.

A much simplified reaction scheme is given by

$$\longrightarrow O_2 + H_2O + 2e \rightarrow HO_2^- + OH^- \quad (4a)$$

$$HO_2^- + H_2O + 2e \rightarrow 3OH^- \quad (4b)$$

$$HO_2^- \xrightarrow{\text{catalytic decomposition}} \tfrac{1}{2}O_2 + OH^- \quad (4c)$$

or

$$O_2 + 2H_2O + 4e \xrightarrow{\text{direct}} 4OH^- \quad (4d)$$

where the HO_2^- ion is either reduced to OH^-, is catalytically converted back to oxygen, which can further react on the cathode surface, or else escapes from the cathode surface into the bulk of the electrolyte, in the immediate vicinity of the cathode.

Examples of complex pathways relating to these reactions have been illustrated by Jacq and Bloch,[29] Wroblowa *et al*.,[30]Appleby and Savy[31] and by Linek *et al*.[27] Although these workers confined their findings to macro-electrodes, there is no reason to suppose that their general conclusions cannot be applied, in part, to the processes taking place at micro-cathode surfaces. The net result of these processes is that the number of electrons, *n*, involved in the reduction of O_2 can vary between the limits $2 \leq n \leq 4$. Hahn *et al*.[32] examined the way in which *n* varied with electrolyte pH and cathode metal, with a rotating ring-disc electrode system. The conclusion was that the amount of HO_2^- reduced as the negative polarising voltage was increased, for all values of pH. The problem with the production of the unwanted HO_2^- ion is that it can build up in the bulk electrolyte if it escapes from the cathode surface. It can then diffuse back to the cathode to be reduced to OH^- ions, and so constitute an unwanted current. This takes the form of a hysteresis effect, when a low P_{O_2} sample has been introduced to the sensor immediately following a high P_{O_2} sample. In this instance, there is a long tail in the sensor response time before it registers the 'true' low P_{O_2} value, and this introduces a long time constant into the measurement procedure. Both the hysteresis and the over-long time constant effects are reduced as $n \rightarrow 4$, as the polarising voltage is increased to high negative values.[32]

Eqns. (4a) and (4b) suggest that two clearly separated O_2 reduction waves should be observed when O_2 is reduced in aqueous electrolyte solutions. However, this is not necessarily the case even for unshielded electrodes. The electrode material, the electrode size and the pH of the electrolyte all play a part in the reduction process scheme, and the presence of a shielding membrane also appears to change the nature of the processes occurring.

For instance, when O_2 is reduced on unshielded gold macro-electrodes, two O_2 reduction waves [corresponding to eqns. (4a) and (4b)] are seen. When shielded with a membrane (as in a Clark sensor), only one wave [corresponding to eqn. (4d)] is seen. When Pt is used as the cathode material, only one reduction wave is seen for both unshielded and membrane-covered cases.

When 20 μm diameter micro-electrodes are employed, the pH of the electrolyte plays a key role in determining the shape and position of the O_2 reduction voltammogram. Fig. 5 shows voltammograms for the same 20 μm diameter Pt micro-disc cathode, shielded by the same 25 μm polypropylene membrane, for O_2 reduction in pH 6.8 and 11.2 electrolyte solutions at 37 °C. The voltammogram with pH 6.8 electrolyte shows no clearly defined plateau at the voltage normally used to poise Clark blood-gas P_{O_2} sensors, namely −0.6 V *versus* Ag/AgCl, whereas with the pH 11.2 electrolyte a clear plateau is seen at polarising voltages more negative than −0.8 V. Furthermore, a large degree of hysteresis is evident between the upward and decreasing sweeps for pH 6.8, and this hysteresis is removed on the plateau part of the wave for pH 11.2.

Despite this evidence, manufacturers to this day use electrolyte solutions with pH ≈ 7 in their Pt microdisc blood-gas P_{O_2} sensors and poise them at about −0.6 to −0.7 V, *i.e.*, sensors appear to work on a voltammogram which does not display a clear diffusion plateau at that voltage.

Cathode size and the stirring problem

The polyethylene membrane covering Clark's electrode not only completely avoided the cathode poisoning problem, and made measurements of gases (and not just liquids) possible (because both cathode and reference electrode were located within a single electrochemical cell) but also, very importantly, the membrane was relatively impermeable to oxygen. Limiting the amount of oxygen consumed by the cathode enabled the sensor to measure the oxygen partial pressure in solution.

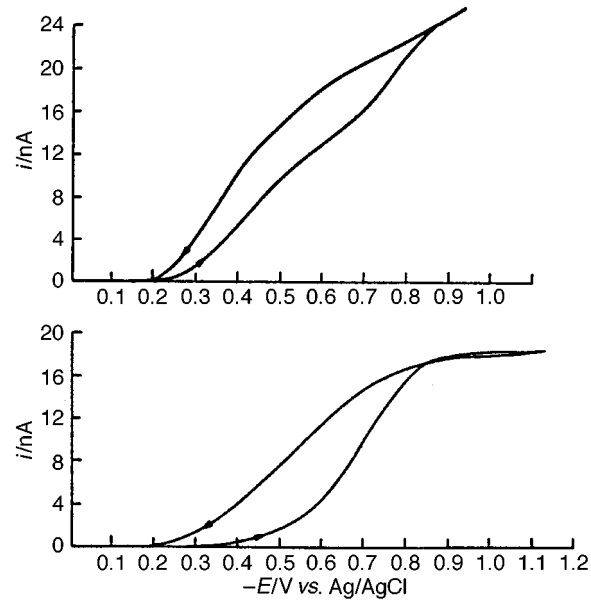

Fig. 5 Voltammograms for a 20 μm Pt micro-electrode, when covered with a 25 μm polypropylene membrane, at 37 °C (normal body temperature), when using a pH 6.8 electrolyte (top) and a pH 11.2 electrolyte (bottom).

However, the first sensor had a relatively large cathode (2 mm diameter) and the sensor reading in stagnant water was 25% too low when the sensor was calibrated with a gas with the same P_{O_2}.[5] This 'liquid–gas' difference, to be called later the 'stirring effect', was enormous. Severinghaus and Bradley[19] immediately saw one solution to this problem, and introduced a magnetic stirring bar in front of the electrode, which greatly reduced the liquid–gas difference. Thereafter, many magnetic stirring systems were described, but alternative methods of solving the stirring problem soon appeared. Some methods used thick polyethylene membranes, some used Mylar (6 μm) as the material, but the disadvantage of tackling the problem by making the membrane more impermeable to oxygen was that the time response of the electrode became unacceptably large (> 2 min).

The final solution of the stirring problem came from attempts to make the electrode small enough for intravascular use or for use with very small *in vitro* samples. The emergence of high-gain current amplifiers made accurate measurement possible for currents of the order of several pA. Staub,[33] in 1961, designed a micro-electrode in order that it might fit into a very small cuvette, and he embedded a 50 μm platinum wire in a glass rod—the first reported such small micro-electrode Clark P_{O_2} sensor. This small electrode drew immediate attention to the possibility of reducing the stirring effect by reducing the cathode diameter and thus the quantity of oxygen consumed by the sensor. It also enabled researchers to use thinner membranes and so reduce the electrode response time to the order of seconds. Very soon, cathodes with diameters of 12–25 μm were produced, and when these were covered with 25 μm polyethylene, the stirring effect was found to be only about 3–5%. This was an acceptable level for clinical and most research uses, without stirring. Other early approaches included mechanically agitating the sample so that it moves to and fro across the membrane face adjacent to the cathode surface, or pulsing the polarising voltage with a fixed duty cycle and thus using pulsed amperometry to control the electrode. Although sufficiently short pulses have the advantage that they can control the expanding diffusion field, emanating from the vicinity of the cathode, in a space of defined diffusion conditions (*e.g.*, within the membrane or with a defined unstirred layer of the solution), the electrode control circuitry is complex. Furthermore, it is only recently (see below) that realistic two or three layer digital simulation models have been developed to describe the complex relationship between the cathode time-dependent current, the cathode diameter and the electrolyte and membrane layer thicknesses and permeabilities.

Therefore, over the past 40 years, blood-gas analyser manufacturers have accepted the blood-gas difference as inevitable, and strike an empirical compromise between the cathode diameter, the type of membrane material and the membrane thickness. This has inevitably led to a 3–5% difference between gas and blood samples which have the same P_{O_2}, but this difference is automatically accounted for in the software of the analyser.

Calibration and quality control

In vitro blood P_{O_2} sensors can be calibrated either with two gases, or with two liquids which have themselves been brought into equilibrium with known gas mixtures. One of these gases (or liquids) will have a zero P_{O_2}, in order to set a 'zero point' for the P_{O_2} sensor. A two-point calibration procedure must be employed, because the quiescent current present in a micro-cathode P_{O_2} sensor is a sizeable fraction of the oxygen reduction current produced when blood samples are introduced into the analyser. Typically, the current generated by a blood-gas P_{O_2} sensor is of the order of 70–100 pA per kPa P_{O_2}. With such a small current sensitivity, it is clear that even a quiescent background current of 10–20 pA has to be 'backed off' by the control circuitry. The 'high' calibration point will typically be set somewhere between a P_{O_2} of 10 and 21 kPa. The other main gas in the two calibration mixtures will be CO_2, with 'low' and 'high' P_{CO_2} values, for calibrating the Stow–Severinghaus CO_2 sensor.

A modern blood-gas analyser will go through an automatic two-point calibration procedure every 2–4 h, to account for the inevitable drift of the output signals from these electrochemical sensors and to correct for changes in their sensitivity to P_{O_2} and P_{CO_2}; however, they will also go through a 'single point' check calibration after each blood sample has been analysed.

Ways in which the accuracy of blood P_{O_2} (and P_{CO_2}) measurements can be assessed has always been controversial, and this subject matter really belongs in the domain of clinical biochemistry. The 'gold standard' should be samples of fresh tonometered human blood, but this is an artform in itself and many laboratories have not had, in the past, the facility for conducting this work. Instead, ampoules of commercial quality control materials became widely available, based on aqueous solutions with O_2 solubilities equivalent to water, haemoglobin-containing products, and perfluorocarbon-containing emulsions with an O_2 solubility several times higher than that of water. According to Hansen and Fiel,[34] it is not unusual to find differences of ⩾20% between model-specific mean P_{O_2} values for a given set of quality control ampoules. In their own work, they found that the perfluorocarbon emulsion materials performed better than the other materials. However, good modern practice now involves the use of tonometered non-human or human blood, with a high quality tonometer sited close to the blood-gas analyser unit, so that the performance of the P_{O_2} and P_{CO_2} electrodes can be checked at will.

Theoretical models

Theoretical models describing the output current–P_{O_2} relationship of the Clark P_{O_2} sensor have changed dramatically over the past four decades. Perhaps the major problem, at least in the early years, arose from the application of one-dimensional diffusion theory to membrane-covered micro-cathode electrodes. Once it had been realised that this was a great mistake, hemispherical and cylindrical diffusion models began to emerge.

All the analytical solutions described below in this section are based on various solutions of the Fick equation:

$$\frac{\delta p}{\delta t} = P\nabla^2 p \tag{5}$$

where P is the oxygen permeability in the medium ($P = \alpha D$, where α is the oxygen solubility and D is the oxygen diffusion coefficient in the medium) and p is the oxygen partial pressure in the medium (written with a lower case p here to avoid confusion with the permeability term, P).

Steady-state models

One-dimensional linear diffusion model. This three-layer model, which only strictly applies to macro-cathodes employed in gas-phase P_{O_2} sensors, is described in Fig. 6. In this model, the constraint is normally made that the cathode surface is polarised sufficiently negatively enough to ensure that all oxygen molecules reaching it are immediately destroyed.

For linear diffusion, eqn. 5 reduced to

$$\frac{\delta p}{\delta t} = P\frac{\delta^2 p}{\delta z^2}$$

and the current at the (disc) cathode surface is given by evaluating the oxygen flux to the cathode.

The steady-state limiting current, i_L, is given by

$$i_L = \frac{nF\pi R^2 p_s}{d_e / P_e + d_m / P_m} \quad (6)$$

where P_m and P_e are the oxygen permeabilities in the membrane and electrolyte layer respectively, p_s is the prevailing P_{O_2} in the sample, n is the number of electrons involved in the reaction, F is Faraday's constant, R is the radius of the disc electrode and d_e and d_m are the thicknesses of the electrolyte and membrane layers, respectively.

Since, in practice, $d_m \approx 20$ μm, $d_e \approx 5$ μm, $P_m \approx 8 \times 10^{-11}$ $m^2\ s^{-1}\ atm^{-1}$ and $P_e \approx 2.7 \times 10^{-9}\ m^2\ s^{-1}\ atm^{-1}$, it is clear that the ratio d_m/P_m is much greater than d_e/P_e and eqn. (6) reduces to

$$i_L = nF\pi R^2 \frac{P_m P_s}{d_m} \quad (7)$$

Eqn. (7) describes adequately the steady-state behaviour of macro-disc gas-phase P_{O_2} electrodes, but the current generated by this equation fails, by at least an order of magnitude, to approach those measured experimentally for *in vitro* blood-gas electrodes which have cathode diameters of the order of 20 μm. In this case, 'edge diffusion' of oxygen towards the cathode surface is of paramount importance, and models employing hemispherical or cylindrical polar coordinates have to be employed.

One-dimensional hemispherical diffusion model. A simplified one-dimensional diffusion model which has been used to describe the behaviour of microdisc electrodes is the semi-hemispherical model. Hahn,[14,35] in 1974, described such a model which included a first-order reaction rate term to describe the reduction of oxygen at the cathode surface, and so allow the model to generate voltammograms. A schematic diagram of this model is shown in Fig. 7, which also describes the sensor parameters. Eqn. (5) in this instance is

$$\frac{\delta p}{\delta t} = P\left(\frac{\delta^2 p}{\delta r^2} + \frac{2}{r} \times \frac{\delta p}{\delta r}\right)$$

and the current is derived from half the oxygen flux at the cathode surface. In the limiting current case, where all the oxygen molecules reaching the cathode surface are immediately destroyed (*i.e.*, the micro-disc cathode is polarised sufficiently negative enough for the reaction current to be diffusion-limited), then the sensor current equation simplifies to

$$i = \frac{2\pi nF p_s}{\frac{d_m}{P_m r_m r_e} + \frac{d_e}{P_e r_e R}} \quad (8)$$

This model is obviously not a true representation of physical reality, but its analytical simplicity enables the effects of varying parameters such as R, d_e, d_m, P_m and P_e to be investigated with ease. The sensor currents generated by this simple theoretical model, for micro-disc diameters of the order of 20 μm, can agree closely with those measured by experiment, if the electrolyte layer thickness (d_e) is assumed to be about 5–10 μm.

This simple hemispherical model (excluding the reaction rate terms) was replicated by Ultman *et al.*[36] in 1981, where it can be seen that their eqn. (5) is identical with that published by Hahn previously.[4,35] They later extended the steady-state hemispherical model to a three-layer model, where a flowing liquid medium constituted the third layer.[37]

Linek *et al.*[27] relaxed the steady-state constraint, previously applied to the three-layer hemispherical model, and developed a series of much more complex time-dependent equations which described the sensor current response to a step-change in the oxygen concentration in front of the sensor membrane (*i.e.*, in the liquid layer). These equations are fully described in their book, and they also describe Clark sensor current relationships (for macro-cathodes) when there is disturbed four-electron stoichiometry of the oxygen reduction process at the cathode surface.[27]

Two-dimensional cylindrical diffusion models. The preceding models, based on oxygen transport by one-dimensional diffusion processes, produce analytical solutions, but they do not describe the real electrochemical situations occurring in sensors with micro-cathodes. The contribution of 'edge', or radial, diffusion towards the micro-cathode is best described by a two-dimensional oxygen diffusion model, which is based on a combination of two one-dimensional diffusion oxygen flows in mutually perpendicular directions. This model involves solving the Fick equation in two separate layers, the first layer comprising the membrane, with diffusion taking place perpendicular to the cathode surface, and the second layer comprising the electrolyte solution, with oxygen diffusing radially towards the micro-cathode surface. This two-layer model is described schematically in Fig. 8, and was proposed by Hahn[14,35] in 1974 as a one-dimensional representation of a micro-electrode oxygen sensor. Again, this early model incorporated a first-order electrochemical reaction, and was used to generate voltammograms describing the overall sensor current–polarising voltage relationship. The success of this model lies in the fact that since P_m is much less that P_e, then the steady-state equations in the two layers can be described by

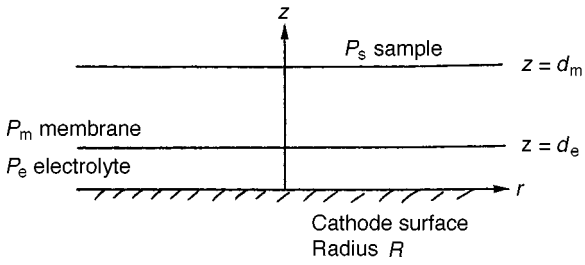

Fig. 6 Coordinate system for a one-dimensional diffusion model for a macro-electrode P_{O_2} sensor, with a large cathode (radius $R \gg d_e$; $R \gg d_m$) and electrolyte and membrane thicknesses d_e and d_m, respectively.

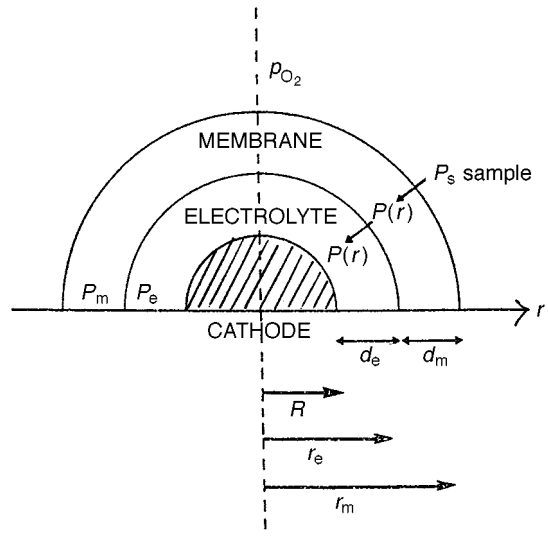

Fig. 7 Schematic diagram of the hemispherical diffusion model for a P_{O_2} sensor, with cathode, electrolyte layer and membrane radii R_c, r_e and r_m, respectively. The electrolyte and membrane layer thicknesses are d_e and d_m, respectively.

$$m + e > z > e: \quad P_m \frac{d^2p}{dz^2} = 0$$

$$e > z > 0: \quad P_e\left(\frac{d^2p}{dr^2} + \frac{1}{r} \times \frac{dp}{dr} + \frac{d^2p}{dz^2}\right) = 0$$

$$z = 0, r \geq R: \quad \frac{dp}{dz} = 0$$

$$z = 0, r \rightarrow \infty: \quad p = p_s$$

$$z = 0\ r \leq R: \quad D_e \frac{dp}{dz} = kp(r, 0)$$

$$z = m + e: \quad p = p_s \text{ all } r$$

$$z = e: \quad P_m\left(\frac{dp}{dz}\right)_m = P_e\left(\frac{dp}{dz}\right)_e$$

where k is the reaction rate at the cathode surface.

The sensor current is given by

$$i = 2\pi nFk \int_0^R p(r, 0) r dr \tag{9}$$

but analytical solutions to these equations are unmanageable, and so the simultaneous equations must be solved by digital simulation techniques. Once more, this type of model was greatly extended and modified by Linek *et al.*,[27] particularly for use with macro-cathodes which could be used for oxygen gas analysis.

Later, Jenson *et al.*,[38] in 1978, made an important contribution to this model when they solved, and balanced, the contributions from the membrane and electrolyte layers, to produce a simplified analytical solution for the electrode current given by

$$i = nF\pi R^2 \frac{P_m}{d_m} p_s \left[1 + \frac{2}{x_R} \times \frac{K_1(x_R)}{K_0(x_R)}\right] \tag{10}$$

The first part of this relationship:

$$nF\pi R^2 \frac{P_m}{d_m} p_s \tag{11}$$

represents the current corresponding to one-dimensional diffusion through the membrane, *i.e.*, eqn. (7). The second part of the equation:

$$2\pi nFR^2 \frac{P_m}{d_m} p_s \frac{K_1(x_R)}{x_R K_0(x_R)} \tag{12}$$

corresponds to the current due to the radial flux of oxygen through the electrolyte, and it is this term which dominates for $R < d_m$. Linek *et al.*[27] have also further modified this relationship to compensate for the possible over-estimation of the importance of the radial contribution to the total oxygen flux by the assumption of an infinitely rapid axial diffusion of oxygen through the electrolyte layer. They circumvented this drawback by introducing a 'resistance' to the diffusion in the *z*-direction, and produced modified equations to describe their compensation technique.[27]

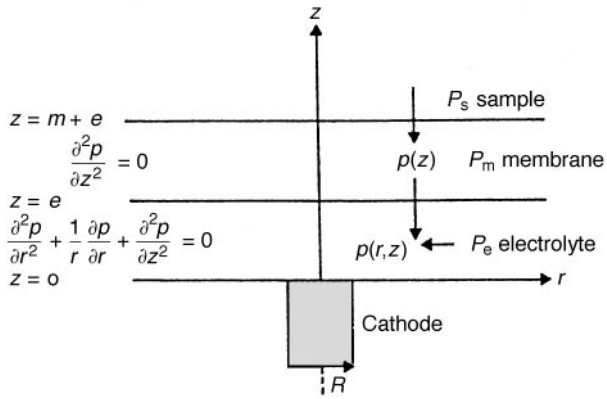

Fig. 8 Co-ordinate system of the cylindrical diffusion model for a P_{O_2} sensor, with micro-disc cathode (radius *R*) and electrolyte and membrane layer thicknesses *e* and *m*, respectively. Diffusion in the membrane is assumed to be one-dimensional.

Time-dependent models

In most situations, the Clark P_{O_2} sensor is continuously polarised and therefore any time-dependence in the Fick equation [eqn. (5)] normally refers to step changes in the sample P_{O_2} at the face of the membrane. However, in other instances, a step change is forced on the polarising voltage (*i.e.*, pulse chronoamperometry), from a potential where oxygen is not reduced to a potential where oxygen is fully reduced. In this case it is assumed that the sample P_{O_2} (p_s) remains constant at the membrane interface, and the Fick equation [eqn. (5)] is then solved, in two or more layers, to produce the sensor pulse amperometry current–time response. These two different time-dependent models will be considered separately.

Step-change in P_{O_2}. In this situation, a step-change in p_s is imposed at the membrane surface, and the Fick equation is (usually) solved for the one-dimensional case (*i.e.*, applying to gas-phase Clark sensors with large cathodes). Hitchman[13] analysed this situation, again making the assumption that the effect of the electrolyte layer may be ignored. His solution for the electrode current at time *t* after a step change in the sample P_{O_2} is given by

$$\frac{i(t) - i_0}{i_\infty - i_0} = 1 + 2\sum_{n=1}^{\infty}(-1)^n \exp\left(-\frac{n^2\pi^2 D_m t}{{d_m}^2}\right) \tag{13}$$

where i_0 is the electrode current immediately preceding the step change in P_{O_2} and i_∞ is the final current reached, *i.e.*, the steady-state current. Considering the simplest example, where the oxygen step change is from zero to p_s, eqn. (13) simplifies to

$$i(t) = nF\pi R^2 p_s \frac{P_m}{d_m}\left[1 + 2\sum_{n=1}^{\infty}(-1)^n \exp\left(-\frac{n^2\pi^2 D_m t}{{d_m}^2}\right)\right] \tag{14}$$

and for large *t* approaches the same steady-state value as given in eqn. (7).

Eq. (14) is the simplest example of a Clark sensor time response for a step change in sample P_{O_2}, and when solving this equation workers normally assume four-electron (*i.e.*, $n = 4$) stoichiometry for the reduction of oxygen at the cathode, and therefore take no account of disturbed stoichiometry or multi-dimensional diffusion. These extra complications have been described, however, in some depth, in the book by Linek *et al.*,[27] where models with hemispherical and cylindrical coordinates are considered. Furthermore, these more complex models have also been adapted by Linek *et al.* to take account of the influence of the various combinations of the electrochemical reactions ($2 \leq n \leq 4$) taking place within the electrolyte layer.

Pulse amperometry. The 'switch-on' current transient, which occurs when the polarising voltage is pulsed, is the most complex of all the models.

An analytical description of the sensor current transient, following the polarising voltage switch-on, for the one-dimensional diffusion model described by Fig. 6, was first described by Mancy *et al.*[39] in 1962. They produced analytical current-time solutions for a macro-cathode membrane-covered

Clark sensor, with a steady sample P_{O_2} at the membrane surface. Their analytical solutions can be broadly divided into three time intervals, namely at very short times when oxygen diffusion was limited to the electrolyte layer alone, at intermediate times when the diffusion layer had entered the membrane and there was joint transport control and, finally, at long time intervals when the diffusion layer had spread right into the membrane and was approaching the outer face of the membrane. By noting that, in practice, $d_e < d_m$ and $D_m \approx 10^{-2} D_e$, the Mancy solutions can be summarised as follows.[14]

(1) At very short time intervals, diffusion in the electrolyte layer alone is rate limiting and the sensor current is given by

$$i = nF\pi R^2 \left(\frac{D_e}{\pi t}\right)^{1/2} p_s \alpha_e \left[1 + 2\sum_{n=1}^{\infty} \exp\left(\frac{-n^2 d_e^{\,2}}{D_e t}\right)\right] \quad (15)$$

where α_e is the oxygen solubility in the electrolyte layer. Since d_e is typically 5–10 μm and d_m is typically about 20 μm, then eqn. (15) will only hold for $t < 0.1$ s, since the diffusion layer must be confined to the electrolyte film. Under these conditions,

$$i \approx nF\pi R^2 \alpha_e \left(\frac{D_e}{\pi t}\right)^{1/2} p_s \quad (16)$$

(2) At short time intervals, when the diffusion layer has entered the membrane (but has not reached the face of the membrane adjacent to the sample) there is joint transport control and the sensor current is given by

$$i = nF\pi R^2 \left(\frac{D_m}{\pi t}\right)^{1/2} \alpha_m p_s \left[1 + 2\sum_{n=1}^{\infty} \exp\left(\frac{-n^2 d_m^{\,2}}{D_m t}\right)\right] \quad (17)$$

This equation can be reduced to a simpler form by noting that typically $b \approx 20$ μm and $d_m \approx 10^{-3}$ m^2 s^{-1}, and thus the sensor current can be approximated to

$$i = nF\pi R^2 \alpha_m \left(\frac{D_m}{\pi t}\right)^{1/2} p_s \quad (18)$$

Eqns. (16) and (18) are similar because, in both cases, diffusional transport is controlling.

(3) At long time intervals, the diffusion layer will have spread right into the membrane and will be approaching the outer face of the membrane. The current will therefore be approaching its steady-state value, and under these conditions Mancy *et al*. gave the current as

$$i = nF\pi R^2 \frac{P_m}{d_m}\left[1 + 2\sum_{n=1}^{\infty} \exp\left(\frac{n^2\pi^2 D_m t}{d_m^{\,2}}\right)\right] \quad (19)$$

and the steady-state current, when the exponential term becomes negligible, is therefore

$$i = nF\pi R^2 \frac{P_m}{d_m} p_s \quad (20)$$

This is exactly the same solution as given by eqns. (7) and (11) when transport in the membrane only is of importance.

Fig. 9 shows a theoretical plot of sensor current against time,[13] together with the details of the section of the time response corresponding to the Mancy equations. The values for the physical parameters of the electrode used in this model are given in the book by Hitchman.[13]

Eqns. (16) and (18) are expressions of the well known Cottrell equation, and Myland and Oldham[40] later reworked the Mancy theory to describe the current–time behaviour and discussed the role of the various geometrical, transport and solubility factors which could affect this behaviour. Again, this analysis was confined to one-dimensional diffusion (*i.e.*, macro-cathodes) and the Myland and Oldham equations were analysed to predict the duration of the sensor Cottrellian behaviour, and the interval before the onset of the steady-state sensor current, for gas-phase or liquid-phase sensors. Although both Mancy *et al*.[39] and Myland and Oldham[40] made it clear that their theory was applicable only to one-dimensional diffusion in all three layers, their theoretical predictions have been mistakenly extended to include membrane-covered micro-disc electrodes for which diffusion is two-dimensional. The reason for this is possibly because both *unshielded micro-disc* electrodes and *shielded macro-disc* electrodes demonstrate a Cottrellian transient, following a step change in potential. This makes it tempting to assume implicitly that the same behaviour will follow when a micro-disc electrode is shielded by a membrane.[41] Unfortunately, this supposition is erroneous, and we cannot extrapolate the (one-dimensional) Mancy theory to the type of microdisc cathode Clark P_{O_2} sensor used in medicine and biology.[41]

This is unfortunate, since inspection of eqn. (16) reveals important potential theoretical advantages for a pulsed Clark sensor. Eqn. (16) shows that the sensor current should be proportional to $t^{-\frac{1}{2}}$ for the short time intervals before the diffusion layer reaches the membrane, and therefore a plot of i against $t^{-\frac{1}{2}}$ should reveal a straight line of zero slope for this time epoch. This epoch should therefore define the time span (after the onset of the pulse) during which the sensor current is independent of the membrane characteristics. If the sensor characteristics were to be confined to electrolyte parameters only (or, at the worst, only weakly dependent on the membrane), then a thin membrane which has a fast time response to changes in oxygen concentration could be employed with the sensor. This ideal and theoretical scenario (*i.e.*, membrane independence) would reduce sensor calibration drift, reduce the problem of membrane fouling, which can ruin sensor performance, and minimise (or theoretically eliminate) the problem of the blood-gas difference effect. Another crucial advantage would have been that the sensor could be used as an absolute measuring device [*i.e.*, current output could be determined from a knowledge of the physical parameters of the sensor contained in eqn. (16)]. It could therefore be calibrated in the gas phase and then used in the blood phase without need of further recalibration, because the current output would be independent of the membrane material or properties such as ageing, stretching.[41] This would be particularly advantageous for intravascular, or *in vivo* sensors, of the type described in the *in vivo* analysis. Unfortunately, all these ideal attributes have proved to be wishful thinking for the micro-cathode Clark sensors used in clinical medicine. There are two reasons for this.

First, membrane-covered Clark sensors, when pulsed in practical situations, produce a non-Faradaic current for an ill-

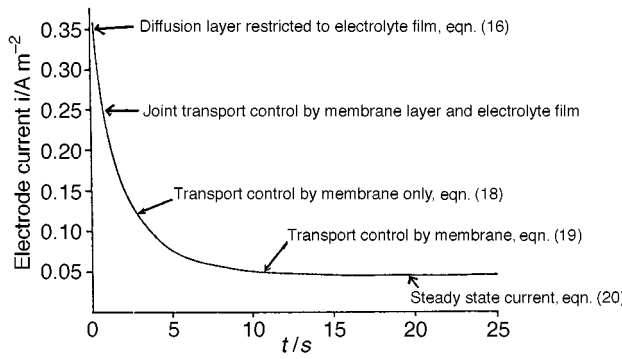

Fig. 9 Variation of current per unit area with time for the one-dimensional macro-cathode P_{O_2} sensor theory. The values for the physical parameters of the electrode model are given in the text. (Taken from ref. 13.)

defined time epoch immediately following the voltage pulse.[41] The important thing to note here is that an *aqueous* solution has been used as the electrolyte and that a four-electron reaction has been assumed to take place. Experiments conducted with either macro- or micro-cathode membrane-covered Clark sensors indicate that the simple 'Faraday current only' assumption, common to all the one- and multi-dimensional mathematical models, breaks down in real practice. This phenomenon has been reported time and again, and various suggestions for this non-Faradaic time current have been put forward, including the electrode resistance–capacitance discharge time constant, sensor geometry and design and the simultaneous electrochemical reduction of some other species in the electrolyte.[14,27,41] The fact that the same micro-disc sensor with the same membrane, but with a non-aqueous solvent such as dimethyl sulfoxide (DMSO) used as the electrolyte solution,[41] does not demonstrate the long-lasting non-Faradaic current phenomena when pulsed might indicate that the problem lies with disturbed four-electron reaction stoichiometry. However, since this long-lasting current is still seen when oxygen is totally excluded from the sensor (*i.e.*, when the sensor is pulsed in the presence of nitrogen only), the problem cannot simply be due to disturbed reaction stoichiometry. The non-Faradaic current problem, observed using aqueous electrolytes, is still unresolved.

Second, and very important, the failure of the micro-cathode Clark sensor to display Cottrellian behaviour at *any* time point during the duration of the voltage pulse is due to the radial diffusion of oxygen to the micro-cathode through the electrolyte layer. Gavaghan and co-workers[41–44] have reworked the three-layer cylindrical diffusion model, using digital simulation techniques to describe the relationship of the sensor current to micro-cathode size and electrolyte and membrane layer characteristics, when the polarising voltage is pulsed. Their solution involved solving the Fick equation:

$$\frac{\delta p}{\delta t} = P\left[\frac{\delta^2 p}{\delta r^2} + \frac{1}{r} \times \frac{\delta p}{\delta r} + \frac{\delta^2 p}{\delta z^2}\right]$$

in the separate electrolyte, membrane and sample layers using similar boundary conditions to those shown in the steady-state cylindrical diffusion model section, with the exception that the partial pressure at the micro-disc cathode was now taken to be zero immediately following the polarising voltage switch-on.

Also, the previous constraint that diffusion was one-dimensional in the membrane layer was relaxed, and diffusion was treated as two-dimensional in this layer—as in the sample and electrolyte layers. All equations, in all layers, were solved numerically and simultaneously as functions of time, *t*. The sensor time-varying current, in this case, is given by

$$i(t) = 2\pi nFP_e \int_0^R \left(\frac{\delta p}{\delta z}\right)_{z=o} r\,dr \qquad (21)$$

Gavaghan and Rollett[45] used an alternating direction implicit (ADI) method to solve the simultaneous equations in a uniform rectangular mesh superimposed on the finite region of Fig. 10, which was then matched to a truncated series solution in the region of a singularity which occurs at the cathode edge. The boundary conditions on $z = 0$ ensure that there is a discontinuity in the first derivative of the oxygen partial pressure at the point $r = R$, $z = 0$, and this forms a 'boundary singularity' at this point. The numerical simulation of these equations show that the i-$t^{-½}$ behaviour for a micro-disc cathode (diameter $< 100\ \mu m$) is decidedly non-Cottrellian for all t values. The conclusion of this work was therefore that analytical solutions to the Fick diffusion equation would not produce predictions which matched practice and that, for the type of micro-disc Clark sensors used in medicine and biology, it is necessary to resort to less transparent numerical computations in order to predict the electrochemical sensor behaviour.

This becomes even more important when predicting the behaviour of a Clark P_{O_2} sensor when it is switched both on and off with a variable duty cycle. Gavaghan *et al.*[44] later extended their work to produce a theoretical computer simulation of this practical situation, and it is clear that future electroanalytical chemistry models describing non steady-state membrane-covered micro-disc cathode behaviour must be based on computer simulation and not on analytical expressions.

In-vivo *analysis*

As stated in the Introduction, there is still disagreement between clinicians over the clinical necessity, or cost effectiveness, of measuring P_{O_2} in the blood with intravascular sensors. Since the use of an *in vivo* sensor necessitates the electrochemical transducer into a patient's artery, such measurements are not undertaken lightly and there must be compelling reasons for performing such measurements. Historically, such a compelling reason arose from the need to monitor, on-line, the arterial P_{O_2} in newborn infants suffering from respiratory distress.[46–48] Arterial P_{O_2} measurements are particularly important in the neonatal period (the first month of life) since a high arterial P_{O_2} (> 11.3–13.3 kPa) will lead to damage to the capillaries of the retina in the eye, producing irreversible blindness (retrolental fibroplasia), and too low a P_{O_2} (< 7.3 kPa) can lead to tissue hypoxia and brain damage. Early intravascular P_{O_2} electrodes were therefore designed with infants, and not adults, in mind.[48] The size of these electrochemical sensors, typically 1.4 and 1.7 mm od, presented no great difficulty in neonatal work, since they could be inserted safely into the umbilical artery of the infant. However, these sensor sizes would present great difficulty for safe insertion into an adult artery. Intravascular P_{O_2} sensors small enough to be inserted into adult arteries (0.6 mm diameter) were produced later,[49] but their use has been severely limited to those centres which have the technical expertise to calibrate them and use them.

The above ethical and practical reservations do not apply to physiological investigations in non-human subjects, and research into the development of *in vivo* animal P_{O_2} sensors began, remarkably, immediately following the original Clark publications. In the late 1950s, Kreuzer and Nessler[50] published a description of a catheter-tip P_{O_2} sensor, using a 0.8 mm diameter cathode in a polyethylene catheter, with a thin Teflon membrane held on the end with a stainless-steel ring. The ring

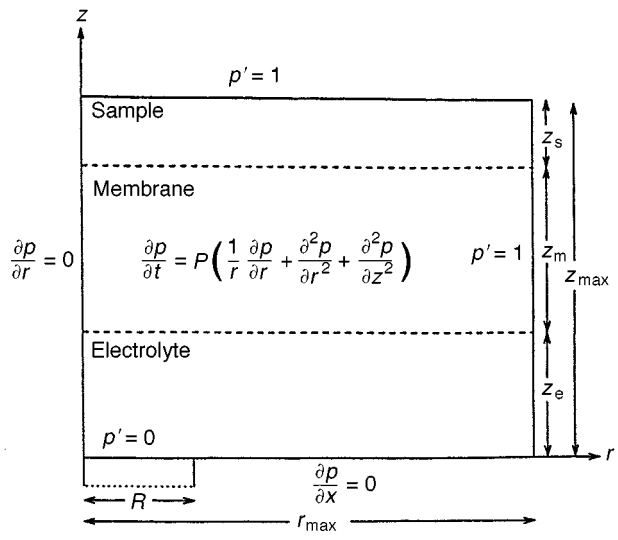

Fig. 10 Two-dimensional solution region and equations used to model the time-dependent oxygen diffusion processes in a membrane-covered micro-disc cathode P_{O_2} sensor. The oxygen partial pressures have been normalised to $p' = p/p_s$, where p_s is the initial sample partial pressure of oxygen.

did not provide enough tension on the membrane, making it sensitive to arterial blood pulse pressure. One year later, Krog and Johanson[51] described an intravascular sensor constructed with platinum and silver electrodes, on the tip of a cardiac catheter, and using Teflon as the membrane. Following these pioneering efforts, a multitude of intravascular P_{O_2} sensors were devised, mainly for use in animals, but sometimes used in human studies lasting several hours.[52] Although these early catheter electrodes were plagued by pressure sensitivity, this sensitivity was finally eliminated by the use of membranes tightly stretched over the cathode. The construction and development of these devices, up to the 1980s, has been described by Kreuzer *et al.*[25] and by Hahn.[15]

There has not been much progress in this field since the 1980s, perhaps owing to a combination of several disparate factors. First, there is the inevitable clinical reluctance to use intravascular devices which can cause blood clotting in an artery and create thrombosis, or introduce a potential source of infection. Other major practical problems include thrombous formation on the sensor membrane itself, which degrades sensor performance, the slow response time of the biocompatible membrane material, sensor sensitivity to blood flow, effects of variations in the patient's temperature on the sensor output characteristics and the sheer magnitude of the problems associated with calibrating the sensor *in vivo*.

Most of these problems, including those of calibration, have still to be overcome. *In vitro* electrochemical sensors, built into bench blood-gas analysers, can be calibrated at will and their performance can be checked and monitored by quality control material. This is not the case for an intravascular electrochemical sensor, which must be calibrated *in situ*. This will involve taking an arterial blood sample from the patient, inserting it into a 'gold standard' bench analyser and then inserting the true P_{O_2} value into the electrochemical control instrumentation controlling the intravascular sensor. Out of sheer necessity, this will involve a 'single-point' calibration, and the clinician will have no idea whether this is adequate or not, or whether the transducer is truly linear in its response to changes in P_{O_2}.

The question of time response is also difficult to deal with. A membrane which is highly permeable to oxygen will give a fast time response (say of the order of 1 s), but this will increase the oxygen flux through the membrane very considerably and will therefore introduce a large blood-gas difference effect. This, in turn, will make the sensor highly sensitive to blood flow across the membrane face. A less permeable membrane will reduce this flow effect, but will inevitably lead to a long time response to changes in arterial P_{O_2}. An inevitable compromise therefore has to be struck, and a material such as polyethylene or polystyrene is chosen as the membrane, in order to minimise the flow effect. The usual consequence of choosing this material is that the sensor response time to a step change in P_{O_2} is typically of the order of 60–90 s to 95% of the final response.[15] This is certainly too slow to follow fast physiological changes, but will provide an indication of slower trends in arterial P_{O_2}, over periods of several hours.

As explained in the mathematical model section, the possibility of using pulse amperometry to control the electrode has not proved successful in practice. Theoretically, pulsing would appear to be an ideal electrochemical control modality, but the non-Faradaic current following the onset on the pulse appears to render the technique useless for present-day intravascular sensors which use aqueous electrolyte layers.[41]

The development of electrochemical intravascular P_{O_2} sensors has therefore remained fairly static over the past decade, with little progress being made either in their utilisation or development. Two sensors which have stood the test of time are those first described by Parker and co-workers[46,47] in 1971 and Mindt and co-workers in 1973[49] and 1979.[53] These are shown in Figs. 11 and 12, since they illustrate two separate approaches to sensor design. Fig. 11 shows the Parker dip-coated catheter P_{O_2} sensor, built into a bi-lumen poly(vinyl chloride) (PVC) catheter. The electrode is at the tip of one lumen of this double-lumen tube, and a rubber-modified polystyrene membrane is dip-coated on to the tip. Prior to this dip-coating, KCl crystals are dip-coated on to the cathode-reference surface and the electrode is sterilised by gamma irradiation after packing and stored dry. The cathode and the anode are silver. The sensor in activated *in situ* by water transport across the membrane, after it has been inserted into the artery. The second lumen is used to obtain blood samples, which are then introduced into the bench blood-gas analyser to calibrate the catheter sensor. The outside diameter of the sensor is either 1.4 or 1.7 mm, making this sensor suitable for insertion into the umbilical artery of an infant. The characteristics and time response of this sensor could best be described by linear one-dimensional diffusion.

A later variation in intravascular sensor design was that of Mindt and co-workers,[49,53] illustrated in Fig. 12. The geometry of this sensor was completely different to that of the conventional end-on sensors. The Mindt sensor obeys cylindrical diffusion, since the catheter body itself forms the membrane, and the end of the sensor is designed to be thick enough to be relatively impermeable to oxygen, as illustrated in Fig. 12. Both the anode and the cathode are fine silver wires, and the electrolyte solution is sealed into the catheter with epoxy resin. Hahn[15] described the steady-state oxygen transport equations for this type of sensor, and derived the sensor current output as

$$i = \frac{2\pi nFLp_s}{\left[\ln(r_2/r_1)/P_e + \ln(r_3/r_2)/P_m\right]} \qquad (22)$$

where r_1 is the wire cathode radius, L is the wire cathode length, r_2 is the inner catheter membrane radius and r_3 is the outer membrane radius.

The simplicity of the Mindt design and the stability of its performance characteristics have ensured its longevity. It is

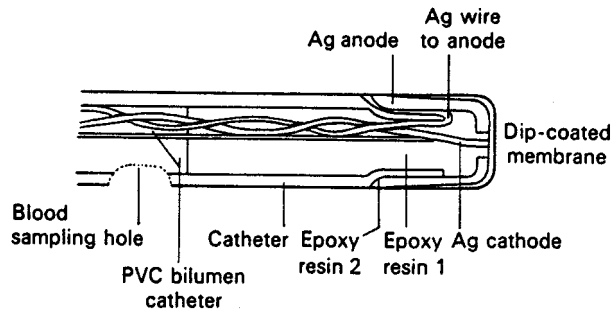

Fig. 11 Dip-coated catheter P_{O_2} sensor, built into a bi-lumen PVC catheter. One lumen houses the electral connections to the sensor and the other lumen is used to obtain blood samples for calibrating the sensor *in situ*.

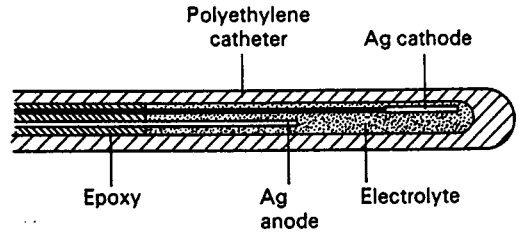

Fig. 12 *In vivo* P_{O_2} sensor which uses a polyethylene catheter as the membrane. Both the anode and cathode electrodes are silver wires, and the electrolyte solution is permanently sealed into the catheter body. A solid 'plug' at the catheter tip ensures that only radial oxygen diffusion reaches the silver wire cathode.

commercially available for use in adult human monitoring, since its outside diameter is 0.65 mm. Its other advantage is that its electrolyte is in liquid form, and the electrode does not need to be activated *in situ*.[53] Sterilisation is achieved by gamma irradiation, as with the Parker design.

One important factor to note about the above two sensors is that they both use silver as the cathode material. This presents another, previously unmentioned, problem concerning the use of intravascular P_{O_2} sensors. Since these sensors are only going to be used, out of clinical necessity, with severely ill patients, it is almost inevitable that they are going to be used in some patients who have recently been anaesthetised, or are undergoing anaesthesia. Two of the commonly used anaesthetic agents, nitrous oxide (dinitrogen oxide) and halothane, have been discovered to be extremely electroactive on silver cathode surfaces, and either of these agents can give rise to a 'pseudo-oxygen' signal on the sensor display system. This therefore causes the sensor to 'fail dangerously', and this topic will be dealt with in the Anaesthetic agents section.

Since the electrochemistry of the catheter tip intravascular sensors is identical with that for the *in vitro* sensors, there is no new electrochemistry to add here. Because the electrochemistry is established, the haemo-compatibility of the intravascular sensor itself remains the key chemical problem to be solved, if these sensors are to realise their clinical potential. The most important unresolved component of an intravascular sensor is the membrane which is in direct contact with the blood. It is therefore important to try to ensure that the various blood components (water, proteins, red blood cells, white blood cells, platelets and ions) do not react with the membrane surface, and so alter its characteristics. This problem has still not been resolved successfully over the past four decades, but perhaps the most significant recent advance in this area has been made by Zhang and co-workers.[54,55] This group has experimented with coating conventional intravascular P_{O_2} sensors with a novel copolymer, poly(MPC-co-BMA), with an electrically neutral head group, 2-methacrylyloxyethyl phosphorylcholine (MPC) copolymerised with butyl methacrylate (BMA), which mimics the lipid bilayer structure of the red cell membrane. This material, and some other biomembrane mimetic polymer surfaces,[55] can significantly reduce protein absorption, platelet adhesion and thrombous formation. Zhang and co-workers[54,55] therefore coated two types of intravascular sensor, one based on the conventional intravascular sensor (Fig. 11) and the other based on the Mindt design (Fig. 12) with poly(MPC-co-BMA) and showed dramatic improvements in sensor performance when compared with similar sensors not coated with the copolymer. Scanning electron microscopy showed that sensors coated with MPC-co-BMA were effectively free of absorption of blood components on their surfaces.[56] Since MPC-co-BMA has poor mechanical properties, it was coated on to conventional intravascular sensor membranes such as polyetherurethane (PU) or PVC. An up-to-date account of the haemo-compatibility of various invasive sensors can be found in a recent review by Benmakroha *et al*.[57] If these recent developments do prove successful, there may well be a resurgence in the interest of developing intravascular P_{O_2} sensors, especially if the sensor response time can be reduced to $\leqslant$1 s by using high-permeability membranes, and then reducing the blood-gas, or flow effect, by means of electrochemical control techniques such as pulse amperometry or high-speed voltammetry, where the electrochemistry only occurs for a defined period and is then switched off during a relaxation period.

Transcutaneous sensors

Gerlach,[58] in 1851, investigated experimentally the O_2 and CO_2 gas exchange between skin and ambient air in animals and humans. One hundred years later, Baumberger and Goodfriend[59] reported the determination of arterial P_{O_2} in humans through the intact skin, by immersing a finger into a phosphate buffer solution at 45 °C and measuring its P_{O_2} using a dropping-mercury electrode. These findings were confirmed in 1957 by Rooth *et al*.,[60] who used a large bare platinum electrode to measure the P_{O_2}.[59] A decade later, Huch *et al*.[61] showed that after drug-induced hyperaemia, P_{O_2} values very close to arterial oxygen partial pressures could be measured with surface P_{O_2} sensors on the skin of newborn babies. Practical P_{O_2} sensors were then developed using electrical heating to warm the skin to 42–43 °C, and these first P_{O_2} sensors were called 'transcutaneous' sensors.[62,63] The sensor (shown schematically in Fig. 13) is basically a Clark sensor housed behind a membrane, which also incorporates an electrical heating element to warm the skin. Two thermocouples measure the temperature of the heating element and the temperature of the skin, to ensure that the patient is not burned.

Later developments of this sensor led to the development of a similar sensor for CO_2[64] (based on the Stow–Severinghaus sensor) and then to the development and testing of combined O_2–CO_2 transcutaneous sensors,[65] as shown in Fig. 14.

Despite the obvious, and attractive, advantages that such sensors do not involve taking a blood sample from the patient or inserting a catheter into an artery, they have proved to be successful only in babies and small children. The potential risk of burning the patient is always present, and the location of the sensor needs to be changed every 2 h or so, to ensure that a blister-forming injury (second degree burn) does not form. Obviously, the incidence of burns is a function of the electrode temperature and the length of time the electrode is left in the same location on the patient's skin. However, the P_{O_2} measured

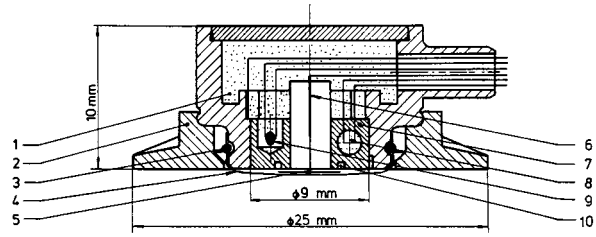

Fig. 13 Schematic cross-sectional diagram of a transcutaneous P_{O_2} sensor, showing the main constituent parts. The platinum cathode is typically 20 μm in diameter. A small heating element and an NTC resistor are located inside the silver anode for measurement and control of the sensor temperature. 1, epoxy resin; 2, retaining ring; 3, O-ring; 4, Teflon membrane; 5, cuprophane spacer; 6, platinum cathode; 7, silver anode; 8, heating element; 9, NTC resistor; and 10, electrolyte chamber. (From Friis Hansen, B., Marstrand-Christiansen, P., Vesterager, P., and Jacobsen, E., *Scand. J. Clin. Lab. Invest.*, 1976, **37**, Suppl., 146.)

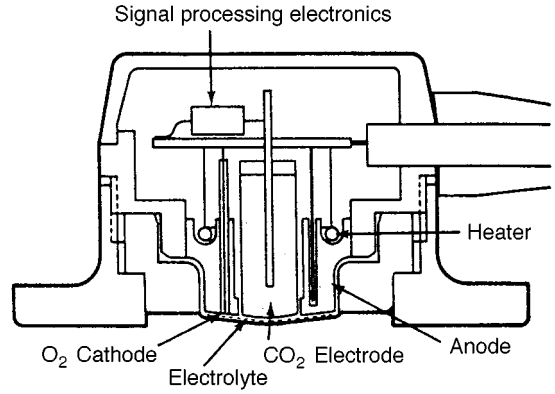

Fig. 14 Schematic diagram of a combined O_2–CO_2 transcutaneous sensor. (From Mahutte, C. K., Michiels, T. M., Hassel, K. T., and Trueblood, D. M., *Crit. Care Med.*, 1984, **12**, 1063.)

by a transcutaneous sensor not only reflects the arterial blood P_{O_2}, but is also influenced by physiological changes such as peripheral blood perfusion and the core temperature of the patient. When the patient is in shock, or is hypothermic, the transcutaneous P_{O_2} measurement no longer correlates with central arterial P_{O_2}, and the device will under-read the arterial P_{O_2}. This can be potentially dangerous with some patients, since the transcutaneous P_{O_2} may indicate that the patient is hypoxic (because the sensor is under-reading the true P_{O_2}) and the patient might then be given added oxygen to breath. For chronically sick patients with some respiratory diseases, this procedure can endanger the patient's life.

Hence, the transcutaneous sensor is not used routinely with adults, and its use has been mainly confined to special care baby units. Since the electrochemical principles of the Clark O_2 and Stow–Severinghaus CO_2 electrodes contained in transcutaneous sensors have already been described in previous sections, no further comments will be made on this particular type of sensor.

Anaesthetic agents

Two inhalational anaesthetic agents, nitrous oxide (dinitrogen oxide; N_2O) and halothane ($CF_3CHClBr$), have been found to be electrochemically active, in aqueous electrolytes, on the cathodes of Clark P_{O_2} sensors, at the polarising voltages commonly used with them. The sensor currents generated by these two anaesthetic agents have been found to be additive to that of oxygen reduction, thereby producing a 'pseudo-oxygen' current which suggests that there is more oxygen present in the sample than there is in reality. The oxygen measuring devices can therefore over-read, and this is a highly dangerous situation since a life-threatening hypoxic episode may be occurring in the patient and yet the O_2 sensor is indicating that the blood oxygen partial pressure is 'normal' or even 'high'. In fact, it was the observation of suspiciously 'high' blood P_{O_2}'s, in the presence of N_2O and/or halothane when there was a much lower inspired oxygen gas partial pressure, which alerted clinicians and scientists to the fact that conventional Clark O_2 sensors were also reducing molecules other than oxygen.[66–73]

Apart from nitrous oxide and halothane, no other inhalational agent has been found to be electroactive using the polarising voltage window available in aqueous electrolytes, and these other anaesthetic agents can be treated as 'inert' gases as far as the sensor aqueous solvent electrochemistry is concerned. Similarly, no inhalational anaesthetic agent has yet been found to interfere electrochemically with the Stow–Severinghaus P_{CO_2} sensor, since its measurement principle is based on potentiometry.

The electrochemical reduction of nitrous oxide and halothane in aqueous solvents appears to depend critically upon the metal used as the working electrode, in addition to the polarising voltage employed. This will be described in the following two sections.

Nitrous oxide

The history of the electrochemical reduction of nitrous oxide in aqueous solutions has followed a staccato pattern over the past three decades.[74–80] Early attempts to reduce N_2O with Pt, Au, Hg or Pb electrodes showed that it was either inactive, or at best gave only a transient reduction current.[81] Much of this early work was conducted in acid solutions, using macro-electrodes and in the absence of oxygen, presumably because the reduction of O_2 might interfere with the interpretation of the results obtained. During this period of initial interest, Johnson and Sawyer[80] specifically examined the electrochemical reduction of N_2O on platinum electrodes in alkaline solutions of pH 10 and 14. Their experiments did not reveal any N_2O electrode activity on Pt surfaces until the surface was deliberately pre-treated. When their platinum electrode had obtained a fresh platinum surface, formed by the prior reduction of a platinum oxide film, the reduction of N_2O was found to proceed at a polarising potential of -0.8 V (pH 14) or -0.65 V (pH 10). Their data clearly established that the N_2O molecule had to be absorbed on the Pt electrode surface before reduction would take place, and that the dependence of the N_2O reduction current on electrolyte composition was due to the change of potential of desorption towards more positive values as pH increased. They therefore proposed that the reduction of N_2O on a PtO surface followed the sequence[80]

$$PtO + 2e^- + H_2O \rightarrow Pt^* + 2OH^- \quad (23a)$$

$$Pt^* + N_2O \rightarrow Pt(N_2O) \quad (23b)$$

$$Pt(N_2O) + 2e^- + H_2O \rightarrow Pt^* + 2OH^- + N_2 \quad (23c)$$

Overall, the process could be expressed as

$$N_2O + H_2O + 2e^- \rightarrow N_2 + 2OH^- \quad (23d)$$

This work clearly indicated that N_2 was formed as a product of the reaction, in aqueous alkaline solutions, their results corroborated those of Zagorski and Suwalski,[79] who had, a year earlier, examined the electroreduction of N_2O with a dropping-mercury electrode in both acidic and alkaline solutions. Although Zagorski and Suwalski failed to produce limiting currents for N_2O reduction, their experimental results showed clear erratic N_2O reduction current oscillations, whose amplitude grew more and more as the potential was driven more negative. They concluded that the average current intensity, at a chosen potential, was proportional to the concentration of N_2O, and we can now deduce that the reduction current oscillations were due to the formation of N_2O bubbles from the reduction process eqn. (23).

Matters seem to have lain at rest at this point, since most Clark sensors used in medicine and biology employed Pt electrodes and aqueous electrolytes with a pH close to 7.0, and Johnson and Sawyer's work had concluded that N_2O would be electrochemically inert on non-pre-treated Pt surfaces. Under these circumstances, N_2O would not be expected to be electrochemically active and, in any case, the solvent decomposition potential would be certainly too low to enable the N_2O molecule to be reduced. It was therefore clearly assumed, at that time, that N_2O was not reducible and would not interfere with blood or gas P_{O_2} measurements. However, a second wave of interest in the electrochemical activity of N_2O was already beginning to emerge, when clinicians reported that P_{O_2} measurements could, in fact, be affected by the level of N_2O in a blood or gas sample.[70,71,73] This 'interference' effect was reported[70] to be intermittently present in conventional Clark Pt cathode P_{O_2} sensors, but was found to be consistently present in an Au cathode respiratory P_{O_2} sensor. This effect, which corresponded to a 'pseudo-oxygen' signal of 7–15% (depending on the polarising voltage) in the presence of pure N_2O gas was confirmed on the examination of a Beckman LB1 O_2 gas analyser, which incorporated a Clark-type sensor with a gold cathode. When pure N_2O was introduced to the gas analyser, the analyser signal read between '1% and 5% O_2'. The most puzzling feature of this N_2O interference effect was that it appeared to occur on gold electrodes used in the clinical environment, but not on gold electrodes used in the electrochemistry laboratory. This dilemma was resolved when it was realised that the clinical sensor Ag/AgCl reference electrode was the culprit, with silver ions from the reference electrode being plated out on the working cathode of the clinical sensor.[70] Silver can be easily plated onto either Pt or Au, but the layer is so thin that it is invisible to the eye. However, this silver layer is certainly very electroactive. Detailed studies soon revealed that N_2O was reduced quantitatively in the potential region

−1.5 to −1.6 V (*versus* Ag/AgCl) on pure silver electrodes, and that when the reduction process proceeded gas bubbles began to appear on the electrode surface. It was found that the half-wave potential was independent of pH indicating that the rate-determining step in the reduction was the transfer of the first electron to N_2O and this reaction scheme was written as follows:

rate determining step:

$$N_2O + e \rightarrow N_2O^-$$

fast steps:

$$N_2O^- + H_2O + e \rightarrow N_2 + 2OH^-$$

and so the overall reaction is

$$N_2O + H_2O + 2e \rightarrow N_2 + 2OH^-$$

agreeing with Johnson and Sawyers' conclusion [eqn. (23d)].

The fact that the reaction took place on Ag, and not on Pt or Au, and that the transfer coefficient was 0.28 (*i.e.*, significantly less than 0.5), showed that the first step was not an outer-sphere electron transfer, but that the N_2O must have been absorbed on the electrode surface probably through a bond between the oxygen atom and silver.[70] This work was conducted in the presence of oxygen, and this highlights the important axiom that electrochemical experiments on clinical sensors must be conducted under those conditions which are expected to be found in clinical practice–for example, experimental results obtained in the absence of oxygen might be totally different to those obtained when oxygen is present in abundance.

These studies on the electrochemical reduction of N_2O and O_2 in the presence of each other revealed clearly separated O_2 and N_2O reduction waves,[70] as illustrated in Fig. 15, which shows voltammograms obtained with a 0.66 mm diameter Ag disc cathode, covered with a 12 μm Teflon membrane, when housed in a Clark sensor body (Fig. 4) at 25 °C. The O_2–N_2O gas mixture was varied between 0 and 100% v/v for each gas, with the other making up the balance. The net result was a series of O_2 and N_2O voltammograms as shown in Fig. 15, each with clearly identified and separated half-wave potentials and good plateaux.

The absolute size of the current is a reflection of the transport properties of the membrane for O_2 and N_2O and, apart from the number of electrons involved, the ratio of the N_2O and O_2 currents is governed by the permeabilities of these two gases in Teflon.

When the limiting currents for O_2 and N_2O (corrected in this case for the O_2 current) were plotted against the percentage of each gas in the mixture, straight line relationships were obtained showing that nitrous oxide concentration (in addition to oxygen) could now be measured electrochemically.

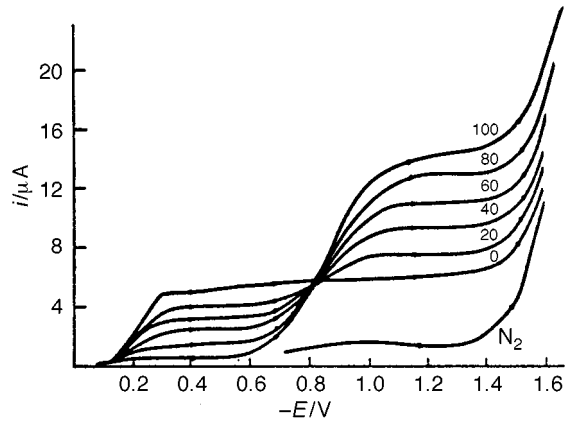

Fig. 15 Voltammograms for O_2–N_2O gas mixtures, obtained with a membrane-covered (12 μm Teflon) 0.66 mm diameter electrode, showing distinct and separate plateaux for O_2 and N_2O reduction.

Following the report which clearly indicated that N_2O could be reduced electrochemically on silver contaminated surfaces,[70] other reports then began to appear on oxygen gas analysers failing dangerously in the presence of N_2O, on blood-gas analysers becoming sensitive to N_2O and so leading to the potential non-diagnosis of arterial hypoxaemia,[71,73] and on an intravascular P_{O_2} blood sensor grossly over-reading in patients breathing O_2 and N_2O gas mixtures.[82,83] The intravascular blood P_{O_2} sensors which were commercially available in the 1970s often employed silver cathodes. Because silver has a much wider electrochemical reduction voltage window than Pt (at the same electrolyte pH), silver cathode P_{O_2} sensors were (and are) exquisitely sensitive to N_2O, if the polarising potential is not kept low enough to escape the rising portion of the second reduction (N_2O) wave (Fig. 15). Clinical reports, published at that time, which used Ag cathode intravascular P_{O_2} sensors in the presence of N_2O, appeared to be unaware of this 'fail-dangerous' phenomenon.[84]

One positive outcome of this work was that it was realised that a Clark-type sensor, employing a silver micro-disc electrode and a conventional aqueous electrolyte solution, could be used for the simultaneous determination of O_2 and N_2O in gas or blood.[70,85] Since the outcome of the N_2O reduction process was the formation of N_2 gas bubbles [eqn. (23d)], which rapidly occluded the electrode surface and prevented any further electrochemistry occurring, the Ag micro-cathode membrane-covered sensor could not be polarised in the conventional constant-potential manner. The solution to this problem was to employ a train of polarising voltages in a pulsing regime (with an appropriate duty cycle) to avoid the build-up of N_2 on the micro-electrode surface.[86] The first voltage pulse was used to reduce O_2 and the second to reduce both O_2 + N_2O. Since the simultaneous O_2 and N_2O reduction processes did not interfere with each other, the current obtained from the second pulse was simply an addition of the O_2 and N_2O reducing currents.[87] An appropriate computer algorithm was therefore employed to deconvolute the O_2 and N_2O current–concentration contributions. The determination of the appropriate duty cycle to employ under these experimental conditions can be obtained from analysis of the digital pulse simulation procedure described in the computer model simulation section.

In 1982, a novel prototype O_2–N_2O 'sandwich electrode' was described,[88] based on a metallised membrane P_{O_2} sensor by Bergman,[89–91] to measure O_2 and N_2O in gas mixtures. The outline design is shown in Fig. 16 and it comprised two separate electrochemical sensors. The first sensor was a Teflon mem-

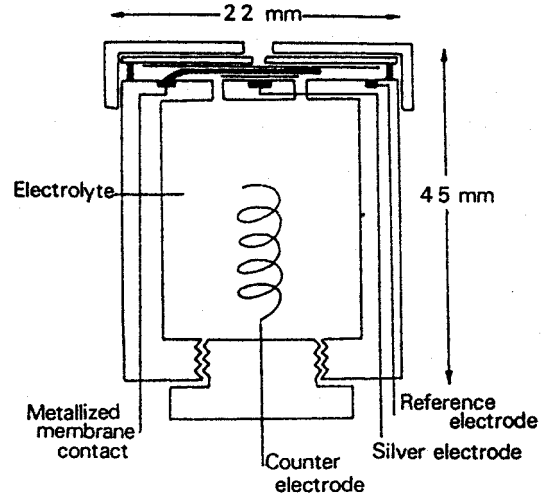

Fig. 16 Schematic cross-sectional view of a composite O_2–N_2O sandwich electrode. A nylon spacer is inserted between the metallised membrane and the inner (second) macrodisc cathode. (Taken from ref. 88.)

brane with one surface coated with a thin layer (5–10 nm) of gold, deposited by vacuum evaporation. This layer, when polarised at -0.6 V formed an oxygen filter, which effectively scrubbed all oxygen from any gas sample diffusing through the membrane. The limiting current generated at the metallised layer was proportional to the P_{O_2} of the gas sample. If the gas sample contained N_2O, these molecules passed through the membrane (since they were not reduced at −0.6 V, on Au surfaces) to the second sensor, which consisted of a silver disc cathode (3 mm diameter) which was pulsed with a regime similar to that described above for the intravascular sensors. Thus, the N_2O concentration in the gas mixture was measured by pulse amperometry at the second cathode.

It was hoped that this design would also facilitate the electrochemical measurement of halothane (see the next section) and so create a composite $O_2 + N_2O$ + halothane sensor, but technical difficulties precluded any further development, despite its success at measuring $O_2 + N_2O$ simultaneously.

More recently, there has been yet another resurgence of interest in the electrochemical reduction of N_2O on metal electrodes, but this more recent work (with one exception) has been confined to the non-clinical area.[92–96] The one exception is the discovery that N_2O can be reduced on gold micro-electrodes in aprotic media, and this will be described further in the non-aqueous section of this review.

Halothane

Halothane was the first satisfactory non-explosive and potent inhalational anaesthetic agent. It is a fluorinated hydrocarbon, liquid at room temperature, with the structural formula $CF_3CHClBr$, and was synthesised in 1951.[97] By the late 1950s, clinical evaluations of halothane showed that it was effective and safe in a wide assortment of clinical conditions and in all age groups, and its great versatility became apparent. Halothane therefore rapidly spread into worldwide use, and it is still in use today despite worries concerning its hepatotoxicity. What was not realised, in the early days following its introduction to clinical practice, was that halothane was electrochemically active on both silver and gold surfaces.

The reasons for not observing this electrochemical activity are as follows. Halothane is a large molecule, with a relative molecular mass of 197, and so the membranes used in conventional Clark blood-gas sensors, such as polypropylene and Teflon, provide a natural barrier to the potential penetration of the halothane molecule to the sensor cathode surface. Furthermore, if a conventional Pt surface is uncontaminated from the deposition of Ag^+ ions, the halothane molecule is 'reluctant' to be reduced on the 'clean' (platinum) surface. It needs a long dwell time of a blood sample, containing halothane, in the analysis chamber of a blood-gas analyser, combined with a membrane reasonably permeable to halothane and a silver-contaminated platinum micro-cathode surface, before electrochemical activity is observed.

However, these particular and necessary requirements *can* be met in clinical practice and Severinghaus *et al.*,[98] in 1971, reported that conventional Pt micro-cathode membrane-covered Clark sensors could become extremely sensitive to the presence of halothane vapour (over-reading the P_{O_2} for room air by up to 1600%), following a 5 min exposure of the sensor to a gaseous sample of halothane vapour in N_2. The sensor was covered with a 25 μm polyethylene membrane but when it was covered with a 25 μm polypropylene membrane the equilibrium with halothane was delayed by about 20 min, probably owing to the reduced permeability of the polypropylene. (When covered with this material, one sensor did eventually over-read by 800%, under the same conditions as before.) Severinghaus *et al.* found that the sensitivity of the O_2 sensor to halothane depended upon the polarising voltage, the membrane material, the electrolyte pH and the deposition of Ag on the Pt cathode surface. Similar studies conducted with oxygenated blood, brought into equilibrium with 2.6% v/v halothane, demonstrated a slow upward drift of the sensor P_{O_2} reading by 100% after 1 min. Thus, an early clear warning was established that the presence of halothane, in clinical concentrations, could cause P_{O_2} sensors to over-read dangerously.

This report was soon followed by further examples. Bates *et al.*,[99] in 1975, reported that intravascular P_{O_2} sensors with gold micro-cathodes and covered with a hydrophilic membrane (Hydron) were sensitive to halothane when polarised at −0.7 V, and the time required to achieve maximum halothane effect was approximately 18 min. Soon after this, Dent and Netter[100] demonstrated that halothane (in the presence of oxygen) could also be reduced on gold micro-electrodes which had been specifically developed for measuring oxygen partial pressures in micro-areas of living tissue during anaesthesia. Since it was apparent from their work that the halothane and oxygen reduction waves overlapped, it was impossible to distinguish between the halothane and oxygen reduction currents, and so this rendered the sensor useless for oxygen tissue measurements in the presence of halothane anaesthesia. Their only solution to this dilemma was the hope that a coating material permeable to oxygen, but impermeable to halothane, might be developed and so permit oxygen measurements to be made with their micro-electrode in the presence of halothane.[100] These reports were soon followed by a clear clinical warning, in a letter to *The Lancet* in December 1978,[72] which highlighted the need for vigilance in exposing commercial blood-gas analysers to blood samples containing halothane. The letter described a gradual upward drift in the analyser P_{O_2} readings, following halothane exposure, and vigilance was emphasised because the errors induced dangerous over-reading of P_{O_2} in critically ill patients. Following this warning, steps were taken either to abrade the cathode surface to remove silver deposition, or else to clean the Pt surface regularly in a nitric acid solution in order to remove potentially harmful Ag deposits. However, the problem of halothane reduction on Ag or Au micro-cathodes used in intravascular sensors still remained unsolved, since these sensors inevitably used hydrophobic membranes which were very permeable to halothane molecules. The only solution was to not use these types of intravascular P_{O_2} sensors in the presence of halothane anaesthesia.

The chemistry of the reduction of halothane is not clear-cut, since both the reaction mechanism and the role of the cathode metal have been a matter of controversy. Early studies were concerned with the reduction of halothane on the dropping-mercury electrode and are of little relevance to clinical sensors.[101–103] The fact that these matters are still unresolved is perhaps due to the way in which studies in different centres have employed different experimental conditions, with large rotating ring-disc electrodes, large stationary wire electrodes or micro-electrodes being employed in some studies, and some conducted in the absence and some in the presence of oxygen. Most of the studies were conducted at unshielded electrodes, with just two reported with membrane-covered micro-disc electrodes.[104,105] Hence it is possible that the wide variety of results and mechanisms proposed are due solely to the particular experimental conditions encountered when the studies were conducted.

Albery and coworkers,[104] in 1981, reported on the amperometric reduction of halothane in 1 M KOH solution using a 1.35 mm diameter silver disc rotating-disc electrode and a membrane-covered 50 μm diameter silver micro-disc electrode, with 25 μm silicone film and 75 μm silastic rubber membranes.[104] Measurements were made both in the presence and absence of oxygen, but their results showed that a micro-disc silver cathode electrode covered with a suitable polymer membrane, such as silicone film, might be used to measure halothane concentration

in the absence of oxygen, but not in the presence of oxygen. The difficulty was that the half-wave potentials for oxygen and halothane were too close, −0.425 and −0.565 V (*versus* SCE), respectively. Fig. 17 shows the reduction wave for halothane at a silver rotating disc electrode in 1 M KOH solution, taken from the work of Albery *et al.*[104] The halothane reduction effect commences early at approximately −0.3 V *versus* SCE and the current is transport limited for potentials more negative than −0.7 V. Thus, the halothane wave overlaps that of oxygen (*cf.*, Fig. 5) in addition to that of nitrous oxide (*cf.*, Fig. 15) in alkaline electrolyte solutions.

Their conclusion was that the reaction of halothane, in the absence of oxygen, is a two-electron reduction:

$$\mathrm{Ag} + \mathrm{e}^- + \mathrm{CHClBrCF_3}$$
$$\downarrow \text{slow}$$
$$(\mathrm{Ag\cdots Br\cdots CHClCF_3})^-$$
$$\downarrow \text{fast, } \mathrm{H_2O}, \mathrm{e}^-$$
$$\mathrm{Ag} + \mathrm{Br}^- + \mathrm{CH_2ClCF_3} + \mathrm{OH}^-$$

The overall mechanism therefore leads to 2-chloro-1,1,1-trifluorethane and bromide, with the overall equation written as:

$$\mathrm{CHClBrCF_3} + 2\mathrm{e}^- + \mathrm{H_2O} \rightarrow \mathrm{CH_2ClCF_3} + \mathrm{Br}^- + \mathrm{OH}^- \quad (24)$$

with the bond split in the reaction almost certainly being the C–Br bond, since bromide is detected as a reaction product. This work suggested that the silver electrode might have a particular affinity for Br.[104]

Later studies suggested that gold was a better cathode material to use,[105] since O_2 and halothane displayed clearly separate reduction waves on gold. Unlike on silver, where oxygen reduction appears as a single diffusion-controlled wave, on gold two plateaux of similar magnitude were observed, attributed to two two-electron steps:

$$\mathrm{O_2} + \mathrm{H_2O} + 2\mathrm{e} \rightarrow \mathrm{HO_2}^- + \mathrm{OH}^- \quad (25a)$$
$$\mathrm{HO_2}^- + \mathrm{H_2O} + 2\mathrm{e} \rightarrow 3\mathrm{OH}^- \quad (25b)$$

A comparison of the current–voltage plots for oxygen and halothane revealed two potential domains of interest, where oxygen alone was reduced between −0.3 and −0.8 V *versus* SCE [eqn. (25a)], and between −1.1 and −1.6 V where the second oxygen wave [eqn. (25b)] was coincident with the halothane reduction. The separation between these two waves therefore appeared suitable for the application of a double potential pulse experiment, of the type already described for the oxygen/nitrous oxide assay on a membrane-covered silver micro-disc electrode. (This approach was also encouraged by the absence of a nitrous oxide reduction wave on gold, under the prescribed experimental conditions.)

However, when these conclusions from the rotating disc experiments were tested with a membrane-covered 125 μm diameter gold disc micro-electrode, current–voltage plots revealed that the magnitude of the current on the first oxygen wave was depressed (at a constant O_2 concentration) in the presence of halothane, as shown in Fig. 18. The depression was non-linear, with the largest reduction being observed between 0 and 1% v/v halothane. Therefore, unlike the rotating disc electrode, the membrane-covered sensor assay of O_2 was not independent of halothane, and the presence of the anaesthetic agent led to large discrepancies in the determination of the oxygen concentration.

Hall *et al.*[105] developed a strategy for assaying halothane in the presence of oxygen by applying a potential step to a polarising voltage where, for short times, the oxygen reduction was under diffusion control whereas the halothane reduction was under mixed kinetic and diffusion control. Oxygen and halothane were thus assayed by the application of a single rectangular wave polarising voltage and current sampling at two points, one at very short times and the other at longer times when both analytes were approaching diffusion control. The resultant current–concentration plot was linear for halothane up to a saturation value, but the sensor still suffered from problems when the gold electrode was contaminated with trace amounts of silver.

A second attempt at the development of a practical halothane sensor, to be used in the presence of oxygen, was reported in 1989, again using a rotating ring-disc electrode for fundamental studies and then a 125 μm gold micro-disc electrode, covered with a 6 μm thick silastic membrane.[106] Again, results with the unshielded and shielded electrodes differed (with one problem being that the reduction of oxygen on a gold electrode behind a membrane with only a thin electrolyte layer appeared as a single wave, in contradistinction to the two waves which appeared at an unshielded electrode), and the membrane-covered sensor appeared to give a linear halothane current response (up to 4% v/v halothane) only as long as the prevailing oxygen concentration was above 60% v/v. As with the previous attempt,[105] the development of a sensor which would unambiguously assay both oxygen and halothane in the presence of each other was proving to be an extremely difficult task.

At the beginning of the 1990s, a third attempt was made, this time by Mount and co-workers,[107,108] to study the electrochemical reduction of halothane and to develop a practical O_2/halothane sensor. Accordingly, the first studies were conducted with silver, platinum, gold and glassy carbon ring-disc electrodes, with silver used as the ring in each case and with copper disc electrodes. In all cases, the diameter of the disc was

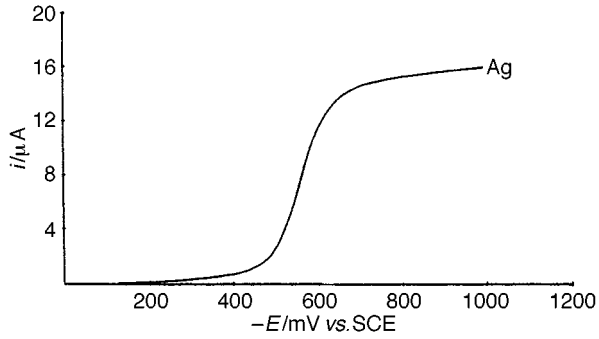

Fig. 17 Current–voltage curve for halothane reduction on a rotating-disc Ag electrode in 1 M KOH electrolyte solution.

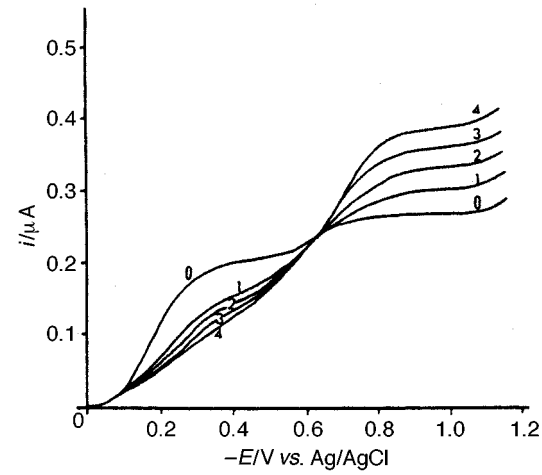

Fig. 18 Voltammograms for 0–4% halothane in oxygen, at a 125 μm diameter gold cathode membrane-covered Clark sensor at a constant P_{O_2}. The depression of the first reduction wave (O_2 reduction) is clearly seen as the halothane concentration is increased.

approximately 7 mm. The mechanistic studies were conducted in the absence of oxygen in an electrolyte solution of 0.1 M potassium hydroxide. Halothane was supplied from an anaesthetic vaporiser in the range 1–4% v/v, and the studies concluded that, for a wide variety of electrode materials, electrochemical reduction of halothane involved two electrons and resulted in the production of a bromide ion at the silver ring electrode. As far as the mechanism was concerned, the conclusion was that the first electron transfer was irreversible and was the slowest of the two, followed by a chemical step which was always fast and never rate-determining, followed by a second electron transfer which might be irreversible or by a subsequent fast chemical step. The following mechanism was postulated:[107]

$$CHClBrCF_3 + e^- \rightarrow (CHClBrCF_3)^{\cdot -} \quad (26a)$$

$$(CHClBrCF_3)^{\cdot -} \rightarrow (CHClCF_3)^{\cdot} + Br^- \quad (26b)$$

$$(CHClCF_3)^{\cdot} + e^- \rightarrow (CHClCF_3)^- \quad (26c)$$

$$(CHClCF_3)^- + H_2O \rightarrow CH_2ClCF_3 + OH^- \quad (26d)$$

and so, overall,

$$CF_3CHClBr + H_2O + 2e^- \rightarrow CH_2ClCF_3 + Br^- + OH^- \quad (26e)$$

agreeing with the conclusions of Albery *et al.*[104] [eqn. (24)].

Because both chemical steps were never rate determining, it was impossible to know the precise sequence of the electrochemical and chemical steps. A further paper by the same group[108] examined the electrochemical reduction of halothane in the presence of oxygen, and this work concluded that silver or gold was the choice of disc electrode metal and that the detection of the bromide ion product from the halothane reduction (at a silver ring electrode) provided a highly successful method for measuring the concentration of halothane in the presence of oxygen, in any mixture of the two gases used for anaesthesia. In this instance, oxygen concentration would be measured at the silver or gold disc electrode. This work also demonstrated that the pH of the electrolyte solution had to be 11, since some oxygen reduction species were also detected on the ring at pH 13. In this technique, both the ring current due to the formation of solid silver bromide and the subsequent reduction charge required for its removal could be used to measure the halothane concentration.[109]

Since it was clear that a clinical sensor needed a single stationary working electrode rather than a ring-disc electrode configuration, Mount and Clark described, in a subsequent paper,[109] an unshielded halothane/oxygen sensor utilising the chronoamperometric measurement of halothane and oxygen concentrations at a silver electrode. The silver disc electrode was 7 mm in diameter and the studies were conducted in a standard electrochemical reaction cell at room temperature, *i.e.*, the sensor was not membrane covered. Their technique employed a triple potential step regime, where the electrode was initially at a potential of −0.15 V. The electrode was then pulsed to −0.85 V, at which point mass transport-limited reduction of halothane (two electrons) and oxygen (four electrons) occurred. The electrode potential was then stepped to a potential between 0.1 and 0.2 V, at which solid silver bromide was formed on the electrode surface from the bromide ion produced by the halothane reduction which took place during the first pulse. Finally, after a time delay, the electrode was pulsed to its starting potential of −0.15 V where the reduction of the solid silver bromide on the electrode occurred according to the reverse reaction

$$Ag(s) + Br^-(aq) \rightleftharpoons AgBr(s) + e^- \quad (27)$$

From their experimental data, it was clear that the current measured during the second pulse was not a good measure of the amount of halothane present, because of complications of either nucleation effects or secondary oxidation reactions. However, the oxidation of bromide which occurs during the second pulse [and which formed the solid silver bromide according to the forward reaction in eqn. (27)] was reduced according to the reverse reaction during the third pulse and this was found to be a good measure of the amount of halothane present. The authors recognised that their system was 'idealised' since the sensor did not have a membrane,[109] and a membrane will always complicate gas transport considerably, as highlighted in the O_2 Clark sensor section of this teaching review. Thus, the Mount-Clark O_2/halothane sensor will have a much more complex current–time response when the sensor is covered by a membrane; transport of the gas in the thin layer of electrolyte will be bounded rather than governed by semi-infinite linear diffusion and the finite rate of transport of gas through the membrane will determine the sensor response. Also, the pH in the thin layer of electrolyte adjacent to the disc may rise more rapidly above pH 11 during the first reduction pulse and then fall more slowly, as radial diffusion of hydroxide ions in the thin electrolyte layer will be less efficient at maintaining the pH at a steady level. However, despite these complications, the triple pulsing regime is clearly a promising way forward, and it should be possible to model this type of sensor using numerical analysis techniques analogous to those described in the Clark O_2 sensor section.

More recently, Langmaier and Samec[110] have questioned the ECEC mechanism proposed in eqn. (26), and have commented that the absence of electrocatalysis in this reaction is surprising. Their criticism is that the mechanism described by eqn. (26) does not account for the strong effect of the nature of the metal on the halothane reduction process. In their examination of the reduction of halothane (in nitrogen) on a wide variety of metal electrodes, they found that the half-wave potentials of halothane reduction at various metal electrodes could differ by several hundred millivolts in both methanol and water. They concluded that such behaviour might be due to the absorption of halothane on the metal surface M, following the scheme

$$(M\cdots S) + CHClBrCF_3 \rightleftharpoons (M\cdots Br\cdots CHClCF_3) + S \quad (27a)$$

followed by uptake of the first electron as the rate-determining step:

$$(M\cdots Br\cdots CHClCF_3) + e^- \rightarrow M + Br^- + (CHClCF_3)^{\cdot} \quad (27b)$$

where S is the absorbed solvent molecule. These steps would then be followed by the fast reactions in eqns. (26c) and (26d). However, their work confirmed the previous conclusions that the electrochemical reduction of halothane proceeded through a two-electron overall scheme yielding 2-chloro-1,1,1-trifluoroethane and bromide as the main product, with the uptake of the first electron by the halothane molecule being the rate-determining step.

The latest word (up to this present time) in this quest to detect halothane in the presence of oxygen must belong to Caruana and Giglio,[111] who have devised a method of measuring halothane by anodic stripping voltammetry, although they appeared to be unaware of the chronoamperometric technique of Mount and Clark.[109] The technique of Caruana and Giglio is different to that of other workers in that they used an acidic electrolyte solution (based on citric acid) with a pH of 4.0. Their electrode was a 0.698 cm diameter gold electrode and cyclic voltammograms were measured in the range −0.3 to +0.9 V. These cyclic voltammograms showed a clear irreversible reduction peak at −0.3 V and a sharp peak at +0.62 V, the height of which was linearly dependent on scan rate. This suggested that the oxidised species was absorbed on the electrode surface, and the oxidation peak only occurred when reduction had taken place on the electrode, confirming that that peak at +0.62 V was due to the oxidation of the absorbed products from the reduction of halothane. Initial calibration plots of halothane concentration against the peak oxidation current showed that the peak current

was independent of oxygen concentration in the electrolyte solution, but the linear range for halothane measurement was between 18 and 155 nM, since beyond 155 nM the response saturated at a constant peak height. Since the equivalent halothane concentration in pH 4.0 buffer for 4% v/v halothane would be 0.44 μM, the cyclic voltammetric technique could not be used to measure halothane under clinical conditions. This problem was obviated by adjusting the sensor sensitivity by using a short duration potential step for 50 ms at −0.7 V, followed immediately by a potential sweep from 0 to +0.8 V at a sweep rate of 50 mV s^{-1}. The result of this pulsing/sweeping regime was that there was a clear linear relationship between oxidation peak current and halothane concentration between 0 and 4% v/v halothane. Furthermore, this linearity was unaffected by the presence of oxygen. Although this new development is obviously very promising, it must be remembered that these experiments[112] were conducted in a reaction cell, and the sensor (like that of Mount and Clark[109]) was not covered by a membrane permeable to halothane. Furthermore, the sensor would not provide a cotemporal measurement of O_2 concentration, unlike the Mount and Clark device.

It is clear from the above that a further (and possibly large) step is required before a practical membrane-covered sensor for halothane can be developed, and this development would be greatly aided by a digital simulation model which incorporated both membrane and electrolyte layers, and which would enable both pulsing and sweep control techniques to be modelled. This stage has yet to be realised.

There is an ironic practical twist to this quest to develop an electrochemical halothane sensor since the increasing availability of alternative volatile anaesthetic agents, and the worries about the pathophysiology of post-halothane hepatic dysfunction, have led to cautionary advice regarding the medico-legal implications of the repeated use of halothane,[112] including calls for it to be made obsolete.[113] Although halothane will no doubt remain popular in undeveloped countries, by virtue of its low cost and high anaesthetic potency, opinion seems to be hardening that the days of halothane anaesthesia in the UK are limited. A recent anaesthetic survey has clearly indicated that a combination of the fear of litigation and the availability of newer 'cleaner' inhalational anaesthetic agents has brought about a sharp decline in the use of halothane, especially amongst anaesthetists in training.[114] Halothane has for years been a reliable agent for use in difficult situations, but as alternative agents are developed for the safe inhalational induction of anaesthesia (a field where halothane has been dominant), then halothane may become little more than an item of historical interest to a new generation of anaesthetists. Unfortunately for the electrochemist, the new inhalational agents, including isoflurane ($CHF_2OCHClCF_3$), enflurane (CHF_2OCF_2CHClF), sevoflurane [$CH_2FOCH(CF_3)_2$] and desflurane ($CHF_2OCHFCF_3$), all appear to be electrochemically inert in aqueous electrolytes. However, all is not lost electrochemically, since it is becoming apparent that most of these agents might be electrochemically active on micro-electrodes in non-aqueous media, as described in the following non-aqueous section.

Non-aqueous solution electrochemistry

The history of the electrochemical measurement of O_2 and CO_2 in non-aqueous solutions is much shorter than that in aqueous solutions, as described in the previous sections, and aprotic solutions have certainly not been used in blood-gas sensors so far. Furthermore, aprotic electrochemistry has not been used for the gaseous determination of O_2, CO_2 or the inhalational anaesthetic agents in clinical medicine, although prototype gas sensors have been developed in recent years. This apparent lack of interest is probably due to the success of the well tried and tested Stow–Severinghaus and Clark aqueous electrolyte sensors, and to the fact that aprotic electrolyte solutions could pose new dangers to the users of blood (or gas-phase) sensors owing to the potential toxicity of these substances. Furthermore, since they would attack the plastic materials currently used in clinical sensors, their use would necessitate new materials and new design procedures.

Why, then, should we bother to design new clinical sensors using aprotic solutions? The answer is that aprotic solutions provide a very wide voltage window (up to −3.0 V) for the electrochemical reduction of potentially reducible clinical gases and vapours. This opens up the possibility of developing a single sensor which could measure a mixture of gases and vapours with a single electrochemical technique. This is a laudable aim, but the journey towards realising this aim has been bedeviled with problems so far, notably the cross-interference of the various reaction products. These problems will be described in the following sections. The non-aqueous electrochemistry will be described taking DMSO as the solvent, since DMSO is already used therapeutically in clinical medicine for urinary disorders and as an anti-viral preparation. It therefore does not pose a toxic hazard to the user provided that standard laboratory precautions are employed.

Because of the way in which this particular electrochemical history has evolved, the reduction of O_2 and CO_2 in aprotic media will be described first, and the following two sections describe the electro-reduction of oxygen and carbon dioxide, in the *absence* of each other, although these conditions are decidedly non-clinical. Special problems exist when these two gases are reduced in the *presence* of each other, and this is considered in the third section. Finally, preliminary results on the electro-reduction of inhalational anaesthetic agents in DMSO are described.

Oxygen reduction

The electrochemical reduction of oxygen in aprotic media was studied in the 1960s by a small group of workers.[115–117] This, and subsequent, work was extensively discussed by Bauer and Beck,[118] in 1972, who reviewed the electrochemical behaviour of O_2 in no fewer than 33 solvents and fused salts. Most of these studies were conducted with either Pt or Au solid macro-electrodes, or with the dropping-mercury electrode, using cyclic-voltammetry. In one instance, controlled amounts of water were added to DMSO and the effect of this on the electrochemical reduction of O_2 in DMSO was examined.[119]

The results of these investigations in DMSO, with tetraalkylammonium salts as the supporting electrolyte, showed that two distinctive, and clearly separated, oxygen reduction waves, as shown in Fig. 19. The first wave is due to the electroreduction of oxygen to the superoxide ion, $O_2^{\cdot -}$ produced by a one-electron process. The second wave is due to the further reduction of $O_2^{\cdot -}$ *via* one electron, to O_2^{2-}. Furthermore, since the first process is reversible, the overall process can be written as

$$O_2 + e \rightleftharpoons O_2^{\cdot -} \quad (28a)$$

$$O_2^{\cdot -} + e \rightarrow O_2^{2-} \quad (28b)$$

If the electrode polarisation is maintained in the vicinity of the first wave, it has been shown that stable solutions of $O_2^{\cdot -}$ can be generated, and that $O_2^{\cdot -}$ undergoes several reactions where it can function as a base, a nucleophile and a one-electron donor.[120,121] In the mid-1990s, renewed interest in the electro-reduction of oxygen in aprotic media led to investigations on the reactivity of $O_2^{\cdot -}$, following the electrochemical reduction of oxygen using cyclic voltammetry and rotating-disc electrodes, and to hydrodynamic chronocoulometric studies to determine the diffusion coefficients and concentrations of oxygen in aprotic media.[122,123] These studies confirmed the earlier conclusions, namely that oxygen is reduced initially in a one-

electron reversible diffusion-limited step to the superoxide ion, which is further reduced to the peroxide, $O_2^{\cdot-}$ This second step was observed as a highly irreversible peak or wave at more negative potentials, indicating that O_2^{2-} is highly unstable.[123]

Although this work is electrochemically interesting, it has had no impact on the design of oxygen macro-electrode sensors, presumably because the conventional Clark O_2 sensor (with aqueous electrolytes) performs so well in clinical practice.

Carbon dioxide reduction

Early work, again conducted in the 1960s and 1970s, on the electroreduction of CO_2 in aprotic media initially revealed ambiguous and conflicting results.[124–126] These early studies were conducted with a variety of electrode materials (including mercury, gold and lead) and with a variety of aprotic solvents including dimethylformamide (DMF), acetonitrile and DMSO. Some results indicated a one-electron reduction step and others a two-electron step. The number of electrons transferred appeared to depend upon the applied potential, the nature of the metal, the solvent employed and the presence or absence of water in the solvent. As with the O_2 reduction studies, a supporting electrolyte such as tetraethylammonium perchlorate was employed in the electrochemical studies.

All these studies revealed a single CO_2 reduction wave in aprotic media when CO_2 was reduced (in the absence of O_2) in N_2. The CO_2 reduction wave was shifted to much more negative potentials (about −2.5 to −3.0 V *versus* Ag/AgCl) than the first O_2 reduction wave. An illustration of this is also shown in Fig. 19, which compares the positions of the O_2 and CO_2 reduction waves relative to each other.

What was clear, from these early and then subsequent studies, was that a complicated set of competing reactions, with differing pathways, were possible. It was reported that the possible reduction products were $C_2O_4^{2-}$ (oxalate), HCO_2^- (formate), carbon monoxide and carbonate, and possibly glycolate under the influence of residual, or purposely added, water.[127–131]

Viewed overall, these reactions, in an anhydrous system, were presented as

$$2CO_2 + 2e \rightarrow C_2O_4^{2-} \quad (29a)$$

$$2CO_2 + 2e \rightarrow CO + CO_3^{2-} \quad (29b)$$

and when water was present as

$$CO_2 + H^+ + 2e \rightarrow HCO_2^- \quad (29c)$$

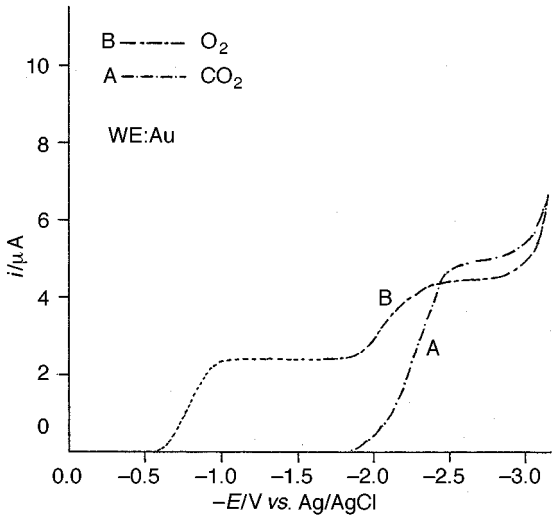

Fig. 19 Linear sweep voltammograms for the independent reduction of O_2 in N_2 and CO_2 in N_2 at an unshielded Au working electrode in DMSO.

However, what was consistent in these early studies was the conclusion that, irrespective of whether water was present or not, the electroreduction of CO_2 first involved a one-electron step to form $CO_2^{\cdot-}$. Thereafter, a variety of competing pathways were possible, and these were summarised by Gressin *et al.*[129] and by Amatore and Savéant[130] in the scheme shown in Fig. 20. Savéant's group further pointed out that the distribution of the products depended strongly upon the experimental operational factors such as current density, concentration and diffusion layer thickness.[130] They also made the important observation that these factors rendered the extrapolation of macro-scale electrolysis results to the context of micro-electrolytic techniques as 'uncertain' (and the opposite argument obviously applies), and this is an important fact to remember when results are obtained with micro-electrodes.

Again, these early studies have had little impact on the development of CO_2 sensors using aprotic solvents, for two different reasons. Firstly, the Stow–Severinghaus sensor is so well established that there has been little room for a competing amperometric CO_2 sensor. Second, and more important, these macro-electrodes studies on CO_2 reduction in nitrogen are rendered 'academic' when they are repeated in the 'real-life' presence of oxygen, as illustrated in the next section.

Reduction of O_2 *and* CO_2 *in the presence of each other*

It is all very well examining the reduction of CO_2 under anaerobic conditions and the reduction of O_2 in the absence of CO_2, but (with the exception of inspired gases which do not contain CO_2) clinical expired gas and blood-gas analysis will always involve a mixture of both gases. At this point, the electrochemistry of O_2 and CO_2, in the presence of each other, becomes decidedly complicated at macro-electrode surfaces. (A clearer picture emerges when micro-electrodes are employed, but this will be considered later.)

On the basis of the evidence presented in Fig. 16, and on the evidence previously obtained when O_2 and N_2O were reduced together in aqueous electrolytes (Fig. 15), it would be expected that O_2 and CO_2 would produce two distinct and clearly separated reduction waves when reduced in the presence of each other. In this theoretical case, the second reduction wave would be expected to be an addition of the CO_2 reduction current and the second O_2 reduction current wave. However, this is not obtained in practice, and the wave shown in Fig. 21 is obtained instead experimentally[132,133] on macro-cathode surfaces. The CO_2 reduction wave has now completely disappeared, and it can be seen that the single 'oxygen' reduction wave has increased somewhat in magnitude from that shown in Figure 19. The conclusion must be that the superoxide anion radical, $O_2^{\cdot-}$, reacts irreversibly with the dissolved CO_2, and is effective in reducing the concentration of dissolved CO_2 prior to its possible reduction at more negative potentials. These experimental studies, which were conducted with macro-electrodes, appeared to confirm that it would be impossible to determine O_2 and CO_2 electrochemically, in the presence of each other, with a simple electrochemical technique such as voltammetry.[133]

However, three attempts have been made to circumvent this superoxide interference effect by devising gas-phase O_2–CO_2 sensors, using macro-electrodes and various degrees of ingenuity. First, Albery and Barron[134] tackled this electrochemical conundrum by attempting to 'scrub' electrochemically the oxygen from a gas sample and then measure the residual CO_2 electrochemically. Accordingly, they modified the O_2–N_2O metallised membrane electrode system described previously,[88] and devised a new double membrane/double solvent layer sensor.[134] The outer membrane consisted of a metallised membrane, polarised at −0.7 V, where O_2 was reduced in an aqueous media electrolyte layer, buffered at pH 5 using a phthalate electrolyte. The current on the metallised

membrane was proportional to the concentration of O_2, and it was found that the metallised membrane filtered the O_2 with 99% efficiency. The remaining CO_2 molecules then passed through the metallised membrane, and through the aqueous electrolyte, to another membrane with an inner compartment containing a non-aqueous electrolyte (usually DMSO) and a silver macro-cathode. The CO_2 was reduced to $CO_2^{\cdot-}$ at this second electrode surface. As is clear from the above description, the electrode construction was complicated and a major problem with this approach was that the two-compartment arrangement introduced a considerable gas diffusion barrier, which manifested itself in a relatively slow response time. Although Albery and Barron[134] published results showing this sensor could be used to determine gaseous O_2 and CO_2 on a breath-by-breath basis, this work could not be replicated by Coombs[132] and Clark,[133] and the sensor appears not to have been developed any further.

In the second attempt to develop a practical sensor, the electrochemical filter technique was abandoned, and a pulsed titration technique was developed in its stead.[135,136] The principle of this approach for the electrochemical analysis of mixed gaseous samples of O_2 and CO_2 is shown schematically in Fig. 22. The method of determination depends on reducing O_2 for a fraction of a second, at a potential where CO_2 is inactive, in order to generate a known amount of the reactive $O_2^{\cdot-}$ anion radical, and thereby initiate the fast $O_2^{\cdot-}$–CO_2 reaction. The amount of $O_2^{\cdot-}$ subsequently left unreacted after a given time was then determined by pulsing the working electrode potential to a more positive potential, and re-oxidising the remaining $O_2^{\cdot-}$ to O_2. The collection efficiency for the O_2–$O_2^{\cdot-}$ redox couple was estimated in the absence of CO_2, and then subsequently with CO_2 present, and the changes in both the generation and recovery transients due to the deactivation of $O_2^{\cdot-}$ and the regeneration of O_2 were observed. By quantifying these observed changes with respect to a theoretical model of the overall system, it was possible to infer the sample concentrations of O_2 and CO_2. In this sensor, DMSO was used as the solvent, and a large Au working electrode, approximately 1.5 mm in diameter, was employed.

The overall reaction scheme for this sensor is shown in Fig. 23, and several practical problems emerge. First, since the sensor design necessitated a gold macro-cathode where the reaction processes could be contained in the thin layer of solvent trapped between the membrane and the cathode surface, the sensor could only be used for gaseous analysis. It had no future for blood-gas analysis. Second, as is clear from the reaction scheme presented in Fig. 23, the very nature of the $O_2^{\cdot-}$–CO_2 reaction pathways regenerates O_2, which is further reduced during the first voltage pulse. This additional O_2 elevates the observed overall O_2 reduction signal. Therefore, a complex mathematical model[136] had to be devised to deconvolute the true O_2 and CO_2 concentrations, and the analysis system became more and more complicated. Because of this complexity, and the impossibility of using this system for blood-gas analysis, the pulse-titration technique made no further progress.

A third attempt was made, this time by Qian *et al.*,[137] to devise an electrochemical method for the simultaneous measurement of CO_2 and O_2 and they combined two separate amperometric sensors. In this technique, a gaseous sample is drawn through an electrolytic cell designed to scrub the oxygen from the gas sample. This device consisted of a Pt-catalysed Teflon-bonded hydrophobic porous electrode, which had been previously developed for fuel-cell applications. The electrolyte was 0.5 M sulfuric acid. Qian *et al.*[137] demonstrated that all the oxygen was virtually completely consumed by electro-reduction, and the remaining gas then passed to a CO_2 sensor consisting of a Pt micro-disc of 60 μm diameter, with a DMSO solution. The gas-permeable membrane employed was either porous Teflon or solid polyethylene. Again, this sensor could

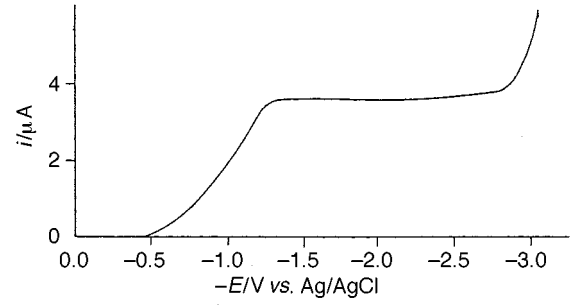

Fig. 21 Experimental linear sweep voltammogram for the mixed electrochemical reduction of O_2 + CO_2 in N_2 under the same experimental conditions as in Fig. 19, showing only one reduction wave. Note the absence of the CO_2 reduction wave and the second O_2 reduction wave, both of which are present in Fig. 19.

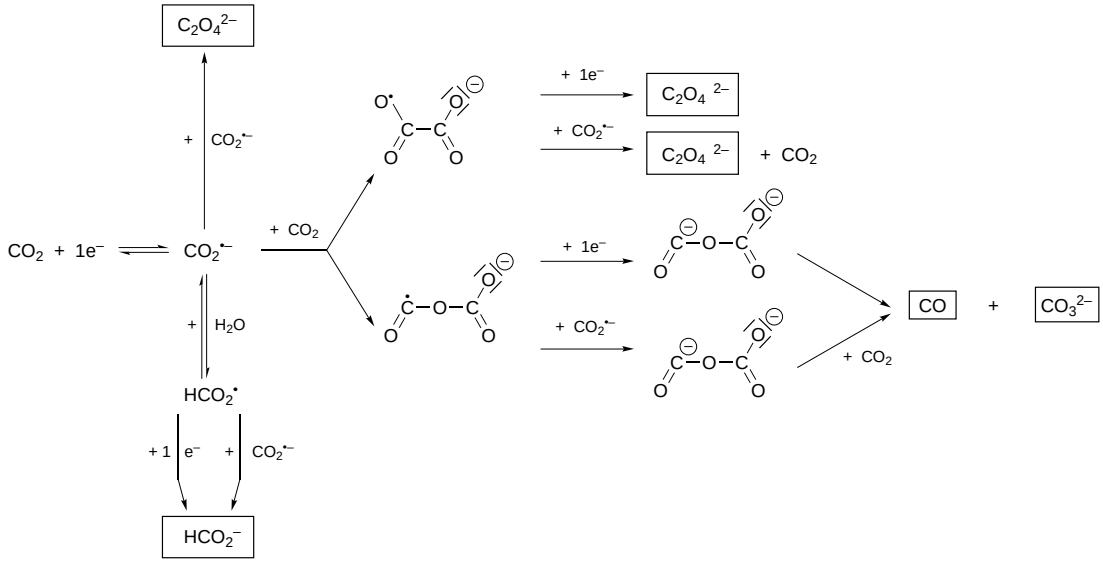

Fig. 20 Possible reduction processes for the electrochemical reduction of CO_2 in aprotic media with and without the addition of water. (Taken from ref. 128.)

only be employed for gaseous samples, and since both the O_2 and CO_2 sensors had different response times (compounded by the transport lag time between the sample gas reaching the two separate cells) the overall system had serious disadvantages. Furthermore, since the quoted response times were 15 and 35 s for 90% changes in O_2 and CO_2, respectively, the combined sensors were far too slow for the required response time (about 0.1 s) for breath-by-breath O_2 and CO_2 determination. As with the two previous design attempts, the sensor system could not be used for the determination of dissolved O_2 or CO_2 in blood.

Reduction of O_2 *and* CO_2 *at micro-electrodes*

The solution to the electrochemical conundrum of the cross-interference of the O_2 and CO_2 reduction processes, in non-aqueous media, finally came by returning to the original electrochemical roots of biology and medicine. By the very nature of their work, biological and clinical scientists use *micro-electrodes* to investigate biological phenomena—as described in the early sections on aqueous amperometric sensors. Micro-electrodes not only perturb (*e.g.*, consume in this case) as little as possible the biological system in which they are measuring, but they also produce a minimal quantity of reaction by-products—which are often unwanted in any electrochemical system, anyway. Hence it could be a sensible proposition to suppose that a micro-disc electrode in either an unshielded, or membrane-covered, system might produce amounts of O_2 and CO_2 reduction products which were too small to interfere significantly with the simultaneous reduction of these two gases in non-aqueous media.

This proposition was tested in a series of studies[138,139] (which have yet to be completed) which analysed the reduction of O_2 and CO_2 in the presence of each other, and in the presence of inhalational anaesthetic agents,[140] at gold micro-cathodes in DMSO. These studies have been conducted with Au micro-disc electrodes of 2–80 μm diameter, with sweep voltammetry rates varying between 0.1 and 5.0 V s^{-1}. It has been demonstrated conclusively that it is most unwise to extrapolate electrochemical results obtained on macro-electrodes to micro-disc electrodes, so confirming the warning by Amatore and Savéant.[130]

Although these micro-disc electrodes replicated the macro-electrode studies, when O_2 was reduced in the *absence* of CO_2 and CO_2 was reduced in the *absence* of O_2, the results were entirely different when O_2 and CO_2 were reduced in the *presence* of each other.[138,139] In this instance, in both the unshielded and membrane-covered cases, clearly separated O_2 and CO_2 reduction waves were obtained. Both produced good linear relationships between the limiting currents of the two waves and the oxygen and CO_2 concentrations. Oxygen plus carbon dioxide voltammograms are illustrated in Fig. 24, showing the reduction waves in both the unshielded and membrane-covered sensor cases. The membrane-covered sensor was a conventional Clark-type sensor, as shown in Fig. 4, and the electrodes were gold wires sealed in glass rods, obtained either from commercial sources or from La Trobe University, Melbourne, Australia. They were 'non-ideal' for sensor design

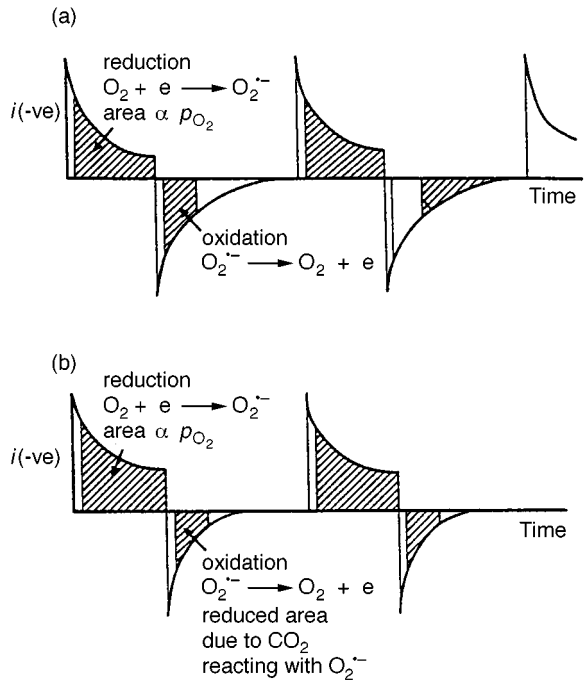

Fig. 22 Pulsing regime for the pulse-titration technique when (a) O_2 only is present and (b) both O_2 and CO_2 are present. It can be seen in (b) that the recovery signal from the oxidation of $O_2^{\bullet-}$ is significantly less in the presence of CO_2 and that the O_2 signal is enhanced in the presence of CO_2, as explained in the text.

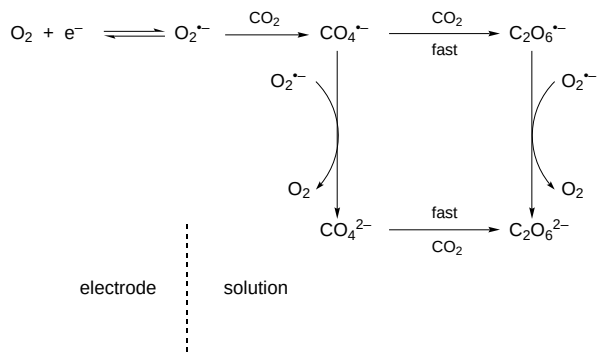

Fig. 23 General reaction scheme for the homogeneous deactivation of $O_2^{\bullet-}$ by CO_2 in DMSO, which occurs in the solvent layer adjacent to the macro-cathode in the pulse-titration sensor.

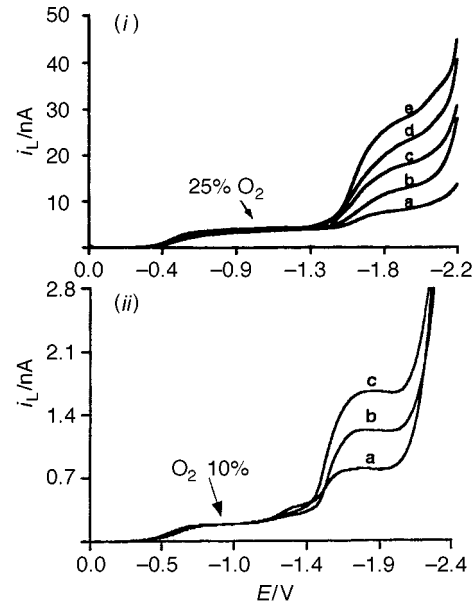

Fig. 24 Voltammograms for the reduction of CO_2 in O_2, with the balance being N_2, for a 10 μm diameter Au microdisc electrode. (*i*) Voltammograms for an unshielded electrode for (a) 3, (b) 6, (c) 9, (d) 12 and (e) 15% v/v CO_2 in 25% v/v O_2. (*ii*) Voltammograms for the gold micro-cathode when covered with a 12 μm PTFE membrane at CO_2 concentrations of (a) 3, (b) 6 and (c) 9% v/v, when the O_2 concentration was kept constant at 10% v/v.

since they had flattened surfaces and were not shaped for a Clark sensor, and yet they gave excellent results. This clearly indicates that specially designed sensors could be designed fairly easily.

The key to the success of this dual O_2–CO_2 system obviously lies in the utilisation of micro-disc electrodes, and it seems reasonable to suppose that the smaller the microdisc the better.

Recalling the reaction scheme of the O_2–CO_2 pulsed titration sensor (Fig. 23):

$$CO_2 + O_2^{\cdot -} \rightarrow CO_4^{\cdot -} \quad (30a)$$

$$CO_4^{\cdot -} + CO_2 \rightarrow C_2O_6^{\cdot -} \quad (30b)$$

$$C_2O_6^{\cdot -} + O_2^{\cdot -} \rightarrow C_2O_6^{2-} + O_2 \quad (30c)$$

the 'feed-forward' process [eqn. (30a)] destroyed the CO_2 present in the DMSO layer adjacent to the gold surface, and the 'feed-back' process [eqn. (30c)] produced O_2, which then elevated the original oxygen signal given by

$$O_2 + e^- \rightleftharpoons O_2^{\cdot -}$$

To eliminate the 'feed-forward' and 'feed-back' mechanisms, which destroy any direct relationship between current and gas concentration, it is necessary to prevent eqn. (30c) from occurring and to generate species such as $C_2O_6^-$ *via* direct electron transfer (heterogeneously at the electrode surface):

$$C_2O_6^- + e \rightarrow C_2O_6^{2-} \quad (31)$$

rather than homogeneously *via* eqn. (30c), so that a new process is available which is proportional to CO_2 concentration. By using micro-disc electrodes, the product $O_2^{\cdot -}$ is produced in sufficiently small amounts that eqn. (30c) does not occur to any substantial extent, and by sweeping the polarising voltage there is also insufficient time under steady-state conditions prevailing at the micro-electrode for this reaction to take place homogeneously in the region of the electrode surface. Hence any $O_2^{\cdot -}$ reaction with CO_2 produces very little O_2 and this does not then elevate the oxygen reduction signal *via* the feed-back process in eqn. (30c).

Micro-disc electrode studies, in the absence of CO_2, have indicated that the reduction of CO_2 provides either a drawn-out single process or two barely resolved processes. However, the limiting current obtained represented the summation of both processes and was well defined. The data can be explained by assuming that the short time-scale and low concentration of $CO_2^{\cdot -}$ lead to conditions where the reactions in Fig. 20:

$$2CO_2^{\cdot -} \rightarrow CO + CO_3^{2-}$$

$$CO_2^{\cdot -} + CO_2^{\cdot -} \rightarrow C_2O_4^{2-}$$

$$2CO_2^{\cdot -} + H_2O \rightarrow HCO_2^- + HCO_3^-$$

are unimportant, *i.e.*, the $CO_2^{\cdot -}$ has a finite existence at the electrode surface and can be directly reduced to a transient CO_2^- moiety:

$$CO_2^{\cdot -} + e \rightarrow CO_2^{\cdot 2-} \xrightarrow{\text{fast}} \text{products} \quad (32)$$

It was clear that the second reduction process in the presence of O_2 and CO_2 mixtures was not simply a summation of the second O_2 reduction process:

$$O_2^{\cdot -} + e \rightarrow O_2^{2-}$$

and the CO_2 reduction processes. In the absence of eqn. (30c), direct reaction of the product of the reaction of CO_2 and $O_2^{\cdot -}$ must occur to give a single multi-electron irreversible process. The exact details of this new process are not known, but direct electron transfer at the electrode surface occurs and O_2 is not generated homogeneously as is the case at macro-electrodes. As a consequence, when O_2 and CO_2 gas mixtures are present in clinical concentrations, the limiting current for the first reduction process was found to be proportional to the O_2 concentration and the limiting current for the second process was directly proportional to the CO_2 concentration. Furthermore, when the membrane-covered sensor studies were repeated after the deliberate addition of 5 and 10% v/v of water to the DMSO solvent, the O_2 and CO_2 reduction results were essentially unchanged, with the only apparent change being that the magnitude of the CO_2 reduction current was decreased on the addition of H_2O to the solvent.[138] Within the measurement error, the O_2 reduction current was essentially the same when either 5 or 10% H_2O was added to the DMSO. In both cases, the sensor responded linearly to changes in CO_2 concentration, and the reduction processes appeared to occur at the same polarising voltages whether the solvent was wet or dry. It is thus clear that 'unexpected' results can be obtained with micro-disc electrodes, and further studies on this new system are required.

So far, the reported studies have used 6 and 12 μm Teflon membranes to cover the sensor, and the gold micro-disc electrodes have varied, in practice, between 2 and 10 μm in diameter. The voltage sweep rates have varied between 0.1 and 5 V s^{-1}. As stated in the Aqueous electrolyte amperometric P_{O_2} sensor section, the use of thin membranes produces conflicting advantages and disadvantages. A thin membrane will yield a fast time response (and hence a fast analysis update) but will inevitably increase the sensor liquid–gas difference by facilitating O_2 transport from the liquid sample to the gas species consuming cathode. This was partly compensated for by employing micro-disc electrodes, but since they were polarised continuously the O_2 consumption process was also continuous, and so a compromise had to be struck between electrode size and membrane permeability and thickness.

However, in the case of the micro-disc O_2–CO_2 sensor described above, the liquid–gas current difference can be obviated by the employment of suitable electrochemical techniques. First, the minute micro-disc electrode will reduce the depletion of the gas species from the liquid sample, and, further, the polarising voltage is swept only for a finite time and can then be switched off. After a suitable quiescent time, the duty cycle can be repeated, allowing the overall diffusion system to relax during the 'pause' period. This sensor therefore lends itself to the type of 'on-off' computer simulation described in the sensor simulation model, where the pulsing regime is replaced by sweep voltammetry, and the characteristics of the membrane and solvent layers are woven into the general equations, together with the size of the micro-disc electrode employed. In this fashion, the actual operating characteristics of a sensor can be optimised by computer simulation to produce the minimum liquid–gas difference effect and yet maximise the analysis update time. Furthermore, the use of an array of micro-disc electrodes, wired in parallel and suitably spaced so that their diffusion-reaction patterns do not overlap each other, would greatly increase the total sensor output signal, and also build 'redundancy' into the sensor performance.

O_2 + N_2O *reduction*

The same micro-disc electrode sensor was tested in the presence of O_2 and N_2O binary gas mixtures.[141] Studies were conducted with both shielded and membrane-covered micro-disc gold electrodes, and similar results were obtained in both instances.

As with the aqueous electrolyte sensor, two distinct and clearly separated reduction waves were observed for the reduction of O_2 and N_2O in DMSO. The addition of 5 and 10% v/v of H_2O to the DMSO did not appear to affect the reduction processes, although the overall voltage window wherein the reduction processes took place was reduced. Also, as with the aqueous electrolyte studies, N_2 appeared to be the

main product of the reduction process, and care had to be taken (as with the aqueous solvents) to avoid the problem of N_2 bubble formation on the micro-disc surface.[141]

Fig. 25 shows the voltammograms (sweep rate 0.1 V s^{-1}) obtained from a 10 μm diameter Au micro-disc electrode, when shielded with a 12 μm PTFE membrane for O_2 and N_2O gas mixtures, where both the O_2 and N_2O concentrations varied from 10 to 90% v/v. Although there does not appear to be an O_2 reduction current in Fig. 25(a), it is in fact there as a 'background current' on the current scale displayed. When this section of the voltammogram is magnified, as shown in Fig. 25(b), the O_2 voltammograms become clearly defined. The steps in the O_2 current are due to the resolution of the potentiostat output, representing the last significant bit in the digital output. The N_2O and O_2 limiting currents from Fig. 25(a) and (b) translate into linear current–gas concentration relationships, and it is therefore clear that a membrane-covered Au micro-disc sensor can be designed to measure O_2 and N_2O in the presence of each other. The design characteristics of this sensor have yet to be optimised in order to facilitate liquid sample measurements of O_2 and N_2O.

Reduction of O_2, CO_2 *and* N_2O *in the presence of each other*

The next complication in the quest for a multi-gas sensor is the determination of O_2, CO_2 and N_2O in the presence of each other.

Results, published only in patent form at the moment,[140] have clearly revealed that three waves are seen on the current–voltage voltammogram when O_2, CO_2 and N_2O are simultaneously reduced in the presence of each other. Fig. 26 illustrates this phenomena clearly, where it is seen that the O_2, CO_2 and N_2O reaction processes are clearly separated and identified by their positions on the voltammogram. Furthermore, the limiting currents, within the gas species concentrations examined so far, have indicated that the three waves produce linear relationships between current and gas concentration for the individual gas species. Clearly, there is still much more work to be done on this new type of sensor, but the indications, so far, are that micro-disc sensors, in aprotic media such as DMSO, clearly hold much promise for the future. The range of solvents examined so far has been severely limited, but a practical sensor might well employ a gel-type electrolyte layer, rather than a liquid layer.

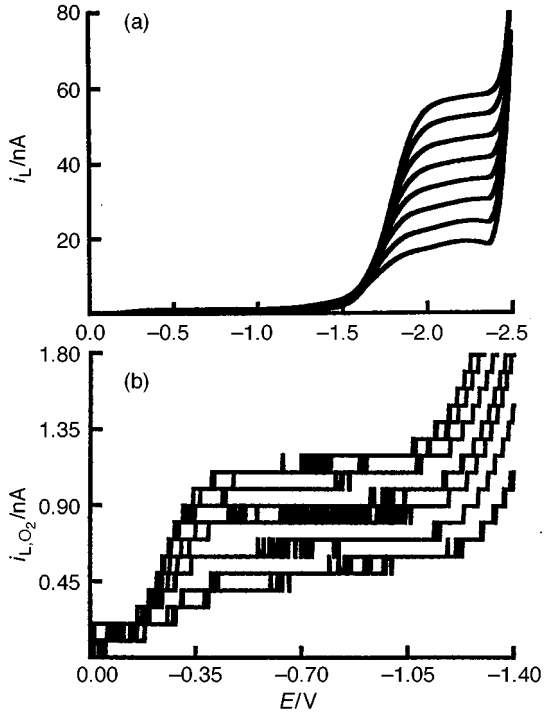

Fig. 25 (a) Voltammograms obtained using a 2 μm diameter Au micro-disc electrode, when shielded with a 12 μm PTFE membrane for O_2 + N_2O gas mixtures. The O_2 concentration varied from 10 to 80% v/v and the N_2O concentration from 90 to 20% v/v. The O_2 reduction current is so minute that it appears as a 'background current' on the current scale displayed here (full scale 80 nA). (b) The greatly amplified O_2 voltammograms for the data displayed in (a), with the exception that the full-scale current is now 1.8 nA and the voltage scale is from 0 to −1.4 V *versus* Ag. The steps in the O_2 current are due to the 'last bit' resolution of the potentiostat output.

Inhalational anaesthetic agents

It is here that a possible clear distinction appears, once more, between electrochemical results obtained with macro- and micro-electrodes.

The first real attempt to devise an electrochemical sensor for an inhalational anaesthetic agent, other than halothane, was made by Compton and co-workers[142,143] in 1988. The agent they chose to examine was isoflurane, a fluorinated ether ($CHF_2OCHClCF_3$), which is now commonly used in anaesthetic practice worldwide. In their first reports, they used a 0.682 cm diameter macro-electrode in a rotating-disc assembly to investigate the electrode activity, or otherwise, of isoflurane at a range of conventional electrode materials.[142,143] Their results, using rotating-disc voltammetry, showed that isoflurane was inert towards reduction at silver, gold, mercury and platinum macro-electrodes, in both aqueous and non-aqueous solutions. The sole exception was on mercury in dimethylformamide solvent, where reduction was observed at around −3.0 V (with reference to a saturated calomel electrode), but this was so close to the potential of the solvent decomposition that any analytical applications were precluded. They therefore directed their attention towards finding a possible mediator for electron transfer. Their investigations demonstrated that the fluoranthene radical anion could mediate the reduction of isoflurane in acetonitrile solution and they then developed a polymer-modified electrode for the reduction of isoflurane based on these observations. In this particular case, the polymer [poly(11-vinylfluoranthene (PVF)] containing electroactive pendant groups was synthesised and deposited on a platinum disc electrode of diameter 0.702 cm. The solvent used was acetonitrile and cyclic voltammetry was employed to investigate the electrochemical processes. Cyclic voltammograms

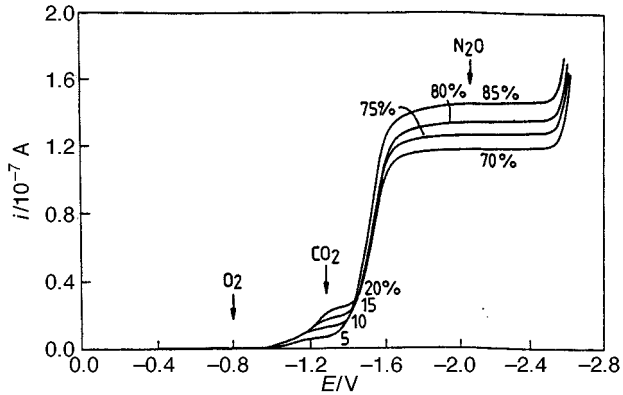

Fig. 26 Voltammograms for an unshielded 2 μm diameter gold micro-disc electrode in a gas mixture containing 10% v/v O_2 and 5, 10, 15 and 20% CO_2, with the balance being N_2O. The oxygen signal is so low compared with the CO_2 and N_2O signals that it appears as a 'baseline' current. Clearly separated diffusion plateaux are seen for CO_2 and N_2O. Diffusion plateaux for oxygen could also be seen if the oxygen reduction current part of the voltammogram was magnified sufficiently (see Fig. 25).

obtained in the absence of isoflurane indicated that the reduction process corresponded to the addition of one electron to the fluoranthene pendant groups in the polymer, particularly since the charged passed corresponded quantitatively to the amount of PVF deposited on the surface. In the presence of isoflurane (in the absence of oxygen and nitrous oxide), typical 'catalytic' behaviour was apparent, with the reduction peak being considerably enhanced and the oxidation peak being significantly reduced. Although these studies showed promise, the further development of such a modified electrode into a practical sensor was severely restricted by the limited lifetime of the polymer coats, because desorption of the polymer occurred on prolonged potential cycling, and after about 40 cycles no recordable voltammogram remained. The other problem with this device was that both oxygen and nitrous oxide displayed electroactivity at the PVF-modified electrode, making it impossible to deconvolute the O_2, N_2O and isoflurane components of the sensor current.

Compton and Northing[144] then made one more attempt to devise an amperometric sensor for the detection of isoflurane and nitrous oxide. In this new work they devised a channel-electrode gas sensor. The conventional channel electrode consists of an electrode set in one wall of a rectangular duct through which electrolyte solution is pumped under laminar flow conditions. The design of Compton and Northing[144] incorporated a gas-permeable membrane upstream of the electrode, and the substrate entered the cell from the gas phase through the membrane, and was then carried by a controlled (and well defined) flow to the electrode surface, where it was detected. Because the flow in the channel could be calculated, the signal observed from the working electrode was related to the concentration of the gas at the membrane–solution interface. This, in turn, gave a direct measure of the levels of the substrate bathing the exterior of the membrane in the gas phase. In order to eliminate all oxygen from the substrate, a second oxygen consuming electrode was located upstream of the membrane to 'scrub' oxygen from the system. Experiments were conducted to examine whether the reduction of anthraquinone (AQ) on the electrode upstream of the membrane could be used to eliminate oxygen interference on the downstream channel electrode. Their conclusion was that transport-controlled reduction of AQ to AQ^{2-} on the upstream electrode (−1.5 V *versus* Ag) did reduce any oxygen interference to acceptably low levels, with the following reaction taking place and 'titrating' any oxygen dissolved in the solution:

$$AQ^{2-} + 2O_2 \rightarrow AQ + 2O_2^{-}$$

These experiments were conducted successfully in the presence of up to 50% v/v oxygen.

The next step was to determine whether nitrous oxide could be detected *via* a mediated reduction by the fluoranthene radical anion in the channel electrode flow cell. Their previous work had already indicated that isoflurane could be detected in this fashion, since it underwent a reduction *via* an EC′ mechanism. Their new studies also showed that N_2O underwent mediated reduction according to the following mechanism:

electrode:

$$F + e^{-} \rightarrow F^{\cdot -}$$

solution:

$$F^{\cdot -} + N_2O \rightarrow F + \text{products}$$

and that a 'classical' EC′ mechanism operated.

The time response of the sensor to changes in isoflurane and nitrous oxide was examined by pulsing the detector electrode between two potentials (−1.5 and -1.9 V *versus* Ag) and allowing the system to reach a steady state at both potentials. A response time of 10 s or less was recorded, corresponding to the time required to establish new anaesthetic concentration profiles within the cell. By modifying the cell geometry to allow very fast flow rates, Compton and Northing expected the time response to improve significantly, with values approaching 1 s being expected.

Their studies also indicated that the catalysis of the fluoranthene reduction of nitrous oxide and isoflurane was additive, and that the two mechanisms proceeded in parallel. Their conclusion was that it would therefore be possible to use the channel electrode sensor to analyse mixtures containing both nitrous oxide and isoflurane, provided that an oxygen scrubber was placed upstream of the channel electrode and that an independent means of measuring one of the two anaesthetic gases, for instance using a separate amperometric nitrous oxide sensor, was available. Thus, a complete measuring system would consist of an oxygen scrubber, a channel electrode sensor and an independent amperometric N_2O detector. This complicated system illustrates how difficult it is to measure electrochemically two or more anaesthetic agents, simultaneously and separately, in the presence of oxygen.

Although the evidence presented above suggests that a simple electrochemical measurement technique is well nigh impossible to devise, some hopes have been raised by the possible use of micro-electrodes and non-aqueous solvents. As stated above, the early work of Compton and colleagues indicated that (at least with macro-electrode surfaces) isoflurane demonstrated a complete lack of electroactivity with a wide range of electrode materials in both aqueous and non-aqueous solvents. Other work, published in patent form,[140] has now indicated that not only isoflurane, but also other inhalational agents such as halothane, enflurane and sevoflurane, can be reduced on Au micro-electrodes in DMSO solution.

The published work reported so far has been conducted with bare Au microelectrodes (with diameters varying between 2 and 10 μm) in DMSO in a reaction cell, with the anaesthetic agents being supplied by conventional anaesthetic vaporisers in clinical concentrations. This work has indicated that, using Au micro-disc electrodes, there is a window where isoflurane, halothane, enflurane and sevoflurane display diffusion-controlled plateaux on the voltammograms.[140] Furthermore, the reduction waves for isoflurane, enflurane and sevoflurane are clearly distinguishable from that of oxygen. Also, similarly to the case of the production of O_2 and N_2O in DMSO with micro-electrodes, the reduction current for 33% oxygen is between one and two orders of magnitude smaller than that of the anaesthetic agents at their usual clinical concentrations (0.6–1.0% v/v). This is illustrated in Fig. 27 for a mixture of 1% v/v enflurane in a 33% v/v oxygen–67% v/v nitrogen mixture. Fig. 28 illustrates a series of voltammograms for enflurane, halothane, isoflurane and sevoflurane, giving a comparison of their respective

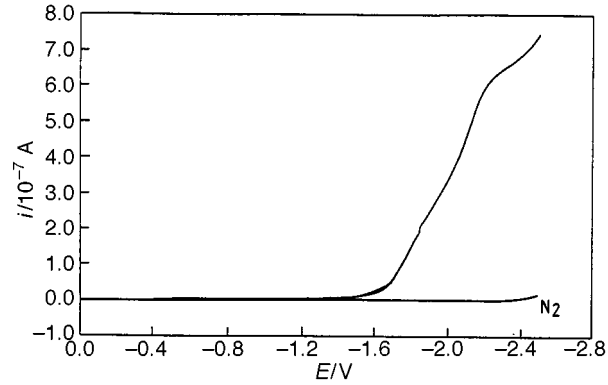

Fig. 27 A voltammogram for the reduction of 1% v/v enflurane in 33% O_2–67% N_2 for an unshielded 10 μm Au micro-disc electrode in DMSO solution.

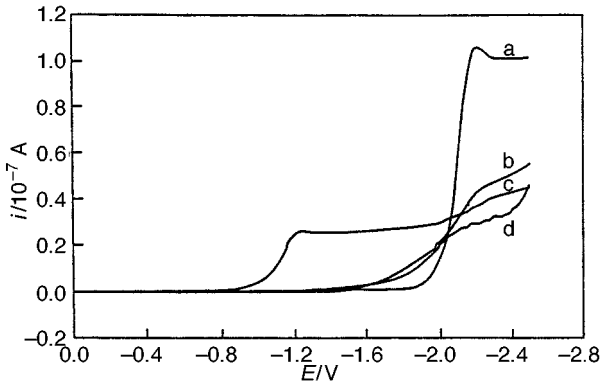

Fig. 28 Voltammograms for the reduction of enflurane, halothane, isoflurane and sevoflurane at an unshielded 12 μm Au micro-disc electrode, all on the same voltage scale, giving a comparison of their respective reduction potentials. (a) 1% v/v sevoflurane; (b) 1% v/v enflurane; (c) 0.6% v/v halothane; and (d) 0.6% v/v isoflurane.

reduction potentials in DMSO solution. Although this work has yet to be reported in detail, it does illustrate that the use of micro-disc electrodes and aprotic solutions may lead to a whole new understanding of the electrochemistry of anaesthetic agents in clinical gas mixtures.

Conclusion

This review has attempted to chart a pathway through the undeniably difficult waters of the electrochemistry of clinical gases and vapours. Despite valiant efforts by many researchers, little real electrochemical progress seems to have been made over the past four decades, following the discovery and demonstration of the ever-popular Clark and Stow–Severinghaus O_2 and CO_2 sensors. It is only, perhaps, when both the electrochemist and the physiologist/clinician turn their eyes back to the roots of electrochemical research in biology and medicine, namely that of the micro-disc electrode, that new progress will be made in this field. As far as this reviewer is concerned, the macro-disc electrode has had its day, perhaps even in the field of gaseous analysis. Although the supply of analyte molecules is essentially 'infinite' in a gas sample, and it is therefore tempting to manufacture macro-electrode sensors, the clear indications are that micro-electrodes allow individual gas species in gas mixtures to be separated electrochemically on the same surface, and thus analysed simultaneously and separately. It is beginning to appear that this process might apply not only to simple gas species such as O_2 and CO_2 but also to the anaesthetic gases and vapours. Only further research and development in this area will prove whether this hypothesis is short- or long-lived.

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