DEVELOPMENT OF A SYSTEM TO MONITOR OXYGEN CONCENTRATION BY PHOSPHORESCENCE QUENCHING: PRELIMINARY RESULTS

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ABSTRACT -- The quenching of phosphorescence lifetime by oxygen can be used as an effective method for monitoring oxygen concentration and hence oxygen partial pressure. This technique presents many advantages over the measurement of fluorescence intensity quenching used by many workers. These advantages are however accompanied by an increase in the measurement system complexity. The development of measurement instrumentation based on the pulsed excitation technique is described, its difficulties and limitations discussed and preliminary results presented.

INTRODUCTION

Many techniques have been developed over the years to monitor oxygen partial pressure (Delpy, 1988), and the use of the corresponding sensors has contributed to the treatment and diagnosis of cardiopulmonary disorders (Hahn, 1987). One of the most frequently employed ways to measure oxygen dissolved in fluids or in the gaseous form is through electrochemical principles (Bacon and Demas, 1987). However, other techniques have been employed (e. g., mass spectrometry) (Hahn, 1987). Electrochemical sensors consume oxygen (Rolfe, 1988) which gives rise to a liquid-gas measurement difference (Bird et al, 1974), and when a short response time is required, reduced accuracy, stability and an increased sensitivity to other parameters also results (Kreuzer et al, 1980). The use of the mass spectrometer to monitor oxygen is mainly restricted to respiratory gas analysis in the specialist laboratory (Scheid et al, 1979). Its use in the measurement of blood gases is hampered by difficulties including a long delay time and a poor response time (Parker and Delpy, 1983). Added to this, the mass spectrometer is a complex, rather fragile and very expensive instrument to purchase and maintain.

Sensors based on optical fibres have been developed in the past few years (Martin et al, 1987). Their potential advantages include: safety (no electrical connection to the patient), no electrical interference on the signal, very small and flexible fibres allowing easy introduction into the body and low cost. Many of the optical sensors developed to measure oxygen use luminescence as the sensing technique. Luminescence is produced in certain molecules when they are excited from the ground state to higher energy states. When the excited electron returns to the ground state, it emits light (fluorescence and/or phosphorescence) at a longer wavelength than the original stimulating light (the Stokes shift). Some luminescent indicators (dyes) are sensitive to oxygen and the intensity variation (quenching) can be used to monitor oxygen (Gehrich et al, 1986).

Vaughan and Weber (1970) showed that the fluorescent dye pyrene butyric acid (PBA) might be used to determine quantitatively oxygen in biological microenvironments. Lübbers and Opitz (1976) developed a fluorescent sensor using PBA dissolved in dimethylformamide (DMF). Using a permeable membrane of teflon (6 μm thick), reproducible results were obtained in the laboratory with a 90 % response time of 2-3 s (Lübbers and Opitz, 1983). Peterson et al (1984) developed a sensor for in vivo PO_2 measurement using fluorescence quenching of perylene dibutyrate. In practice, this optic

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PO_2 sensor suffered from a decrease of sensitivity with time, thrombus formation around the tip and, interference from volatile or gaseous anaesthetics. Other workers have also developed optical sensors, again based on fluorescence quenching. Lee et al (1987) for example, synthesised one indicator (5-(4-bromo-1-naphthoyl) pentyltrimethylammonium bromide) ($BNK5^+$) immobilized on γ-CD (cyclodextrins) cellulose which was capable of measuring gas-phase oxygen. However, the system was not suitable for use in aqueous samples because the humidity almost completely quenched the luminescence.

Although the above examples of optical sensors show some positive results, many difficulties (including response time, lifetime and stability) must be overcome before they can enter routine use. Added to these difficulties, the technique of luminescence intensity quenching has inherent problems. These include: (a) variations in the amplitude of the light signal caused by photodecomposition of the lumiphore or, changes in the gain of the associated electronics or, fluctuation in the excitation light intensity; (b) intensity changes due to optical interferences e. g. alteration in the optical path, light scattering and absorption (Vanderkooi and Wilson, 1986).

PHOSPHORESCENCE LIFETIME MEASUREMENT

In 1985, Vanderkooi and Wilson (1986) introduced a new optical method for measuring oxygen in biological systems based upon phosphorescence quenching. Their method differed from the previous luminescence quenching because they used the measurement of the lifetime of the phosphorescence, and not its intensity to monitor oxygen concentration.

Phosphorescence is a spin forbidden process which involves a change in electron spin. It occurs at a low probability, thus phosphorescence has a longer lifetime than fluorescence (Roberts, 1982). Oxygen quenching is diffusion limited, and its effect on lifetime is described by a modified Stern-Volmer (1919) relationship valid both for fluorescence and phosphorescence lifetimes:

$$\tau_0/\tau = 1 + k_q \tau_0 [O_2] \quad (1)$$

where: τ_0 and τ are the lifetimes in the absence of oxygen and at the measured oxygen concentration $[O_2]$, respectively. The quenching rate constant k_q is dependent upon the physical constants of the system and is given by:

$$k_q = 4 \pi N p (D_D + D_A) \times 10^{3} \quad (2)$$

where: N is Avogadro's number, p is a factor related to the probability of quenching and other interactions between the probe (donor) and oxygen (acceptor) (typically its value is about 1/9). D_D and D_A are the diffusion coefficients for the donor and the acceptor respectively. Under defined experimental conditions, k_q can be considered constant. To measure oxygen one must first obtain the values of τ_0 and k_q by measuring τ at two different oxygen concentrations, e. g. zero and equilibrated with air (Wilson et al, 1987). Then other values of τ measured can be used to calculate the oxygen concentration. Vanderkooi et al (1987) applied the method to measure oxygen concentration in solutions in reactions catalysed by glucose oxidase and by cytochrome c oxidase. The observed lifetimes (obtained from averaged decay curves) were found to fit a single exponential function which facilitated subsequent calculation.

The main advantages of the new technique are: changes in the concentration of the probe due to photodestruction do not disturb the lifetime; the oxygen concentration of the sample is minimally affected by the measurement; once τ_0 and k_q are determined for a fixed experimental system, the method does not require recalibration and; great sensitivity to oxygen concentration, allowing measurements to be made down to nanomolar levels (Vanderkooi et al, 1987). With regard to the instrumentation requirements, the pulsed excitation method can be used resulting in a relatively inexpensive detection system.

DEVELOPMENT

The aim of this development was to produce a clinically acceptable instrument to both excite the probe (dye) and to record and process the desired information. This instrument should also be as inexpensive as possible, and in its final version suitable for use as a bedside monitor. This article therefore deals with the development of the prototype of the measurement instrumentation based upon the pulsed technique for detection of phosphorescence lifetime. In this technique light pulses of a duration much less than τ excite the probe and the resulting decay profile is then measured. A time delay before measurement can be used to separate fluorescence from phosphorescence or, an optical filter can be employed since they have different wavelengths.

Following some preliminary work, the system shown in figure 1 was developed. A xenon flash lamp unit was built and is used as the optical excitation source. A Bentham monochromator (model M-300) is used to select the most effective wavelength to excite the dye. A photo multiplier tube (PMT) (Hamamatsu R 928) with a wide spectral sensitivity (185 nm $\leq \lambda \leq$ 930 nm) is used as the detector of the emitted light (positioned at 90° in relation to the incident light, so that less of the excitation light reaches it), and the electrical signal is stored in a digital sampling oscilloscope (Gould Ltd., model OS 4040). A sample chamber (representing the optical sensor at this stage) was also constructed. It consists of a light proof box which contains a sample holder into which a standard spectroscopy cell (CXA-145-150S from Gallenkamp Ltd.) of one cm path length is fitted. The chamber also contains a lens and an optical filter. The aspherical lens (39 AF 50 from Comar Ltd.) is used to improve the collection efficiency of the luminescent light from the cell. The optical filter (Schott type - 05 GB 50 from Comar Ltd.) helps to eliminate the undesired excitation light (high pass filter, $\lambda \geq$ 570 nm). In addition, a data acquisition system (CED 1401 from Cambridge Electronics Design Ltd.) is used. This is an intelligent peripheral that generates and receives waveforms, produces digital and time signals using its own processors, clocks and memory and which can interface to a personal computer (PC).

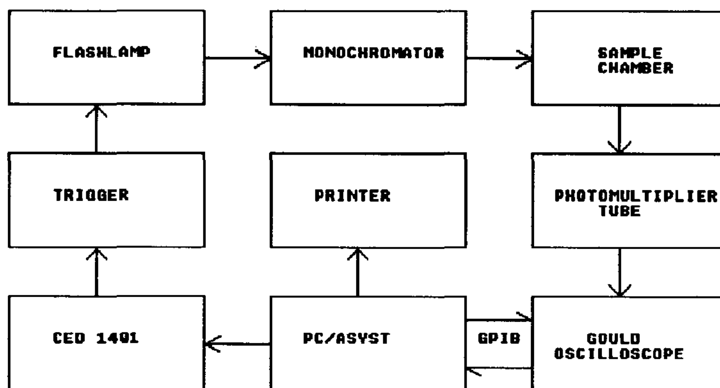

Figure 1. Measurement system using computer control.

The key element of the system is the Gould oscilloscope having a sample rate of 10 MHz (i. e., 100 ns sample interval) and capable of storing 5120 data points. This instrument also incorporates a General Purpose Interface Bus (GPIB). The whole system is controlled by the PC using the Asyst software package (MacMillan Software Ltd.). This software allows: graphics generation, sophisticated mathematical analysis, data acquisition and, control of scientific instrumentation through the GPIB data bus. A GPIB interface (Capital Equipment Ltd.) is installed in the personal computer. The CED 1401 is compatible with Asyst, and was therefore initially used for convenience to trigger the flash lamp.

RESULTS

The characteristics required of the xenon flash lamp were: a pulse duration of about 2 μs, energy per flash up to 1 J, maximum repetition rate of 50 Hz and a broad emission spectrum including the near UV. The best option for cost, characteristics and reliability was a xenon flash lamp bulb type (FX-249 from EG & G Ltd.). This flash lamp is enclosed in a metal can, and has a 1.5 mm arc length. The other main characteristics of the lamp were: maximum energy per flash (7 J) and spectral output from 310 to about 1100 nm .

To operate the flash lamp an FY-712 Lite-pac (EG & G Ltd.) is used to provide a series trigger for the lamp. The Lite-pac consists of a ceramic socket (incorporating a pulse transformer) into which the lamp fits. The total inductance of the discharge circuit is stated by the manufacturer to be about 0.5 μH. To calculate the approximate pulse width at 1/3 peak height, the equation below is used:

$$t_{1/3} = \pi \, (L\ C)^{1/2} \qquad (3)$$

The xenon flash lamp unit was tested using a charging voltage of 700 V and a storage capacitor of 1 μF/1000 V giving calculated values of E= 0.245 J and $t_{1/3}$= 2.2 μs. The measured pulse duration shown in figure 2, was about 3.5 μs (half maximum) or 7.5 μs ($t_{1/3}$). Considering that the wire lengths of the circuit were not optimized (adding inductance to the discharge circuit), the results were considered satisfactory at this stage.

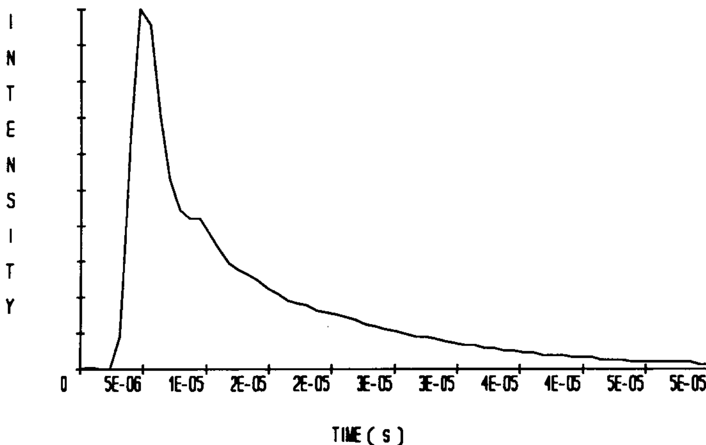

Figure 2. Optical pulse obtained with the xenon flash lamp.

Using the Asyst software, a control programme for the instruments was written. This programme had to be capable of: (a) triggering the flash lamp via the CED 1401, (b) storing the data in the Gould scope, (c) reading the stored data from the Gould scope (8 bits x 5120 points) via the GPIB data bus and, (d) analysing the data using Asyst. The preliminary version of the programme developed is divided into two main parts with respective menus. The first part involves the control of the instrumentation and the acquisition of data using the GPIB. The second part deals with the manipulation and analysis of data (averaging of the curves, fitting to a single exponential and calculating the lifetime).

Using the system of figure 1, and the flash lamp described above, experiments with dyes were started. A probe, Pd-coproporphyrin was obtained from Porphyrin Products Ltd. (Logan, Utah, USA), and a small quantity was dissolved in distilled water and

dimethylformamide. To observe the possible phosphorescence emission of the probe, it should ideally be excited at 380 nm, its peak absorption (figure 3). This was not however possible using the Bentham monochromator, since it uses a grating of 1200 lines/mm (blaze of λ= 750 nm) which has a poor response below 450 nm. However, using another excitation wavelength of the probe at 535 nm, phosphorescence emission was observed. This was possible because, although the probe is much less sensitive at 535 nm, the response of the monochromator is near its maximum. As was expected the maximum phosphorescence emission occurred at 670 nm.

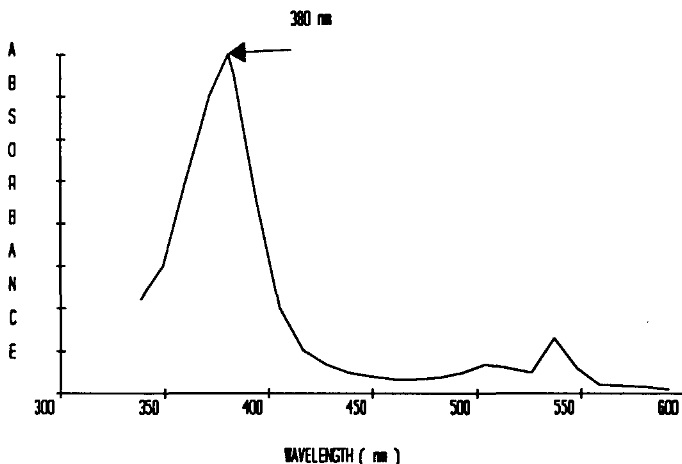

Figure 3. Absorption spectrum of Pd-coproporphyrin (modified from Vanderkooi et al, 1987).

Data points from 10 curves collected and averaged are presented in figure 4 together with the calculated lifetime for two different oxygen concentration [a] and [b] (concentration [a] > concentration [b]). The optical density of the sample was adjusted to 0.1 before the addition of bovine serum albumin (to make the quenching independent of the concentration of the dye (Vanderkooi et al, 1987)) and sodium dithionite (to consume oxygen). The curves shown are the best fit for single exponentials and the correlation coefficient for both was > 0.99 (using ANOVA).

DISCUSSION

A system for measurement of phosphorescence lifetime has been built. Although the results obtained so far are encouraging, it is necessary to optimise the characteristics of the system and complete the development of the sensor.

A problem, not mentioned yet, is the slow data acquisition, transfer and processing time of the overall measurement system due to limitations in the software. The acquisition of one single curve takes about 4 seconds to complete. The recording of the 10 averaged curves (figure 4) took, for example, more than 40 seconds. Added to this, some more time is required to process and analyse the data and one must bear in mind that the current version of the programme does not yet calculate the concentration or partial pressure of oxygen, the final aim of this development. Although data has not yet been obtained with this system, results from other workers (Vanderkooi et al, 1987) have shown that the linearity of the Stern-Volmer relationship (lifetime x oxygen concentration) is held for Pd-coproporphyrin in aqueous solution over a wide range of oxygen concentrations of clinical interest.

A possible way to increase the speed of data collection is to replace the storage

oscilloscope by using the CED 1401 as the A/D converter. Software is available with the CED which enables it to acquire and average data. Several parameters of the A/D operation can be controlled by the programmer such as: number of sweeps, number of data points, sampling rate, trigger type, clock period (which set the clock and generates TTL pulses that can drive, for example, the flash lamp), automatic filing and others. The use of the 1401 will however require additional amplification of the output current of the PMT.

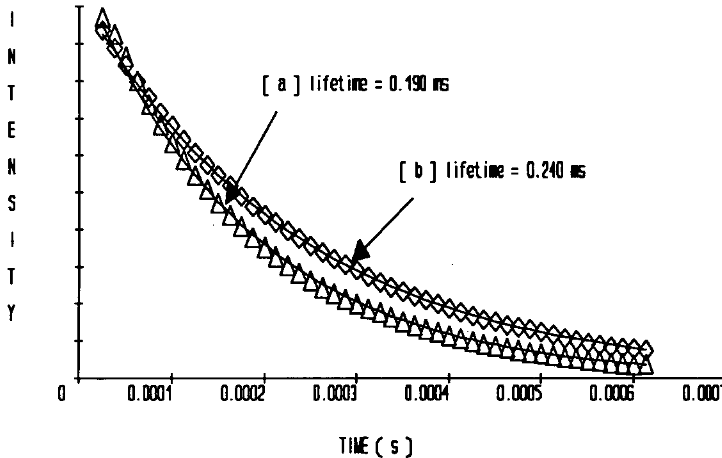

Figure 4. Phosphorescence lifetimes obtained with system and programme developed.

Regarding the other instrumentation, the monochromator in figure 1 will be substituted by an optical interference filter that transmits in the near UV region, since a better signal to noise ratio for the phosphorescence can be obtained by excitation at these wavelengths. A screened housing for the xenon flash lamp unit must also be constructed to minimise RF interference caused by the discharge pulse, and further work must be done to eliminate the effects of any interference on the functioning of the other instruments. This may involve gating the photomultiplier (i. e., turning the PMT off while the discharge spike is present) (Wardle, 1982).

Later, the optical sensor based upon an oxygen permeable film matrix must be constructed. This matrix will be formed by immobilising the probe in a suitable polymer substrate. As an example of this technique, Bacon and Demas (1987) have already successfully used a luminescent probe immobilized in silicone rubber. Also important to the final optical sensor design are the parameters of the optical fibre such as core/cladding ratio, numerical aperture (NA), transmission losses, material and mechanical ruggedness. In case of a fibre bundle, the right packing configuration is essential for optimal light collection. Finally, the performance of the optical sensor must be evaluated including the action of possible interferents.

CONCLUSION

In this article a new optical method of measuring oxygen partial pressure has been described. Suitable instrumentation to use with the sensor has been built. The instrument can produce an optical pulse of 3.5 μs duration (recently improved to 2 μs) and hence can measure phosphorescence lifetime of a probe (Pd-coproporphyrin) in a sample chamber. The performance and limitations of this instrument have also been presented and discussed.

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REFERENCES

Bacon, J. R., Demas, J. N. (1987). Determination of oxygen concentrations by luminescence quenching of a polymer-immobilized transition-metal complex. **Anal. Chem.,59**,2780-2785.

Bird, B. D., Williams, J., Whitwam, J. G. (1974). The blood gas factor: a comparison of three different oxygen electrodes. **Br. J. Anaesth.,46**,249-252.

Delpy, D. T. (1988). Developments in oxygen monitoring. **J. Biomed. Eng.,10**,533-540.

Gehrich, J. L., Lübbers, D. W., Opitz, N., Hansmann, D. R., Miller, W. W., Tusa, J. K., Yafuso, M. (1986). Optical fluorescence and its application to an intravascular blood gas monitoring system. **IEEE Trans. Biomed. Eng.,BME-33(2)**,117-132.

Hahn, C. E. W. (1987). Blood gas measurement. **Clin. Phys. Physiol. Meas.,8**,3-38.

Kreuzer, F., Kimmich, H. P., Brezina, M. (1980). Polarographic determination of oxygen in biological materials; in **Medical and Biological Applications of Electrochemical Devices** (ed. J. Koryta), p. 217. John Wiley & Sons.

Lübbers, D. W., Opitz, N. (1976). Quantitative fluorescence photometry with biological fluids and gases. **Adv. Exp. Med. & Biol.,75**,65-68.

Lübbers, D. W., Opitz, N. (1983). Optical fluorescence sensors for continuous measurement of chemical concentrations in biological systems. **Sensors and Actuators,4**,641-654.

Lee, E. D., Werner, T. C., Seitz, W. R. (1987). Luminescence ratio indicators for oxygen. **Anal. Chem.,59**,279-283.

Martin, M. J., Wickramasinghe, Y. A. B. D., Newson, T. P., Crowe, J. A. (1987). Fibre-optics and optical sensors in medicine. **Med. & Biol. Eng. & Comput.,25**,597-604.

Parker, D., Delpy, D. T. (1983). Blood gas analysis by invasive and non-invasive techniques; in **Measurement In Clinical Respiration Physiology** (eds. G. Laszlo and M. F. Sudlow), p. 75. Academic Press.

Peterson, J. I., Fitzgerald, R. V., Buckhold, D. K. (1984). Fiber-optic probe for in vivo measurement of oxygen partial pressure. **Anal. Chem.,56**,62-67.

Roberts, D. (1982). Block 4: Physical properties of excited states: in **Photochemistry: Light, Chemical Change and Life.** p. 7. Milton Keynes: Open University Press.

Rolfe, P. (1988). Review of chemical sensors for physiological measurement. **J. Biomed. Eng.,10**,138-145.

Scheid, P., Meyer, M., Piiper, J. (1979). Arterial expired PCO_2 differences in the dog during acute hypercapnia. **J. Appl. Physiol.,47(5)**,1074-1078.

Stern, O., Volmer, M. (1919). Über die Abklingungszeit der Fluoreszenz. **Physik. Zeitschr.(XX)**,183-188.

Vanderkooi, J. M., Wilson, D. F. (1986). A new method for measuring oxygen in concentration in biological systems. **Adv. Exp. Med. & Biol.,200**,189-193.

Vanderkooi, J. M., Maniara, G., Green, T. J., Wilson, D. F. (1987). An optical method for measurement of dioxygen concentration based upon quenching of phosphorescence. **The Journal of Biol. Chem.,262(12)**,5476-5482.

Vaughan, W. M., Weber, G. (1970). Oxygen quenching of pyrene butyric acid fluorescence in water. A dynamic probe of the microenvironment. **Biochem.,9(3)**,464-473.

Wardle, R. (1982). **Gating of Photomultipliers.** Middlesex: Thorn EMI Electron Tubes Ltd.

Wilson, D. F., Vanderkooi, J. M., Green, T. J., Maniara, G., Defeo, S. P., Bloomgarden, D. C. (1987). A versatile and sensitive method for measuring oxygen. **Adv. Exp. Med. & Biol.,215**,71-77.