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DETERMINATION OF THE SPECIFIC HEAT CAPACITY OF HEMOGLOBIN– AND METHEMOGLOBIN–WATER MIXTURES USING AN ADIABATIC CALORIMETER*

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ABSTRACT

The specific heat capacity of bovine hemoglobin–, methemoglobin–, and thermally denatured hemoglobin–water mixtures were measured in the temperature range from 10 to 80°C. The partial specific heat capacities for mass fractions of the protein between 0 and 1 were computed. Significant differences of the partial quantities were obtained for the native, respectively denatured state of protein and for the protein in the native state in fluid mixtures, respectively in rather dry mixtures. For mixtures with protein mass fractions up to 0.45, exceeding the value in living human red cells, partial specific heat capacities of either components are found to be constant. The accuracy of the used adiabatic calorimeter will be described briefly.

I INTRODUCTION

In this laboratory a precise adiabatic micro-calorimeter has been developed[1–4] for measuring continuously the specific heat capacity of small liquid or solid samples in the temperature range from 10 to 80°C subject to an instrumental uncertainty of less than 6 mJ K^{-1} or about 0.3% for a typical sample mass of 0.6 g water. The precision and accuracy achieved are required in order to evaluate partial specific heat capacities of the components in a mixture of water with proteins and other components of biological interest. The values of the partial quantities should give some information about the interactions of protein with water, especially at high protein concentrations in the range of 20 to 40 wt.% resembling the mass fraction of proteins in the cytoplasm of living cells. From the partial specific heat capacity of water in such simple model systems, we hope to obtain information about the state of water in living cells[5, 6].

Micro-calorimeters for the same purpose but of somewhat different design were described by Privalov[7] and by Suurkuusk and Wadsö[8].

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II THE CALORIMETER ASSEMBLY[4]

The calorimeter—defined as that part of the apparatus in which heat is accurately accounted for—is a copper tube of 10 mm outer diameter and of 50 mm length. In the calorimeter a small glass ampoule with the sample was placed. The sample mass ranged between 0.3 and 1 g.

The temperature difference between the calorimeter and the adiabatic jacket was measured by an 8-junction thermopile. The adiabatic jacket temperature was measured by a quartz thermometer 2801 A of Hewlett-Packard using a Hewlett-Packard quartz probe 2850 A. The resolution of the digital thermometer amounted to $1 \cdot 10^{-5}$ K with a sample time of 100 s. Due to the presence of a second and third temperature-controlled jacket, it was possible to keep the amplitude of the temperature oscillations between calorimeter and adiabatic jacket below $3 \cdot 10^{-5}$ K. The difference between time and average temperature was smaller because of the rather symmetric temperature oscillations. The relative accuracy when determining a temperature interval of typical 0.2 K in 100 s, corresponding to a rate of temperature increase of 8 K per hour, was about $5 \cdot 10^{-4}$.

The potential difference at the heater and the heater current was measured by a classical potentiometer assembly of high precision instruments obtained from Leeds and Northrup. The relative determination accuracy of the electric power in the calorimeter heater was better than $2 \cdot 10^{-4}$.

The relative overall determination accuracy of the specific heat due to the determination uncertainty of electric power and temperature increase with time was better than $1 \cdot 10^{-3}$. The corrections of systematic errors introduced an uncertainty of the same order of magnitude. The experimentally determined overall accuracy of about 0.3% with a typical liquid sample of about 0.6 g is based on the known small irreproducibility of the charging and discharging procedure. All errors are conservatively stated. Details will be described in ref. 4.

In order to demonstrate the reproducibility and the accuracy of the calorimeter, two measurements from the specific heat capacity of about 0.6 g water samples in the temperature range between about 17 and 80°C are shown. The values deviate from the standard values[9] by about 0.1% (Fig. 1).

III RESULTS

Bovine oxyhemoglobin (BHb) was prepared from fresh blood and purified as described in ref. 10. Bovine methemoglobin (BMetHb) was prepared by lyophilizing BHb. Samples with different protein mass fraction w were filled into the glass ampoules mentioned above and introduced into the calorimeter. The protein mass fraction w as determined by dry mass determination with a relative uncertainty of a few permill.

Figure 2 shows the computer plot of a calorimeter scan of a 19.12 wt.% Hb–water mixture. In the temperature range of interest: between 10 and 50°C the data points were fitted by the function $c_p = a_0 + a_1 \cdot T + a_{-2} \cdot T^{-2}$, where T is the

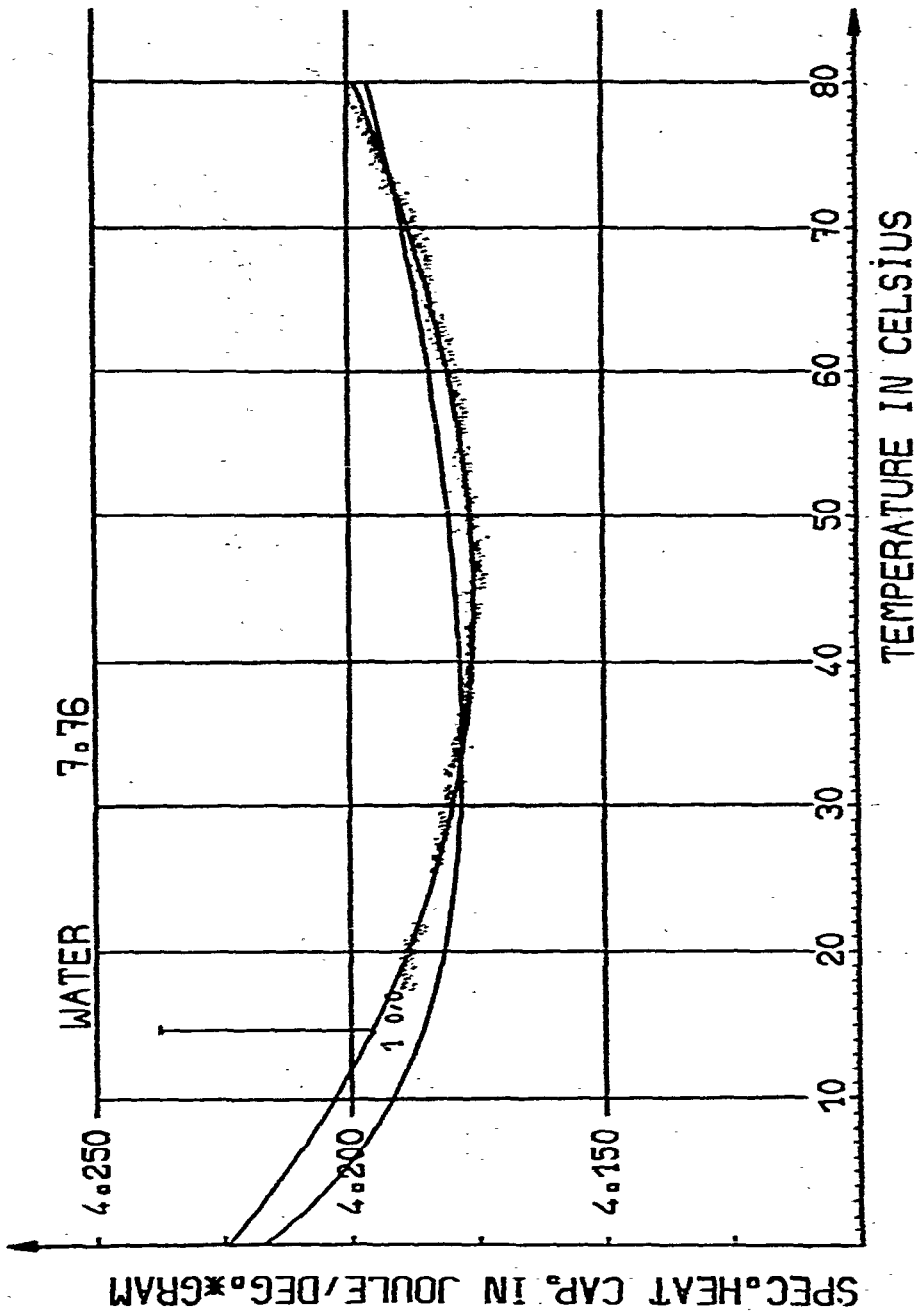

Fig. 1. Specific heat of water in J $g^{-1}K^{-1}$ vs. temperature in °C. Data points of two scans of the same sample are shown on the original computer plot after processing (Teletype output, punch tape, CDC 3300). The data points of the two scans deviate relatively less than $5 \cdot 10^{-4}$. They were fitted with the function $a_0 + a_1T + a_{-2}T^{-2}$, where $a_0 = 2.0656$ J $g^{-1}K^{-1}$, $a_1 = 4.4346 \cdot 10^{-3}$ J $g^{-1}K^{-2}$, $a_{-2} =$ 70743 J $g^{-1}K$. The second smooth curve gives the present standard values for c_p of pure water, according to Landolt–Börnstein Tables[9]. The largest difference between the two curves (at 17°C) is 0.2%. The mean deviation is less than 0.1%.

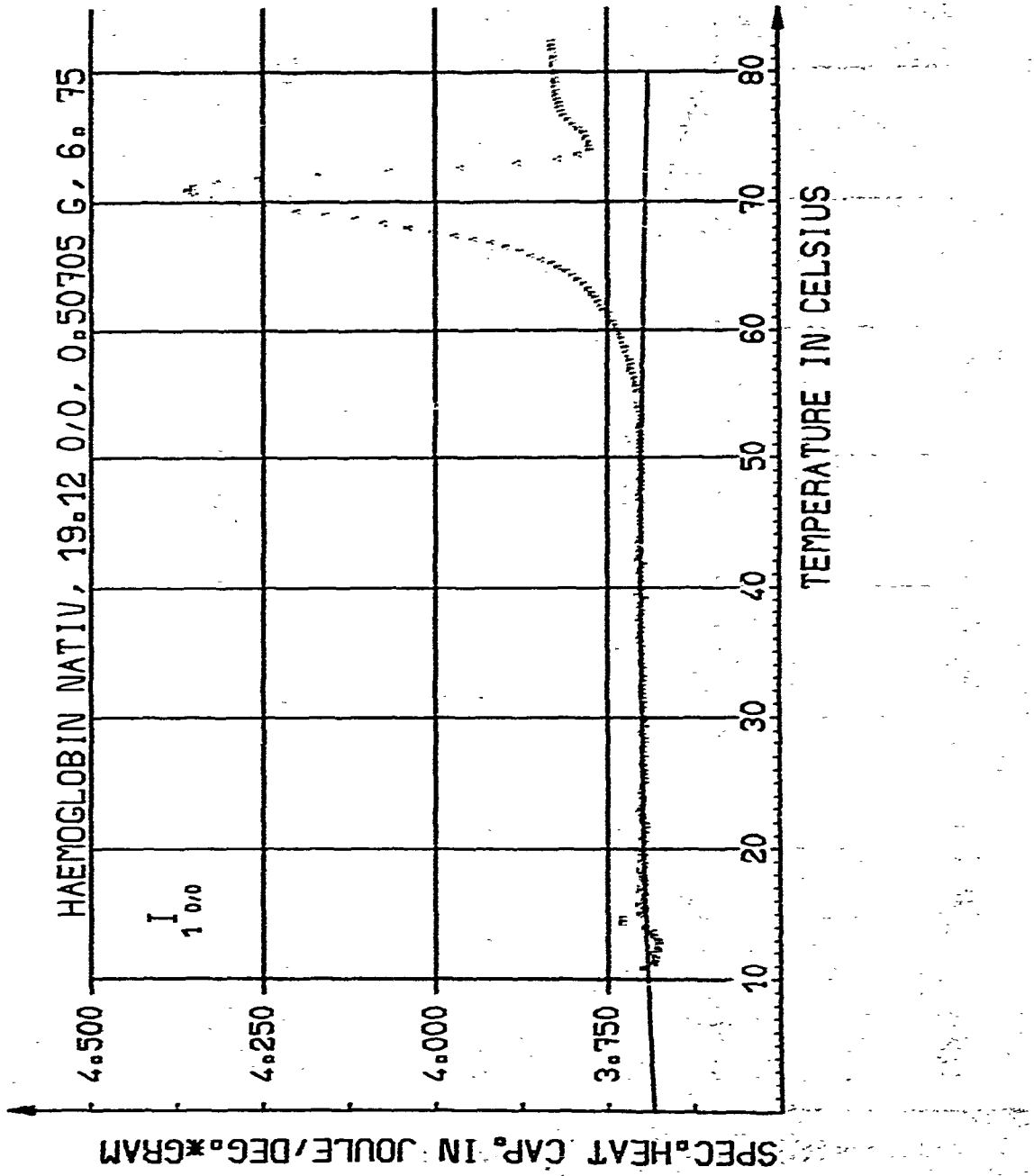

Fig. 2. Specific heat capacity in $J\,g^{-1}K^{-1}$ of a 19.12 wt.% bovine hemoglobin–water mixture versus the temperature in °C. The smooth curve corresponds to the function $c_p = a_0 + a_1T + a_{-2}T^{-2}$ fitting the data points between 10 and 50°C. Above 50°C denaturation heat becomes visible.

temperature in K and a_0, a_1, and a_{-2} are constants, depending on the mass fraction w of protein in the protein–water mixtures.

The partial specific heat capacity of water c_{p1} was obtained from the function $c_p(w, T)$ using the equation $c_{p1} = c_p - w(\partial c_p/\partial w)_{T,p}$, where $(\partial c_p/\partial w)_{T,p}$ is the slope

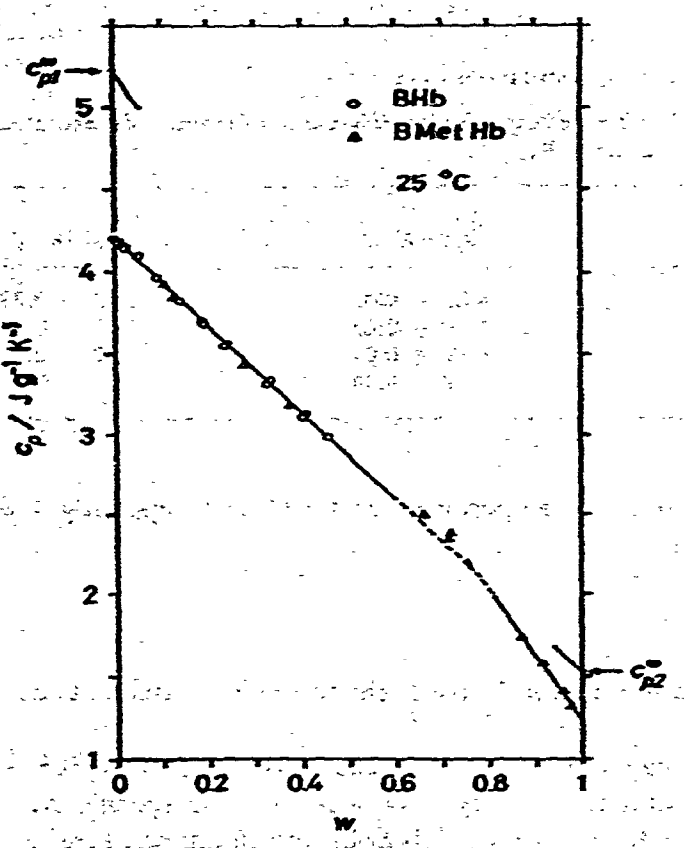

Fig. 3. Specific heat capacity c_p of BHb and BMetHb in J g^{-1}K^{-1} versus w at a temperature of 25°C. The smooth curve for $w < 0.5$ is a linear least square fit using the BHb data points. The straight line in the right range is the least square fit using the four BMetHb data points shown in the Figure. The partial specific heat capacity of water in the rather dry range ($w > 0.8$) c_{p1}^{∞} is indicated at the left ordinate scale, the partial specific heat capacity of BHb(and BMetHb) at "infinite dilution" c_{p2}^{∞} is indicated at the right ordinate scale.

of the function $c_p(w, T)$ at a specified and constant temperature and at constant pressure[11]. The partial specific heat capacity of the protein (BHb, BMetHb or thermally denatured BHb) c_{p2} was obtained from $c_p(w, T)$ using the equation $c_{p2} = c_p + (1 - w) \cdot (\partial c_p/\partial w)_{T,p}$ (ref. 11).

Figure 3 represents the data points for c_p of BHb and BMetHb–water mixtures at a temperature of 25°C versus the mass fraction w of protein from pure water ($w = 0$) to nearly dry protein ($w = 1$). It can be seen that there are two w-ranges, the "solution range" up to $w \approx 0.5$ and the "rather dry protein range" above $w = 0.8$. In the range $w > 0.6$, no BHb data points are experimentally obtainable, because BHb was not stable in this rather dry state. In the intermediate range $0.5 \leq w \leq 0.8$ (the range between the liquid mixture and the hydrated protein powder) the c_p values are less reproducible, but are always located above the two straight lines.

In Table 1, the values for the partial quantities at a temperature of 25°C are given. The table includes values for mixtures of heat denatured BHb, obtained by

TABLE 1

PARTIAL SPECIFIC HEAT CAPACITIES OF WATER c_{p1} AND PROTEIN c_{p2}

BHb = Bovine hemoglobin; BMetHb = Bovine methemoglobin; BHb den. = thermally denaturated BHb. The errors indicated are twice residual errors[12].

Component	*Mass fraction*	c_{p1} $(J\,g^{-1}K^{-1})$	c_{p2} $(J\,g^{-1}K^{-1})$
BHb	$w < 0.5$	4.20 ± 0.01	1.53 ± 0.03
BMetHb	$w < 0.5$	4.19 ± 0.02	1.47 ± 0.08
BHb den.	$w < 0.5$	4.19 ± 0.01	1.82 ± 0.03
BMetHb	$w > 0.8$	5.32 ± 0.34	1.24 ± 0.03

scanning a BHb–water mixture up to a temperature of 80°C and repeating the scanning procedure.

IV DISCUSSION

From the graph in Fig. 3 and the data in Table 1, the following statements can be made:

(1) The partial specific heat capacity of water in the range $0 \leq w < 0.5$ is constant and, within the experimental uncertainty, identical with the specific heat capacity of pure water of 4.179 J g^{-1} K^{-1} at 25°C. This holds for all temperatures up to at least 50°C.

(2) The value 5.32 J g^{-1} K^{-1} at 25°C of the partial specific heat capacities of water in the rather dry BMetHb powder ($0.8 < w < 1$) exceeds that in the range $0 \leq w < 0.5$ by 27%. This finding is in good agreement with the literature[1, 13, 14] and reflects a structure change of the water in the vicinity of the protein surface as a result of hydrophobic interaction[15].

(3) Corresponding to statement 2, the partial specific heat capacity of BMetHb in the left linear range ($0 \leq w < 0.5$) c_{p2}^{∞} is larger than the (extrapolated) value c_{p2}° of 1.24 J g^{-1} K^{-1} of the pure dry methemoglobin by about 19%.

(4) There is no significant difference in c_p of the BHb and BMetHb solutions at the same protein mass fraction w.

(5) c_{p2}^{∞} for heat denatured hemoglobin significantly surmounts the value for hemoglobin in the native state by about 19%. This increase can probably be interpreted as a result of the exposure of new hydrophobic protein surface in the process of denaturation.

Conclusion: Even very high concentrated protein solutions with protein mass fractions exceeding the physiological range of the protein concentration in erythrocytes behave like a pseudo-ideal mixture in the sense, that only the partial quantities of the solvent are identical with the corresponding specific properties of the solvent in the pure state.

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