Optical properties of human whole blood - Changes due to slow heating

Nilsson, A. M. K; Lucassen, G. W; Verkmysse, W; Andersson-Engels, Stefan; van Gemert, Martin .j. C.

Published in:
LASER-TISSUE INTERACTION AND TISSUE OPTICS II, PROCEEDINGS OF

DOI:
10.1117/12.260746

1996

Optical properties of human whole blood - changes due to slow heating

Annika M. K. Nilsson[1], Gerald W. Lucassen[2], Wim Verkruysse[2], Stefan Andersson-Engels[1], and Martin J. C. van Gemert[2]

[1]Department of Physics, Lund Institute of Technology,
P.O. Box 118, S-221 00 Lund, Sweden
annika.nilsson@fysik.lth.se
[2]Laser Centre, Academic Medical Centre, Meibergdreef 9,
1105 AZ, Amsterdam, The Netherlands

ABSTRACT

Optical properties of human whole blood were measured *in vitro* at 633 nm with a double integrating sphere set-up. The blood was kept at constant flow through a flow cell while slowly heating the blood from approximately 25°C to 55°C in a heat exchanger. The results show a small but distinct decrease in the g-factor of 1.7±0.6% and a similar increase in the scattering coefficient, μ_s, of 2.9±0.6% at approximately 45-46°C. When studying the thermal effect on the blood cells under a white-light transmission microscope, the changes in the scattering properties could be correlated to a sudden change in the shape of the red blood cells, from disc-shaped to spherical, at approximately the same temperature. Furthermore, a continuous manifest increase in the absorption coefficient, μ_a, was seen with temperature rise, on average 83.8±68.1% when reaching the temperature 50°C. This might be due to heat-induced haemolysis of the red blood cells, resulting in free light absorbing haemoglobin in the surrounding plasma and thus higher effective light absorption.

key words: optical properties, human whole blood, double integrating sphere set-up

1. INTRODUCTION

Most clinical laser treatment modalities, such as laser surgery, hyperthermia and obliteration of port-wine stains, are based on the photothermal effect induced by laser light, *i.e.* transformation of light to thermal energy[1]. The optical properties of blood, being one of the main light absorbers in tissue, are in this respect important parameters. They constitute, together with the geometry of the tissue, the input parameters for theoretical models used to simulate light and thermal distributions, finding conditions for optimal treatment[2,3]. The optical properties of blood are constant throughout the course of the simulation, although it has been shown that heat-induced injuries to several other types of tissues are accompanied by changes in optical properties[4,5]. The aim of this study was to investigate whether thermally induced injuries to the blood cells, as changes in cell shape

0-8194-2325-4/96/$6.00

and haemolysis described by others[6-9], affect the optical properties of the blood. If so, this should be accounted for when modelling the light distribution for accurate photothermal treatment dosimetry.

2. MATERIAL AND METHODS

The optical parameters were measured on human venous blood *in vitro* from nine healthy volunteer donors. The blood was withdrawn into EDTA-tubes to prevent coagulation resulting in sixteen blood samples each containing 28-49 ml, depending on how much blood had been available from the donor. The samples were routinely analysed with respect to concentration of red and white blood cells, haemoglobin, haematocrit, mean corpuscular volume, concentration of platelets, platelet distribution width and other standard blood parameters prior to each integrating sphere measurement. The blood was gently stirred and kept flowing through the flow set-up for about five minutes immediately after the venipuncture in order to obtain constant oxygenation level.

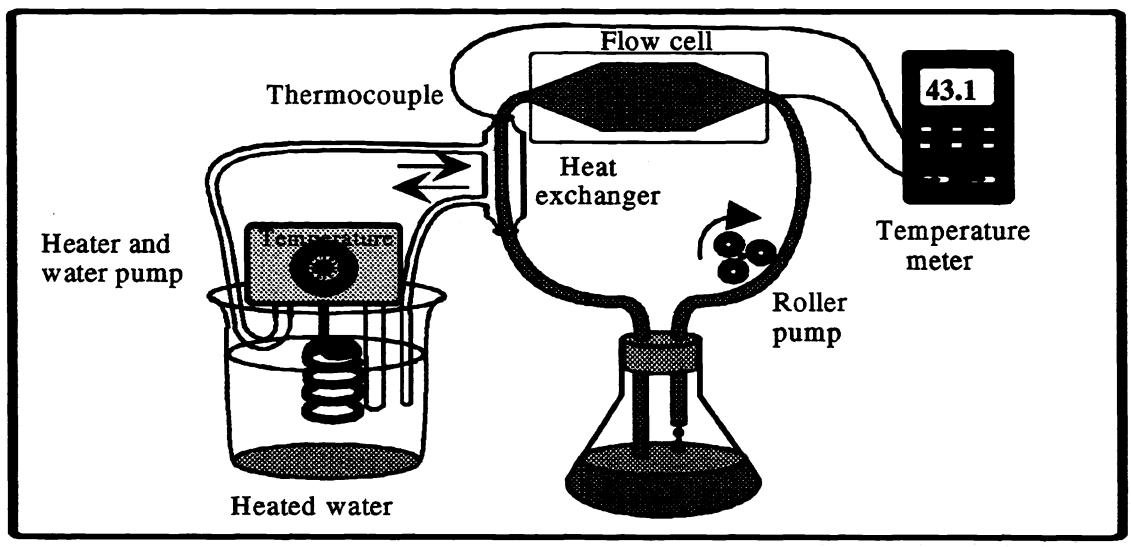

Fig. 1 The flow and heat set-up used to keep the blood constantly flowing through a flow cell and to control the blood temperature, measured before and after the flow cell. The blood was heated in a heat exchanger, with flowing warm water, located immediately before the flow cell.

The flow and heat set-up used for the experiments is illustrated in Fig 1. The main part of each blood sample was kept in a gently stirred glass flask. Surgical tubes with an inner diameter of two millimetres were leading the blood to and from this blood reservoir. The blood was pumped by a roller pump at a flow rate of 10.7 ml/minute. Immediately before reaching a thin flow cell (total thickness of approximately 2.5 mm, 65×34 mm) made of glass, the blood was passing a heat exchanger with warm water. By slowly increasing the temperature of the water, the temperature of the blood was increased from approximately 25 to 55°C at rates between 0.2 and 1.1°C/minute. The heat exchanger, the tubes and the flask were wrapped with aluminium foil in order to minimise heat radiation losses to obtain blood temperatures high enough to cause non-reversible damages to the blood cells. The temperature of the blood was measured with thin well-calibrated (±0.1°C)

thermocouples at the outflow of the heat exchanger and the flow cell, respectively. The optical measurement site was located on the central part of the flow cell yielding a blood sample thickness of 0.48±0.02 mm. The flow and heat set-up was thoroughly cleaned after each measurement sequence with detergents dissolved in water. Hydrofluoric acid (3-5%) was also used to clean the flow cell and the heat exchanger in order to completely remove the thin coating of coagulated blood on the inner glass surface. The entire flow set-up was finally richly rinsed with saline before new fresh blood was filled into the system.

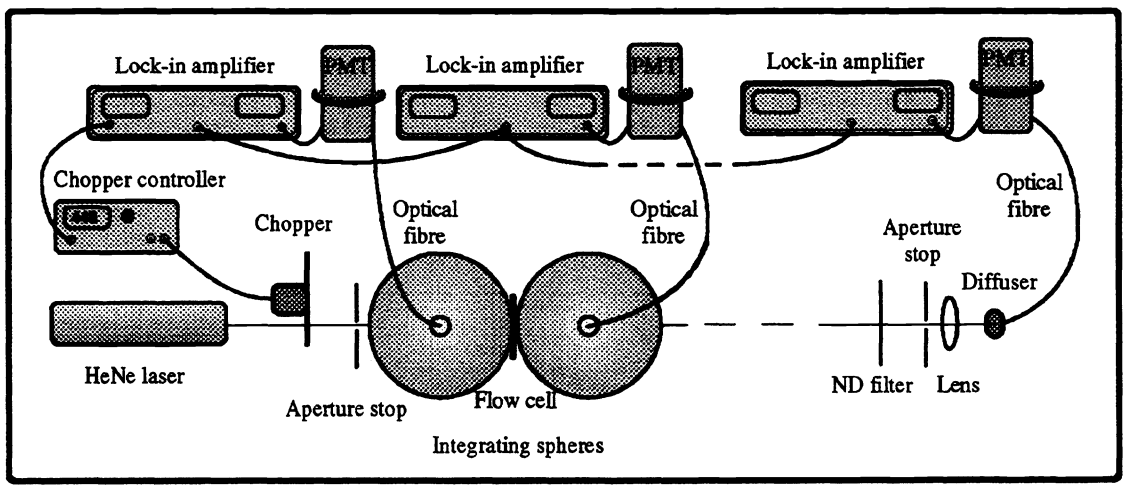

Fig. 2 The optical set-up with a double integrating sphere used to measure heat-induced changes in optical properties of blood. The blood was flowing through the flow cell located in-between the spheres.

The optical set-up, illustrated in Fig 2, was composed by a He-Ne laser as light source (633 nm) yielding a collimated beam with a beam diameter of 4 mm at the sample position, and two integrating spheres (Labsphere IS-060) with the flow cell placed in-between. The spheres were optically connected by 600 μm-fibres to two photomultiplier tubes (Hamamatsu, R928) utilised to measure diffuse reflected light (sphere number one) and diffuse transmitted light (sphere number two) from the sample in-between. The collimated transmitted light was passing through the exit port of the second integrating sphere and was probed by an optical fibre at a distance of approximately 1.5 m, connected to a third photomultiplier tube. A chopper/lock-in amplifier technique (EG&G Parc, 5209) was used to amplify the signals from the photomultiplier tubes and to suppress the influence of background light present. The signals from three lock-in amplifiers (diffuse reflected and transmitted light flux, and collimated transmitted light flux) were recorded by a computer every 30 seconds. The optical properties in terms of the absorption coefficient, μ_a, the scattering coefficient, μ_s, and the g-factor were derived from the measured light powers utilising the inverse adding-doubling method[10] compensating for light losses in the integrating spheres and for the interaction between the light fluxes in the two spheres by applying integrating sphere theory[11]. The double integrating sphere set-up and the theory behind the inverse adding-doubling method are more extensively described elsewhere[10,11].

Four of the sixteen blood samples, **sample number 1-4**, were used for control measurements, *i.e.* to measure the optical properties without heating the samples. This was

done during 60-75 minutes for each sample, while the blood was flowing through the flow set-up at room temperature in order to check the influence of mechanical stress on the blood cells and possible changes in oxygenation level caused by the flowing and pumping in the set-up. Small blood samples, about 1 ml, were taken from the flowing control blood sample every 15 minutes and all blood parameters listed previously were routinely measured. One of the sixteen blood samples, **sample number 5**, was diluted to a haematocrit of approximately 1% in order to be able to distinguish single erythrocytes under a white-light transmission microscope and study how their cell shapes were affected by heating when flowing in the flow set-up used. This low haematocrit was obtained by separating blood cells from plasma, diluting a small amount of the separated blood cells with the total volume of separated plasma. The remaining eleven blood samples, **sample number 6-16**, were slowly heated from room temperature until the blood started to coagulate, approximately at 55°C, and the optical properties were recorded every 30 seconds versus the blood temperature at the outflow of the heat exchanger. A complete measurement series lasted for about one hour.

The results were evaluated by calculating the relative change in the three optical properties. This was for the absorption coefficient accomplished by taking the difference between the absorption coefficient obtained when the blood temperature was 50°C, $\mu_a(50°C)$, and the lowest registered value of the absorption coefficient during the measurement, $\mu_a(\text{minimum})$ and divide the result by the minimum value.

$$\Delta\mu_a/\mu_a = 100[\mu_a(50°C) - \mu_a(\text{minimum})]/\mu_a(\text{minimum}) \quad [\%] \quad (1)$$

The relative change in the scattering coefficient and the g-factor was obtained by calculating average values before the heat-induced change, $\bar{g}$ (before) and $\bar{\mu}_s$(before), and after, $\bar{g}$ (after) and $\bar{\mu}_s$(after), and divide the difference by the average values before the change.

$$\Delta g/g = 100[\bar{g}\,(\text{after}) - \bar{g}\,(\text{before})]/\bar{g}\,(\text{before}) \quad [\%] \quad (2)$$

$$\Delta\mu_s/\mu_s = 100[\bar{\mu}_s(\text{after}) - \bar{\mu}_s(\text{before})]/\bar{\mu}_s(\text{before}) \quad [\%] \quad (3)$$

The temperature related to the heat-induced change, a so-called critical temperature, was defined as the temperature in the heat exchanger at which the g-factor and the scattering coefficient reached statistically significant values of three standard deviations from the $\bar{g}$ (after) value and the $\bar{\mu}_s$(after) value, respectively.

3. RESULTS

The analyses of the blood samples taken regarding standard parameters as for example haemoglobin, haematocrit, mean corpuscular volume, concentration of platelets, and platelet distribution width before all measurements (samples 1-16) and during the control measurements every 15 minutes (samples 1-4) all showed normal values ensuring that healthy blood was handled during the experiments. Thus, the mechanical stress from the pumping and flowing of blood did not have any significant influence during the period of time - approximately one hour - used for the measurements. The haematocrit of the blood from the nine volunteer donors was on average 44.5±2.6%.

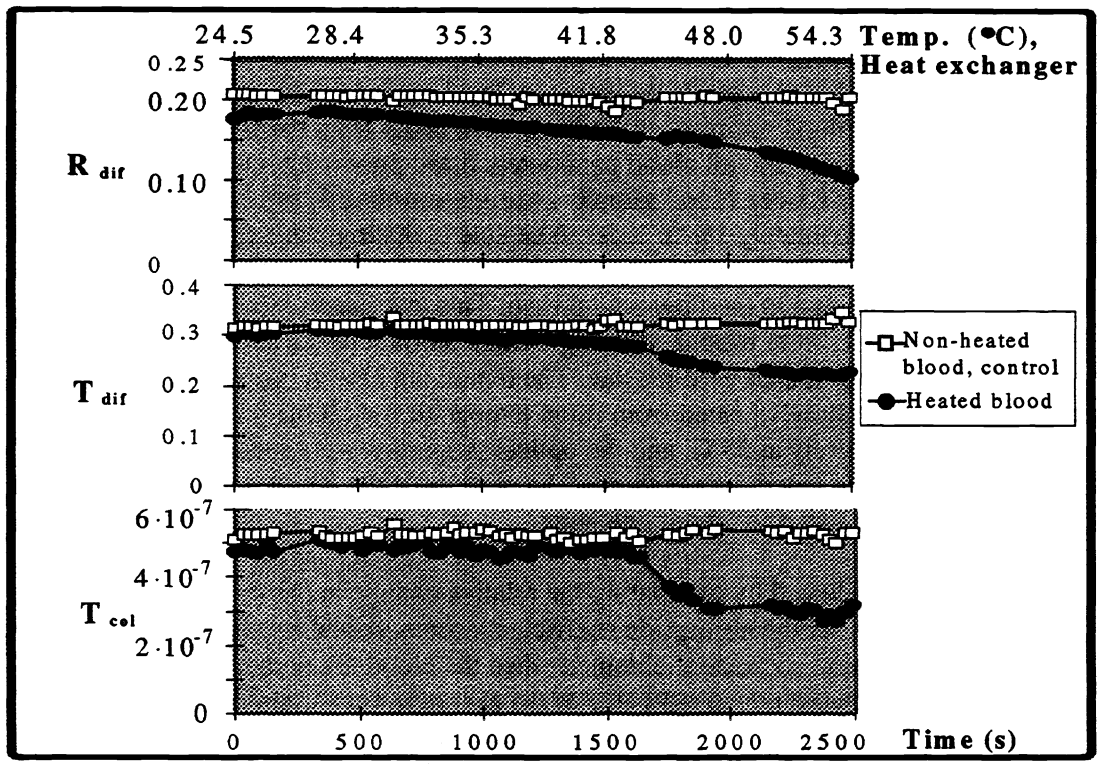

Fig. 3 The originally recorded data in terms of diffuse reflectance, diffuse transmittance and collimated transmittance versus recording time. The open squares represent control measurements and the filled circles show the results from a slowly heated sample with the blood temperature measured at the outlet of the heat exchanger indicated at the top.

Recorded raw data, related to calibration measurements using a reflectance standard, of two typical measurements are shown in Fig. 3 in terms of diffuse transmitted, collimated transmitted and diffuse reflected light versus time. The open squares represent data from one of the control measurements without heating (samples 1-4) showing stable values not only for the 2500 seconds flowing shown in the figure but also during the remaining 1700 seconds of the measurement, in all lasting for approximately 4200 seconds. When the samples were heated (samples 6-16), changes in the recorded data were induced as shown by example number two in Fig. 3, represented by filled circles. The data is plotted versus time and the blood temperature measured at the outlet of the heat exchanger is labelled at the top of the graphs. What can be observed here is a slight continuous decrease in the diffusely reflected light followed by a final steeper decrease, a rather sudden decrease in the diffusely transmitted light and an abrupt decrease in the collimated transmitted light often followed by an increase at even higher temperatures (the latter not completely shown in this example). These characteristic phenomena were registered for all eleven samples (samples 6-16). Transforming the raw data into optical properties utilising the inverse adding-doubling method results in curves shown in Fig 4. The stability of the example from the control measurements, samples 1-4, can be recognised here as well (open squares plotted versus time). In numbers, the absorption coefficient has a standard deviation on the mean value of 1.5% and the g-factor and scattering coefficient standard deviations on approximately 0.1%. The heat-induced changes in raw data of example

number two representing samples 6-16 correspond in Fig. 4 (filled circles) to a continuous increase in the absorption coefficient at an increasing rate with higher temperature, a small but distinct decrease in the g-factor and an increase in the scattering coefficient at a certain temperature, here called the critical temperature. The g-factor increased again at higher temperatures, sometimes along with a decrease in the scattering coefficient. The time is indicated at the bottom of the graphs in Fig. 4 and the blood temperature at the top. The scattering properties can also be summarised by just one parameter, the so-called reduced scattering coefficient $\mu_s' = \mu_s \times (1-g)$. This parameter is presented in Fig. 5 for the same measurements as in Fig. 4, open squares represent the control measurement and filled circles the heated blood measurement. The measured blood temperature of the latter is again shown at the top of the graph and the recording time of both curves is shown at the bottom. Both curves show constant values in the beginning, but while the non-heated control measurement continuous this way, the heated sample exhibits a distinct increase at a critical temperature followed by a decrease at higher temperatures.

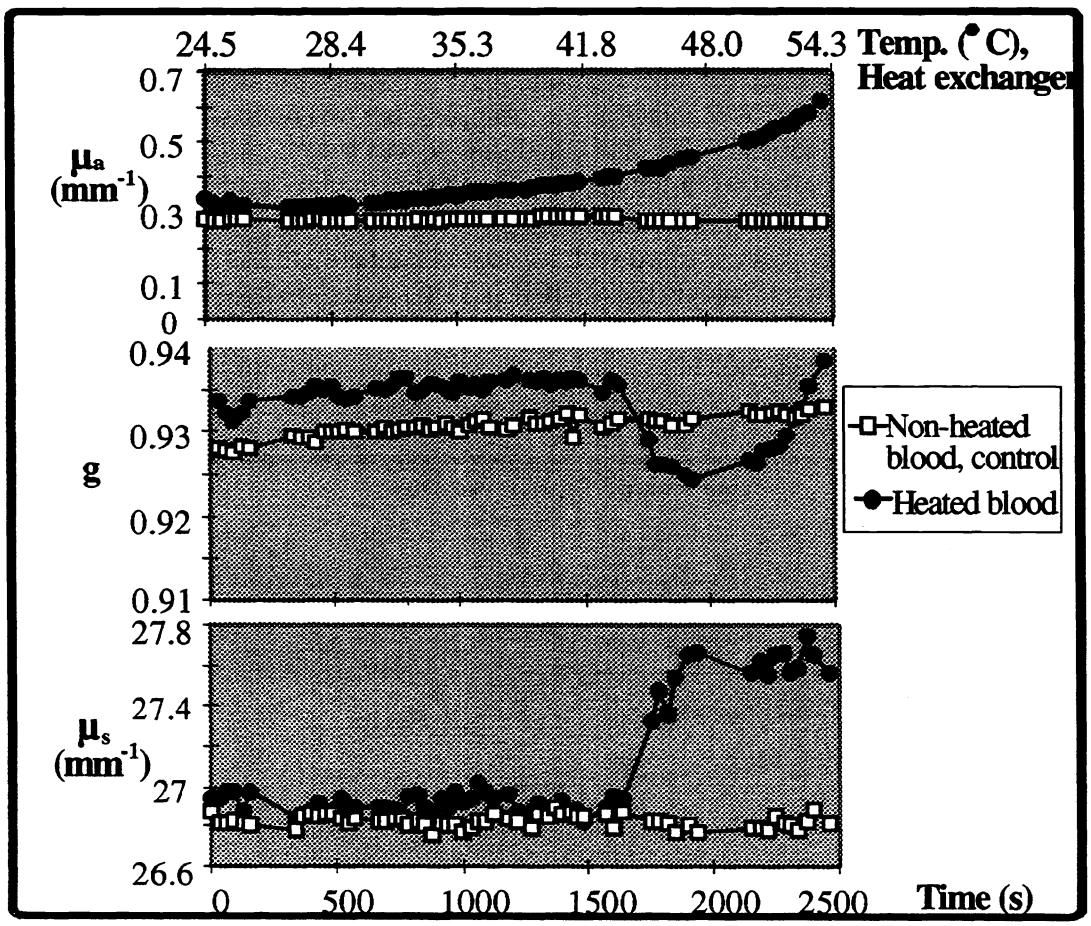

Fig. 4 The absorption coefficient, g-factor and scattering coefficient of whole blood versus recording time. The open squares show a non-heated control sample and the filled circles a slowly heated sample with the blood temperature indicated at the top. The parameters are deduced from the originally recorded data shown in previous figure.

Average values, $\overline{\mu}_a$(before), $\overline{g}$ (before), and $\overline{\mu}_s$(before), and standard deviations of optical properties of highly oxygenated flowing blood were obtained from the initially recorded values of samples 6-16, before the heat had started to influence the optical properties. The absorption coefficient was 0.26±0.06 mm^{-1}, the g-factor 0.95±0.02 and the scattering coefficient 26.7±0.6 mm^{-1}, all measured at 633 nm. The heat-induced relative changes in these optical properties (samples 6-16) were calculated using equations (1)-(3) and the critical temperatures of the scattering properties in connection with the abrupt heat-induced changes were obtained as the blood temperature three standard deviations before the scattering parameters had reached the $\overline{g}$ (after)- or $\overline{\mu}_s$(after)-value, respectively. The absorption coefficient increased by 83.8±68.1% (mean value±standard deviation), the g-factor decreased by 1.7±0.6% at a critical temperature of 46.4±3.3°C and the scattering coefficient increased by 2.9±0.6% at 45.3±3.4°C. The reduced scattering coefficient, $\mu_s'=\mu_s\times(1-g)$, including the two former parameters increased on average by 39.3±12.4%.

When studying the shape of the erythrocytes in the diluted sample number 5 under a white-light transmission microscope, we initially observed normal biconcave shapes of all red blood cells. Abnormal appearance as for example glass and rouleaux effects described elsewhere[12,13] was not noticed. When the blood sample was heated, the normal disc-shaped appearance of the erythrocytes lasted until the blood temperature reached a temperature just below 45°C. The disc-shaped erythrocytes then all rather abruptly turned spherical. The size of the spherocytes increased with increasing temperature and cell fragmentation including disruption of cell membranes started at 47°C. At approximately 49°C all cells were destroyed. The shapes of the erythrocytes versus the blood temperature are illustrated in Fig. 5, along with the obtained reduced scattering coefficient from one of the samples 6-16.

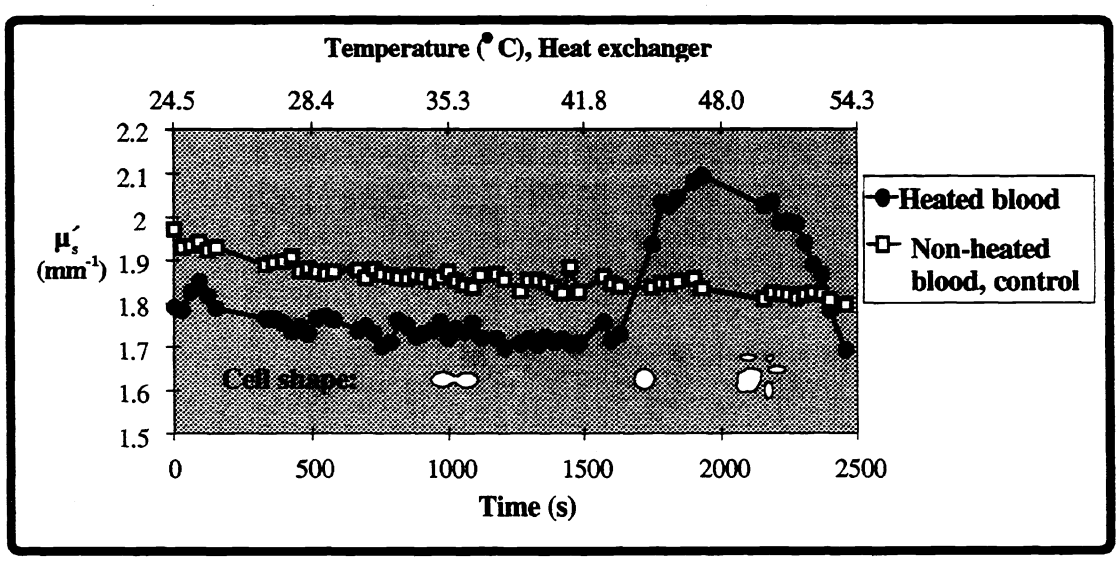

Fig. 5 The reduced scattering coefficient, obtained from a control measurement (open squares) and from a slowly heated sample (filled circles) versus recording time. The blood temperature of the heated sample is indicated at the top and the shape of the erythrocytes observed under a white light transmission microscope at corresponding temperatures are illustrated in the lower part of the graph.

4. DISCUSSION

The aim of this study was not to measure and determine correct absolute values of the optical properties of blood, as this is very difficult with a double integrating sphere set-up measuring all three parameters at the same time. Blood is highly absorbing and thin samples are thus often employed to be able to record any transmitting light. The transmitting light is very forward-directed, though, which makes it difficult to separate the influence from the diffusely transmitted light on the collimated transmitted light in a double integrating sphere set-up. It also makes it difficult to form Lambertian light fluxes inside the spheres, which is assumed by the integrating sphere theory[11]. These are likely explanations to the deviations in the scattering parameters obtained here ($g=0.95\pm0.02$, $\mu_s=26.7\pm0.6$ mm^{-1}) from those measured and theoretically calculated elsewhere[14,15]. It is, however, uncertain if it is correct to compare whole blood measurements (a distance between the cells in the order of one radius) with measurements all performed on diluted blood (haematocrit<3%) and calculations assuming independently scattering spheres when a distance greater than three times the cell radius is needed to consider the cells being separate, inducing independent light scattering[16]. However, the purpose of this study was to investigate heat-induced *changes* in optical properties.

When one wants to monitor the influence of a parameter on the optical properties, as here the temperature, all other parameters of optical importance have to maintain constant during the measurements. This is particularly important in conjunction with measurements of optical properties of blood, as there are so many features affecting the sensitive well-balanced biochemistry of the blood and thus the cell membranes/cell shape (optically influencing the scattering) and the haemoglobin contents inside the cells (optically influencing the absorption). Several precautions concerning the blood osmolarity were taken as for example by only using non-diluted whole blood and flushing the flow set-up with physiological saline before use. The routine blood analyses during the control measurements (samples 1-4) showed normal blood parameters (haemoglobin, haematocrit, mean corpuscular volume, concentration of platelets, platelet distribution width etc.) confirming no influence of the artificial environment in the flow set-up including the mechanical stress caused by flowing and pumping. The monitoring of optical properties during the non-heated control measurements are also essential to be able to extract changes in optical properties caused by heating from those caused by other parameters. The stable appearance of the control measurement in Fig. 3 (raw data) and 4 (deduced optical properties) verifies that the experiments were done without changing any optically important properties, including the flow rate of the blood[17] and the oxygen saturation (would clearly affect the absorption coefficient at a wavelength of 633 nm). However, the blood was, as previously mentioned, flowing for approximately five minutes (300 seconds) before the measurements started, in order to stabilise the oxygenation level of the blood. These 300 seconds are not included in figures 3 and 4. Stable values were often recorded here as well, but the absorption coefficient sometimes showed a slight decrease - in well accordance with the lower absorption coefficient of oxy-haemoglobin compared to that of deoxy-haemoglobin (and in contrast to the heat-induced change

resulting in an increase). Three hundred seconds were always enough to obtain constant absorption coefficients.

The major change in optical properties caused by heating was observed for the absorption coefficient. It was on average increased by 83.8% when reaching a blood temperature of 50°C. The increase occurred mainly in the end of the measurements, after the so-called critical temperature. Considering that haemoglobin is the main absorber of blood, it is likely that the increase in μ_a is related to the haemoglobin contents and distribution. Tan *et al.*[18] have measured increasing amount of extracellular haemoglobin with higher temperatures which goes well along with the shape transformation and fragmentation of the cells observed in our microscopy study (sample 5) and by others[6,8]. Thus, the measured increase in μ_a can be explained by increasing amount of extracellular haemoglobin with higher temperatures. A homogeneous distribution of absorbers absorbs more efficiently than a discrete distribution when the cells are intact[19], as the innermost absorbers then are shaded by the surrounding ones. This means that a suspension with a homogeneous distribution of absorbers has a higher effective absorption coefficient than one with a discrete distribution of absorbers, in well accordance with our measurements.

The heat caused relatively small (on average 1.7-2.9%), but distinct, changes in the two scattering properties, the g-factor and μ_s, at a critical temperature of approximately 45-46°C. When evaluating the relative change in the scattering expressed by one parameter, *i.e.* the reduced scattering coefficient, it exhibits a higher average value of 39.3% mainly as a consequence of the (1-g)-factor when the g-factor is close to one. We can see the characteristic temperature dependent behaviour of the scattering parameters in Fig. 4 and 5, starting with values as stable as and as close to those of the control measurement at lower temperatures, followed by an almost step-like response at a critical temperature inducing a deviation from the control values. The reduced scattering coefficient of the control measurement and the heated blood measurement differ also before the critical temperature shown in Fig. 5, although both samples are taken from the same donor. An explanation to this can be the less apparent difference in the g-factor shown in Fig. 4 originating from insignificant differences in the raw data shown in Fig. 3. A small difference in the g-factor influences the reduced scattering coefficient considerably when the g-factor is close to one, again due to the (1-g)-factor. However, the samples are taken from the donor at two different occasions and the difference in the g-factor is within the obtained standard deviation (0.95±0.02). The characteristic heat-induced behaviour can be correlated with the changes in cell shape seen during our microscopy study (sample 5) and by others[6,8]. During the first constant phase of the reduced scattering coefficient shown in Fig. 5 (analogous to the behaviour of the g-factor and scattering coefficient shown in Fig. 4), the erythrocytes were normal and disc-shaped. The shape transformation into spherocytes occurred at approximately 45°C, seen in μ_s' as an abrupt increase at the so-called critical temperature (a decrease in the g-factor and an increase in the scattering coefficient). Fragmentation and disruption of the cells at 48-49°C observed under the microscope caused a decrease in the reduced scattering coefficient mainly corresponding to an increase in the g-factor. What we see here is how clearly the cell shape influences the scattering properties.

In conclusion one can say that the increase in the absorption coefficient and reduced scattering coefficient most likely influences light distributions during photothermal laser treatment and should thus be accounted for when theoretically modelling the light and heat distribution for correct dosimetry.

5. ACKNOWLEDGEMENTS

The authors would like to thank Dr E. J. van den Dool at the Department of Haematology, AMC, for the invaluable help, doing all the venipunctures and blood analyses. We would also like to express our gratitude to all the students and co-workers at the Laser Centre, AMC, who generously donated some of their blood to this study. The valuable support of Professor Sune Svanberg is also gratefully acknowledged. This work was financially supported by the Dutch Technology Foundation STW (AGN 33.2654) and the Swedish Research Council for Engineering Sciences.

6. REFERENCES

1. S. Thomsen, "Pathologic analysis of photothermal and photomechanical effects of laser-tissue interactions," Photochem. Photobiol. **53,** 825-835 (1991).

2. G. W. Lucassen, W. Verkruysse, M. Keijzer, and M. J. C. van Gemert, "Light distributions in a port wine stain model containing multiple cylindrical and curved blood vessels," Lasers Surg. Med. **18,** 345-357 (1996).

3. C. Sturesson and S. Andersson-Engels, "A mathematical model for predicting the temperature distribution in laser-induced hyperthermia. Experimental evaluation and applications," Phys. Med. Biol. **40,** 2037-2052 (1995).

4. S. Jaywant, B. C. Wilson, L. Lilge, T. Flotte, J. Woolsey, and C. McCulloch, "Temperature dependent changes in the optical absorption and scattering spectra of tissues: correlation with ultrastucture," in *Laser-tissue interaction IV,* , SPIE **1882,** 218 - 229 (1993).

5. J. W. Pickering, S. Bosman, P. Posthumus, P. Blokland, J. F. Beek, and M. J. C. van Gemert, "Changes in the optical properties (at 632.8 nm) of slowly heated myocardium," Appl. Opt. **32,** 367-371 (1993).

6. T. H. Ham, S. C. Shen, E. M. Fleming, and W. B. Castle, "Studies on the destruction of red blood cells. IV," Blood **3,** 373-403 (1948).

7. H. Karle, "Effect on red cells of a small rise in temperature: in vitro studies," Brit. J. Haemat. **16,** 409-419 (1969).

8. N. Mohandas, A. C. Greenquist, and S. B. Shohet, "Effects of heat and metabolic depletion on erythrocyte deformability, spectrin extractability and phosphorylation," The red cell(New York, USA , 1978) pp. 453-477.

9. C. Prinsze, K. Tijssen, T. M. A. R. Dubbelman, and J. van Steveninck, "Potentiation of hyperthermia-induced haemolysis of human erythrocytes by photodynamic treatment," Biochem. J. **277,** 183-188 (1991).

10. S. A. Prahl, M. J. C. van Gemert, and A. J. Welch, "Determining the optical properties of turbid media by using the adding-doubling method," Appl. Opt. **32,** 559-568 (1993).

11. J. W. Pickering, S. A. Prahl, N. van Wieringen, J. F. Beek, H. J. C. M. Sterenborg, and M. J. C. van Gemert, "Double-integrating-sphere system for measuring the optical properties of tissue," Appl. Opt. **32,** 399-410 (1993).

12. L. E. G. Eriksson, "On the shape of human blood cells interacting with flat artificial surfaces - the 'glass effect'," Biochim. Biophys. Acta **1036,** 193-201 (1990).

13. M. Bessis, *Living blood cells and their ultrastructure* (Springer Verlag, 1973).

14. W. F. Cheong, S. A. Prahl, and A. J. Welch, "A review of the optical properties of biological tissues," IEEE J. Quant. Electr. **26,** 2166-2185 (1990).

15. J. M. Steinke and A. P. Shepherd, "Comparison of Mie theory and the light scattering of red blood cells," Appl. Opt. **27,** 4027-4033 (1988).

16. H. C. van de Hulst, *Light scattering by small particles* (Wiley, New York, 1957).

17. L.-G. Lindberg and P. Å. Öberg, "Optical properties of blood in motion," Opt. Eng. **32,** 253-257 (1993).

18. O. T. Tan, J. G. Morelli, D. Whitaker, J. Boll, and G. Murphy, "Ultrastructural changes in red blood cells following pulsed irradiation in vitro," J. Invest. Dermatol. **92,** 100-104 (1989).

19. W. Verkruysse, G. W. Lucassen, J. F. de Boer, D. J. Smithies, J. S. Nelson, and M. J. C. van Gemert, "Modelling light distribution: homogeneous versus discrete absorbers in light irradiated turbid media," submitted to Phys. Med. Biol. (1996).