

Photoacoustic measurements of red blood cell oxygen saturation in blood bags *in situ*

Ruben N. Pinto^{ab}, Karan Bagga^{ac}, Alexandre Douplik^{ab}, Jason P. Acker^{de}, and Michael C. Kolios^{ab}

^aInstitute of Biomedical Engineering, Science and Technology, Toronto, Canada

^bDepartment of Physics, Ryerson University, Toronto, Canada

^cDepartment of Mechanical and Industrial Engineering, Ryerson University, Toronto, Canada

^dCentre for Innovation, Canadian Blood Services, Edmonton, Canada

^eDepartment of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Canada

ABSTRACT

Red blood cell (RBC) transfusion is a critical component of the health care services. RBCs are stored in blood bags in hypothermic temperatures for a maximum of 6 weeks post donation. During this *in vitro* storage period, RBCs have been documented to undergo changes in structure and function due to mechanical and biochemical stress. Currently, there are no assessment methods that monitor the quality of RBCs within blood bags stored for transfusion. Conventional assessment methods require the extraction of samples, consequently voiding the sterility of the blood bags and potentially rendering them unfit for transfusions. It is hypothesized that photoacoustic (PA) technology can provide a rapid and non-invasive indication of RBC quality. In this study, a novel PA setup was developed for the acquisition of oxygen saturation (SO₂) of two blood bags *in situ*. These measurements were taken throughout the lifespan of the blood bags (42 days) and compared against the clinical gold standard method of the blood gas analyzer (BGA). SO₂ values of the blood bags increased monotonically throughout the storage period. A strong correlation between PA SO₂ and BGA SO₂ was found, however, PA values were on average 3.5% lower. Both techniques found the bags to increase by an SO₂ of approximately 20%, and measured very similar rates of SO₂ change. Future work will be focused on determining the cause of discrepancy between SO₂ values acquired from PA versus BGA, as well as establishing links between the measured SO₂ increase and other changes in RBC *in situ*.

Keywords: Blood bag, blood gas analyzer, hemoglobin, oximetry, oxygen saturation, photoacoustics, red blood cell, red cell concentrate, storage lesion, 2,3-DPG

1. INTRODUCTION

Red blood cell (RBC) transfusion is the most common procedure carried out in transfusion medicine and is an integral part of health care. RBC transfusion is primarily implemented for the augmentation of a compromised oxygen carrying capacity, attributed to acute and chronic situations including surgery, trauma and anemia¹. From donated blood, RBCs are separated into a sterile blood bag that contains a prescribed amount of preservative solution to aid in prolonging the *in vitro* shelf life². Storage in blood banks or hospitals of the so called red cell concentrate (RCC) occurs at hypothermic environments (1-6°C) for a maximum of 6 weeks³.

The RBC cytosol contains approximately 270 million molecules of hemoglobin, each composed of a tetramer structure that can bind up to 4 diatomic oxygen molecules⁴. Hemoglobin exists primarily in either an unbound deoxygenated state (Hb) or an oxygenated state (HbO₂). The oxygen saturation (SO₂) of RBCs is defined as the fraction of oxygenated molecules:

$$SO_2 = \frac{[HbO_2]}{[HbO_2 + Hb]} \quad (1)$$

The affinity of hemoglobin to oxygen is heavily regulated by the presence of 2,3-diphosphoglycerate (2,3-DPG)⁴. At regular *in vivo* conditions, 2,3-DPG exists in nearly equimolar intracellular concentrations to that of hemoglobin⁵. By favourably attaching to unbound hemoglobin, 2,3-DPG facilitates an enhanced net release of oxygen⁶. A lack of intracellular 2,3-DPG would result in an unregulated increase in hemoglobin's affinity for oxygen and consequently an increase in SO₂⁶. Furthermore, this abnormally high affinity for oxygen would reduce the ability to effectively deliver oxygen to deprived environments.

RBCs stored in blood bags *in vitro* have been shown to undergo reversible and irreversible changes in biochemistry, functionality and morphology during the 6-week storage period⁷⁻¹⁰. Collectively termed as the RBC storage lesion, these changes reduce the RBC's *in vivo* functionality when reinfused and increase in severity over the storage duration⁸. One consequence of the RBC storage lesion is an increased affinity to oxygen over time due to the rapid degradation of 2,3-DPG¹⁰. Low storage temperature slows down the rate of glycolysis and creates a shortage of available ATP for basic cellular upkeep. To compensate, phosphatases are activated to target and break down 2,3-DPG into components that are used to drive the glycolytic pathway towards producing more ATP^{6,11}. Under constant partial pressures of oxygen (pO₂), an increased oxygen affinity results in a monotonic increase in SO₂. Due to the link between 2,3-DPG consumption, poor ATP production and declining cell viability, SO₂ changes could serve as a marker for *in vitro* RBC storage lesion growth.

Blood banks and clinical laboratories routinely use the blood gas analyser (BGA) to acquire several blood-related parameters¹². Considered the gold standard technology, BGAs incorporate a spectrophotometer to determine the SO₂ of a sample. The optical technique of near infrared (NIR) absorption spectroscopy used in the BGA does not chemically alter the sample; however, the required sample extraction infringes on the sterility of the blood bag and therefore renders them unfit for transfusion.

Currently, no analytical method exists that can monitor the quality of blood bags without compromising their sterility for transfusions. In light of this issue, optical technologies can potentially be used for non-invasive, non-ionizing measurements of blood bags *in situ*¹³. By taking advantage of the dominant absorption profile of hemoglobin within the visible/near-infrared (NIR) electromagnetic (EM) spectrum¹⁴, optical technologies can measure the optical properties of the RCC within a blood bag. Within this optical range, Hb and HbO₂ can be differentiated due to the differences in their absorption spectra. Using a 2-wavelength oximetry principle, SO₂ can be monitored non-invasively¹⁵.

Optical technologies employing the oximetry method are limited by poor penetration depth, especially if illuminating a sample of RCC that contain a high concentration of strong chromophores¹⁵. Photoacoustic (PA) technology has the potential to overcome this limitation, as it receives acoustic rather than optical signals which encounter 2-3 orders of magnitude less scattering^{16,17}. Under an assumption of negligible fluence profile changes between both illumination wavelengths, the PA SO₂ can be computed as¹⁸

$$SO_2 = \frac{\Phi^{\lambda_2} \cdot \epsilon_{Hb}^{\lambda_1} - \Phi^{\lambda_1} \cdot \epsilon_{Hb}^{\lambda_2}}{\Phi^{\lambda_1} \cdot (\epsilon_{HbO_2}^{\lambda_2} - \epsilon_{Hb}^{\lambda_2}) - \Phi^{\lambda_2} \cdot (\epsilon_{HbO_2}^{\lambda_1} - \epsilon_{Hb}^{\lambda_1})} \quad (2)$$

where, for illumination wavelength λ , $\epsilon_{Hb/HbO_2}^{\lambda}$ represents the tabulated molar extinction coefficient of each state of hemoglobin, and Φ^{λ} represents the measured PA amplitude.

This paper introduces a novel, customized PA setup developed for the acquisition of SO₂ of RCC blood bags *in situ*. Results collected using the PA setup are compared against values obtained from a BGA, the current clinical gold standard for SO₂ measurement. Time series data are collected to observe the change in *in vitro* RBC SO₂ during storage. Bland Altman analysis is used to compare data acquired from the novel and gold standard technique¹⁹.

2. METHODS

2.1 RCC blood bags

Two RCC blood bags were transported from Canadian Blood Services (CBS) blood-for-research center (netCAD, Vancouver, Canada) to the Institute of Biomedical Engineering, Science and Technology (iBEST, Toronto, Canada).

Throughout the 15-20 hour transit period, the bags were insulated in standard blood shipment containers which are comprised of a styrofoam /cardboard box filled with ice packs to maintain hypothermic temperatures. The RCC provided in blood bags were separated from anticoagulated whole blood using a top/bottom manufacturing method, leukoreduced and stored in up to 110 ml of saline-adenine-glucose-mannitol (SAGM) preservative solution³. Upon reception, blood bags were promptly stored in a designated laboratory fridge until six weeks after the production date.

2.2 PA Setup

PA measurements were performed with a Vevo LAZR probe that contained a coupled laser source and transducer; experiments were conducted within a Vevo LAZR photoacoustic housing container (FUJIFILM Visual Sonics, Toronto, Canada). Laser illumination was generated from a Q-switched, Nd:YAG laser, with a repetition frequency of 20Hz, pulse width of 6 ns, pulse energy of approximately 30mJ and a spot size of 24mm² (1mm×24mm). The transducer consisted of a linear array of 256 piezoelectric elements, having a center frequency of 21MHz. The focal depth of the transducer was adjusted to match the fixed focal depth of the laser source at 11mm.

The PA setup used for SO₂ measurements is displayed in Figure 1. A rectangular plexiglass tank (25×14×12cm) was built to keep blood bags underwater, stationary and flat to reduce PA signal scattering. The bags were held in place using a plexiglass lid, containing a central elliptical hole (major:minor axis 10:7cm) allowing for the Vevo LAZR probe to scan the bag. Prior to PA measurement, an RCC blood bag was immersed in the tank that was pre-filled with cold distilled water, followed by fastening the lid on top of the bag. The probe was placed at a distance of approximately 10.5mm above the bag such that the optical focal zone (11mm) occurred under the blood bag plastic surface. Real-time, coregistered US images provided verification of distances and bag flatness, as shown in Figure 2.

2.3 PA SO₂ acquisition

An in house module (*oxy-hemo*) of the Vevo LAZR photoacoustic system was used to generate real time SO₂ maps that were formed by applying the PA oximetry principle (Equation (2)) to each image pixel of PA images acquired at 750nm and 850nm. After 10 image frames were collected, an ROI was traced over the central, homogenous part of the SO₂ map of the first frame. An average of the SO₂ values of all pixels within the selected region was produced. An example of this procedure is displayed in Figure 2. This image ROI was applied to the other 9 frames, producing 10 SO₂ values for that particular measurement. This procedure was repeated for two other locations on the RCC blood bag, accumulating (in total) 30 SO₂ values per RCC blood bag per PA measurement. PA measurements of each bag were taken approximately every 10 days throughout the lifespan of the RCC.

2.4 BGA SO₂ acquisition

Before the first extraction of sample for BGA analysis, bags were fitted with a sampling site coupler that contained a natural rubber sleeve stopper for needle penetration. Immediately before PA experiments, 1.5ml aliquots of RCC were extracted with 20-gauge needles via the coupler. This method was repeated to collect 3 individual samples (per bag) into *safe*PICO syringes, that contained a vented cap to expel air within the sample and seal the sample from the external environment (Radiometer, Copenhagen, Denmark). The 3 syringes were placed into designated placeholders on the ABL800 Flex BGA for automated aspiration and acquisition of SO₂ (Radiometer, Copenhagen, Denmark).

3. RESULTS/DISCUSSION

Figure 3 displays PA and BGA time series measurements of SO₂ values acquired from two RCC blood bags (labelled 1 and 2). During the lifespan of bags 1 and 2, PA measurements recorded an SO₂ increase of 19.1% and 20.3% respectively, while the BGA measured an increase of 18.0% and 20.9%, respectively. Guerrero et al. has measured a larger total SO₂ increase²⁰. This suggests that there can be variation not only in initial SO₂ value (as shown in Figure 3), but also the percent SO₂ change for the lifespan of the blood bag. Future studies will concentrate on quantitatively investigating links between the variation in total SO₂ increase and the variation in other characteristics of RBC storage lesions. Establishing such a correlation could indicate a relationship between magnitude of SO₂ increase and severity of the RBC storage lesion. The standard deviation of the BGA measurements of bags 1 and 2 were found to be 0.72% and 0.88% respectively, while the standard deviation of the PA measurements was 1.53% and 1.13% for each respective bag (Figure 3). The larger

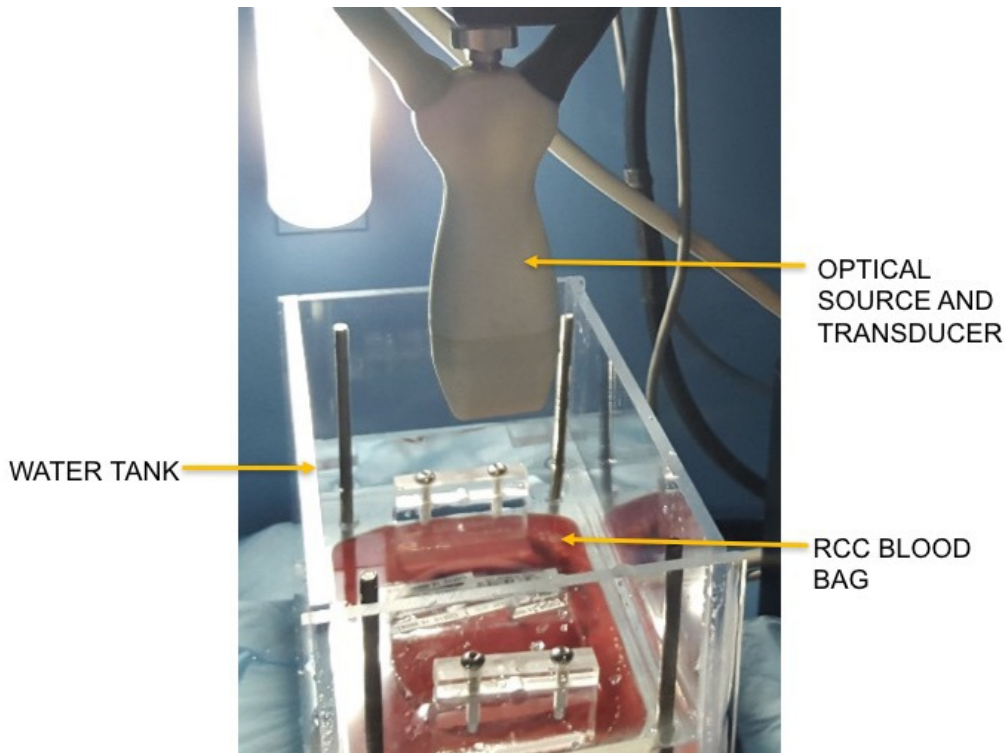


Figure 1: PA setup for SO_2 acquisition. The 21MHz optics/transducer apparatus was immersed into a water tank containing an RCC blood bag.

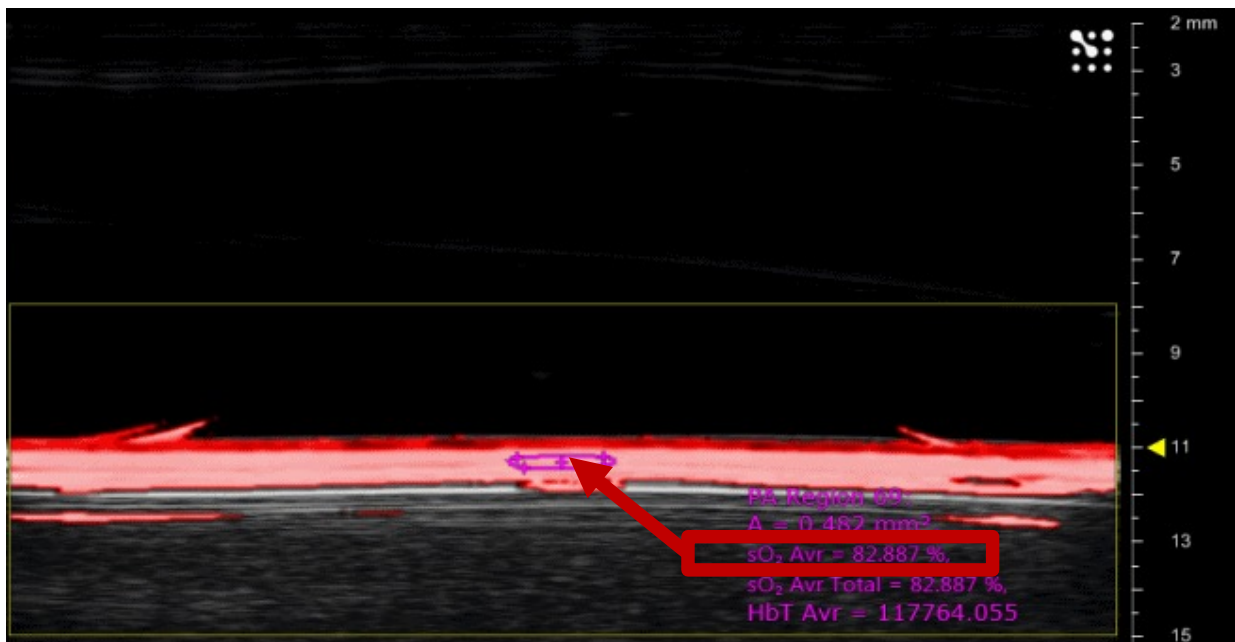


Figure 2: An SO_2 color map of a blood bag using the Vevo LAZR oxy-hemo module. A coregistered US image can be seen in the background (white), which was used for position the surface of bag just above 11mm (yellow arrow) and to verify bag flatness for optimal light penetration. An ROI boundary (purple) can be seen in the central, homogenous part of the SO_2 color map. The corresponding average SO_2 for this ROI was found to be 82.89% (red box).

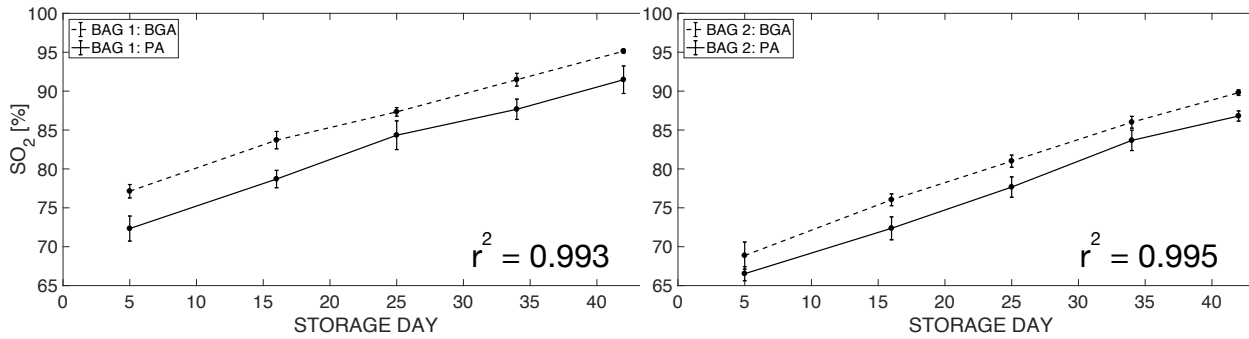


Figure 3: PA (solid line) and BGA (dashed line) SO₂ values of RCC blood bags 1 (left) and 2 (right) as a function of storage day. Error bars represent the standard deviation of 3 and 30 values acquired from ABL800 FLEX BGA and Vevo LAZR PA respectively. r^2 calculated as the square of the correlation coefficient.

standard deviations observed in PA measurements are small compared to the magnitude of the SO₂ changes measured. The correlation between both measurement techniques was found to be very strong, with an r^2 of 0.993 and 0.995 for blood bags 1 and 2 respectively.

Figure 3 illustrates that the calculated PA SO₂ values were consistently lower than the measured BGA SO₂ values. A Bland-Altman plot was generated using the average and difference between PA and BGA SO₂ values of bags 1 and 2. As displayed in Figure 4, BGA SO₂ values were on average $3.5 \pm 0.90\%$ higher than PA SO₂ values, with a distribution within the 95% confidence interval. One hypothesis proposed to explain the higher BGA values is that the extraction of RCC from the blood bag may unavoidably increase SO₂ due to direct contact with the external environment, even if minimized by the *safePICO* syringe. Future work will test this hypothesis, as it suggests that *in vitro* RBC SO₂ is more accurately acquired through non-invasive *in situ* measurements.

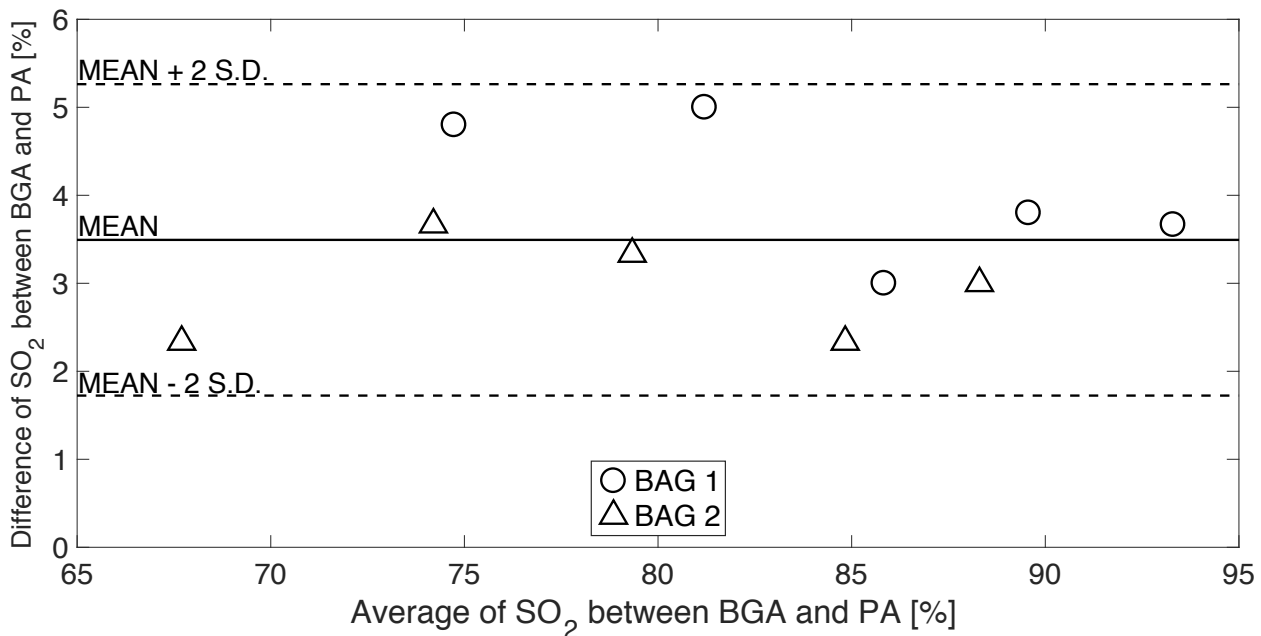


Figure 4: Bland-Altman plot of the average and difference between PA and BGA SO₂ values for bags 1 (circle) and 2 (triangle). S.D. represents the standard deviation calculated for all difference values.

The strong correlation found between the two methods (Figure 3) suggests that the rate change in SO₂ values would not incur a significant bias. A Bland Altman plot using the average and difference between BGA and PA SO₂ changes is shown in Figure 5. The mean difference between the SO₂ changes was found to be negligible (0.07%), and the distribution was found to be enclosed within the 95% confidence interval. The lack of bias confirms that although there is a consistent discrepancy between PA and BGA SO₂ values, their changes over time are not subject to this bias.

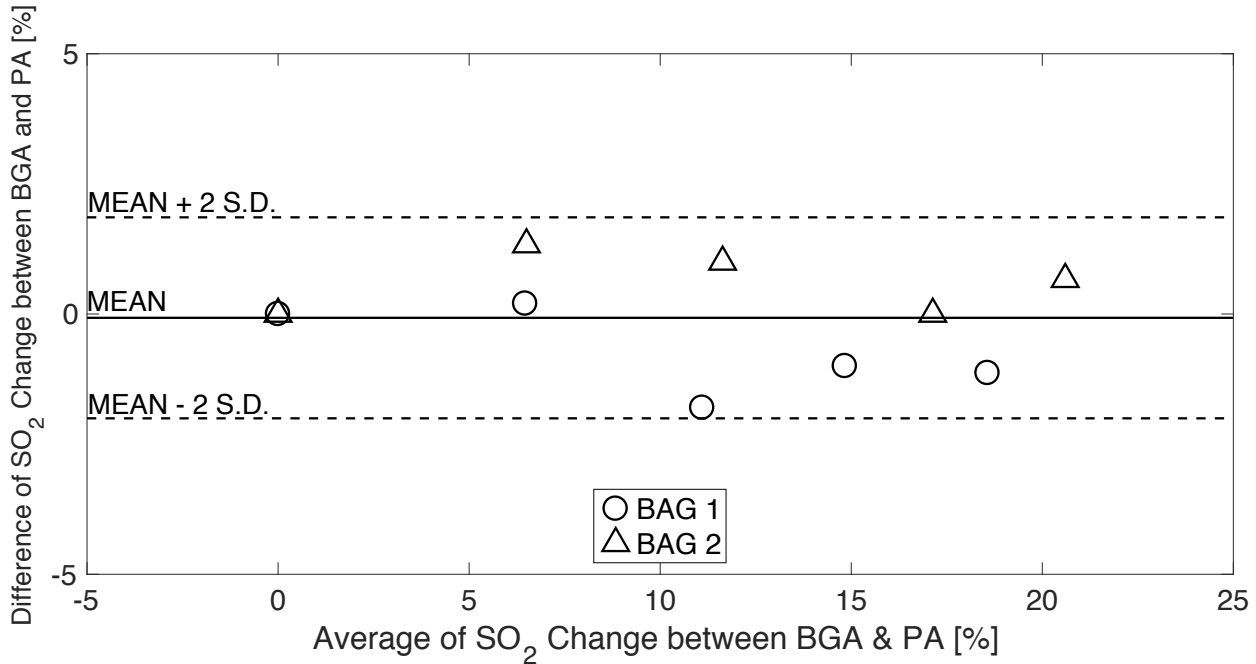


Figure 5: Bland-Altman plot of the average and difference between PA and BGA SO₂ changes for bags 1 (circle) and 2 (triangle), w.r.t. the initial value (Day 5). S.D. represents the standard deviation calculated for all difference values.

4. CONCLUSIONS

This paper presents a PA method as a non-invasive optical technique to acquire the SO₂ of blood bags *in situ*, in contrast to the current clinical gold standard method which requires sample extractions from the blood bag. PA and BGA measurements showed that SO₂ values of two RCC blood bags increased by approximately 20% over the 42-day storage period. The standard deviation of the PA measurements (1.13-1.53%) was larger than standard deviation of the BGA measurements (0.72-0.88%), however, this was an order of magnitude smaller than the changes observed in SO₂. Bland-Altman plots of BGA and PA data showed that PA SO₂ values are consistently lower than values acquired from the BGA by an average of $3.53 \pm 0.90\%$. The SO₂ changes over time did not show such a bias, with a mean difference of $0.07 \pm 0.99\%$ between the two measurement methods. The results suggest that the developed PA SO₂ setup could potentially replace the current gold standard for a non-invasive monitoring method of SO₂ change over storage life. Future work will concentrate on investigating the small discrepancy between PA and BGA SO₂ measurements, as well as establishing quantitative links between total SO₂ increase and other functional and morphological characteristics of the RBC storage lesion.

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