

# VISIBLE AND NEAR-INFRARED ABSORPTION properties of blood from sickle cell patients and normal individuals

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## Background

Sickle cell disease (SCD) is a genetic blood disorder characterised by red blood cells that assume an abnormal and rigid shape.<sup>1</sup> A point mutation in the beta globin chain of haemoglobin results in glutamic acid to be replaced with valine at the sixth position. The abnormal haemoglobin (HbS) leads to the distortion of red blood cells in certain conditions, such as low oxygen tension, and leads to sickling.<sup>1,2</sup> Sickling decreases the flexibility of red blood cells and causes microvascular occlusion, which may manifest as stroke, acute chest syndrome, pulmonary hypertension or organ damage.<sup>1</sup> SCD occurs primarily among people of sub-Saharan African, Mediterranean, Middle Eastern and Indian descent.<sup>3</sup> Of note, sickle cell anaemia refers to people who are homozygous for the mutation causing HbS, while sickle cell trait refers to heterozygotes who have one normal haemoglobin gene and one sickle cell gene. Approximately 250,000 children worldwide are born each year with sickle cell anaemia. According to the Gulf Genetic Center (GGC), abnormal haemoglobin was detected in 44.35% of neonatal samples in Bahrain. Of those, 18.1% had sickle cell trait and 2.1% had SCD. Additionally, the GGC reported that in the non-neonatal cases, the overall frequency of SCD was found to be 10.44%.<sup>4</sup>

Several techniques are used to screen for sickle cell trait or SCD, such as high-performance liquid chromatography (HPLC), haemoglobin electrophoresis and DNA sequencing. HPLC uses ultraviolet rays to detect the difference in shape and surface area between the normal blood cells and the sickle cells.<sup>5</sup> Haemoglobin electrophoresis differentiates between the haemoglobin forms based on charge, while DNA sequencing of the haemoglobin gene can detect the presence of the single amino acid substitution implicated in SCD. First reported in 1942, five different forms of haemoglobin (oxyhaemoglobin,

carbonyl haemoglobin, methaemoglobin, reduced haemoglobin and metcyanhaemoglobin) were detected based on marked differences in the absorption spectra in the visible (380nm-760nm) and near-infrared (760nm-2,500nm) region of the electromagnetic spectrum.<sup>6</sup> No such investigation comparing the absorption spectra of normal adult haemoglobin (HbA) and sickle cell haemoglobin (HbS) has been conducted in the visible and near-infrared region. Such is the aim of this brief study.

## Methods

Four whole blood samples were obtained from the haematology laboratory in the Bahrain Defense Force (BDF) Hospital. 400ml each of two normal samples and two sickle cell samples were identified and collected in EDTA anticoagulant tubes. The samples were diluted with 2ml of normal saline solution and 0.4ml of diluted blood from each sample was inserted into a 1mm-thick tube. A light source of halogen broadband spectrum, with a wavelength between 250nm and 2,500nm, was focused on the sample using an arrangement of lenses. Lens L1 was used to collimate the light, lens L2 was used to focus the light on the sample and lens L3 was used for collecting the light from the sample, which was then focused onto the charge-coupled-device (CCD) spectrometer through the fibre. The spectrometer measured the absorbance at various wavelengths and the collected data was plotted by the computer software Origin 6.1 (Figure 1).

## Results

The absorption spectra of HbA and HbS in the visible near-infrared region are demonstrated in Figure 2. This difference in absorption spectra of the HbS versus normal HbA was not evident at a

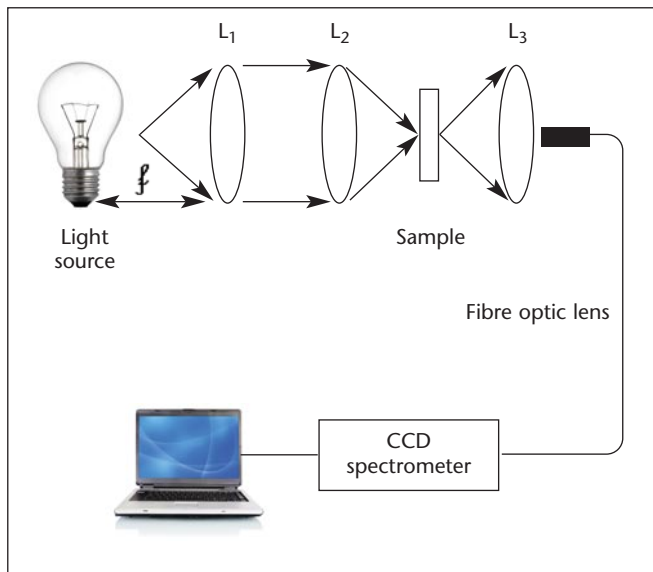


FIGURE 1: A schematic of the experimental set-up. A light source, L1, is a collimation lens, while L2 and L3 are focusing lenses.

wavelength range between 400 and 500nm but is clearly detected in their absorption pattern beyond 500nm. Specifically, HbS shows a higher absorption pattern than normal HbA, especially at the range between 700nm and 900nm, which lies in the near-infrared region. In addition, the two forms of haemoglobin differed with respect to their absorption peak. The absorption peak of HbS was found to be in the region of  $600\text{nm} \pm 10\text{nm}$  whereas the peak for HbA was approximately  $500 \pm 10\text{nm}$ .

## Discussion

The variation in optical properties shown between the two forms of haemoglobin could be due to the difference in size, shape, or three-dimensional structure.<sup>7-9</sup> Based on these results, the estimation of the absorption spectra of HbA and HbS blood samples

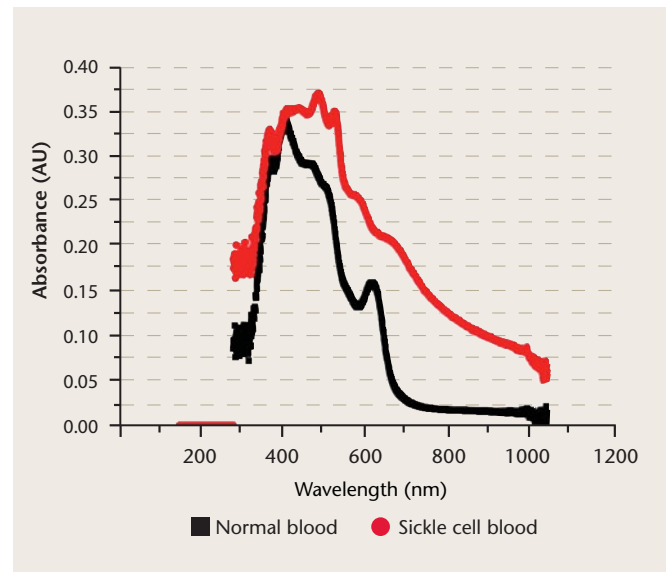


FIGURE 2: Absorption spectra of normal HbA and HbS blood samples in the visible near-infrared region show a significant difference in the absorption properties beyond 500nm. Note the peak absorption for HbA at  $500 \pm 10\text{nm}$  compared with  $600\text{nm} \pm 10\text{nm}$  for HbS.

could provide a quick and affordable assay of blood products for SCD. Currently, SCD can be diagnosed by methods of comparable accuracy such as HPLC, electrophoresis and DNA sequencing. However, further research is required with other variants of haemoglobin such as haemoglobin C (lysine group attached to the haem group), foetal haemoglobin (HbF), and oxygenated and non-oxygenated haemoglobin S. Furthermore, research is required to determine whether visible and near-infrared spectroscopy would be a reliable method for diagnosing SCD in comparison with the gold standard diagnostic methods.

## Acknowledgements

We would like to thank Dr Wahid Ali Agab and Dr Khalil Jassim for the provision of blood samples and equipment used in this study.

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