

DEVELOPMENT AND EVALUATION OF A NONINVASIVE HEMOGLOBINOMETER

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ABSTRACT

Measurement of hemoglobin concentration is among the most common clinical procedures. It is necessary in screening for anemia and is a means of providing a general assessment of physical well-being. Current methods for measuring hemoglobin concentration are invasive, requiring blood access. This paper describes a novel technique for measuring hemoglobin concentration noninvasively. The method takes advantage of the unique optical properties of hemoglobin to make the measurement. A vascularized tissue bed is transilluminated with a narrow linewidth optical source at an isobestic wavelength of oxy- and deoxy-hemoglobin. The transmitted light intensity is measured as the tissue is compressed, which causes an increase in the transmitted optical signal by forcing blood out of the optical path. The Lambert-Beer Law, modified to include optical scattering by tissue, provides the basic principle upon which the technique is based. The theory is discussed and an experimental prototype device is described. Preliminary analysis of experimental results shows a correlation between measurements obtained using the noninvasive tissue compression/transillumination method and hemoglobin concentrations measured by a clinical (invasive) hemoglobinometer. However, noise in the data are cause for concern, and may limit the clinical utility of the technique. Possible explanations for the variability in experimental results are given, along with suggestions for possible improvements to the device and technique.

1. INTRODUCTION

Blood is the liquid of life. In the conduct of the body's moment-to-moment business, blood serves a multitude of purposes. It is the conduit for nutrients, water, and waste products. It assists in the regulation of body temperature. In blood's milieu the body maintains a militia of infection fighters, ready to mobilize to defend against foreign bodies and disease.

Among blood's most important functions, however, is the transport of the essential metabolite oxygen from the lungs to tissues and the subsequent transport of the metabolic byproduct carbon monoxide from the tissues back to the lungs. The highly specialized molecule charged with this essential transport function is hemoglobin. In the normal individual, over a quarter million hemoglobin molecules are contained within every erythrocyte. Normal ranges for adult hemoglobin concentration are given in Table 1.

Table 1. Normal ranges for adult hemoglobin concentration in whole blood.

Adult Males	13.5 – 17.5 g/dl
Adult Females	12.0 – 16.0 g/dl

The measurement of total hemoglobin in blood is one of the most common procedures conducted when assessing an individual's overall health status. Current methods for determining the concentration of hemoglobin and other blood constituents require direct blood access. There are numerous disadvantages associated with obtaining a blood sample from a patient, including pain and inconvenience to the patient as well as the more serious risk to both patient and caregiver of blood-borne disease transmission. There may also be morbidity associated with repeated sampling from a patient requiring chronic blood constituent monitoring. In cases where the blood is transferred to a laboratory for analysis, there is a risk of sample mix-up or loss causing false reporting of results. For these reasons, it is desirable to have a means of measuring blood constituents without requiring blood access. The goal of this project was to evaluate a novel technique for measuring the concentration of hemoglobin in blood noninvasively.

The unique optical properties of hemoglobin make it particularly suitable for noninvasive interrogation. The visible to near-infrared region in which hemoglobin absorbs light most strongly (350 –

1100 nm) is a sort of optical window into biological systems. At shorter wavelengths, photons are absorbed strongly by the epidermis and dermis, preventing penetration into the subdermal vascular space. At longer infrared wavelengths above 1100 nm, the optical density of water overshadows that of other optical absorbers. Pulse oximeters, which have become a mainstay in operating rooms, take advantage of this optical window to monitor a patient's arterial blood oxygenation level (ref). The noninvasive hemoglobinometer also takes advantage of this optical window to measure hemoglobin concentration.

2. THEORY

The principle of the noninvasive hemoglobinometer is based loosely on the Lambert-Beer Law, which states that the optical absorbance of a substance is dependent on the concentration of absorbers in the substance and the optical pathlength through the substance (Eq. 1).

$$\ln(I_0/I) = \epsilon(\lambda)Cx \quad \text{Lambert-Beer Law} \quad (1)$$

where $\epsilon(\lambda)$ is the wavelength-dependent extinction coefficient C is the absorber concentration, and x is the optical pathlength.

The noninvasive hemoglobinometer operates by transilluminating a vascularized tissue bed with monochromatic (laser diode) light at 807 nm, an isobestic wavelength (i.e., a wavelength at which two or more absorbers have identical optical extinction coefficients) for the two most abundant hemoglobin species, oxy- and deoxy-hemoglobin. The optical signal is attenuated as it passes through the tissue and sampled on the opposing end with a silicon photodiode.

The Lambert-Beer Law as defined in Eq. 1 applies when there is no fluorescence of the sample and no scattering of photons as they transit through the sample. While the photons in the wavelength range of interest for measuring hemoglobin induce little or no fluorescence in tissues, photons in this range are strongly scattered by cells, subcellular organelles and extracellular matrix components. It is therefore necessary to modify the Lambert-Beer Law to include a scattering term (μ_s) when considering biological tissues:

$$\ln(I_0/I) = [\epsilon(\lambda)C + \mu_s]x \quad (2)$$

In describing the operation of the device, the vascularized tissue is described by a two-compartment model comprised of a vascular (blood) compartment

and a tissue compartment (Figure 1). With this model, Eq. 2 can be rewritten as:

$$\ln(I_0/I) = [\epsilon_{Hb}C_{Hb} + \mu_{s,bl}]x_{bl} + [\mu_{a,tiss} + \mu_{s,tiss}]x_{tiss} \quad (3)$$

where:

- ϵ_{Hb} = extinction coefficient of hemoglobin (807 nm)
- C_{Hb} = intravascular concentration of hemoglobin
- x_{bl} = pathlength through the vascular compartment
- $\mu_{s,bl}$ = scattering coefficient of blood
- x_{tiss} = pathlength through tissue compartment
- $\mu_{a,tiss}$ = absorbance coefficient of non-blood tissue
- $\mu_{s,tiss}$ = scattering coefficient of non-blood tissue

In order to determine the concentration within the vascular space, it is necessary to (1) obtain information about the pathlength through the blood and (2) account for the scattering and absorbance by the non-blood tissue. This is achieved by compressing the tissue. The tissue compartment is somewhat constrained by nature of the structural components of its cells and the extracellular matrix. The blood compartment, however, is free to move within the vascular space, and will do so when the surrounding tissue pressure exceeds the intravascular pressure. As the external pressure increases, the venous vessels (venules and small veins) will be the first to collapse, followed by capillaries and arterioles. Ultimately, the external pressure will cause the tissue to deform. A basic assumption of the theory is that there will exist a tissue compression region where closure of the opposing plates will correspond directly to a change in the optical pathlength through blood (Figure 1). Based on this assumption, Eq. 3 can be used to derive an expression for the hemoglobin concentration.

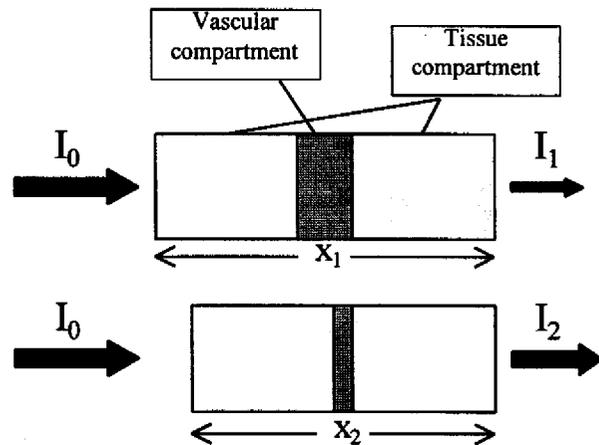


Figure 1. Two-compartment tissue model.

$$\ln(I_0/I_1) = [\epsilon_{Hb}C_{Hb} + \mu_{s,bl}]x_{bl,1} + [\mu_{a,tiss} + \mu_{s,tiss}]x_{tiss,1} \quad (4)$$

$$\ln(I_0/I_2) = [\epsilon_{Hb}C_{Hb} + \mu_{s,bl}]x_{bl,2} + [\mu_{a,tiss} + \mu_{s,tiss}]x_{tiss,2} \quad (5)$$

Subtracting (4) from (5) yields

$$\ln(I_1/I_2) = [\epsilon_{Hb}C_{Hb} + \mu_{s,bl}](x_{bl,2} - x_{bl,1}) + [\mu_{a,tiss} + \mu_{s,tiss}](x_{tiss,2} - x_{tiss,1}) \quad (6)$$

Since blood comprises only a small percentage of the total tissue volume (less than 5% in most tissues), it can reasonably be assumed that the contribution of the vascular compartment to optical scatter is small relative to that of the tissue compartment:

$$\mu_{s,bl}x_{bl} + \mu_{s,tiss}x_{tiss} \cong \mu_{s,tiss}x_{tiss} \quad (7)$$

Furthermore, if the compression distance covers the range where blood is the only mobile component, then

$$x_{tiss,2} - x_{tiss,1} = 0 \quad (8)$$

Thus Eq. 6 is reduced to

$$\ln(I_1/I_2) = \epsilon_{Hb}C_{Hb}(x_{bl,2} - x_{bl,1}) \quad (9)$$

Finally, optical scattering will lengthen the optical pathlength through the tissue. Thus the change in optical pathlength through blood should be proportional to the overall change in tissue thickness:

$$x_{bl,2} - x_{bl,1} = f(x_2 - x_1) \quad (10)$$

This yields a general expression that can be used to relate the hemoglobin concentration (C_{Hb}) to one known constant (ϵ_{Hb}) and two measurable quantities (x, I):

$$C_{Hb} \propto \epsilon_{Hb} \partial[\ln(I)]/\partial x \quad (11)$$

3. METHODS

3.1 Device Description

Within the tissue interface tool of the noninvasive hemoglobinometer reside the necessary components for tissue compression, optical signal generation and detection, and position sensing (Figure 2). A DC motor drives a captive screw mechanism to cause linear relative motion between the source and detector. A laser diode ($\lambda = 807$ nm) provides the source of optical radiation. A silicon photodiode positioned on the opposing surface of the tissue interface (aligned linearly with the laser diode) serves

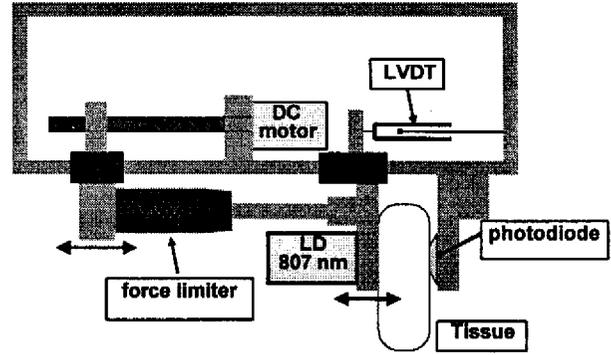


Figure 2. Diagram of the tissue interface tool.

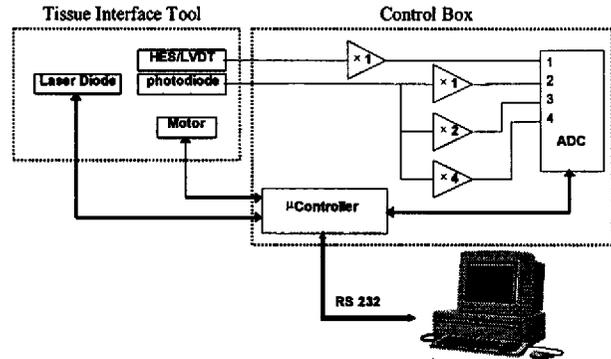


Figure 3. Overall block diagram of the noninvasive hemoglobinometer.

as the optical detector. During operation, vascularized tissue is interposed between the laser diode and the photodiode. An LVDT positioned between the mobile laser diode mount and a point fixed relative to the photodiode provides a measure of the source-detector separation distance. A force limiter protects against tissue damage caused inadvertently by excessive tissue compression.

The tissue interface tool is connected to a microprocessor-based control box (Figure 3). The microcontroller (PIC 16C65) provides motion control to the tool via pulse-width modulation of the power signal that drives the DC motor in open-loop fashion. It also paces the analog-to-digital conversion of the photodiode and LVDT signals. The laser diode drive signal is chopped, providing a measure of the contribution of ambient light to the optical signal. The ambient signal is subtracted from the "laser diode on" signal to obtain the desired net optical signal. The photodiode signal is routed through three parallel amplifier stages, each with a different gain setting, allowing optimization of the signal-to-noise ratio and the dynamic range for a given experiment. The control box is linked to a PC through a serial (RS 232)

connection. Motion control parameters (rate of tissue compression and decompression, number of compression cycles, etc.) are passed to the microcontroller prior to the start of data acquisition. During data acquisition, sampled data (optical and position signals) are transferred to the PC at ~ 84 Hz per signal (168 Hz total data transfer rate) for recording to file.

3.2 Animal Preparation

The canine tongue was used as the experimental tissue model. Five mongrel dogs were used in the study. At the start of an experiment, the test animal was placed under general anesthesia. Splenectomy was performed to reduce the animal's ability to restore hematocrit during hemodilution. The animal's hemoglobin concentration was incrementally reduced by removing blood and then infusing a combination of colloid (hydroxyethyl starch) and crystalloid (lactated Ringer's) solutions to restore blood volume. Hemoglobin concentration was reduced in this manner from a mean initial value of 17.2 g/dl (s.d. 1.3) to a mean low value of 5.3 g/dl (s.d. 2.2) in 5 (n = 3) or 6 (n = 2) steps. Reference hemoglobin measurements were taken periodically with the HemoCue hemoglobinometer. Animals were euthanized by intravenous potassium chloride injection at the conclusion of data acquisition. All animal experiments were conducted following a protocol approved by the University Hospital Institutional Review Board (protocol #96-09012).

3.3 Experiment Protocol

The tissue interface tool was positioned toward the rostral tip of the canine tongue, offset from the midline, for data collection. At each hemoglobin increment, data were collected for several compression profiles, which included oscillatory motion profiles of various amplitudes and frequencies and "ratchet" profiles (oscillatory motion superimposed on a ramp function). Several hemoglobin reference measurements were taken with the HemoCue hemoglobinometer at each hemoglobin level to track drift in the animal's hemoglobin concentration caused by fluid exchange between the intra- and extravascular space.

3.4 Data Analysis

Signal analysis routines were written in the Matlab script language to process the data. Data corresponding to the compression regions of the multi-cycle compression/decompression curves were extracted from each data set. Piecewise linear least

squares regression was performed to calculate average light intensity as a function of source-detector separation distance. The slope of the light intensity vs. source-detector separation curves was also calculated in this fashion.

4. RESULTS

Representative plots of light intensity and slope of light intensity vs. source-detector separation distance are shown in Figures 4 and 5. These plots represent a single compression profile performed on a single animal across a range of hemoglobin concentrations. In some experiments, the shapes of the light intensity and slope curves were fairly constant across the range of hemoglobin concentrations. In many cases, however, the curve shapes showed high variability, making it difficult to identify a particular source-detector

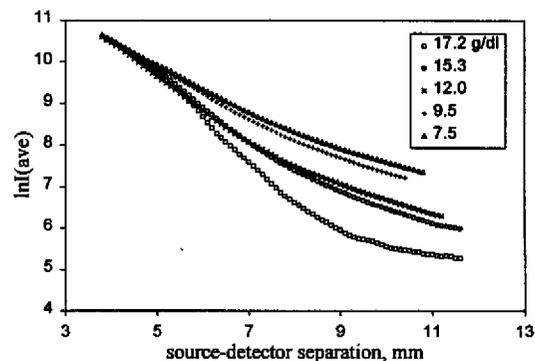


Figure 4. Average optical intensity data obtained from canine tongue using the noninvasive hemoglobinometer at various hemoglobin concentrations.

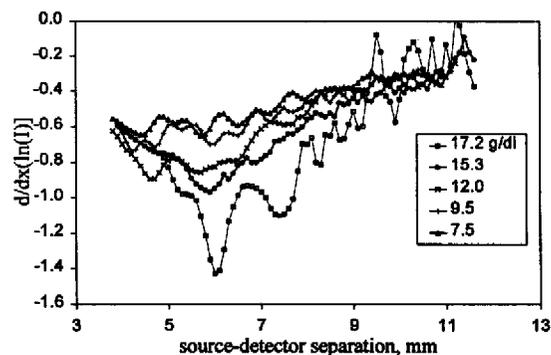


Figure 5. Slope of the optical intensity vs. source-detector data of Figure 4.

separation difference at which to make comparisons of the slopes with their corresponding hemoglobin values. In order to facilitate comparison of slope data with invasively measured hemoglobin concentration, minima in the slope curves were identified and plotted against the reference hemoglobin measurements (Figure 6).

5. DISCUSSION

Results from the animal experiments indicate that the noninvasive approach to measuring hemoglobin described in this paper has some merit. In one of the five studies, the decrease in slope of the light intensity data was quite obvious, with a steady decrease toward steeper (more negative) slopes at the higher hemoglobin values. In most studies, a general trend toward steeper slope of the light intensity data at the higher hemoglobin levels could be detected by observing the data qualitatively, but the trend was much less obvious and was not consistently monotonic. High variability in the data made quantitative analysis of the data difficult. Plotting slope minima (these are the steepest values since the slopes are negative) as a function of invasively measured hemoglobin levels provided a means of comparing the device performance across several test animals.

There are several possibilities for the variability in the data. First, it is possible that the canine tongue is not the most suitable tissue model. While this tissue is highly vascularized, it is very flaccid. Thus the tissue deforms readily upon compression. Tissue deformation results in a change in the scattering properties of the tissue. In the current model, tissue scattering can only

be accounted for if it is highly repeatable. Furthermore, the flaccid tissue made it difficult to maintain a constant position on the tongue over the several hours it took to conduct an experiment. An additional problem with the canine tongue is that there are several large veins and arteries in the tissue with numerous arteriovenous shunts which dogs utilize for temperature regulation. If the device were positioned over a large vessel, a greater change in optical intensity would be observed during tissue compression which would distort the measurement. A tissue with more homogeneous distribution of the vasculature would be more suitable for device evaluation.

Even with a more rigid and uniform tissue such as the finger, the linear compression approach may not provide an adequate means of removing blood from the optical path. Strong scattering in the tissue will cause photons to pass into the tissue immediately adjacent to the compression region. Blood in this region will absorb the scattered photons, reducing the optical signal that passes to the detector and distorting the measurement.

To overcome these potential problems, a next generation device should provide a more accurate means of measuring the change in blood volume in the transilluminated tissue bed. Furthermore, the device could take advantage of tissue scattering of the optical signal by surrounding the tissue with optical detectors, thus maximizing the information obtained from the optical signal.

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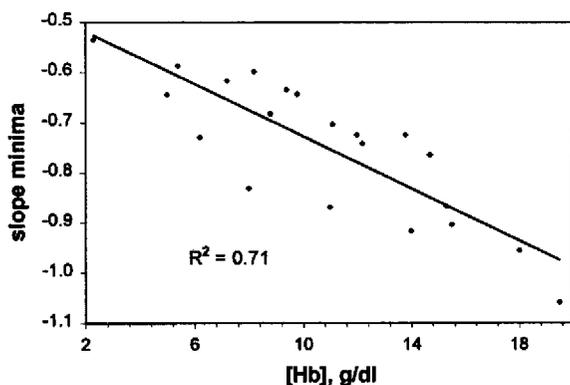


Figure 6. Slope minima of noninvasive hemoglobinometer data vs. invasive hemoglobin measurement (outliers culled).