Use of a wedge cuvette in this layer photometry and its application to oximetry

Citation for published version (APA):

DOI:
10.1007/BF01063864

Document status and date:
Published: 01/01/1977

Document Version:
Publisher’s PDF, also known as Version of Record (includes final page, issue and volume numbers)

Please check the document version of this publication:

• A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
• The final author version and the galley proof are versions of the publication after peer review.
• The final published version features the final layout of the paper including the volume, issue and page numbers.

Link to publication

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license above, please follow below link for the End User Agreement:
www.tue.nl/taverne

Take down policy
If you believe that this document breaches copyright please contact us at:
openaccess@tue.nl
providing details and we will investigate your claim.

Download date: 17. Sep. 2023
Use of a Wedge Cuvette in Thin Layer Photometry and Its Application to Oximetry*

J. A. E. SPAAN1, L. J. GARRED2, and P. VAN DE BORNE

1 Department of Physiology and Physiological Physics, University of Leiden, Wassenaarseweg 62, Leiden, The Netherlands
2 Lakehead University, Thunder Bay, Canada

Abstract. A wedge cuvette was constructed by fixing 2 glass plates at a known angle with a spacer at one end. This resulted in a thin layer with thickness varying from 0 to 250 μm. By measuring the intensity of a beam of light through the thin layer as a function of distance along the wedge (and thus layer thickness), the absorption coefficient at the light wavelength used could be obtained without a separate measurement of \( I_0 \), the reference light intensity. In addition, the difficult problem of determining accurate layer thickness as encountered in conventional thin layer photometry has been avoided.

Tests of the wedge cuvette method with Evans Blue and Malachite Green serial dilutions as well as with haemoglobin solutions at several oxygen saturations demonstrate that accuracy of the order of 1% can be obtained. Application of the wedge cuvette in experiments on oxygen uptake by layers of haemoglobin solution are discussed.

Key words: Photometry — Cuvette — Haemoglobin — Oximetry — Evans blue.

INTRODUCTION

The wedge cuvette is constructed of two high quality glass plates fixed at a known angle (Fig. 1). If the light path of a photometer is moved perpendicular to the axis of the wedge the measured intensity \( I \) will be a function of the position \( x \). From the measured relationship between \( \log I(x) \) and \( x \) the absorption coefficient of the solution for the light used can be obtained directly.

In conventional spectrophotometric measurements of a solution one uses a cuvette constructed of 2' plane parallel plates with a well known path length of the light. Before a cuvette with sample is introduced into the spectrophotometer, a reference intensity (100% transmittance) must be measured with water or other reference solution in a second cuvette. This use of more than one cuvette necessitates regular determination of cuvette corrections. More difficulties are encountered if thin layers are required. The cuvettes generally used are 1 mm cuvettes with thin plane parallel glass plates inserted to obtain a thickness down to 50 μm; however, the extremely accurate measurement of layer thickness is a difficult procedure (Van Assendelft, 1970).

Our particular problem and motivation for the technique described in this paper was the investigation of oxygen transfer in very thin layers (50—200 μm) of haemoglobin solution. This process was studied colorimetrically by means of a specially designed photometer using two Light Emitting Diodes (LED's) having peak emission wave lengths at 900 nm and 660 nm respectively. The following 2 experimental oxygen transfer studies were performed.

1. The oxygenation of an initially deoxygenated haemoglobin solution in a stationary thin layer (Spaan, 1976). During the oxygenation process the light path of the photometer through the layer remains fixed. The layer thickness and increase in oxygen saturation are determined from the measured change in light absorption at 660 nm.

2. Steady state oxygen uptake by a thin film of haemoglobin solution streaming down a glass plate from an oxygen free reservoir. The light path of the photometer traverses along the falling film. The layer thickness and average oxygen saturation as a function of position are measured via the light absorption at 660 nm and 900 nm.

The application of the photometer required values of the light absorption coefficients of the haemoglobin solution in the oxygenated and deoxygenated states for...
the spectral distribution of each LED. Moreover, the possible presence of haemoglobin which is unable to bind oxygen (e.g. methaemoglobin) requires, as will be explained below, a check of these absorption coefficients for each haemoglobin solution used.

The wedge cuvette technique was evaluated experimentally by using Evans Blue solutions at different concentrations and a hemoglobin solution at different oxygen saturations. Since Evans Blue shows almost no light absorption at a wavelength of 900 nm and the light absorption of a haemoglobin solution depends strongly on oxygen saturation at 660 nm, only measurements are reported obtained with the LED having a peak emission wavelength of 660 nm. The photometer used will not be described in detail since it has special features relevant only to the particular oxygen transfer experiments mentioned and not to the use of a wedge cuvette as such. Details of the entire apparatus may be found elsewhere (Spaan, 1976).

APPARATUS
A wedge has been constructed with a length of 70 mm over which the length of the light path varies from 0 to approximately 250 μm. Thus μ is in the order of 3.7 × 10⁻³ which corresponds to a wedge angle of 0.2°. High quality glass with a thickness of 6 mm (± 1 μm) was used. The glass plates are held in a specially constructed cuvette holder. The sides of the wedge are sealed but an inlet and outlet remain so that it can be refilled without dismounting. The cuvette volume is in the order of 0.3 ml. The cuvette can be filled anaerobically from a syringe. A sample present in the cuvette can be rinsed out completely by 3 ml of a new sample. A Van Abbe measuring device (manufactured by Carl Zeiss, W. Germany) was used to measure the thickness of the glass plates and the thickness change and thus the angle of the
wedge. The value $\mu$ could be determined in this manner with an error of less than 0.5%. The wedge is used with a single beam photometer built in our laboratory. Glass fibers of 2 mm diameter conduct the light to the wedge from two light emitting diodes (Fig. 1).

The peak light intensity of the spectral distribution of one LED is at 660 nm, and of the other LED at 900 nm. The width of both distributions at 50% peak intensity amounts to 40 nm. The photometer is mounted in a device which automatically traverses the wedge length. The light intensity is recorded on a digital data logger at 2 mm intervals.

TESTING EXPERIMENTS

In order to avoid problems specifically related to the use of haemoglobin solutions Evans Blue was used to test our device. Four different pH-buffered dilutions of dye were introduced into the wedge one at the time and two passes of the wedge length were made with the photometer, giving identical results. Repeat measurements were made for the concentration series. The $\log I$ data for each of the Evans Blue dilutions (Fig. 2) form a straight line function of $x$, as predicted. A linear regression was applied to the data and analysis of variance showed the 95% confidence limits to be in the order of 1% of the slopes of the curves.

The intersection point of the lines is close to $x = 0$ and corresponds to the first term of the right hand side of eq. (3). This value agrees with the measured $\log I$ when the cuvette was filled with water. This could be expected since $[\sinh(\frac{1}{2} e \mu w)]^{\frac{1}{2}} e \mu w$ remained between 1 and 1.001 in all our experiments.

The light absorption coefficient $e$ is determined from the slope of these plots and knowledge of $\mu$. In Figure 3 the values of $e$ are plotted against the original concentrations. The result is a straight line passing through the origin, the slope of which yields a value of 0.398 $\text{lg}^{-1} \text{m}^{-1}$ for $e$. A value of 0.408 $\text{lg}^{-1} \text{m}^{-1}$ was obtained for $e$ at 660 nm from measurements with a standard 1 cm cuvette on a Cary spectrophotometer. The small difference (2.5%) between both values for $e$ may be explained on the one hand by an uncertainty concerning the emission spectrum of the LED and on the other hand by the steep slope of the $e-\lambda$ curve of Evans Blue at 660 nm.

APPLICATION OF THE WEDGE CUvette TO OXYGEN TRANSFER MEASUREMENTS

We utilized the wedge cuvette to determine the absorption coefficient of haemoglobin solutions in both the oxygenated and deoxygenated state. As explained in the introduction these absorption coefficients were needed for the interpretation of oxygen transfer exper-
ments. The basic equation used for the interpretation of both these experiments is

\[
\log \frac{I}{I_0} = -\left[\varepsilon_{\text{Hb}} C + (\varepsilon_{\text{HbO}_2} - \varepsilon_{\text{Hb}}) Cy + e_i\right] l
\]  

(4)

where

- \(\varepsilon_{\text{Hb}}\) = specific extinction of pure haemoglobin
- \(\varepsilon_{\text{HbO}_2}\) = specific extinction of pure oxyhaemoglobin
- \(C\) = the concentration of haemoglobin capable of binding oxygen
- \(e_i\) = the cumulative absorption coefficient for the impurities present
- \(l\) = layer thickness
- \(y\) = fraction of \(C\) within the light path with bound oxygen.

Eq. (4) is derived easily from Lambert-Beer's law and takes into account the light absorption by haemoglobin and oxyhaemoglobin present in the solution as well as other absorbing species such as methaemoglobin.

Eq. (4) may be rewritten as follows

\[
\log \frac{I}{I_0} = -e_f l = -\left[e_0 + (e_1 - e_0)y\right] l
\]  

(5)

where

- \(e_0 = \varepsilon_{\text{Hb}} C + e_i\)
- \(e_1 = \varepsilon_{\text{HbO}_2} C + e_i\)
- \(e_y\) = absorption coefficient of the solution at oxygen saturation \(y\).

Thus \(e_0\) and \(e_1\) represent the absorption coefficient of the haemoglobin solution in the deoxygenated and oxygenated state respectively.

The validity of eq. (5) was tested with the wedge cuvette and using a haemoglobin solution with approximately 120 g/l total haemoglobin and 5% metahaemoglobin.

The haemoglobin solution was obtained from packed red blood cells (human blood group O"). The cells were rinsed three times with saline and subsequently lysed by adding 60 ml of distilled water to 100 ml packed cells. The red cell stroma was removed by adding another 40 ml of toluene (C\(_6\)H\(_8\)) to the solution. After shaking the mixture the toluene and stroma were separated from the solution by centrifuging (6000 g, 20 min).

The absorption coefficient \(e_y\) was determined from samples having different oxygen saturations. From the samples the oxygen content \(V_{O_2}\) was determined using a Lex-O\(_2\)-con, a commercially available instrument. Since the amount of dissolved oxygen is small compared to the amount of bound oxygen in our samples, except the one with a \(P_{O_2} = 700\) mm Hg, one may expect a linear relationship between \(e_y\) and \(V_{O_2}\) (=\(y\)C). This is clear from the following

\[
e_y = e_0 + (e_1 - e_0) \frac{V_{O_2}}{C}
\]  

(6)

Eq. (6) results from eq. (5) and the definition of oxygen saturation of the solution. Figure 4 shows a fair correlation between both measuring techniques even though the hemoglobin solution contained 5% methaemoglobin.

**DISCUSSION OF APPLICATION**

**OF THE WEDGE CUvette**

The main advantage in utilizing a wedge cuvette becomes clear from eq. (3). There is no need for calibrating the instrument before each measurement. This calibration is also avoided using a double beam spectrophotometer although the matching of both cuvettes used has to be checked regularly. In principle there is no need to use a wedge cuvette in order to avoid calibrating the photometer. By using a cuvette in which two known distinct liquid layer thicknesses are formed, rather than one continuous changing layer thickness as within the wedge, the same results may be obtained (Longhurst, 1967). However if thin layer cuvettes are needed, a wedge shaped cuvette is easier to build since only smooth surfaced glassplates are required. In our specific problem the wedge method had a major advantage compared to plane parallel cuvettes. Since in our oxygen uptake experiments a light source of finite bandwidth and haemoglobin solutions of high concentration were used, the calibration with the wedge demonstrated the law of Lambert-Beer to hold for the layer thicknesses used in the oxygenation experiments.

The wedge cuvette has been utilized in the laboratory of the Eindhoven University of Technology since
Regression analysis applied to the wedge experiments on Evans Blue presented here yield 95\% confidence limits in the order of 1\%. In later experiments these confidence limits decreased to 0.5\%. Furthermore, the detailed construction of the cuvette changed during the years of application. During the earlier work the construction was such that the cuvette could be dismounted for cleaning; remounting the cuvettes necessitated a recalibration of the wedge angle. However, it appeared that by rinsing successively with detergent, water and alcohol, the cuvette could be cleaned perfectly. Therefore it was decided to construct permanent cuvettes. In our experiments the wedge angle $\mu$ was determined by measuring the thickness as a function of position $x$ outside the wedge. Obviously the value of $\mu$ can also be determined by use of a solution with a known absorption coefficient and eq. (3). However, we have been unable to find a dye having a plateau in its absorption spectrum at 900 or 660 nm.

Although the wedge cuvette method was developed to overcome problems in thin layer oximetry as discussed above, clearly it may find more general applications, for example in spectrophotometry. As is obvious from eq. (3), there is, at least in principle, no limitation with regard to beam-width or wedge-angle.

Acknowledgements. We gratefully acknowledge the dedicated and professional technical assistance of Diana Garred in this work.

REFERENCES


Received June 13, 1977