Thermochimica Acta, 141 (1989) 235–243
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

THERMOPHYSICAL PROPERTIES OF PROTEIN CRYSTALS: THERMAL DIFFUSIVITY MEASUREMENTS OF METHEMOGLOBIN SINGLE CRYSTALS USING A DIELECTRIC HEATING TECHNIQUE

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(Received 11 May 1988)

ABSTRACT

A method for determining the thermal diffusivity of thin solid samples is described. This method, using a pulsed dielectric heating technique, is applied to methemoglobin single crystals with volumes of less than 10^{-3} mm^3. The value for thermal diffusivity tensor element corresponding to heat flow perpendicular to the $a-b$ plane of the monoclinic methemoglobin single crystals is determined as 0.215 ± 0.015 mm^2 s^{-1}.

INTRODUCTION

Dynamic methods for measuring thermal diffusivity in materials can generally be grouped into continuous wave techniques and pulsed techniques. The technique presented here is a pulsed technique. The principle behind this technique lies in generating a heat pulse within the sample and observing the thermal relaxation. The thermodynamic parameters of the sample can then be calculated from this response. The most widely used pulsed technique is the flash technique [1–3]. In this technique a flash of radiant energy is applied to the surface of a homogeneous slab and the temperature evolution at the opposite surface is observed. The diffusivity is calculated from parameters of the resulting temperature–time curve, such as the time taken for the temperature to reach a given percentage of its maximum value. The temperature is measured by contacting a thermocouple at the centre, or by optical means. The result may need to be corrected for finite pulse time effect, heat losses and non-uniform heating [4]. Other methods for metallic material involve measuring resistive self-heating of the specimen in the form of a thin foil or wire [5], or deriving heat pulses from the Joule heat generated by a current pulse passing through a planar electrical resistance [6].

The pulsed technique we present differs from the preceding techniques in that a temperature difference between the sample and the surroundings is generated by a short microwave pulse which heats sample and surroundings differentially. The amount of this temperature difference between the sample and the surroundings depends on their relative dielectric permittivities. The geometrical and heating parameters are chosen such that the respective temperature distributions in the sample and the surroundings are homogeneous. The temperature history of the sample after the pulse is observed by optical means.

This method has been used to determine the thermal diffusivity of methemoglobin single crystals of space group C2. Methemoglobin crystallizes in plates along the plane (001). These thin plates have areas of between 100×100 and 500×500 μm^2 and thicknesses of between 10 and 100 μm. Data for the diffusivity tensor element corresponding to through-plate heat flow were determined. These measurements are of great interest because diffusivity of protein crystals has to be established in order to carry out investigations using temperature jump [7] and photoacoustic methods [8].

Using this method with single crystals of methemoglobin is only one example of its application. The method should be generally applicable to thin sheets of material with similar optical properties.

The remainder of this paper presents a description of the experimental techniques, discussion of the data analysis method, results and conclusions.

MEASUREMENT TECHNIQUES

Experimental set-up

A block diagram of the experimental set-up is shown in Fig. 1. The whole apparatus may be divided into three basic components. These are the sample holder and heating system, the detection and amplification system, and the recording and analysis assembly.

The heating system used here is known from dielectric heating temperature jump techniques [9]. The sample cell containing the sample is positioned in a tunable TE 101 microwave cavity. An adjustable short circuit allows the cavity to be matched to the frequency of the microwave source. This source is a low-cost 2M186 magnetron operating at 2.5 GHz and capable of delivering a pulse in excess of 0.2 J in 100 μs, thereby producing a temperature rise of about 2 K. A home-made magnetron power supply produces pulse durations of between 10 and 1000 μs.

The temperature of the sample is monitored using an optical detection system. It is known that methemoglobin in solution shows a fast relaxation process [10], which has also been observed in methemoglobin single crystals [7,11]. This relaxation process is accompanied by a change in the extinction of the sample, which may be detected by following the change in absorbance of a light beam passing through the crystal. For $\lambda = 633$ nm the relaxation

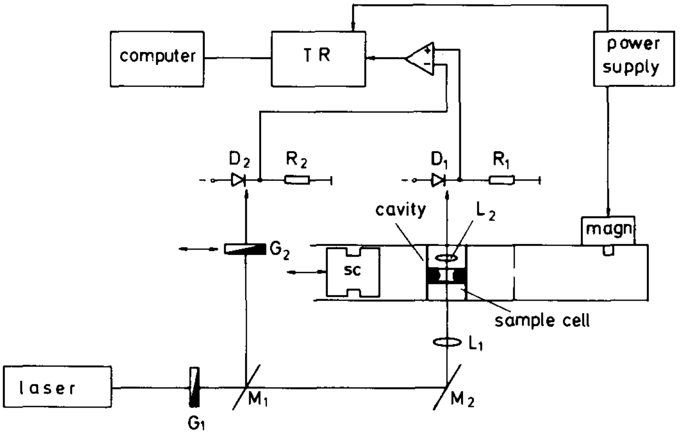

Fig. 1. Simplified block diagram of the experimental set-up: G, wedge filter; M, mirror; D, photodiode; L, lens; sc, short circuit; cavity, TE101 microwave cavity; magn, magnetron; TR, transient recorder.

amplitude has been determined as $\Delta E/(E\Delta T) = (1.9 \pm 0.2) \times 10^{-3}\ \mathrm{K}^{-1}$. The relaxation rate is greater than $10^5\ \mathrm{s}^{-1}$. Assuming that this fast relaxation process is the only process detectable in the time range under investigation, the time course of the absorbance change $\Delta E(t)$ should directly follow the temperature change in the crystal.

A He–Ne laser (model 124 B, Spectra Physics) with a maximum output power of 20 mW serves as a light source. After the light beam has passed through the wedge filter it is split, at the mirror M1, into two beams of equal intensity. One of these beams is reorientated at the mirror M2, and focussed on the sample by the lens L1. The diameter of the beam reaching the sample depends on the focal length of L1 and is adjusted to 20–50 μm, depending on the area of the sample. The lens L2 collects the light passed through the sample and focuses it on the receiver diode D1 (BPW 34). The second half of the beam coming from M1 passes through a wedge filter G and reaches the reference diode D2. The signals from D1 and D2 are subtracted by a differential amplifier (Tektronix AM 502) to achieve a reduction in laser mode interference noise of about 30 dB. A Biomation transient recorder serves as both an analogue-to-digital converter and a data buffer. The transient recorder is pre-triggered by the current driving the magnetron. With the system described here it is possible to detect sample absorbance changes of as low as $\Delta E/E = 10^{-4}$ with an amplifier band-width of 30 kHz. The relaxation amplitude $\Delta E/(E\Delta T)$ of the fast relaxation effect mentioned above is $1.9 \times 10^{-3}\ \mathrm{K}^{-1}$, leading to a temperature resolution for the average temperature $\langle T(t)\rangle$ of about 5×10^{-2}K (depending on the thickness of the crystal under investigation). The data processing is carried out with the help of a CBM 8296 computer system and a Cyber 855 (Control Data).

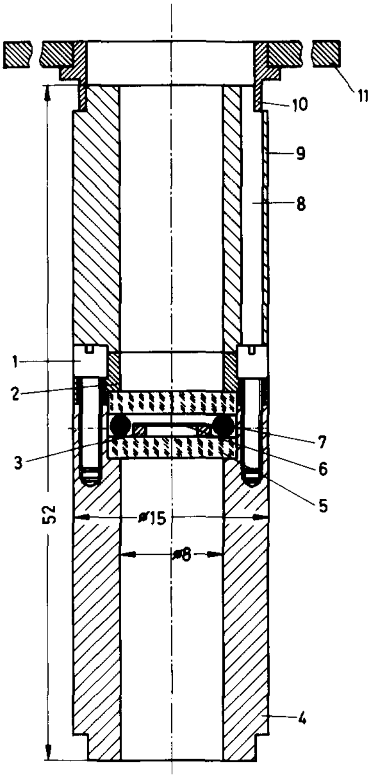

Fig. 2. Sectional drawing of the sample cell. Components (2), (4) and (9), made of polycarbonate (macrolon), make up the cell body. Two windows (5), held pressed against an O-ring (7) by screws (1), enclose the sample cavity. The sample lies on a nylon meshed network (6) supported by a macrolon ring (3). A bored hole (8) allows determination of the temperature of the sample environment. A brass ring (10) connects the graduated disc (11) with the cell body.

The sample cell is shown in Fig. 2. The sample cavity, which is mounted in a tube of polycarbonate (macrolon), consists of two windows and an O-ring, defining a volume of 1×10^{-2} ml. The sample lies in the middle of the sample cavity on a nylon meshed network with a mesh aperture of about 0.2–0.4 mm. A sketch of the irradiation geometry is shown in Fig. 3. The whole cavity is filled with mother liquor (2.8 M $(NH_4)_2SO_4$ solution, pH 7). This arrangement of the sample guarantees that the crystal is covered with aqueous solution on all sides. A hole bored in the macrolon tube allows the temperature of the sample environment to be determined. The whole sample cell may be turned to adjust the crystal axes of the sample relative to the electric vector of the plane polarized light beam, the degree of turn being controlled by means of a graduated disc. The whole microwave cavity is held at a constant temperature by means of a thermostatted N_2 gas stream.

The lens L2 produces a real image of the sample together with the focussed laser beam and the meshed network, which may be examined to

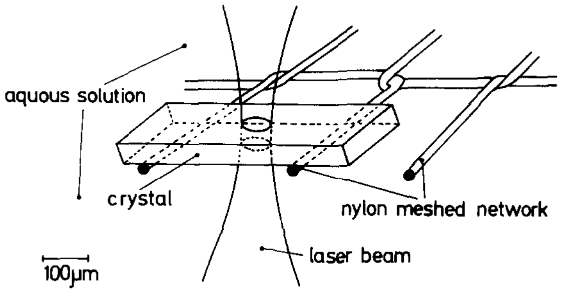

Fig. 3. Sketch of a methemoglobin single crystal lying on the meshed network and irradiated by a focussed laser beam.

check that the distances between the laser beam and the edges of the crystal, and between the laser beam and the nylon filaments are as great as possible.

Preparation of methemoglobin single crystals

Preparation of the oxyhaemoglobin from fresh horse blood followed the methods of Benesch et al. [12]. Oxidation of the haemoglobin to methemoglobin was achieved by the addition of a threefold amount of $K_3(Fe(CN)_6)$. The sample was desalted by being run through a column of Sephadex G-25. Single crystals were prepared by mixing the solution of methemoglobin with buffered ammonium sulphate solutions, according to the method of Perutz [13]. Optically clean crystals were selected from the preparations after two or three weeks and washed several times with 2.8 M $(NH_4)_2SO_4$ solution. The pH of this solution was adjusted to 7 using a phosphate buffer.

The extinction E_a of the crystals was determined as a function of crystal thickness d using plane polarized light with the electric vector parallel to the a-axis at a wavelength of 633 nm [14]. These measurements were taken for a range of d of between 100 and 300 μm, where the d values could be determined with a mean error of less than 1%. According to Lambert–Beer's law, the extinction E_a appears to be proportional to the crystal thickness d, yielding a coefficient $\Delta E_a/\Delta d = 8.00 \pm 0.10$ mm^{-1}. The thickness of crystals with $d < 100$ μm was then calculated from their E_a values.

THEORY AND DATA ANALYSIS

In the experimental conditions described above, the heat propagation is essentially one-dimensional, perpendicular to the plane given by the crystal axes a and b. The heat conduction equation for one dimension

$$D(x)\delta^2 T(x, t)/\delta x^2 - \delta T(x, t)/\delta t = e(x, t)\ D(x)/\beta(x) \qquad (1)$$

has to be solved. $D(x)$ is the thermal diffusivity, $T(x, t)$ the temperature in the sample coordinate x at time t, and $\beta\ (x)$ the heat conductivity. The term $e(x, t)$ represents the externally added heat energy density per time, and is thus the so-called source term in the heat conduction equation. The boundary conditions for the microwave pulse starting at $t = 0$ are

$$T(x, 0) = 0 \tag{2}$$

$$T(x, t) = 0 \qquad \text{for } t \geq 0, |x| \geq 100d \tag{3}$$

$$D(x) = D_{\mathrm{C}} \qquad \text{for } -d/2 \leq x \leq d/2 \tag{4}$$

$$D(x) = D_{\mathrm{M}} \qquad \text{for } |x| > d/2 \tag{5}$$

$$\begin{aligned} D(x)e(x, t)/\beta(x) &= T_{0\mathrm{C}}/t_{\mathrm{dur}} && \text{for } 0 \leq t \leq t_{\mathrm{dur}} \\ & && \text{and } -d/2 \leq x \leq d/2 \\ &= T_{0\mathrm{M}}/t_{\mathrm{dur}} && \text{for } 0 \leq t \leq t_{\mathrm{dur}} \text{ and } |x| > d/2 \\ &= 0 && \text{otherwise} \end{aligned} \tag{6}$$

where d is the thickness of the crystal, and the subscripts C and M of the term $D(x)$ indicate the different diffusivities of the crystal and the surrounding mother liquor. Here we are assuming that convection in the mother liquor does not contribute to heat transport in the time range under investigation. This assumption will be justified in the Results and Discussion section below. Boundary condition (2) takes into account the fact that the walls of the sample cavity are held at a constant temperature by a thermostat. Equation (6) considers finite pulse duration. $T_{0\mathrm{C}}/t_{\mathrm{dur}}$ and $T_{0\mathrm{M}}/t_{\mathrm{dur}}$ take into account the differential heating of the crystal and the mother liquor respectively, throughout the microwave pulse of duration t_{dur}.

The heat conduction equation (eqn. (1)) is solved numerically by the 'implicit' method, as described in standard textbooks [15,16]. This method will be sketched here only briefly, for the case of equally spaced points $x_{\mathrm{i}} = (i - 1)\Delta x$. The temperature values $T_{n,i} = T(x_i, t_n)$ at time t_n are given. The values $T_{\mathrm{n+1,i}} = T(x_{\mathrm{i}}, t_{n+1} = t_n + \Delta t)$ of the temperature distribution at a later time t_{n+1} are to be determined. Replacing the differential quotient by the quotient of finite differences gives, approximately,

$$\frac{D(x)(T_{i-1,n+1} - 2T_{i,n+1} + T_{i+1,n+1})}{(\Delta x)^2} - \frac{T_{i,n+1} - T_{i,n}}{\Delta t} + \frac{D(x)e(x, t)}{\beta(x)} = 0 \tag{8}$$

These are implicit equations for T_{n+1}. Iterative application to a given temperature distribution yields the function at any later time. A Fortran program for the numerical calculation of $T(x, t)$ and the averaged value

$$\langle T(t) \rangle = 1/d \int_{-d/2}^{+d/2} T(x,t)\,\mathrm{d}x \tag{9}$$

follows the procedure described above to calculate iteratively the temperature distribution at times $t > 0$ from that at $t = 0$ according to boundary conditions (2–6). A least-squares fitting program [17] varies the initially chosen values of T_0 and D_C iteratively until a minimum of the chi-squared value is obtained.

RESULTS AND DISCUSSION

The temperature profile of the sample cavity during and after the microwave pulse was investigated extensively. The sample cavity was filled with an aqueous solution of buffer and litmus. From the known temperature coefficient $\Delta E/\Delta T$ of this solution the time course of the temperature profile was determined using a laser beam of 0.1 mm diameter. The temperature relaxation of the solution in the centre of the cell to the temperature of the thermostatted cell walls has a half-life of about 5 s. No indications of convection have been detected where the temperature rise in the sample cavity resulting from the microwave pulse is less than 3 K.

A typical transient record of a temperature jump and the thermal relaxation of a horse methemoglobin single crystal is shown in Fig. 4. The time course shown results from the conversion of optical absorbance change to temperature change. Throughout the microwave pulse of 400 μs duration the average temperature $\langle T(t)\rangle$ of the crystals increases in an approximately linear way with time, reaching a maximum of about 2.4 K. After the pulse the average temperature of the crystal relaxes to the temperature of the surrounding mother liquor with a half-life of about 10 ms. The temperature rise in the mother liquor resulting from the microwave pulse can be estimated to be 0.8 K. The temperature relaxation of the mother liquor to the temperature of the thermostatted cell walls has a half-life of about 5 s, so

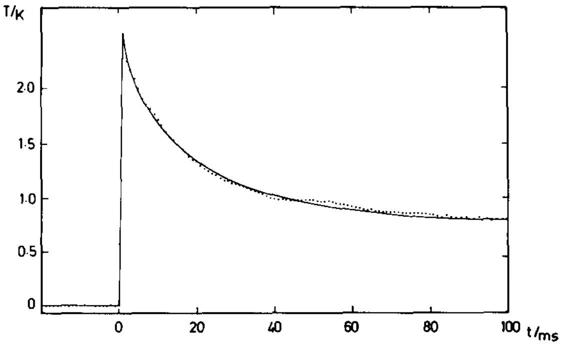

Fig. 4. A typical transient record of a temperature jump and the thermal relaxation of a horse methemoglobin single crystal of thickness $d = 103$ μm. The curves show the change of the average temperature $\langle T(t)\rangle$ according to eqn. (9): ·, experimental data points; ———, least squares fitting with $D_C = 0.22$ mm^2 s^{-1}.

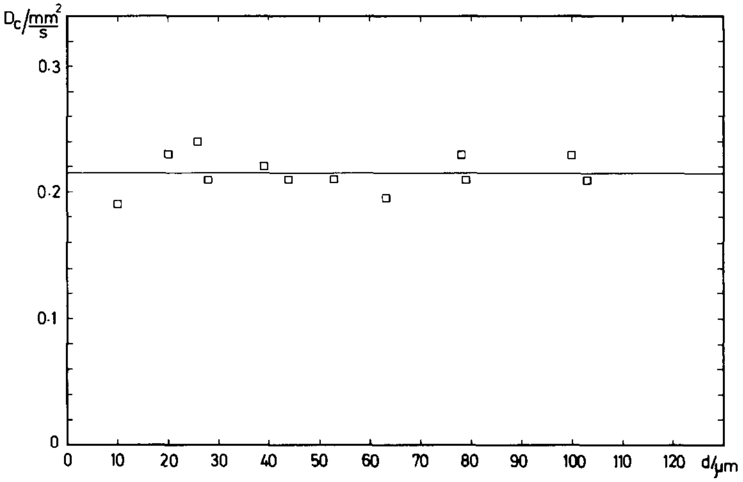

Fig. 5. The fitted values of D_C as a function of crystal thickness d.

this relaxation does not interfere with the temperature relaxation of the crystals. The fitted temperature course for the temperature relaxation of the crystals is also shown in Fig. 4. Excellent agreement is obtained between this calculated plot of $\langle T(t) \rangle$ and the experimental time course. The fitted value obtained for D_C for the crystal shown is 0.22 mm^2 s^{-1}. The value of D_M was set to 0.14 mm^2 s^{-1}. Crystals of different thickness in a range of 10–105 μm were investigated at $T = 293$ K. The observed relaxations of the samples yield half-lives of 0.5–10 ms. For crystals thicker than 100 μm the condition of a one-dimensional temperature diffusion may be violated because of an unfavourable ratio of crystal length to thickness. Figure 5 shows a plot of the determined values of D_C as a function of crystal thickness. No significant correlation of D_C with crystal thickness is observed, confirming that optical analysis of the temperature relaxation is not interfered with by further chemical relaxations changing the extinction coefficient of the hemoglobin in the time range under investigation. A mean value of D_C is calculated from the values shown in Fig. 5: $D_C = 0.215 \pm 0.015$ mm^2 s^{-1}.

CONCLUSION

Agreement between the calculated and the experimentally observed temperature relaxation is very good. No correlation of the determined values of D_C with crystal thickness was observed. These results indicate that the method presented here is applicable to determination of the thermal diffusivity of small thin samples. The thermal diffusivity of methemoglobin single crystals could therefore be determined for the first time. One field of application for this method should be determination of the thermal diffusivities of thin films. The required optical properties, similar to those of the methemoglobin crystals, could be obtained by means of thermal paint or similar thermal indicators.

ACKNOWLEDGEMENTS

Special thanks are due to Prof. Dr. A. Redhardt, who supported this work with numerous inspiring conversations. The assistance of R. Seyfarth and H. Lehky in the installation of the apparatus is gratefully acknowledged.

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